Physiological Entomology (1997) **22**, 316–324

Helicoverpa zea males (Lepidoptera: Noctuidae) respond to the intermittent fine structure of their sex pheromone plume and an antagonist in a flight tunnel

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Abstract. We investigated the behavioural response of male *Helicoverpa zea* (Boddie) to the fine-scale structure of an odour plume experimentally modified in a wind tunnel by using an air-pulsing device. Male *H. zea* flew upwind to pulsed filaments of a binary pheromone blend of (*Z*)-11-hexadecanal (Z11–16:Ald) and (*Z*)-9-hexadecanal (Z9–16:Ald) in the ratio of 20:1. Sustained upwind flight in experimentally altered intermittent plumes was dependent on concentration, as well as the frequency of generation of odour filaments. At a loading of 10 μg of the major pheromone component, Z11–16:Ald, which gave an emission rate of approximately that released by a female *H. zea*, sustained upwind flight and source contact correlated positively with filament delivery rate, becoming significant at a minimum filament delivery rate of 2/s. Decreases in upwind progress and source location were recorded at a loading of 1 μg of Z11–16:Ald. At this suboptimal dosage, a high filament generation rate of 10/s was necessary for significant upwind progress and source contact. When an interspecific compound: (*Z*)-11-hexadecenyl acetate (Z11–16:OAc), was added to the attractive pheromone binary aldehyde blend of *H. zea* at a proportion of 10% of the major pheromone component, and pulsed from the same source, there was a significant reduction in sustained upwind progress and source location by males, indicating that Z11–16:OAc is antagonistic to the upwind progress of *H. zea*. However, Z11–16:OAc was less antagonistic when its filaments were isolated and alternated with pheromone filaments, indicating a strong effect of the synchronous arrival of odour filaments on the antenna needed for antagonism of upwind flight.

Key words. Corn earworm, *Helicoverpa zea*, sex pheromone, filament, antagonist, inhibitor, flight behaviour, flight tunnel.

Introduction

Due to turbulence and eddies at the source, an odour emanating from a point source is not homogeneous, but instead consists of pockets of clean air intermixed with strands of odour-laden air known as filaments (Wright, 1958; Murlis & Jones, 1981). This intermittent structure is maintained far downwind of the source of an odour plume (Murlis & Jones, 1981; Baker & Haynes, 1989; Vickers & Baker, 1992), and has been shown to be critical to the successful location of an odour source upwind of a male moth (Willis & Baker, 1984; Vickers & Baker, 1992, 1994, 1996; Mafra-Neto & Cardé, 1994).

The importance of the filamentous structure of a pheromone plume to behaviour is well-accounted-for in two models of orientation proposed by Baker (1990) and by Kaissling & Kramer (1990). In these quite similar models, an orienting male moth is hypothesized to respond to individual filaments of odour, as well as to each pocket of clean air. Contact with a filament would suppress the visible expression of the counter-turning component (zigzagging) while causing an upwind surge, whereas each pocket of clean air would cause upwind flight to cease and counter-turning to be expressed, ultimately changing the flight track into crosswind casting flight after a more prolonged exposure to clean air. The behavioural

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responses predicted by these models were observed in experiments by using two species of moth, *Heliothis virescens* (Vickers & Baker, 1994) and *Cadra cautella* (Mafra-Neto & Cardé, 1994). Males of both species surge upwind in response to contact with single filaments of pheromone, and more frequent contact with rapidly generated filaments causes straighter sustained upwind flight tracks with little counter-turning-based zigzagging flight. The two models differ slightly in that the Baker (1990) model interprets straight upwind flight as being due to an excessively high frequency of counter-turning that makes the expression of counter-turning during flight (zigzagging) impossible to observe. Kaissling & Kramer (1990) propose that during the surge, counter-turning itself is inhibited. Further research will be needed to resolve these two responses.

Other studies have revealed that the shapes of flight tracks of males of different species are dictated by each species' latency of behavioural response to the interception of pheromone filaments and clean air (Baker & Haynes, 1989; Vickers & Baker, 1996). In male moths with shorter response latencies (e.g. *Grapholita molesta*), the counter-turning component will inevitably be expressed in the abundant clean air between filaments, and upwind flight will nearly always have a zigzag appearance due to this expression of casting. At similar filament generation frequencies, males of species with longer response times, on the other hand, will travel a straighter path upwind because the next filament will arrive before casting flight can be expressed. This latency of response to filaments will undoubtedly determine the minimum pulse rate that can sustain upwind progress in different moths in experimentally generated plumes. It follows, therefore, that moths with longer response latencies will need lower pulse rates to sustain significant upwind progress than moths with shorter response latencies.

Vickers & Baker (1992) recorded for male *H. virescens*, a close relative of *Helicoverpa zea* (Boddie), a mimimum pulse generation rate of 4 filaments/s for the maintenance of upwind flight in significant numbers, suggesting that their latency of response should be ≈ 0.25 s. Indeed, this latency value was later confirmed in another study in which it averaged 0.27 s (Vickers & Baker, 1994, 1996). Vickers & Baker (1992) also demonstrated the importance of the simultaneous arrival of a complete pheromone blend on the antenna for optimal response of sustained upwind flight. They recorded a significant reduction in upwind flight of male *H. virescens* when a behaviourally important component, (*Z*)-9-tetradecanal (Z9–14:Ald), was isolated into its own filaments and pulsed alternately in a staggered fashion against filaments of the remaining five components.

In the current study an odour plume consisting mainly of *H. zea* binary sex pheromone blend (Z11–16:Ald + 5% Z9–16:Ald) was experimentally generated in the wind tunnel and altered with respect to filament generation rate, concentration and composition. The change in composition consisted of tainting the pheromone filaments with Z11–16:OAc. Although Z11–16:OAc is not known to be produced by female *H. zea*, receptors tuned to it have been located on the antennae of *H. zea* males (J. L. Todd *et al.*, personal communication), and interneurones in *H. zea* male antennal lobes respond specifically to this compound (N. J. Vickers & T. A. Christensen, personal communication). These findings suggest that Z11–16:OAc might be important in the behavioural response of males of the species. The sympatric heliothine species *Heliothis subflexa* (Teal *et al.*, 1981; Klun *et al.*, 1982) as well as some noctuid moth species of related genera (Underhill *et al.*, 1977; Cork *et al.*, 1992) use Z11–16:OAc as a pheromone component. This acetate also has been isolated from the hairpencils of male *H. virescens* (Teal & Tumlinson, 1989).

Materials and Methods

Moths

A modified pinto bean diet was used to rear *H. zea* larvae in the laboratory (Shorey & Hale, 1965). Insects were sexed at the pupal stage after which the males were held in a separate environmental chamber from females in a LD 14:10 h light/dark cycle at 25°C and 55 ± 5% r.h. Upon emergence, adult males were supplied with a 10% sugar solution *ad libitum*. Adult males aged 2–5 days were used for the flight bioassays. Approximately 1 h before a daily flight test, individual males were placed under red light into 6 × 6 cm wire screen cages. The cages were held in plastic trays and transferred into the wind tunnel to allow for acclimatization of the moths to the flight tunnel conditions. Flight bioassays were conducted between the fifth and eighth hour of scotophase. A male was scored only once and then discarded.

Flight tunnel and filament generation

The wind tunnel apparatus was 2.4 × 1 × 1 m and modified after Miller & Roelofs (1978). Moths were flown under a mixture of red and white light (measuring about 0.5 lux) at a wind speed and temperature of 50 cm/s and 25°C, respectively. Males were released individually at a height of 23 cm above the floor, 170 cm downwind of the pheromone source. Each male was held in the plume such that it received at least thirty pheromone filaments before release, and was then allowed 2 min to take off from its cage.

Odour filaments were generated using a mechanical pulsing device known as stimulus-flow controller or puffer (Syntech, The Netherlands) described in Vickers & Baker (1992). The flow rate and pulse duration were held constant at 5 ml/s and 0.02 s, respectively. Pipettes containing odour sources were held in a holding device within the tunnel with the tip of each pipette pointing upward. The holding device could hold one or more pipettes at the same time and the distance between two pipettes could be adjusted. Visualization of the filaments generated by the puffer using smoke plumes of $TiCl_4$ suggests that filaments tended to remain as separate entities during their passage through the wind tunnel at all tested filament generation rates.

Semiochemicals

Of the five known constituents of the sex pheromone gland or gland emission of *H. zea*, only two, Z11–16:Ald (the major component) and Z9–16:Ald, are necessary for sustained upwind flight of males (Vetter & Baker, 1984). The attractive pheromone blend used in this study consisted of a binary mixture of Z11–16:Ald and Z9–16:Ald formulated in a stock solution in the ratio of 20:1 and diluted serially in 10-fold increments. Ten microlitres of either a 1 μg/μl or 0.1 μg/μl solution was applied evenly over the surface of square-ending filter paper using a micropipette and, having allowed for the evaporation of the hexane solvent, placed inside a glass pasteur pipette. Where necessary, filter papers containing 10 μl of hexane were used as hexane-solvent controls. The final loading dosage of the major component was either 10 μg or 1 μg. In addition to this attractive binary blend, two three-component blends containing 1% or 10% Z11–16:OAc relative to the major pheromone component also were tested. All chemical compounds were made from neat materials maintained in our laboratory and each was found to be >98% pure by capillary gas chromatography (GC). All tested blends were checked on the GC for correct concentration and ratio.

Effect of pulse generation rate

To investigate the frequency of filaments required to sustain upwind flight in *H. zea*, males were flown to a 10-μg loading of the binary sex pheromone blend (Z11–16:Ald and Z9–16:Ald in the ratio of 20:1) at five different filament generation regimes: 1, 2, 4, 5 and 10 filaments/s. In this and the subsequent experiments, the puffer was set to produce multiple pulses over a period of time and the measured behavioural parameters included upwind flight (at the level of plume), orienting moths reaching midway (85 cm) to the source or nearing the source (15 cm to source), as well as the number of moths reaching the source. To check for contamination and leakage, males were released in the presence of a hexane control at 5 filaments/s pulse rate and a binary blend source with no air pulses being generated, respectively. At least thirty-six males were released to each treatment. Significant differences within behavioural categories in this and the subsequent experiments were established using a χ^2 2 × 2 test of independence with Yates' correction of continuity (Parker, 1979).

Effect of pheromone loading

The emission rate of 10 μg of Z11–16:Ald loading, 2.2 pg/pulse (A. Cossé, H. Fadamiro & T. Baker, unpublished data), was approximately that which is emitted by the forcibly extruded sex pheromone gland of a *H. zea* female, 0.42 ng/min (Pope *et al.*, 1984). At this loading, significant upwind progress was recorded, even at 2/s (see Results). To test if the optimal pulse rate was dependent on pheromone loading, we compared the upwind progress to 10-μg and 1-μg sources at different pulse rates. The emission rate of 1 μg of Z11–16:Ald loading was approximately 50-fold less than the emission rate of the 10 μg loading (A. Cossé, H. Fadamiro & T. Baker, unpublished data).

Effect of addition of Z11–16:OAc

To investigate the effect on upwind flight of the addition of Z11–16:OAc to the binary blend (10 μg Z11–16:Ald loading), a 3 × 3 randomized block design experiment was carried out. Male *H. zea* were released to three different odour blends: binary, binary plus 1% Z11–16:OAc and binary plus 10% Z11–16:OAc, at three pulse generation rates of 1, 2 and 5 filaments/s. Twenty-five males were released for any particular treatment.

Effect of temporal partitioning of pheromone and Z11–16:OAc filaments

Using an optimum pulse rate as well as an antagonistic level of Z11–16:OAc established in the third experiment, we proceeded to investigate the effects on upwind progress of separating out the binary mixture and the antagonist, and pulsing them alternately. For this purpose, three treatments were compared: pheromone filaments alone generated at 5 filaments/s; pheromone filaments generated at 5 filaments/s, alternated with 10% Z11–16:OAc filaments also generated at 5 filaments/s; and filaments containing both pheromone blend and 10% Z11–16:OAc generated from the same pipette at 5 filaments/s.

All treatments were generated using the dual channel puffer in the 'alternate' mode at a frequency of 10 filaments/s, such that each of two pipettes emitted filaments at a frequency of 5/s in a staggered fashion. In the first and third treatments, the second pipette contained only 10 μl of hexane, and thus a blank pipette was paired with the odour-containing pipette source. Two pipette sources in a treatment were held at the same height, but 1 cm apart along the wind line. Using this set-up, there was a *c.* 4 or 6 cm clean air gap between the two different filaments and a 10 cm clean air gap between two successive filaments of the same kind.

Results

Effect of pulse rate

Significant differences in response were recorded for moths exposed to filaments from the 10-μg source generated at different frequencies (Fig. 1). These differences occurred mainly in response to the filament delivery rate of 1 filament/s compared with each of the other pulse regimens. Males exposed to a filament delivery rate of 1 filament/s did not sustain upwind flight or locate the source in great numbers. Significant upwind response was recorded at a filament generation rate of 2 filaments/s. Further increases in upwind progress were recorded at higher filament delivery rates of 4,

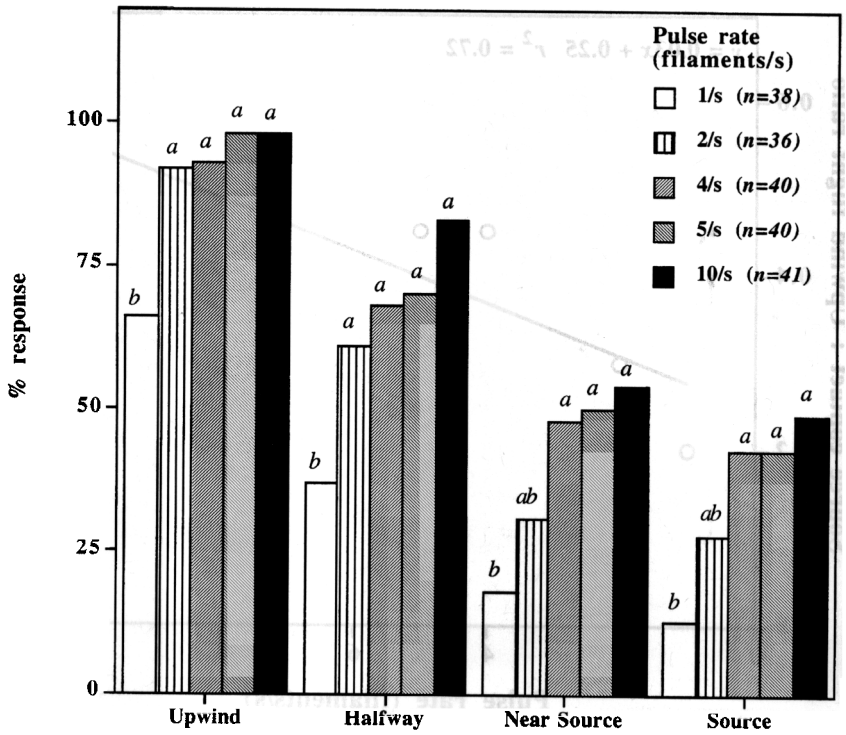

Fig. 1. Response of male *Helicoverpa zea* to pheromone filaments generated at different pulse regimens. Pheromone was 10 μg Z11–16:Ald + 5% Z9–16:Ald. Bars in the same behavioural category (*x*-axis) having no letters in common are significantly different at $P < 0.05$.

5 and 10 filaments/s, but these numbers were not significantly greater than at 2 filaments/s. Behavioural observations and data analysis showed that although males exposed to a pulse delivery rate of 1 and 2 filaments/s successfully locked-on to the plume and headed upwind, they soon began casting approximately midway to the source. A comparison of the proportion of males initiating upwind flight that made it to the source at the different pulse rates (source contact/upwind flight) revealed that a greater proportion of such males did not reach the source at the lower pulse rates compared with the higher (Fig. 2). Of the moths that headed upwind to a 1 filament/s pulse rate, only 20% made it to the source, compared with 30% and 50% at 2 filaments/s or 10 filaments/s, respectively. No significant levels of upwind flight (0% and 0%) and source contact (0% and 4%) were recorded to the hexane-pulse ($n = 20$) and pheromone-no-pulse ($n = 23$) controls, respectively, indicating minimal leakage from, or contamination of, the pipette tips.

Effect of pheromone loading

There was a marked effect of concentration on the response of males to pheromone filaments pulsed at different rates (Fig. 3). Although upwind flight (80%) and source contact (33%) occurred in response to the 10-μg pheromone loading delivered at 2 filaments/s, a much higher pulse rate of 10 filaments/s was necessary for comparable upwind flight (60%) and source contact (30%) in response to the 1-μg pheromone loading. A decrease in upwind flight (33%) and source contact (3%) was recorded in response to the filaments of the 1-μg pheromone loading generated at 2 filaments/s.

Effect of addition of Z11–16:OAc on response

At the optimal filament delivery rates of 2 filaments/s or 5 filaments/s, male *H. zea* flew upwind to point-source plumes of a binary blend of the sex pheromone produced by the female. At both the 2/s or 5/s filament generation rates, the addition of 10% Z11–16:OAc (relative to the dosage of Z11–16:Ald) resulted in significant reductions in the proportion of males flying towards, or landing on, the source compared with binary blends lacking Z11–16:OAc (Table 1). Blends containing 1% Z11–16:OAc, however, resulted in a slight but generally insignificant reduction in response. At a suboptimal filament delivery rate of 1 filament/s, the proportion of males exhibiting upwind flight to the odour blend containing 10% Z11–16:OAc was significantly lower (28%) than that to the blend containing 1% Z11–16:OAc (72%) or 0% Z11–16:OAc (76%). However, no significant differences were recorded in 'near source' responses and source contact to the three treatment blends at this suboptimal filament delivery rate.

Effect of temporal partitioning of Z11–16:OAc filaments

When Z11–16:OAc was blended with pheromone at 10% of Z11–16:Ald dosage and pulsed from the same source, males

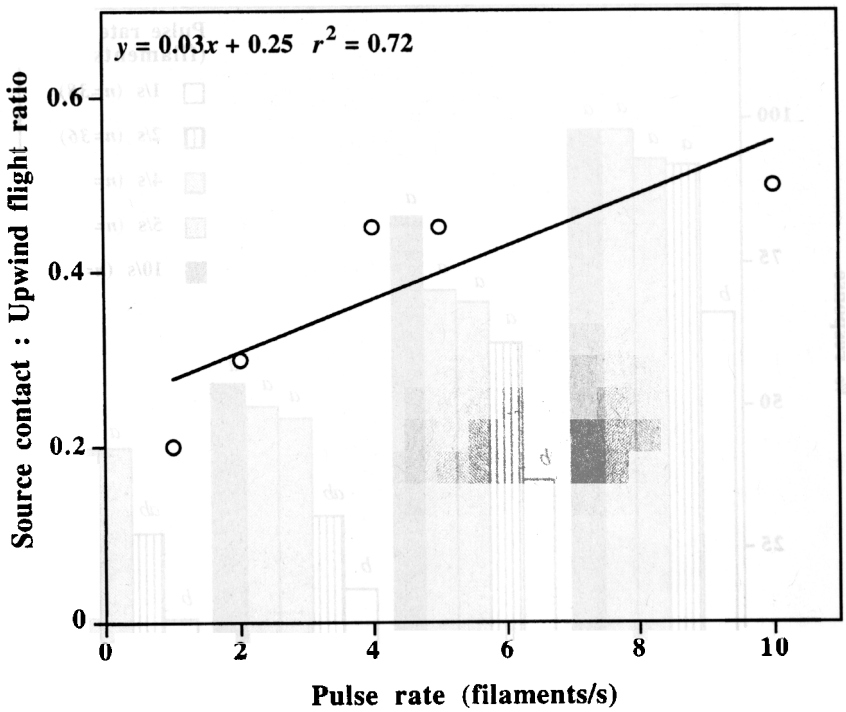

Fig. 2. Effect of filament generation rate on the tendency of flying moths to deviate from the odour plume. The significant linear relationship between the proportion of upwind bound males that made it to the source and filament generation frequency is an indication of increased loss of plume upwind by males flying at the lower pulse regimens.

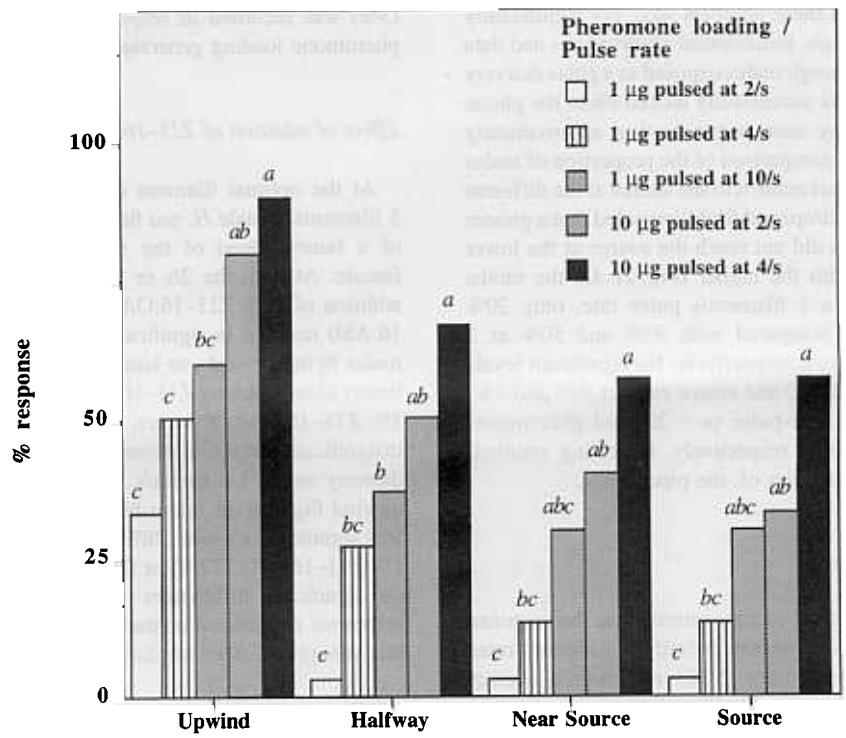

Fig. 3. Effect of concentration on the response of male *H. zea* to pheromone filaments generated at different frequencies. Pheromone emitted per pulse from the 10-µg loading was approximately equal to that emitted by a female *H. zea*. A total of thirty moths was released in each treatment. Bars in the same behavioural category (*x*-axis) having no letters in common are significantly different at $P < 0.05$.

Table 1. Response of male *H. zea* to pheromone filaments containing varying amounts of Z11–16:OAc (0–10%) at different pulse regimens. The table shows the percentage of males exhibiting the important behavioural categories to odour blends made up of a mixture of 10 μg Z11–16:Ald + 5% Z9–16:Ald and different amounts of Z11–16:OAc relative to Z11–16:Ald. A total of twenty-five males was flown in any particular treatment. Percentages in the same column having no letters in common are significantly different at $P < 0.05$.

Blend composition (% of Z11–16:OAc)	Number of filaments generated/s	Upwind flight (%)	Midway to source (%)	Near source (%)	Source contact (%)
0	1	76 ^{ab}	36 ^{bc}	24 ^{bc}	16 ^{c}
0	2	80 ^{ab}	68 ^{ab}	64 ^{a}	56 ^{ab}
0	5	96 ^{a}	84 ^{a}	76 ^{a}	64 ^{a}
	1	72 ^{ab}	40 ^{bc}	32 ^{bc}	28 ^{bc}
	2	72 ^{ab}	40 ^{bc}	32 ^{bc}	28 ^{bc}
	5	92 ^{a}	80 ^{a}	56 ^{ab}	48 ^{ab}
10	1	28 ^{c}	12 ^{c}	4 ^{c}	4 ^{c}
10	2	60 ^{bc}	32 ^{c}	20 ^{c}	20 ^{c}
10	5	48 ^{bc}	32 ^{c}	12 ^{c}	12 ^{c}

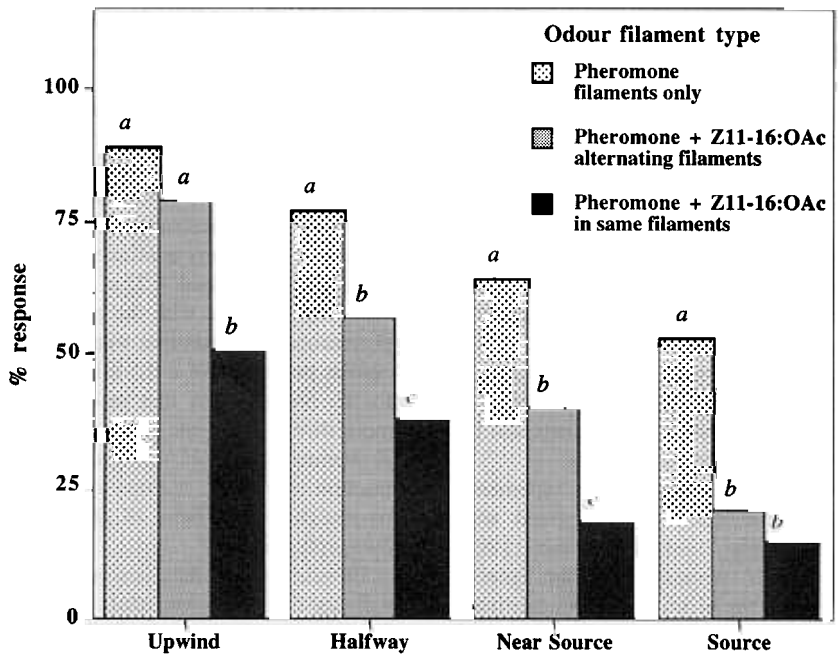

Fig. 4. Effect of temporal partitioning of antagonist (10% Z11–16:OAc) filaments on the response of *H. zea* to its pheromone filaments (10 μg Z11–16:Ald + 5% Z9–16:Ald). A total of forty-seven males was released for each odour treatment at the 5 filaments/s pulse regimen. Bars in the same behavioural category (*x*-axis) having no letters in common are significantly different at $P < 0.05$.

exhibited significantly reduced upwind flight (51%), sustained upwind progress midway to source (38%) and source contact (15%). However, when filaments of Z11–16:OAc were isolated and alternated with pheromone filaments, separated in time by *c.* 0.1 s, significantly higher upwind flight levels (79%) and upwind progress midway to source (57%) were recorded in this staggered regimen compared with when the Z11–16:OAc was emitted from the same pipette with the pheromone. When alternated with pheromone, however, Z11–16:OAc significantly reduced source contact (21%), compared with the higher responses to the pheromone filaments alone: upwind flight (89%), progress midway to source (77%) and source contact (53%) (Fig. 4).

Discussion

The addition of 10% Z11–16:OAc relative to Z11–16:Ald, the major component to the pheromone blend of *H. zea*, significantly reduced upwind flight and source location by males. This indicates that Z11–16:OAc is a behavioural antagonist of the upwind flight pheromone response of male *H. zea*. The antagonistic effects on the upwind flight of male moths of certain compounds, when added to the pheromone blend of a given species, have been reported previously, although little is known about the mechanism of antagonism of upwind flight. Vickers & Baker (1997) recently reported the antagonistic effect of Z11–16:OAc on the upwind flight of

male *H. virescens*. They recorded stunted upwind surges and more casting for males intercepting single filaments of pheromone containing the antagonist, Z11–16:OAc at levels of either 1% or 10% of the major pheromone component. Z11–16:OAc constitutes 15–25% of the total composition of the pheromone of *H. subflexa* (Teal *et al.*, 1981; Klun *et al.*, 1982), which is the only sympatric heliothine species of *H. zea* and *H. virescens* in North America known to produce the acetate as a component of its sex pheromone. This suggests that this acetate may be playing an important role in the prevention of interspecific attraction in these heliothine species. Teal & Tumlinson (1989) also identified Z11–16:OAc from the hairpencil extract of male *H. virescens*, but no information is yet available on its behavioural effects. We are not aware of any similar studies on the identification of compounds from the hairpencils of male *H. zea*.

At the neurophysiological level, receptor neurones and interneurones tuned to antagonistic compounds have been identified in males of different species. Almaas & Mustaparta (1990) and Berg *et al.* (1995) identified receptors on the antennae of male *H. virescens* that are highly responsive to both Z11–16:OH and Z11–16:OAc, two known antagonists of pheromone-mediated upwind flight in this species (Hartstack *et al.*, 1980; Vickers & Baker, 1997). A.A. Cossé *et al.* (personal communication) recently recorded from three sensillar types on the antenna of male *H. zea*, one of which contains a neurone tuned to the behaviourally antagonistic compounds, Z11–16:OH (Teal *et al.*, 1984), Z9–14:Ald (Vickers *et al.*, 1991) and Z11–16:OAc (present study). Using an activity-specific cobalt-lysine neuroanatomical staining technique, J. L. Todd *et al.* (personal communication) showed that this antagonist-tuned neurone projected into a compartment of the macroglomerular complex (MGC) that was not innervated by neurones tuned to the two pheromone components.

The poorer suppression of upwind flight in the experiment in which the Z11–16:OAc filaments were staggered with pheromone filaments is intriguing. Vickers & Baker (1992) recorded a similar result for *H. virescens* with the agonist, Z9–14:Ald, and concluded that the blend of components must arrive on the antenna simultaneously for optimal evocation of upwind flight. Liu & Haynes (1992), using continuous point-source plumes, recorded a poorer suppression of upwind flight of *Trichoplusia ni* when a known antagonist, Z7–12:OH was released from a separate point source placed 5 cm crosswind on both sides of a pheromone source or 10 cm upwind of the source, compared with when the antagonist and the pheromone were released from the same source. The results of the present study, in which we alternated filaments of pheromone with antagonist filaments, is striking in that the distance between two filaments was as close as 5 cm, and temporally separated in arrival (on the antennae) by only *c.* 0.1 s (if the male was stationary, but faster still if the male was flying upwind), and yet males could still discriminate between the two different types of filament arrays. Projection interneurones in the antennal lobe that are selective to different pheromone blends have been reported for some moths, including *Manduca sexta* (Christensen & Hilderbrand, 1988). It would seem plausible that a type of these blend-specific neurones, which relies on the synchronous arrival of pheromone blend and antagonist (if present in *H. zea*), is likely to mediate the antagonism of upwind flight. It will be of interest to identify a resolution threshold where two different filaments could be perceived as one unit of information by a male. Questions relating to the thresholds of temporal or spatial resolution of filaments by a male as a single unit of information could ultimately be addressed at the olfactory level.

In the current study, both the filament generation rate and the concentration of pheromone-containing filaments influenced the response of male *H. zea*. At an optimal pheromone loading of 10 μg, which gave a release rate (7–16 pg of Z11–16:Ald/pulse) approximating to that emitted by a female *H. zea* (0.42 ng/min of Z11–16:Ald; Pope *et al.*, 1984), a significant response was recorded when pheromone filaments were delivered at a frequency of 2/s. Using the data obtained with the binary pheromone blend at 10 μg, we predict a 0.5 s latency of response to interception of pheromone filament or clean air for male *H. zea*. Of the few moths (e.g. *G. molesta*, Baker & Haynes, 1989 and *H. virescens*, Vickers & Baker, 1996) for which latencies of response to interception of pheromone filaments and clean air have been measured, only *H. virescens* was flown to a similar point-source pheromone plume generated by a pulsing device. For this moth, a minimum pulse rate of 4 filaments/s was recorded for significant sustained upwind flight to its sex pheromone (Vickers & Baker, 1992). Vickers & Baker (1996) later confirmed that its latency of response to clean air was indeed 0.27 s. Although a direct comparison could not be made with the present results because different pheromone concentrations were used in both experiments, our current prediction of a 0.5-s latency of response to clean air for *H. zea* indicates that male *H. zea* respond significantly more slowly than male *H. virescens* to the onset and offset of pheromone odour. If this prediction holds true, the slower reaction of male *H. zea* may mean that their flight tracks will be straighter than those of *H. virescens* at a given filament interception rate.

However, the marked upwind flight recorded at the filament delivery rate of 2 filaments/s should not be overemphasized. The increase in response at higher pulse rates and the dramatic reduction in response at a lower pulse rate of 1 filament/s suggest that the pulse rate of 2 filaments/s was at the borderline. Furthermore, the significant linear increase in the ratio of source contact to upwind flight with increasing rates of filament delivery indicates a significantly increased loss of plume midway to source by males flying in response to the lower pulse rates of 1 filament/s and 2 filaments/s. Less concentrated filaments generated at the 1-μg pheromone loading produced an almost negligible response at 2 filaments/s, and at this suboptimal loading a high pulse rate of 10 filaments/s was necessary to record responses comparable with those when the 10-μg source was pulsed at 2 filaments/s. These results are not necessarily surprising because the release rate of the 1 μg Z11–16:Ald loading was about 50-fold less then the pheromone emitted by a female. The highest response was recorded at the highest filament generation rate of 10 filaments/s, and indicates that the male's antennae are capable of disadapting fast enough to respond to repeated pulses at this rate. There is evidence

that males' peripheral receptor cells and central projection interneurones are capable of responding to rapidly delivered pulses. For instance, Almaas *et al.* (1991) demonstrated that *H. zea* male receptor neurones could follow 20-ms pulses delivered at up to 9.8 Hz.

The results of the current study show that male *H. zea* are capable of sustaining upwind flight in response to multiple filaments of odour, experimentally pulsed to mimic the temporal fine structure of a natural plume similar to that which has been carried out with *H. virescens* (Vickers & Baker, 1992, 1994, 1996). Investigations into the possible response of male *H. zea* to single pheromone filaments are necessary to confirm our current prediction of a 0.5 s latency of response to pheromone or clean air for this species.

Acknowledgements

We thank Allard A. Cossé and Julie L. Todd for technical assistance and comments, and Paul Shaputis for maintaining moth colonies. Funding was provided by USDA NRI competitive research grant 95–37302–1805 awarded to T.C.B.

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Accepted 26 June 1997