

ORIGINAL PAPER

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Latencies of behavioral response to interception of filaments of sex pheromone and clean air influence flight track shape in *Heliothis virescens* (F.) males

Accepted: 27 November 1995

Abstract 1) Male *Heliothis virescens* moths flew upwind to pulsed pheromone plumes. Upon truncation of the pulsed plume males flew into clean air, turning their tracks crosswind ($> 60^\circ$ relative to directly upwind direction at 0°) within an average of 0.27 s, and were casting, perpendicular to the wind-line (90°), within 0.43 s.

2) The characteristic casting flight in clean air consisted of left-right crosswind reversals, continuing for many seconds without further pheromonal stimulation. Males intercepting a single strand of pheromone during casting flight responded by surging upwind (track angles $< 60^\circ$). The phasic surge lasted only 0.38 s before reverting to crosswind flight ($> 60^\circ$).

3) Average templates of responses in two and three dimensions were created. Males controlled their vertical deviations very tightly when in contact with pheromone but upon entering clean air, lateral and vertical excursions became much greater.

4) Males failed to sustain upwind flight to repetitively pulsed plumes generated at < 4 filaments/s. At the threshold frequency of 4 pulses/s we show that upwind flights were composed of reiterated surges followed by crosswind casting. As the pulse frequency increased, the tracks became straighter and the single filament cast-surge-cast template could be viewed only sporadically when, for example, a male apparently failed to intercept filaments.

Key words *Heliothis* · Noctuidae · Lepidoptera · Plume structure · Behavior

Introduction

Odor, arising from a point source in a natural or artificial arena, is dispersed mainly by the wind. Due to turbulence within the air mass and eddies in an air-stream created in the lee of an object, such as an odor source, shearing promotes the formation of a non-homogeneous odor plume. The plume formed from such a point source is filamentous in structure, pockets of clean air intermingling with strands of odor-laden air as first observed by Wright (1958). These odorstrands are referred to as filaments and the intermittent nature of the plume has been shown to be maintained far downwind of the source (Murlis and Jones 1981; Murlis 1986; Baker and Haynes 1989; Murlis et al. 1990).

Experimental evidence has demonstrated that not only does the plume from a female (Vickers and Baker, unpublished observations) or a synthetic point source (Baker and Haynes 1989) appear filamentous to a stationary moth electroantennogram preparation but also that a flying male relies upon this intermittency in order to locate the female. Maintenance of upwind progress ceases in an homogeneous cloud of pheromone (Kennedy et al. 1981; Willis and Baker 1984; Baker et al. 1985) but is resumed when a point-source is placed within the cloud. Even more convincingly when a cloud is pulsed, introducing bands of clean air between the swaths of odor-laden air, males are once again able to sustain upwind progress (Baker et al. 1985).

Baker (1990) proposed a two-part mechanism by which male moths may locate a female source. As a male encounters a filament of odor he surges upwind. Over open ground or a short grassy canopy each packet of pheromone-bearing air is likely to be displaced from the source in a straight line, and therefore

Journal Paper No. J-16064 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa Project No. 3188

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such an upwind path will take the male directly toward the female (Ludlow 1984; David et al. 1982; 1983; David and Birch 1989). Each pocket of clean air would contribute to the quick waning of the upwind surge and the expression of the second part of the mechanism (Baker 1990), counterturning (Kennedy 1983, 1986). Here the male reverses his direction across the wind line at a tempo inversely related to the time after pheromone loss according to an endogenous motor program giving the program the label, 'self-steered' (Kennedy 1983; Kuenen and Baker 1983). In addition to the changes in counterturning, the wind-steered (anemotactic) component of the behavior is also altered to result in the track angle becoming more crosswind. The combination of slower reversals and increased groundspeed results in the male flying for longer distances before reversing his direction across the wind-line (Marsh et al. 1978; Baker and Haynes 1987). The eventual behavior observed is called casting (Kennedy and Marsh 1974; Marsh et al. 1978), the track leg (the part of the track between crosswind reversals) being at approximately 90° with respect to the wind direction. Baker (1990) hypothesized that if, however, another filament of odor was contacted before the waning of the last surge then the moth may reiteratively surge upwind before the overt counterturning behavior is witnessed. Thus the moth is thought to be constantly fluxing between surging upwind and casting across the wind line (Baker 1990). Therefore, moths slow to respond to odor onset and offset will fly a straighter path because the next filament arrives before casting commences. In faster responding moths such as *Grapholita molesta* (Baker and Haynes 1987), the resultant path taken by the moth is thought to have a more zigzag shape because the surge would be quickly extinguished following odor contact, almost always lapsing into casting flight before the next filament is reached.

It behooves males to respond to each and every packet of clean air caused by fine-scale turbulence (Baker 1990), because as a plume meanders with the air mass each packet could also turn out to be a large packet formed by a big wind-swing due to large-scale turbulence. This shift in wind direction will take the moth on a path off-line from the source and away from the pheromone-laden air if the moth continues to fly upwind for even a short period of time after pheromone is lost.

Other explanations for the zigzag shape of flight tracks have been put forward, most notably by Preiss and Kramer (1986). They suggest that the zigzag shape of the flight track is caused by an inability of males to steer directly into the wind (0°) following pheromone contact. The males correct for their imprecision by turning when the error reaches a certain detectable threshold, thus producing the zigzag track shape. Support for this idea has not materialized and the original hypothesis has been criticized because it was based on

data from tethered flight experiments. The process of tethering the moths in a flight simulator removes their ability to experience all the degrees of rotational freedom that would normally be encountered during free upwind flight in a pheromone plume (David and Kennedy 1987).

Vickers and Baker (1992) reported on the ability of male *H. virescens* to sustain upwind flight to puffs of odor, artificially pulsed to mimic the intermittent structure of a natural plume. Males sustained upwind flight at filament generation rates of 4/s or greater. This suggested that their latency to ONs and OFFs within the plume must be about 0.25 s as plumes created of 2 filaments/s were not as successful in generating sustained upwind flight. These initial papers (Baker 1990; Vickers and Baker 1992) pointed to the need to study the responses of male moths to encounters with single pulses of odor.

Two recent, independent studies (Mafra-Neto and Cardé 1994; Vickers and Baker 1994) have provided evidence in support of Baker's (1990) model. These studies showed that males of two different species, after casting in clean air, are capable of an upwind surge following contact with a single strand of pheromone. Vickers and Baker (1994) also demonstrated that the response to a single filament is reiterated during upwind flight to plumes consisting of four filaments/sec, the threshold of filament generation needed to promote upwind flight in *H. virescens*, and a feature of the dual mechanism proposed by Baker (1990). We report here changes in behavior that are observed in male *H. virescens* as they encounter a truncated pheromone plume and fly into clean air. We then show how the casting behavior manifested following pheromone loss can be quickly changed to an upwind surge following an encounter with a filament. By utilizing three-dimensional templates that were generated from responses to single filaments we demonstrate how these behaviors appear to be repeated during upwind flight in multiple pulse plumes.

Methods and materials

Moths

Heliothis virescens (F.) larvae were reared on a modified pinto bean diet (Shorey and Hale 1965). Following pupation the moths were separated according to sex and placed in separate environmental chambers on a 14:10 h L:D cycle. Emerging adults were supplied with a 10% sugar solution ad libitum. Adults were periodically aged and were used between 3 and 8 days. On the day that they were to be flown, before scotophase, individual male moths were placed into small wire screen cages (6 cm diameter cylinder by 6 cm high). The cages were inverted to prevent the moth escaping and were then placed on a plastic retaining tray, 20 cages per tray. These trays were returned to the environmental chamber until the 4th hour of scotophase (at the latest) when they were removed once again and placed in the wind-tunnel. The moths were flown between the 5th and 8th hours of scotophase, consequently they were given at least

one hour to acclimate to the ambient conditions within the wind-tunnel

Chemicals

A mixture of the six components known to be released by *H. virescens* females and used by the males in upwind flight are maintained in the laboratory of T.C.B. These compounds, Z11-16:Ald, Z9-14:Ald, 16:Ald, 14:Ald, Z7-16:Ald, and Z9-16:Ald were present in the ratio of 100:2.5:50:5:1:1. Before mixing, the chemicals were shown to be 99% pure by gas chromatographic methods. The hexane solution containing the mixture of six components was loaded onto a thin strip of filter paper (Whatman No. 1) 3.5 cm long by 0.5 cm wide. The loading rate of the major component, Z11-16:Ald, was 100 µg with other components present in their respective ratios. Ten µl of the 10 µg/µl solution was loaded by means of a micropipette and the hexane solvent was allowed to evaporate before the filter paper strip was introduced into a glass pipette (Fisher Scientific, Catalog # 13-678-6A). Loaded pipettes were allowed to sit in a fume hood for 24 h prior to use.

Wind-tunnel

I. Single pulse experiment

The U.C. Riverside wind-tunnel used in this study was based on the design of Miller and Roelofs (1978), slightly modified by Kuenen and Baker (1982). Its dimensions were 3 m by 1 m by 1 m. The wind-speed was held constant at 64 cm/s. Conditions within the wind-tunnel room were as follows: 0.5 lux (mixture of red and white light), 25°C, and 60% R.H. The outer shell and the floor of the tunnel were scattered with 6 cm and 9 cm diameter black dots to provide cues for visual feedback used by the moth in monitoring upwind progress (Marsh et al. 1978). At 2 m from the release point for the moths, two Sony rotary shutter cameras were positioned looking up through the clear plexiglas floor of the wind-tunnel. One camera pointed directly up and the other was set at an angle of 30° relative to the vertical, allowing resolution of the moth into 3 dimensional coordinates (Baker and Haynes, unpublished observations). The cameras' field of view encompassed approximately 45 cm of the flight track (if a moth were to fly directly upwind at the level of the plume). Each camera's signal was processed by a FOR. A time/date generator, whose stopwatch facilities were triggered simultaneously, allowing precise synchronization (to 1/100th of a second) of the views from the two cameras. From the time/date generators the signals of the two cameras passed to two Sony SLO 340 video tape decks where they were recorded for later analysis. The ceiling of the wind-tunnel in the view of the cameras was covered from outside with a square of white cloth. A silhouette of the moth against the background could then be easily observed during play-back of the videotapes. The dots providing visual cues in this area were underneath the cloth and, hence, still visible to the moth.

Also placed inside the wind-tunnel, at the level of the pulsed plume, was a series of L.E.D. lights that were programmed to flash sequentially. The lights were red in color and did not disturb the moths since moths are less sensitive to light at the red end of the spectrum. The speed with which the lights flashed down the wind-tunnel was set to match the windspeed such that the delivery of a single pulse (odor ON) or the passing of the last possible filament interception following plume truncation (odor OFF) could be followed visually on the angled camera. The synchronization of lights with windspeed was checked daily prior to experimentation by using pulsed puffs of TiCl₄ smoke.

II. Multiple pulse experiment

The wind tunnel used for the multiple pulse experimentation was of exactly the same design as that used for the single pulse study but was located at Iowa State University. Also, the tunnel was 1.2 m shorter than the U.C.R. chamber. The same equipment was used to record and analyze moth behavior. A single camera (1 m by 0.75 m field of view) was used in this instance and was positioned above the wind tunnel but at the same distance downwind of the source as the cameras used in the single pulse experiment.

Plume generation

I. Single pulse experiment

A plume of distinct pheromone filaments was created by a two-channel pulsing device (Syntech, Hilversum, The Netherlands) described by Vickers and Baker (1992). Filaments of pheromone were produced at the rate of 10 pulses/s, each channel being pulsed alternately. Males were able to fly upwind toward the source without any apparent difficulty when exposed to this pulsed plume. As the moth approached the start of the cameras' field of view an observer, tracking the moth, notified the pulsing-device operator. The second operator truncated the plume by pushing a reset button on the pulsing device. At exactly the same time the flashing lights were started via a handheld button. Consequently the last filament and the lights travelled together down the wind-tunnel at the same speed. The timing was such that both the moth flying upwind and the lights marking the passage of the last filament downwind would pass in opposite directions, within the field of view of the cameras. Due to the loss of odor the moth started to cast, turning its track across the windline to $\pm 90^\circ$ and then drifting downwind while continuing to counternturn. In the meantime, following the truncation of the pheromone pulses the second operator had turned the pulse-generation switch to 'single-pulses' and, using a foot-switch, generated single pulses, each accompanied during its downwind progress by a train of lights generated simultaneously with the pheromone filament. If the moth casted downwind and out of the cameras' field of view following pheromone loss, the second operator was notified by the first to recommence pulsing at 10 filaments/s and the process was repeated.

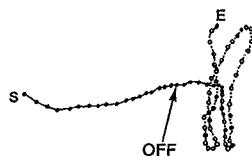
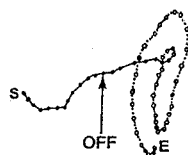
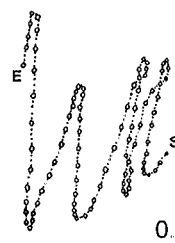
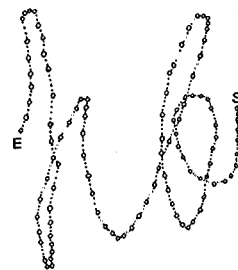
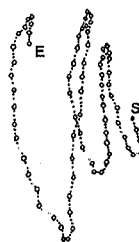
II. Multiple pulse experiments

Plumes consisting of pulses delivered at frequencies of 1, 2, 4, 5, and 10/s were generated by the pulsing device. Moth tracks from both single and multiple pulse experiments were analyzed as outlined below.

Analysis of the tracks

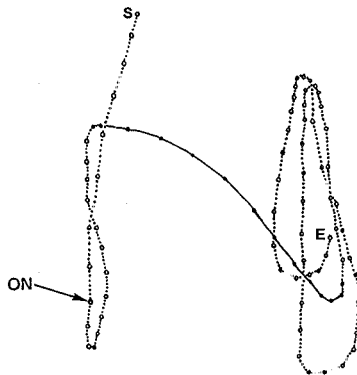
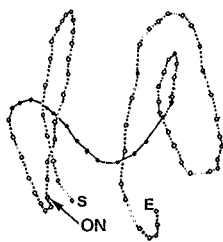
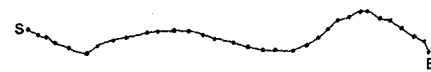
Synchronized tracks from the two cameras were played back using a Sony slow motion video analyzer (SVM1010) and relayed to a Panasonic monitor. The moth's position every 1/30th s was marked on a Mylar sheet placed over the screen. This procedure was performed for the tracks from both cameras. Starting and finishing points of the tracks were noted. From the Mylar sheet transcript of the track, each point was measured using a ruler calibrated for use with the 3-D analysis program and an ordinary protractor. Data points were entered into a RadioShack computer and using these distances and angles the computer calculated the moth's position in 3-D space. From movements in the horizontal plane, the program also calculated a triangle of velocities upon each vector, and hence the moth's course angle, track angle, airspeed, and groundspeed every 1/30th s (Marsh et al. 1978).

Fig. 1A-D The tracks of three male *H virescens* that responded to the truncation of a pheromone plume (*Pheromone-On-Off*) by entering into casting flight following a latency that averaged 0.27 s (± 0.1 S.D., $n = 10$). **B** If allowed to develop, the casting behavior continues for many seconds (up to 3.5 s for the tracks shown) in clean air (*Pheromone-Off*), the moths frequently drifting downwind. **C** The casting flight is interrupted if a filament is intercepted by the casting male (*Pheromone-Off-On-Off*). A short upwind surge ensues following a latency of about 0.3 s (± 0.16 S.D., $n = 13$). This is followed by a reversion to casting flight 0.38 s (± 0.12 S.D., $n = 13$) later. **D** A fourth group of control males was allowed to continue their upwind progression without truncation of the pulsed plume (*Pheromone-On*). In **A-D** S indicates the start of the track and E denotes the end of the track. Dots along each track represent the position of the moth each 1/30th s. Dotted lines show when the male is casting (track angle $> 60^\circ$) in **A**, **B**, and **C**. Solid lines show when the male is heading upwind (track angle $< 60^\circ$) in **A** and **C**. Hence the solid lines in **C** indicate the 'surge' part of each track.

A 'Pheromone-ON-OFF'**B 'Pheromone-OFF'**

Wind Direction

0.1m

C 'Pheromone-OFF-ON-OFF'**D 'Pheromone-ON'**

Wind Direction

0.1m

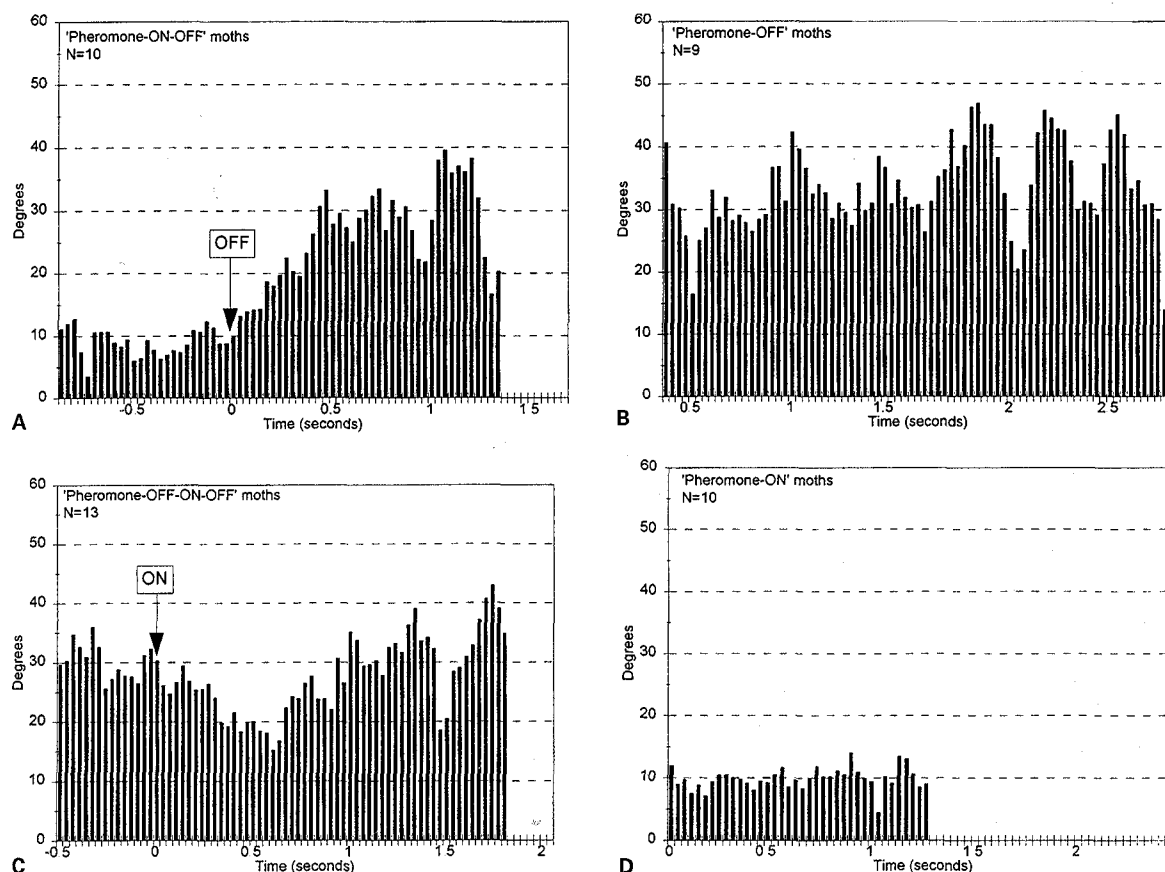


Fig. 2A–D Course angles steered by the four groups of males depicted in Fig. 1. A–D. Males steered a course of about 10° when in a continuous plume of 10 filaments/s (A prior to OFF, and D). Moths casting in clean air (B) never consistently generated average course angles this low, fluctuating most of the time between 30° and 50°. Moths casting in clean air exposed to a single filament of pheromone (C) steered a course of 30° prior to stimulation and then after intercepting the filament made an upwind movement by steering more into the wind and maintaining their courses at 20° or less for 0.37 s

The tracks were synchronized by odor event (e.g. for moths exposed to a single filament by the point at which males intercepted the pulse) and with the tracks aligned, averages for each 1/30th s for the triangle of velocities variables were calculated. For course and track angle, values can be positive or negative depending upon which side of the wind-line the moth is flying, absolute values were used as the direction of reversal (track leg) was not considered to be an important factor, males surging or beginning casting with about equal frequency to the left or right of the wind-line.

Casting flight versus upwind flight

Casting flight is characterized by males reversing from left to right about the wind line with track angles of $\pm 90^\circ$. The start of a response to a filament of pheromone was declared when vector values declined below 60° (0° being directly upwind) and remained there for five or more consecutive 1/30th s vectors. Any response was declared as having finished when vector values became greater than 60°, again for five consecutive vectors.

Results

Single pulse

All moths that had been progressing upwind responded to the truncation of the pheromone plume by entering into casting flight following a brief latency period. Ten males that remained in the center of the tunnel and continued upwind for a short distance following the pheromone-off light signal (Fig. 1A, 'Pheromone-ON-OFF') were utilized to calculate the latency from upwind to crosswind flight caused by the loss of pheromone. The average latency between last possible contact with the final filament of pheromone issuing from the pipettes and the initiation of casting behavior was 0.27 s (± 0.1 S.D., $n = 10$) (60°, Fig. 3A). Full casting as evidenced by track angles of approximately 90° with respect to the wind-line was established after 0.4 s (± 0.1 S.D., $n = 10$). As a control comparison some of these moths were monitored during casting without a filament of pheromone being presented (Fig. 1B, 'Pheromone-OFF'). Also, for control purposes, we analyzed the tracks of moths that flew through the field of view during continuous pulsing (Fig. 1D, 'Pheromone-ON').

Approximately 32% ($n = 192$) of the moths that flew into the field of view and remained there long enough

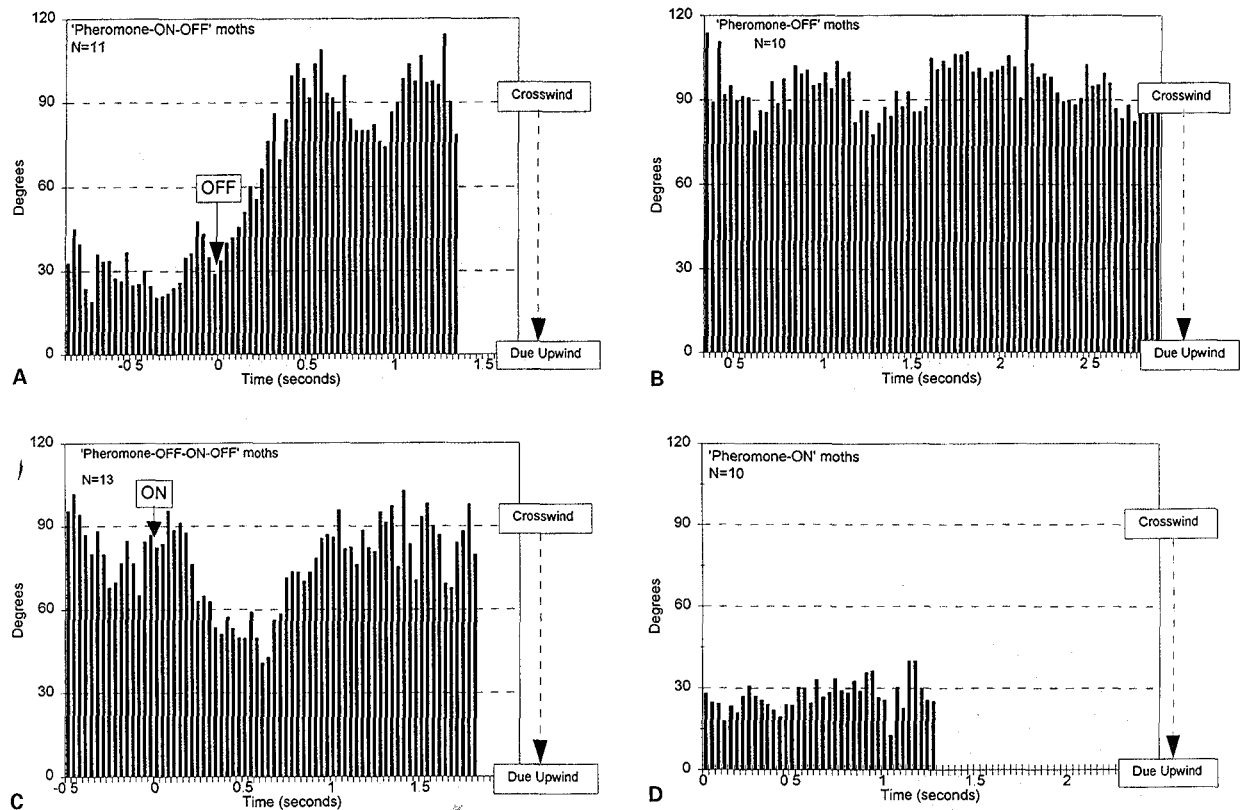


Fig. 3A–D Resultant track angles from the four groups of males in Fig. 1. Following truncation of the plume (A), the resultant track angles quickly reach 90°, characteristic of casting flight. Casting flight continues at about 90° for many seconds following truncation of the plume (B). Upwind flight in the plume results in track angles of about 30° (A prior to OFF, and D). A single filament causes casting males to briefly turn into the wind (C). The resultant track angles do not reach the sustained level of around 10° seen in moths flying upwind in continually pulsed plumes (A prior to OFF, D), but they do approach that level for a brief time before returning to 90° and casting flight.

for the first (or following) single filament of pheromone showed some observable response to the odor (Fig. 1C). Only 7% of these 'Pheromone-OFF-ON-OFF' moths were utilized for further behavioral analyses as many of the observed moths' responses quickly took them upwind out of the field of view before a reversion to crosswind casting flight could be recorded. These moths were not used in the behavioral analysis because an objective of this experiment was to understand the response to the passage of a filament, which is in the same instance both an ON and OFF. The 68% of males that did not apparently respond exhibited no surge whatsoever, with tracks similar to those of control moths in clean air (Fig. 1B).

The average latency of response to contact with an odor filament, based upon the criteria outlined in the methods and materials, was 0.3 s (± 0.16 S.D., $n = 13$). This upwind surge response quickly waned into casting

across the wind-line (Fig. 1C) after an average surge duration of 0.38 s (± 0.12 S.D., $n = 13$). The average upwind displacement was calculated to be 13.4 cm (± 6.2 S.D.). Males never made any upwind movement unless a filament was presented.

Thus, some moths did respond by making an upwind movement following contact with a single strand of pheromone. The results of the triangle of velocities analysis (Kennedy 1940), which would reveal the maneuvering changes that cause the resultant upwind movement shows that those moths responding to the cessation of pheromone filaments (Fig. 2A, 'Pheromone-ON-OFF') first steered their course angles at an average of only 10° off either side of the wind-line while in pheromone. However, 0.4 s following contact with the last possible filament these moths turned their courses to ca. 30° off the wind-line such that the resultant tracks were now at an average of ca. 90° with respect to the wind-line (Fig. 3A), characteristic of casting flight. In the absence of further pheromonal stimulation, casting persisted as males continued to steer their courses between 30° and 50° (Fig. 2B) with the resultant track angles being between 90° and 120° (Fig. 3B). Those moths that responded to a single filament (Fig. 2C, 'Pheromone-OFF-ON-OFF') were initially casting, achieved by steering a crosswind course of between 30 and 40° comparing them favorably with moths responding to clean air in the other groups (Figs. 2A following OFF and 2B, flight in clean air in the absence of any pheromone). During

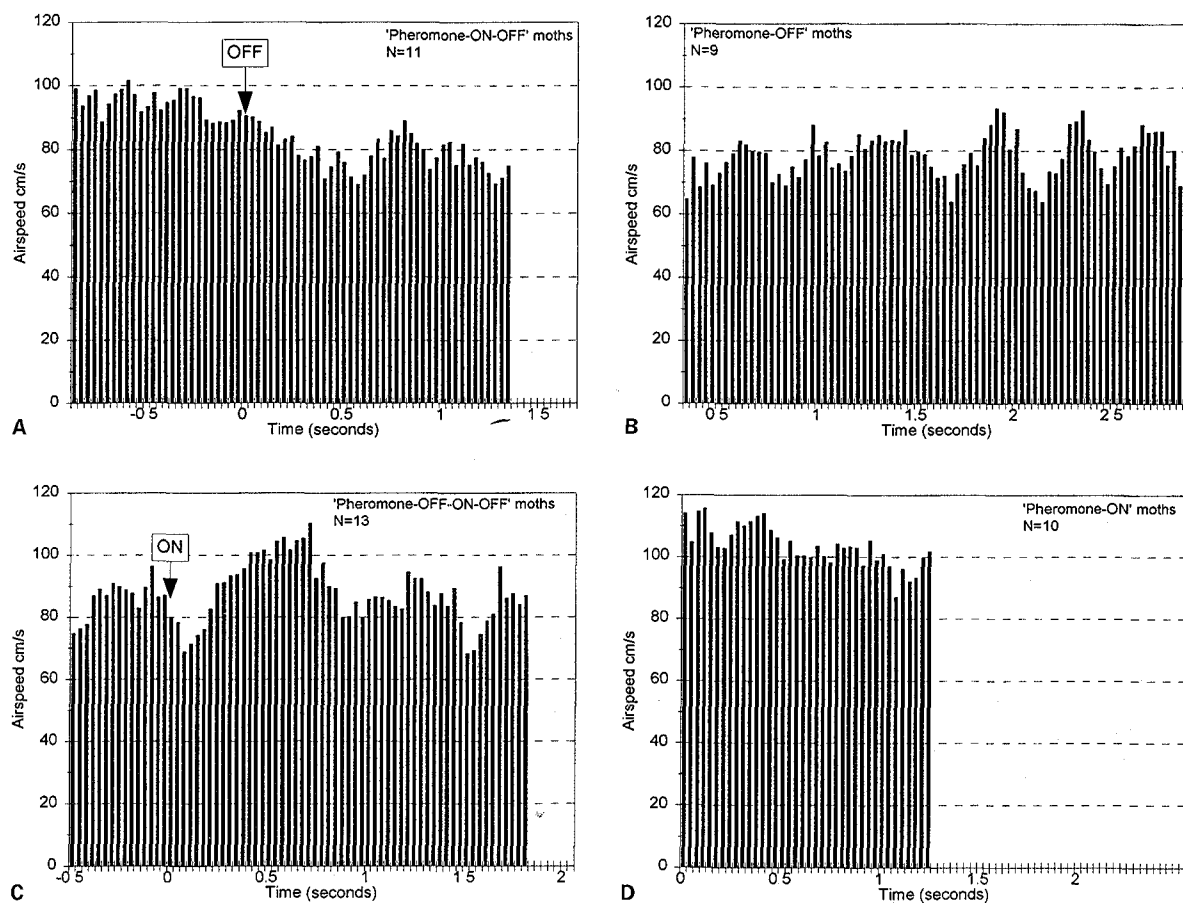


Fig. 4A-D Air speeds of the four stimulation regimens illustrated by the tracks in Fig. 1. Males flew with faster airspeeds when heading upwind in pheromone and slower when they steered more crosswind, in the absence of pheromone

the upwind surge, course angles became less than 20° (Fig. 2C). As a consequence resultant track angles were more into the wind, between 40° and 60° off the windline (Fig. 3C). In contrast, those moths that flew through the field of view in an uninterrupted train of pulsed filaments continued to do so with course angles about 10° and resultant track angles of about 30° with respect to the wind direction (Figs. 2D and 3D respectively). Sustained course angles of about 10° were achieved in both groups that were flying to uninterrupted chains of pulses generated at a frequency of 10 filaments/s (Fig. 2A and D). Moths that responded to a single pulse did not steer as closely to the windline.

Course angles steered are not the only variable that accounts for the resultant groundspeed and track angle. Airspeed generated by the moth thrusting through the air is also an important determinant of the moth's speed and direction of progress. The 'Pheromone-ON-OFF' moths flew at an airspeed of between 90 and 100 cm/s while in continuous filaments, and this airspeed dropped following the sustained loss

of odor to between 70 and 90 cm/s (Fig. 4A). The resultant groundspeed (Fig. 5A) showed a slight increase as the airspeed dropped owing to the more crosswind direction steered by the moth (Fig. 2A). 'Pheromone-OFF' moths, receiving no further filaments, also maintained a constant airspeed of between 70 and 90 cm/s while their groundspeed varied mostly between 40 and 60 cm/s (Figs. 4B and 5B, respectively).

Prior to the delivery of the filament the 'Pheromone-OFF-ON-OFF' group were casting across the windline at speeds between 70 and 90 cm/s (Fig. 4C), similar to males from the 'Pheromone-ON-OFF' group (following OFF, Fig. 4A) and the 'Pheromone-OFF' group (Fig. 4B). However, immediately following passage of the filament there was a drop in airspeed and then, at about 0.25 s, a short-lived increase in airspeed reaching up to and over 100 cm/s (Fig. 4C). The groundspeeds remain relatively constant throughout this period (Fig. 5C) owing to the fact that the moths were steering their courses more into the wind (Fig. 2C). Those moths flying in the uninterrupted pulses of pheromone registered an airspeed of about 100 cm/s (Fig. 4D) and had a fairly constant groundspeed of between 40 and 50 cm/s (Fig. 5D).

Utilizing the averages of track angles and groundspeed for each 1/30th s (Figs. 3 and 5) to create vector information (track angle conferring direction of vector

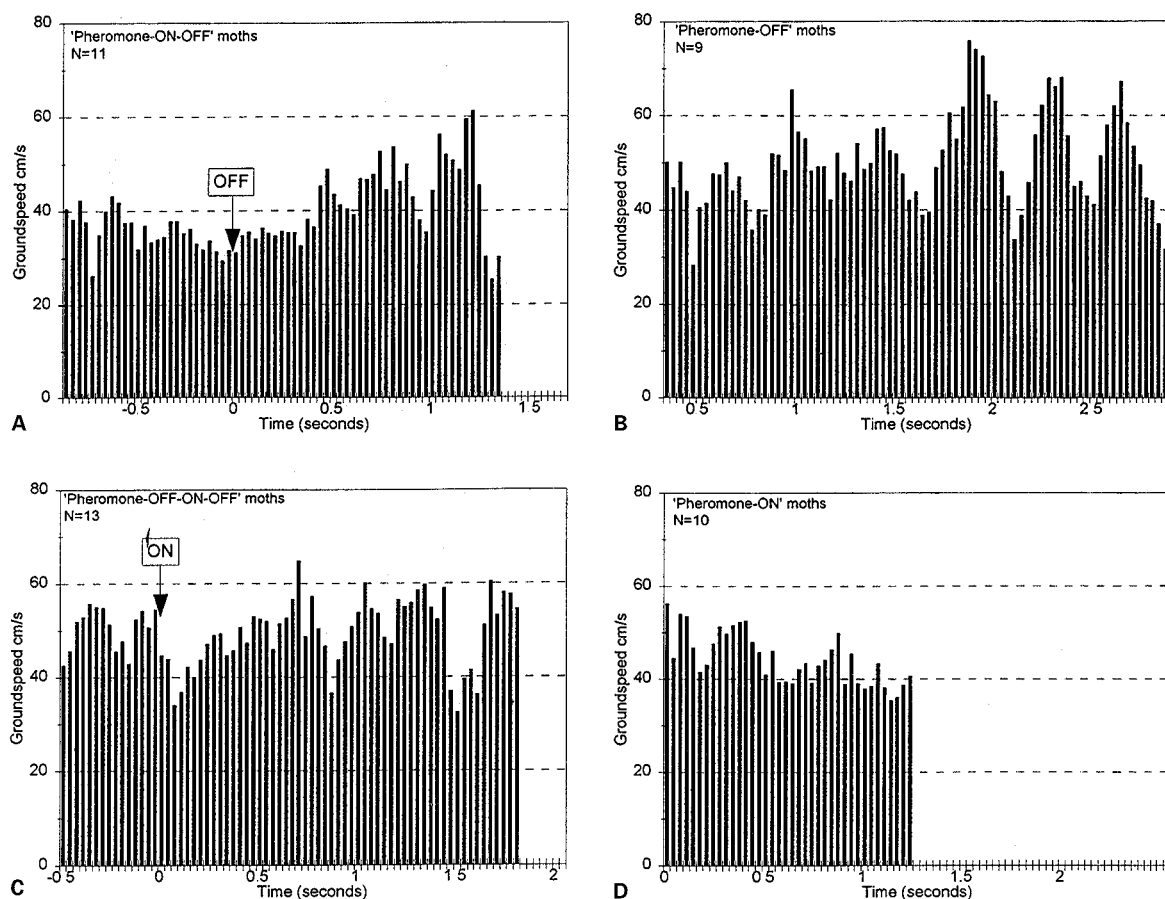


Fig. 5A–D Resultant groundspeeds were generally slower in males flying more into the wind during repeated contact with pheromone filaments. Casting flight was characterized by faster resultant groundspeeds

and groundspeed the distance between points) we calculated average tracks for the three experimental situations where odor stimulation history of the moth was known ('Pheromone-ON-OFF', 'Pheromone OFF-ON-OFF', and 'Pheromone-OFF'). Due to the fact that absolute track angle values had been used in the averaging process we used counterturning information (Table I) to impose left-right turns upon the average tracks. Each averaged track represents a template of *H. virescens* male elapsed-time response to a known odor stimulus event. The templates for the three different odor-stimulation situations are shown in Fig. 6.

With the use of two cameras beneath the wind tunnel, positions and coordinates of each moth were calculated in three dimensions. Individual tracks for each treatment are illustrated in three dimensions in Fig. 7. As the tracks demonstrate, males allow little altitudinal deviation during flight in pheromone (Fig. 7A, prior to OFF, and 7D). As well as making large lateral movements, casting males express large vertical motion as seen in Fig. 7A (following OFF) and 7B during their flight in clean air. Males that responded to the encounter with a single strand of pheromone ('Pheromone-OFF-ON-OFF', Fig. 7C) by surging upwind did so by eliminating vertical change and reducing side-to-side movement during the most upwind section of the response (the surge).

For each moth track, altitude change from the previous vector was given an absolute value and average

Table 1 Counterturning data. Average time for track legs in seconds (\pm S.D.) of moths in each pheromone treatment prior to (–1),

during (ON/OFF), and following presentation or removal of the odor stimulus (1–3). *N* for each treatment is indicated in brackets

Pheromone stimulus (<i>N</i>)	–1	Counterturning leg ON/OFF	1	2	3
ON-OFF (11)	3.45 ^{on} (\pm 0.38)	2.86 ^{off} (\pm 0.37)	3.1 ^{off} (\pm 0.25)	2.94 ^{off} (\pm 0.15)	2.78 ^{off} (\pm 0.17)
OFF (10)		3.57 ^{off} (\pm 0.29)	3.13 ^{off} (\pm 0.19)	2.56 ^{off} (\pm 0.10)	2.38 ^{off} (\pm 0.14)
OFF-ON-OFF (13)	2.38 ^{off} (\pm 0.45)	3.33 ^{on} (\pm 0.51)	2.38 ^{off} (\pm 0.45)	–	–
ON (10)	3.7 ^{on} (\pm 0.37)	3.57 ^{on} (\pm 0.25)	3.85 ^{on} (\pm 0.27)	–	–

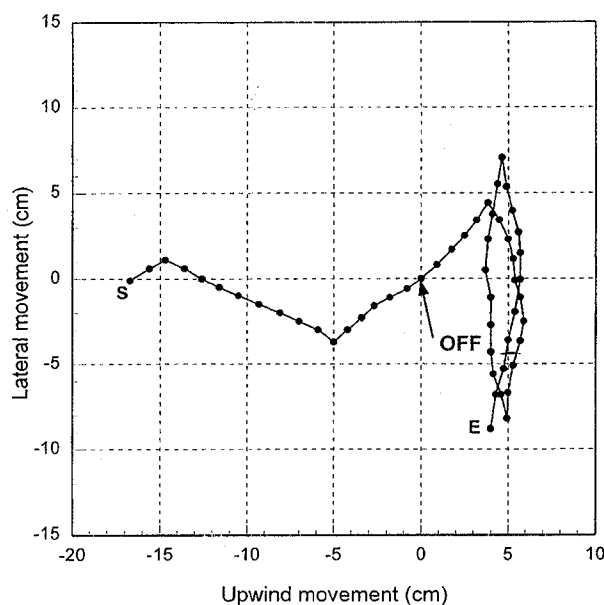
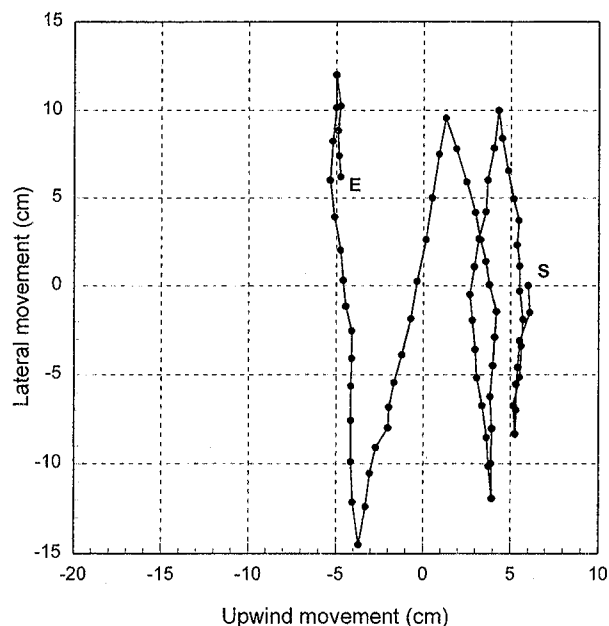
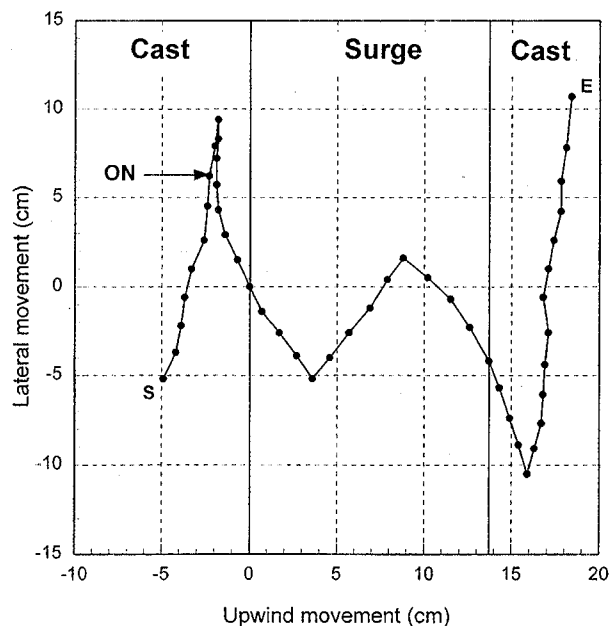
**A 'Pheromone-ON-OFF':
Latency to Casting Template****B 'Pheromone-OFF':
Casting Template****C 'Pheromone-OFF-ON-OFF':
Cast-Surge-Cast Template**

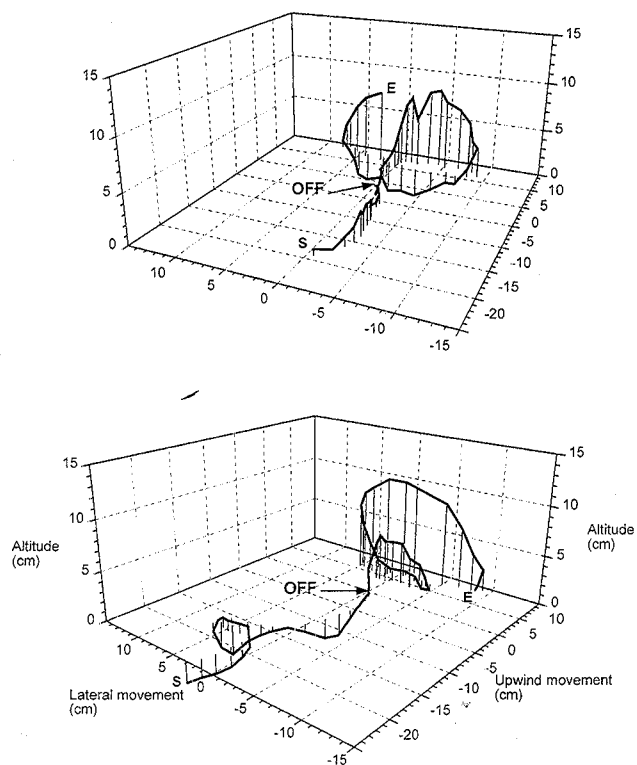
Fig. 6A-C From the combination of the triangle of velocities analysis of the resultant track angles and groundspeed we were able to create time-elapsd average responses of a typical male to the various pheromone stimulations used in this study. **A** Average response to truncation of the pulsed plume. **B** A template average of the continued casting that occurs in the absence of a pulse in clean air. **C** The cast-surge-cast template represents the average response of the males to the interception of a single filament of pheromone. Counterturning averages were also integrated into the templates

altitudinal change for each 1/30th s was calculated. The altitude information was then incorporated into the two dimensional templates by studying each individual track and determining when altitude gain became loss and vice versa. Average positions along the template track were then calculated to reflect the altitude changes expressed by the majority of the moths. The three dimensional templates are shown in Fig. 8. It is

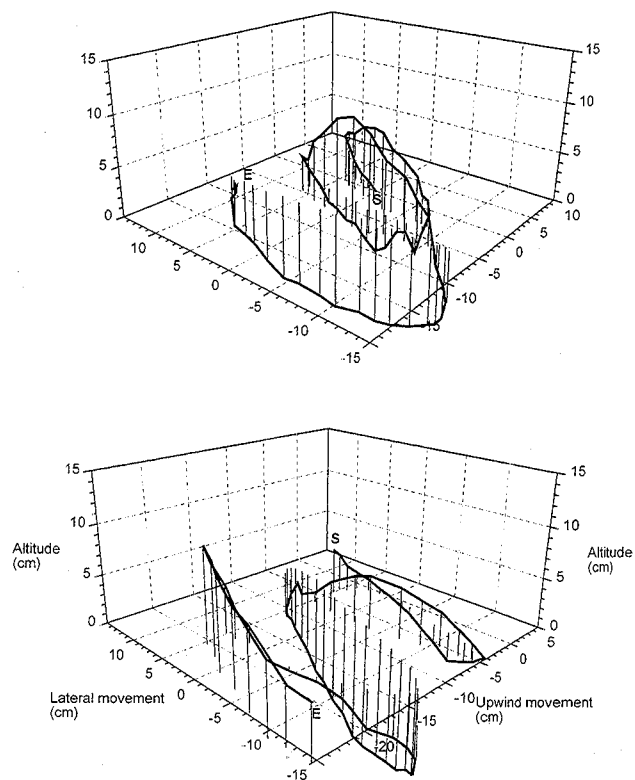
clear from the templates that the moths regulate not only their lateral excursions but also their vertical displacement according to time since odor contact. When stimulated by pheromone (Fig. 8A and C) males stringently regulate their altitude such that their flight forces result in mostly horizontal upwind progress with little altitudinal fluctuation. Following pheromone-OFF (Fig. 8A) vertical displacement becomes more

Individual Tracks

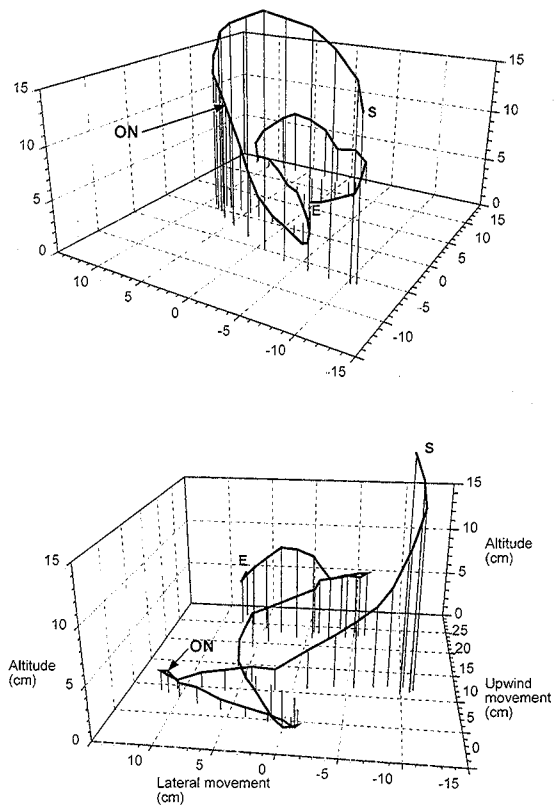
A 'Pheromone-ON-OFF'



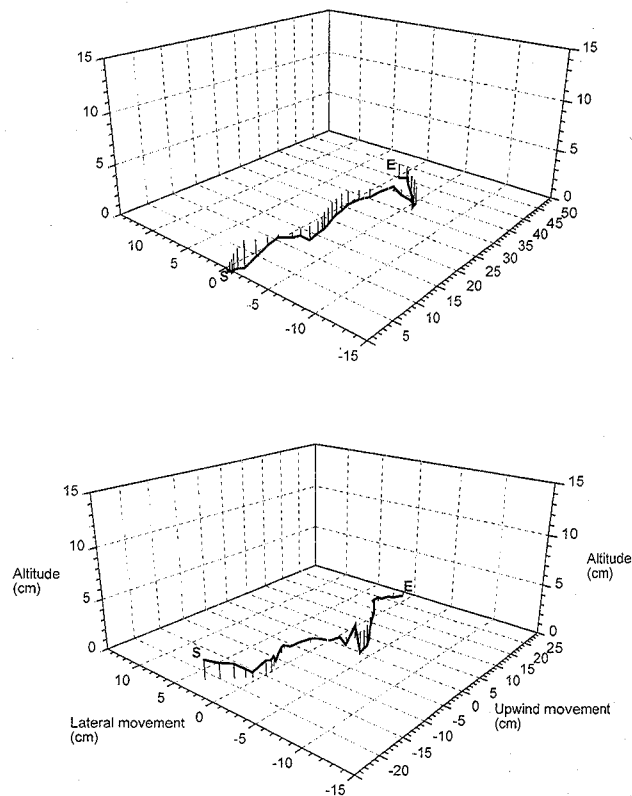
B 'Pheromone-OFF'



C 'Pheromone-OFF-ON-OFF'



D 'Pheromone-ON'



pronounced along with lateral casting movements, as upwind progress ceases. If allowed to develop into full-blown casting, the vertical excursions become as large as the lateral left-to-right movement (Fig. 8B). As the moths cast both vertical and lateral horizontal movements increase in magnitude and are often combined with a slow downwind displacement. However, if a filament of pheromone is intercepted during casting, and an upwind surge results, there is very little side-to-side or altitudinal deviation (Fig. 8C). This results in the moth making maximal horizontal progress towards the source. No template for the 'Pheromone-ON' treatment is presented because the actual positions along a given track where a male encountered pheromone cannot be determined.

Multiple pulse

Males flying upwind to pulsed plumes failed to do so unless pulses were generated at a rate of at least 4 filaments/s. Tracks of males to 4 filaments/s plumes were often tortuous, with upwind activity being interspersed with bouts of casting flight (Fig. 9A). At higher pulse frequencies the tracks became straighter with far fewer crosswind track legs (Fig. 9B and C). At 10 filaments/s, the highest pulse generation rate, the tracks often had extended portions of upwind flight (Fig. 9C). These parts of the tracks were characterized by track angle vectors $< 20^\circ$ with track leg typically longer in duration than the average counterturn period. Frequently, it was also difficult to discern where males turned across the wind line along these extended legs. Analysis of the vectors produced by the three groups of males showed that track angles were unimodally distributed at the highest pulse frequency (Fig. 9C), whereas the lowest pulse frequency capable of sustaining upwind flight showed a bimodal distribution of track angles

(Fig. 9A). Track angle vectors of moths flying to five filaments/sec were intermediate between the other two distributions. Counterturning data for the three pulse frequencies was also calculated: 4 filaments/s 2.61 turns/s (± 0.15 S.D.); 5 filaments/s 3.16 turns/s (± 0.24 S.D.); 10 filaments/s 3.26 turns/s (± 0.18 S.D.).

The actual tracks at the threshold of upwind flight activity (4 filaments/s) appear to be comprised of single responses (cast-surge-cast template: Fig. 6C) evoked reiteratively (Fig. 9A). Even at the higher pulse frequencies elements of the various templates are exposed. For example in Fig. 9C, the male apparently loses the pulses and enters into casting flight (cf. latency to odor-OFF template, Fig. 6A) and then when his track takes him into contact with the pulsed plume again the first part of the cast-surge-cast template is evoked. Evidently the crosswind component expressed in this very first surge following re-contact with odor is later dampened out and the male's path is directed more upwind once again.

Discussion

We have demonstrated that 32% of male *H. virescens*, casting following loss of a pulsed plume of pheromone, respond to interception of a single filament of odor by making an upwind movement. We define this surge toward the source as occurring when the male maneuvers in such a way that the resultant track angle achieved is $< 60^\circ$, sustained on average for 0.38 s followed by a reversion to casting flight at approximately 90° with respect to the wind-line (hence, cast-surge-cast). The change in track is achieved by the male steering a course more into the wind and by an increase in airspeed (to levels of 100 cm/s or greater) to maintain groundspeed and make progress toward the source during the upwind turn. Many males (68%) apparently did not respond to the filament presumably because they did not intercept it. However, we cannot preclude the possibility that some males may have intercepted a filament or some part thereof and failed to respond in any observable way.

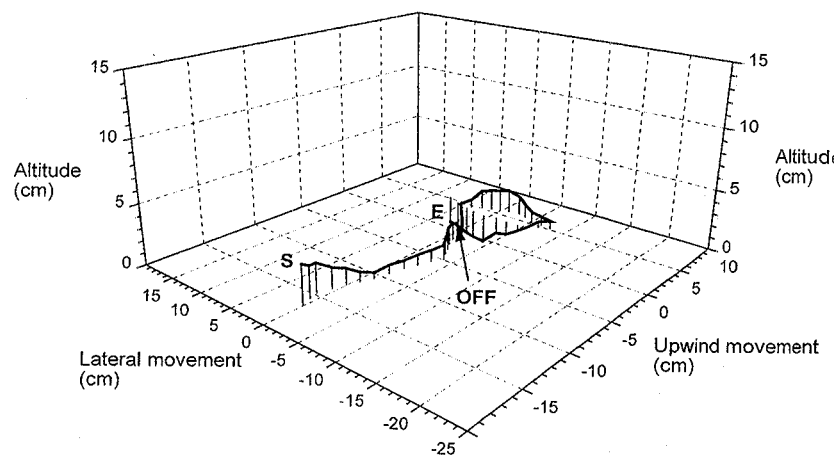
By averaging the responses of males to: 1) the truncation of a plume ('Pheromone-ON-OFF'); 2) continued absence of pheromone following plume truncation ('Pheromone-OFF') and 3) presentation of a single filament of pheromone ('Pheromone-OFF-ON-OFF'), we have created two- and three-dimensional templates for the behaviors expressed (Figs. 6A-C and 8A-C). Furthermore, utilizing these templates has enhanced our understanding of the flight behavior observed during upwind progress in multiple-pulse and, hence, point-source plumes (Fig. 9A-C).

Measurements of the latency of response by male moths to the truncation of a pheromone plume ('Pheromone-ON-OFF') have been made in a few species.

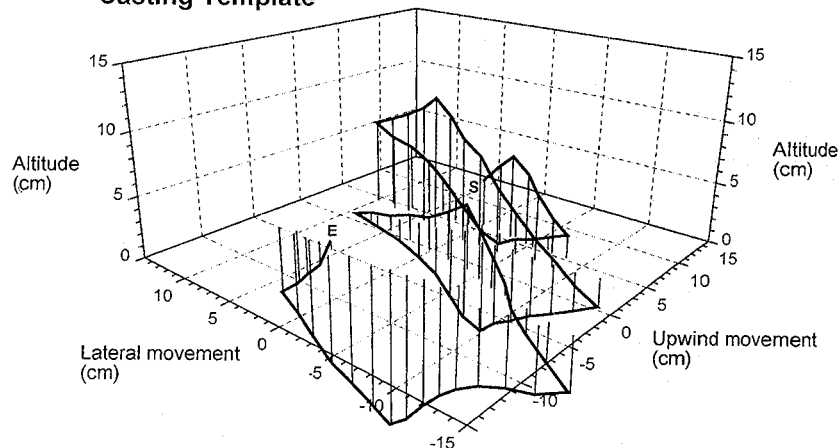
Fig. 7A-D Individual tracks of males presented in three dimensions. Thin vertical bars (every 1/30th s) indicate the altitude of the male above or below an arbitrary level. **A** 'Pheromone-ON-OFF': males control their altitude well while flying in the pulsed plume prior to truncation. Shortly following the last possible encounter with a filament, lateral and altitudinal deviations become more apparent as upwind progress ceases. **B** 'Pheromone-OFF': males casting in clean air display large side-to-side and up-and-down movements. The spiralling displayed in the upper track is a frequent feature of male tracks during casting flight and might indicate that there is some phase relationship between horizontal and vertical movements. **C** 'Pheromone-OFF-ON-OFF': shortly following ON, males make a brief upwind surge where the side-to-side and vertical movement characteristic of casting flight are curtailed. **D** 'Pheromone-ON': in a continually pulsed plume, reiterated contacts with filaments of pheromone lead to a flight track where lateral and vertical excursions are minimized. In **A-D** S indicates the start of the track and E the end-point. Axes are the same for all graphs (even though the perspective varies a little) and axis labels are indicated on the lower graph of each pair of example tracks.

Fig. 8A–C Three dimensional coordinates of the males' position allowed calculation of the males' altitude at any given time. This information was incorporated into the 2-D templates, as well as information concerning the timing of changes in altitude. *Thin vertical bars* (every 1/30th s) indicate position up or down of the moth above or below an arbitrary level. In **A** males kept a level course while in pheromone but this changed, as casting began, into more altitudinal movement. As casting develops the altitudinal changes become marked (**B**), the males essentially circling, in spirals of increasing circumference as they drift downwind. Interception of a filament interrupts this process (**C**) and altitude is strictly regulated during the surge allowing the males to horizontally progress towards the source. Following the surge, as casting begins once again, the changes in altitude become more prevalent

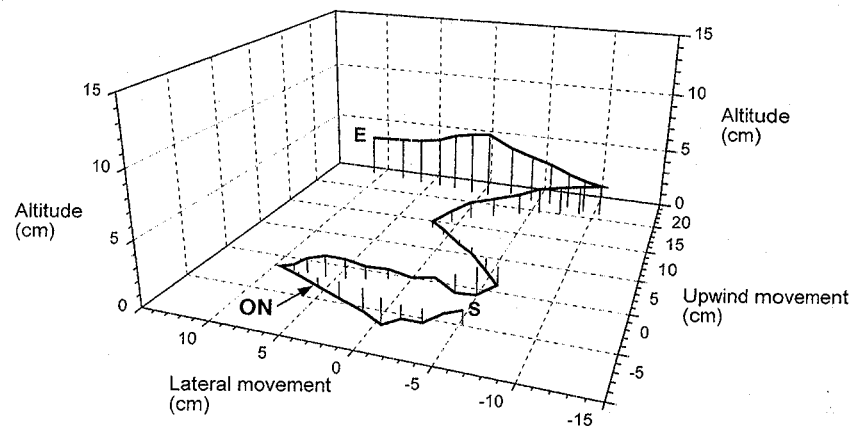
A 'Pheromone ON-OFF':
Latency to Casting Template



B 'Pheromone-OFF':
Casting Template



C 'Pheromone OFF-ON-OFF':
Cast-Surge-Cast Template

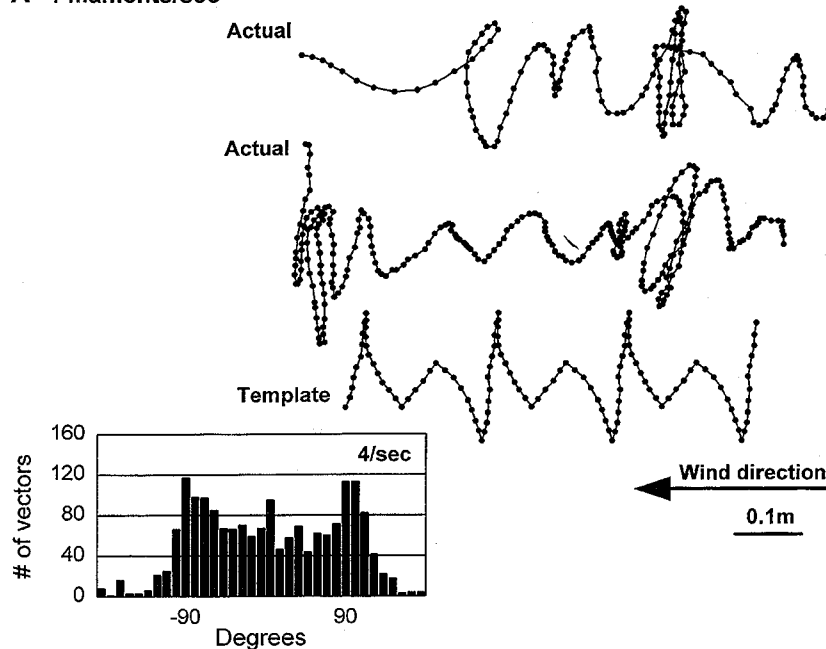


These species include *Grapholita molesta* (0.15 s) (Baker and Haynes 1987), *Antheraea polyphemus* (0.3–0.5 s) (Baker and Vogt 1988), *Amyelois transitella* (0.4 s) (Haynes and Baker 1989), *Manduca sexta* (0.5 s) (Willis

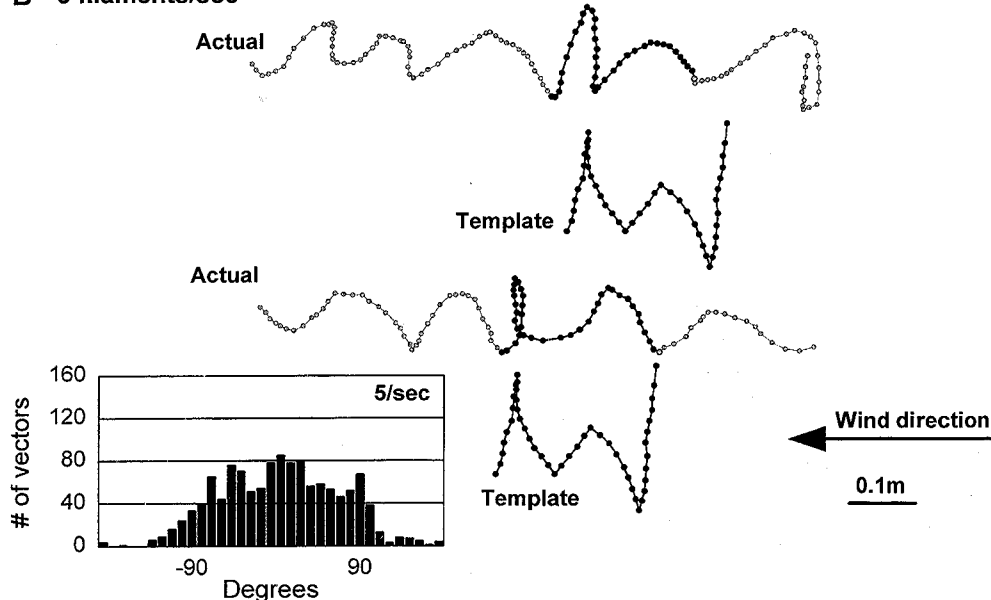
and Arbas 1991), *Callosamia promethea* (0.3–0.5 s) (Vickers et al. unpublished), and *Heliothis virescens* (0.27 s) (Vickers and Baker 1994, this study). However, only in two species have the latencies of response to

Fig. 9A–C Tracks of flying *H. virescens* males in response to plumes consisting of filaments generated at three different frequencies. All tracks start to the left of the page and move upwind, to the right. Interval between dots along a track are 1/30th s. In **B** and **C** actual tracks are highlighted (in bold) when they appear to have similar components as the single response cast-surge-cast template, or parts thereof. **A** Males cannot sustain flight to plumes generated at less than 4 filaments/s. The Actual tracks bear a very close resemblance to a Template track of individual cast-surge-cast templates (Fig. 6C) linked end-to-end. As such, Actual tracks in response to plumes at this threshold generation rate appear to be comprised of reiterated individual responses to encounters with single filaments of pheromone. **B** Tracks in response to a higher generation rate (5 filaments/s) are more directly upwind compared to the lower pulse delivery rate (**A**) and single cast-surge-cast templates are viewed less frequently, and often only in part. **C** At the highest pulse frequency for generation of pulsed plumes, the Actual tracks are often directly upwind for extended periods. The single cast-surge-cast response is only seen fleetingly or partially here when, for example, the moth momentarily loses contact with the plume. More frequent encounters with odor appear to canalize the upwind surging behavior, the clean air in-between filaments being of insufficient duration to allow expression of even the beginning of casting flight. Inset in **A**, **B**, and **C** are distributions of track angle vectors for males making complete flights through the field of view in response to each treatment ($N = 10$). As the frequency of filament generation increases, the distribution of these vectors shifts from clearly bimodal, peaks centered about $\pm 90^\circ$ (**A** indicative of more time spent in crosswind flight) to clearly unimodal, peak centered around 0° (**C** indicative of more time spent flying directly upwind)

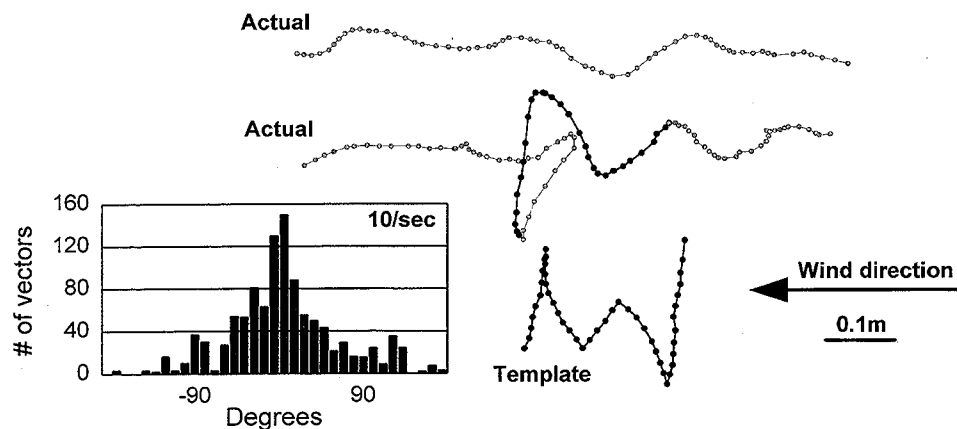
A 4 filaments/sec



B 5 filaments/sec



C 10 filaments/sec



pheromone ONs and OFFs been measured. Of these two species, *G. molesta* has a much faster response time of 0.15 s (Baker and Haynes 1987) to both ON and OFF compared to *H. virescens*. If male *G. molesta* are similarly responsive to encounters with single filaments of odor then their fast response time could explain their more zig-zag track shape as casting would begin in-between filaments (Baker 1990) which do not arrive frequently enough in a point-source plume to prevent turns across the windline (Baker and Haynes 1989). In *H. virescens* the olfactory latency to OFF and ON was found to be 0.27 s and 0.3 s respectively (Vickers 1992; Vickers and Baker 1994; this study). These response latencies dictate that the flight tracks of *H. virescens* males will be straighter than those of *G. molesta* males in a plume of identical structure, provided that the latter also respond to single filaments. The slower reaction of *H. virescens* to clean air between filaments means that there will not be enough time for casting flight to be expressed before the next filament arrives and re-elicits an upwind surge in the already surging males.

The time course of responses to pheromone ON and OFF have been hypothetically linked to the presence of certain types of neuron residing within the central nervous system of various species of moth (Baker 1990). The short-lived surge occurring after a brief contact with a pheromone pulse may be linked to the existence of neurons that have a phasic response profile when stimulated with a blend of pheromone components (*Manduca sexta*, Christensen et al. 1989a,b). Neurons with a tonic response profile, on the other hand, may be important in mediating long-lasting responses such as the continued counterturning that occurs during casting flight in clean air. These types of neurons have been discovered in two species *Helicoverpa zea*, (Christensen et al. 1991) and *H. virescens* (Christensen et al. 1995), and interestingly they are also blend-sensitive. The above examples are derived from recordings of projection interneurons residing in the antennal lobe but tonically-firing descending interneurons have also been described in *M. sexta* (Kanzaki et al. 1991). The tonic activity of these neurons is briefly inhibited during stimulation with pheromone and they may also play a role in mediating counterturning during casting flight. The exact nature of the neural underpinnings of the behavior remains elusive but the presence of these neurons is particularly intriguing given our current understanding of the behavior.

The surge created in response to interception of a single filament (Fig. 6B) mapped out very well onto tracks generated in response to multiple filaments at what appeared to be the threshold generation rate of 4 filaments/s (Fig. 9A). However, during the surge a typical moth heads more into the wind, effectively reducing the time gap between filaments as a function of his airspeed. Hence, pulses generated at a rate of 4/s

have a 0.25 s gap between them at the time of the creation and this gap is rendered smaller by the surging male. If the measured latencies are about 0.25 s or greater (as our results show), then should we expect the males to have straighter tracks to 4 filaments/s rather than the repeated iterations that appear? The answer to this question probably lies in the incomplete extinction of casting that occurs during the first surge. Most of the surges recorded are not directly upwind: they still retain a large crosswind or zigzag component. As the filaments do not have a large crosswind diameter then it is likely that any crosswind movement on the part of the male will remove him from the line where he would intercept the next filament. The male enters into casting flight once more in the clean air behind the missed filament. This hypothesis could easily be tested by exposing the males to very broad puffs such that even there was a crosswind component to the initial surge the possibility of intercepting successive filaments would be enhanced. In fact the results of such experiments have already been hinted at in previous studies. Baker et al. (1985) showed that males resume upwind flight when clouds of pheromone are pulsed such that swaths of clean air are interspersed with swaths of pheromone, compared to the casting flight that results from exposure to an homogeneous cloud of pheromone.

Mafra-Neto and Cardé (1994) demonstrated that male *Cadra cautella* flew straighter to turbulent plume compared to a narrow "ribbon" plume. Presumably these straight upwind tracks (slight deviation from directly upwind combined with difficult-to-discern counterturning) are a response to the increased filament encounter rate that males would experience in the turbulent plumes with their broader crosswind filaments.

Clearly, as more filaments are generated the chances are improved that the upwind orienting male will intercept successive filaments during the straight part of the upwind surge and this part of the surge will be repeated. This appears to happen to some extent at 5 filaments/s and to an even greater extent at 10 filaments/s, where the crosswind aspect of the surge is almost completely extinguished as the male's activity becomes canalized upwind. Thus the templates are only fleetingly viewed here when, for example, the pulsed plume is lost and then recaptured by the flying male (Fig. 9).

Kennedy and co-workers (Kennedy et al. 1980, 1981, Kennedy 1983) viewed zigzagging upwind flight and crosswind casting flight as a continuum, with pheromone turning on a combination of both counterturning and upwind anemotaxis to result in zigzagging upwind flight, and with clean air causing counterturning plus a more crosswind oriented anemotaxis to result in casting. In contrast, Baker (1990) predicted that nearly directly upwind surges caused by contact with pheromone filaments should result in a

straight-upwind track if the frequency of filament contacts was high enough, and that zigzagging upwind flight would result if the frequency of contacts was lower, resulting in a hybrid state of behavior somewhere between straight upwind surging and casting, due to the greater amount of clean air between filaments that would allow the beginning of casting to be expressed. This model was superficially similar to the one that Kennedy and Marsh (1974) and Marsh et al. (1978) had proposed, but which was later abandoned by Kennedy (1983). Among other things, Baker's (1990) model differed from the earlier, discarded Kennedy view in that it incorporated a program of self-steered counterturning that was integrated with anemotaxis, whereas Kennedy's model (Kennedy et al. 1981) was purely anemotactic, including the mechanism "reversing anemomenotaxis" in addition to positive anemotaxis. The results of the current study, as well as those of Mafra-Neto and Cardé (1994) support the Baker model, demonstrating in two quite different species of moth that males can fly straight upwind with little deviation from the wind line and exhibit scant evidence of counterturning if the filament frequency is sufficiently high. Zigzagging is absent in large stretches of flight tracks, perhaps as a result of 'suppression' of the counterturning motor program by other neural circuits during repeated and frequent contact with pheromone filaments. Kennedy et al. (1980, 1981), may have been unable to see straight upwind tracks in their moths because they did not attempt to create a situation where moths would intercept pheromone frequently enough to eliminate the initiation of crosswind flight in-between filaments. The filament-frequency dependent straight tracks, both in our species and in *C. cautella*, reveal that there must be a component of neural circuitry that enables direct upwind flight without crosswind zigzags, i.e., without allowing counterturning to be visibly expressed, as per Baker's (1990) model.

The shape of a flight track can be influenced by a number of factors. Two critical factors are the concentration of the pheromone and the actual composition of the pheromone blend (Linn et al. 1986, 1988; Willis and Baker 1987; Charlton et al. 1993). Shifted ratios of compounds or the total absence of a compound altogether can radically alter the shape of the flight track (Linn et al. 1986, 1988; Willis and Baker 1987). Clearly, as the results of the current study show, moment to moment contact with filaments constituting the plume can also influence the resultant behavior of the moth. Witzgall and Arn (1990) suggested that straighter tracks flown by male *Lobesia botrana* responding to a calling female compared to synthetic sources were the result of less error in the upwind course steered by the male, according to the model for zigzagging upwind flight proposed by Preiss and Kramer (1986). However, a more heuristic explanation exists in that the synthetic blend could have lacked

some of the components present in the natural pheromone blend, emitted at an optimal rate by a calling female, causing more of the synthetic filaments produced to be below threshold compared to those derived from a calling female. Hence changes in dosage or composition of the pheromone could manifest themselves as a reduction in the number of detectable filaments. Thus, the perceived structure of the plume in the female versus synthetic blend might be very different. Clearly the results of the current study, as well as other studies, show plume structure can influence track shape.

Other experiments with walking moths support the claim that a high frequency of pulse interception can cause a straightening of the track, even when synthetic pheromone sources are used (Kramer 1986; Kanzaki et al. 1994) or plumes consisting of a pheromone mimic and an inhibitor (Kramer 1992). Working with walking *Bombyx mori* males, Kramer (1986) demonstrated that fast arriving pulses resulted in a more upwind path. If the clean air gap between pulses was increased then males turned their tracks more across the wind-line following the upwind movement and responded to each sequentially arriving pulse by turning more upwind once again.

The only other study that has looked in detail at the effect of single and multiple pulses upon the flight track behavior of free-flying male moths was conducted by Mafra-Neto and Cardé (1994). The results of their study are, in essence the same as those shown here and by Vickers and Baker (1994). Male *C. cautella* casting in clean air responded to a filament of pheromone by surging upwind and then lapsing into casting flight once again. For the four tracks illustrated by Mafra-Neto and Cardé (1994), we calculated the latency between odor onset and the surge, duration of the surge, and the physical length of the surge. *C. cautella* males had a cast-to-surge latency of between 0.1 s and 0.17 s. The duration of the *C. cautella* surge varied from between 0.3 and 0.7 s and the physical upwind distance travelled by the moth was between 6.4 and 14.8 cm. These figures are similar to those measured with *H. virescens* and as both studies were conducted completely independently lend support to the Baker model (1990). Furthermore, tracks of males flying to fast-pulse plumes were essentially directly upwind whereas slow-pulse plumes resulted in tracks that were tortuous. This result was unchanged even when the dosages of pheromone loading were 100-fold different. When analysed the track angles show a bimodal distribution at the slow pulse rates whereas a unimodal distribution can be seen at the higher production frequency (Mafra-Neto and Cardé 1994).

By reducing altitudinal deviation during the surge *H. virescens* males are optimizing their upwind progress. In many open-field situations each filament moves away from the source in a straight line (David et al. 1982, 1983) and the narrowing of the track in the

vertical plane serves to optimize the moths' horizontal displacement in the toward-source direction. The initial small decrease in altitude following the commencement of the surge would appear to be due to an increase in the thrust vector by the moth partitioning more of its wing force into increased airspeed (David 1986). As the moth adjusts its pitch to create more airspeed without increasing total wing force there is an accompanying loss of lift and the moth descends.

In *H. virescens* loss of pheromone following either truncation of the plume (Fig. 7A), an upwind surge (Fig. 7C), or during continued exposure to clean air (Fig. 7B) results in an increase in vertical movement as well as the typical increase in lateral movements that occur in the horizontal plane during casting flight. Similarly, in other species increased altitudinal changes have been linked to more zigzagging during upwind flight (*L. botrana*, Witzgall and Arn 1990) as well as during casting flight (*G. molesta*, Von Keyserlingk 1984).

The exact maneuvers used by a male moth to increase his airspeed during the surge remain the subject of speculation. Further detailed investigations of the mechanisms by which males control their flight in filamentous point-source or pulsed plumes will be required to understand the complete nature of this remarkable feat.

Acknowledgements We are grateful to Rick Vetter and Eric Meador for providing technical assistance in the maintenance of moth colonies. Part of this study was conducted as a component of the Ph.D. dissertation by N.J.V. while at the University of California, Riverside. This work was initiated under U.S. Department of Agriculture Competitive Grant 87-CRCR-1-2577 and completed with support from U.S. Department of Agriculture National Research Initiative Competitive Research Grant 92-37302-7636, both awards to T.C.B.

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