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Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist

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Abstract Addition of (*Z*)-11-hexadecenyl acetate (Z11-16:Ac) into a normally attractive binary blend of *Heliothis virescens* pheromone components resulted in a suppression of upwind flight and source location by males. Male response was reduced even at the lowest dosages of Z11-16:Ac tested but upwind flight and source location were most clearly reduced when the loading of Z11-16:Ac reached 10% or more of the (*Z*)-11-hexadecenal (Z11-16:Ald) loading (the major component present in the binary blend). Similar patterns of suppression in response were noted when Z11-16:Ac was added to binary blends of pheromone components at both 10 and 100 μg loadings of Z11-16:Ald. Males in casting flight following upwind flight in a mechanically generated pulsed plume, responded to the interception of a subsequent, single binary-blend filament by making a toward-source upwind surge. Responses of males to a single filament that was tainted by a level of Z11-16:Ac that had allowed some reduced level of upwind flight and source location to occur in the previous plume experiments were diminished compared with their control counterparts. Analysis of the flight tracks revealed that the surges in response to single tainted filaments were stunted because males made fewer significant changes in course angles steered, airspeeds generated, and in the tempo of counterturns executed.

Key words Sex pheromone · Flight · *Heliothis virescens* · Noctuidae · Lepidoptera

Abbreviations *Z11-16:Ac* (*Z*)-11-hexadecenyl acetate · *Z11-16:Ald* (*Z*)-11-hexadecenal · *Z9-14:Ald* (*Z*)-9-tetradecenal · *Z11-16:OH* (*Z*)-11-hexadecenol · *Z7-12:OH* (*Z*)-7-dodecenol · *Z5-10:Ac* (*Z*)-5-decenyl acetate · *Z5-10:OH* (*Z*)-5-decenol · *MAD* median absolute deviation · *MGC* macroglomerular complex

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Introduction

A calling female moth releasing pheromone into the airstream evokes a stereotypical sequence of behaviors from a conspecific male coming into contact with the plume downwind of her. Over the past few decades the behaviors evoked during pheromone-mediated upwind flight have been carefully studied by a number of workers (Kennedy and Marsh 1974; Marsh et al. 1978; David et al. 1982, 1983; Baker et al. 1985). Our current understanding of this flight is that males use a combination of two behavioral mechanisms in order to locate the female: optomotor anemotaxis (Kennedy and Marsh 1974; Marsh et al. 1978) and counterturning (Kennedy 1983, 1986). Both systems are mediated by encounters with the pheromonal odor and may last for many seconds, even after the male has lost the odor plume. During flight in the plume the male's track reverses from left to right and back at a frequent rate (Baker and Haynes 1987; Willis and Arbas 1991). These reversals, coupled with an upwind bias in the anemotactic system, result in a track with a net upwind displacement. Following odor loss, alterations in both mechanisms occur. Firstly, the anemotactic response is changed (through shifts in the course steered by the male and the airspeed that he is generating) to result in a track that is now oriented perpendicular to the wind-line. Secondly, the reversals continue but the time between reversals from one side of the wind-line to the other becomes greater. Hence, there is a decrease in the tempo of counterturning, defined as the rate of track reversal from one side of the wind-line to the other (Baker and Haynes 1987).

Small-scale turbulence within an air mass moving a plume away from the source is responsible for breaking

the plume down into a series of odor-bearing packets (called filaments) and clean air pockets such that a male flying upwind in the plume is exposed to an intermittent signal that may be formed of many such Ons and Offs every second. Measurement in both plumes created of ions (Murlis and Jones 1981; Murlis 1986; Murlis et al. 1990) and actual pheromone (Baker and Haynes 1989) revealed this to be the case. Baker and Haynes (1987) calculated the latency between crosswind casting and upwind flight of male *Grapholita molesta* to pheromone On and Off in a plume made to swing through an arc by changing the wind direction at the source. This led to the realization that males were capable of responding to individual Ons and Offs that occur within the plume (Baker 1990).

Two groups have recently, independently confirmed that male moths in casting flight can respond to a single encounter with a pulse of odor by briefly surging upwind before lapsing into casting flight once again (Mafra-Neto and Cardé 1994; Vickers and Baker 1994, 1996).

Furthermore, in pulsed plumes generated at a threshold rate of four filaments s^{-1} *H. virescens* males appeared to repeatedly respond with a surge to single filaments, indicating that sustained upwind flight could be a reiterative process (Vickers and Baker 1994, 1996). At higher pulse frequencies the tracks became more directly upwind, evidently due to the repeated evocation of only the straightest part of the surge. Both Mafra-Neto and Cardé (1994, 1995) and Vickers and Baker (1994, 1996) showed that such tracks were almost directly upwind and that counterturning became difficult to discern, supporting the notion that counterturning is in some way inhibited or suppressed during high-frequency filament contact. Zigzagging upwind tracks in point source plumes thus may be the result of a hybrid state of behavior, somewhere between reiterative upwind surging and crosswind casting, produced as a function of both the males' response latency to On and Off as well as the frequency of filaments present in the plume, as outlined in detail by both Baker (1990) and Kaissling and Kramer (1990). In contrast to the recent experiments with flying male moths (Mafra-Neto and Cardé 1994, 1995; Vickers and Baker 1994, 1996), Kramer (1986) had previously demonstrated with walking *Bombyx mori* that a straightening of track occurred at high frequencies of pulse interception. Males followed a more upwind track after each pulse, and turned crosswind between pulses if the frequency of delivery was slowed. Clearly, anything that inhibits males from responding optimally to each contact with a strand of odor will diminish the sustainability of upwind flight in an adverse fashion.

As a completely different hypothesis, Preiss and Kramer (1986) proposed that zigzagging upwind flight arose from an error correction mechanism in a system designed to hold a due upwind course, a hypothesis that was criticized because conclusions were based upon the use of tethered and not free-flying animals (David and Kennedy 1987). Witzgall and Arn (1990) found that male *Lobesia botrana* zigzagged more in synthetic point-source plumes compared with the more directly upwind flights observed in plumes released by calling females, which led them to conclude in support of the Preiss and Kramer (1986) hypothesis. An alternative explanation for the phenomenon observed by Witzgall and Arn (1990) was put forward by Baker and Vickers (1996) and Vickers and Baker (1996), who suggested that the straighter flights might be due to the presence of many above-threshold filaments in the female-released plume compared with the synthetic plume where an off-ratio or absence of components might cause some filaments to be below threshold. As proposed by both Baker (1990) and Kaissling and Kramer (1990), fewer detectable filaments would result in tracks with more of a casting component and hence a more zigzag appearance.

Of the compounds released by female *H. virescens* two, Z11-16:Ald and (*Z*)-9-tetradecenal (Z9-14:Ald), are sufficient to evoke most of the behaviors associated with upwind flight and source location (Roelofs et al. 1974; Tumlinson et al. 1975). It was therefore surprising that receptors were recently characterized that respond specifically to Z11-16:Ac (Berg et al. 1995), which is not known to be released by conspecific *H. virescens* females. These receptor neurons appear to project to a specific compartment within the sexually dimorphic macroglomerular complex (MGC). Receptor neurons responsive to other conspecific pheromone components appear to innervate other regions of the MGC (Hansson et al. 1995). Central projection interneurons also were discovered that were specific for this compound (Christensen et al. 1995). We were therefore prompted to assay the effects of the addition of this compound into an otherwise attractive two-component blend. Having discovered that addition of small amounts of Z11-16:Ac into the blend suppresses upwind flight and reduces source location, we then presented males with single filaments of pheromone similarly tainted with the inhibitor in order to investigate the effect of initial contact with the blend plus antagonist upon the surge responses of males.

Materials and methods

Wind tunnel

The design of the wind tunnel is based upon that of Miller and Roelofs (1978). The plexiglas wind tunnel measured 1 m in diameter by 2 m in length. Examination of flow velocity and turbulence within the tunnel using $TiCl_4$ smoke revealed that the plume created by a point source moved along the center of the tunnel and had a typically fenestrated structure. The wind speed used in all experiments was 60 cm s^{-1}. A floor pattern of 10-cm-diameter red dots randomly placed on white cloth was used to provide proximal visual feedback to the upwind orienting moths.

Moths

H. virescens larvae were reared on a pinto-bean diet (Shorey and Hale 1965). Males were separated from females at the pupal stage and allowed to eclose in a separate environmental chamber. Adults

were provided with an 8% sucrose solution ad libitum and were kept in a controlled-environment chamber with a 14:10 h L:D cycle at a temperature of 30°C with humidity between 50 and 80%. Males were used in behavioral experiments between 3 and 8 days following eclosion. Prior to the onset of scotophase on the day of experimentation, individual males were placed into cylindrical wire screen cages (6 cm diameter × 6 cm high). These cages were placed onto plastic trays (20 per tray) that were returned to the environmental chamber. The trays were transferred to the darkened wind tunnel at least one hour prior to the beginning of the experiment. Males were flown between the 5th and 8th hours of scotophase (Vetter and Baker 1983).

Plume experiments

For experiments involving behavioral assays in response to point source plumes, a two-component mixture was used as a positive control. Male *H. virescens* will respond with upwind flight and source location to a binary mixture of Z11-16:Ald and Z9-14:Ald in a 40:1 loading ratio (Pope et al. 1982; Vetter and Baker 1983). Varying amounts of Z11-16:Ac were added to this attractive blend from 0.1 to 100% (such that 100% equaled a loading of equivalent amounts of Z11-16:Ald and Z11-16:Ac). The purity of compounds as checked by capillary GC was found to be greater than 99%. Dilutions were made of each of the three compounds such that the final dosage required was achieved by adding 10 µl of test solution to a 1-cm-diameter filter paper disc (Whatman No.1). In experiments where only two compounds were used, 10 µl of hexane also was added to the filter paper disc. A small crocodile clip was attached to the bottom of the disc to provide stability in the wind tunnel and the disc and clip were placed on a metal platform 15 cm above the wind tunnel floor.

Two identical series were tested with a tenfold increase in the amount of Z11-16:Ald dosage such that the final loading was either 10 or 100 µg. Males were released 2 m away from the source and were assessed for their ability to (1) take flight, (2) lock on to the plume, (3) progress upwind, (4) progress greater than half-way to the source, and (5) land on the source. Responses of males were video-recorded from above the wind tunnel using a Sony RSC 1050 rotary shutter camera. The camera's field of view encompassed 1 m of the length of the wind tunnel and 0.75 m of its width. The field of view was such that neither the take-off nor source platforms were visible. The audio channel on the video tape was utilized during the behavioral experimentation to aid in determining the entire sequence of behaviors exhibited by any individual moth.

Single-pulse experiment

Pulsed plumes were generated by an air pulsing device (Syntech) that has been used successfully before in other experiments designed to study the effect of intermittent plumes upon flight behavior (Vickers and Baker 1992, 1994; Mafra-Neto and Cardé 1994). The operation of this device has been described previously (Vickers and Baker 1992), with air pulses being generated by solenoid valves within the device and the resulting pulses being directed from the machine via a length of tygon tubing through a standard Fisher Scientific pipette. Pulses were of 20-ms duration at a flow rate of 50 ml s^{-1}, giving each pulse a volume of 0.1 ml. The pipette contained a 3.5 cm × 0.7 cm filter paper wick (Whatman No.1) laced with a binary mixture of Z11-16:Ald and Z9-14:Ald. Pulses were generated at a rate of 5 s^{-1} and males were released at the downwind platform and allowed to initiate upwind flight. Each pulse was accompanied by a flash of light from a low-light red diode placed within the video camera's field of view. When a male was close to entering the field of view of the video camera the plume was truncated by resetting the pulsing device to a zero pulse rate. The timing was intended to allow the male to encounter the last possible filament generated by the pulsing device while flying within the camera's field of view. A second pulsing device set up to generate single pulses was attached to a treatment pipette, and activated by use of a foot pedal. Each pulse from this device also was accompanied by a flash of red light from a separate diode. Treatments within the single-pulse pipette consisted of either a hexane blank, a binary blend of Z11-16:Ald and Z9-14:Ald (creating filaments identical to those that the male had encountered during upwind flight in the 5 s^{-1} pulsed plume), or the same binary blend laced with either 0.1 or 1% Z11-16:Ac. Responses of males to plume truncation and interception of single pulses were video-recorded (as described above).

Behavioral analysis

Video tapes were replayed on a Sony SLO 340 video tape deck. For both plume and single pulse experiments flight tracks were recorded onto a Sony SVM1010 motion analyzer and replayed frame-by-frame (60 frames s^{-1}). The male's position every two frames (1/30th s) was transcribed onto a sheet of acetate. Tracks were then digitized on an Hitachi digitizing pad (Puma Plus). Analysis of tracks was completed by subjecting the digitized data to a triangle of velocities program that allows calculation of a male's course angle, track angle, airspeed and groundspeed (Marsh et al. 1978).

Data analysis

Plume experiments

For the two dosage series males' responses were grouped into the sequential behavioral categories outlined above. Track angles, calculated each 1/30th s, from those males that made complete flights through the field of view at 100-µg dosage of Z11-16:Ald were subjected to a simple frequency analysis by placing angle values into 10° bins from −180° to +180°.

Single-pulse experiment

Tracks from many males were aligned with each other about the relevant odor stimulus event (OFF, for males responding to truncation of the plume; ON, for males responding to a pulse of pheromone). For each male the triangle of velocities program calculated the male's track and course angles and air and groundspeeds every 1/30th s. In order to perform statistical analyses concerning changes in behavior the data were condensed by grouping sets of three consecutive 1/30th-s intervals together and taking an average. Triangle of velocities information thus was consolidated for each 0.1 s. For track and course angles where negative and positive values can exist depending upon which side of the wind-line the moth is moving, absolute values were used because there did not appear to be any bias in the direction of flight (i.e., left or right side of the wind-line) prior to or following any of the odor-stimulus events.

Statistical analysis

Plume Experiments

Male responses falling into each sequential behavioral category were compared across experimental treatments using an adjusted χ^2 2 × 2 test of independence.

Single-pulse experiment

Median values (± median absolute deviations, MAD) across the population of responses in any given odor stimulus group were calculated for each 0.1 s (as outlined above). In each case the triangle of velocities parameters (course and track angles; air and groundspeeds) prior to a defined odor event (either OFF or ON) were used to establish a baseline against which subsequent behavioral events were compared. For those males responding to OFF (pheromone-ON-OFF males) an average for each male for the 0.9 s

preceding OFF was calculated. These individual measures then were used to calculate a median (±MAD) for the population prior to OFF and this value was compared against subsequent 0.1-s values using a one-tailed Wilcoxon matched-pairs signed rank test. For those males responding to encounter with a single pulse of pheromone (Pheromone-OFF-ON-OFF males) the same procedure was utilized with the exception that the baseline was established from averages for 0.3 s prior to ON. To correct for the number of comparisons, significance was determined at $P < 0.01$. The median values prior to OFF or ON were not tested (unshaded bars in the figures) against the established baseline but are included in figures to show the stability of the behavioral performance prior to the odor stimulus event.

For counterturning data the median value (±MAD) of the track leg prior to OFF or ON was used as the basis of comparison for track legs during and following OFF/ON. As fewer comparisons were made, significance was determined at $P < 0.02$.

Results

Plume experiments

Males responded to point-source plumes of a binary blend generated from a filter paper source by taking flight, locking-on to the plume, and flying upwind. The same proportion of males took flight to all treatments but the addition of as little as 1% Z11-16:Ac (relative to the dosage of Z11-16:Ald) resulted in a significant reduction in the proportion of males locking-on and initiating upwind flight in the plume (Fig. 1A, B). Thus, the ability of males to lock onto the plume was impaired by the presence of small amounts of Z11-16:Ac. This trend was established even at the lowest levels of Z11-16:Ac tested in these experiments. However, in response to blends containing 0.1% Z11-16:Ac males still performed the sequence of behaviors in significant numbers. The addition of 1% Z11-16:Ac to the blend resulted in a significant reduction in all behaviors except taking flight, although some males persisted in making upwind flights particularly at the 100-μg dosage (Fig. 1B). As the amount of Z11-16:Ac added to the blend increased to 10% of the Z11-16:Ald dosage, fewer males exhibited any behavioral activation, other than taking flight. The trends for both 10- and 100-μg Z11-16:Ald loadings were similar, although fewer males located the source in response to the control blend at the 100-μg loading (Fig. 1B). In response to blends containing 1% Z11-16:Ac, many males took flight and attempted to lock-on to the plume above the take-off platform. However, comparatively few of the males made any further progress in the sequence of behaviors compared with the binary blend and 0.1% Z11-16:Ac treatments. The effectiveness of the blend in sustaining upwind flight thus also was impaired by the addition of Z11-16:Ac, that appears to act as an effective antagonist, even at relatively low concentrations. Blends containing 10% and higher of Z11-16:Ac acted to completely eliminate upwind flight responses and source location at 100-μg dosage of Z11-16:Ald and, in all but a few individuals, at the 10-μg dosage.

Flight tracks of those males that made a complete flight to the source revealed that relatively direct upwind progress could be made by those males responding to the control blend at the 100-μg loading of Z11-16:Ald (complete flight made by six males) (Fig. 2A). Blends tainted by the presence of 0.1% Z11-16:Ac did not appear to elicit such direct upwind progress, the tracks appearing somewhat more tortuous (Fig. 2B).

A plot of track angles vectors, placed into 10° bins (Fig. 2A–C) revealed that the distribution of track angles for males responding to a control blend was unimodal, with a large peak centered at 0° (Fig. 2A). In contrast, the males responding to blends containing 0.1% Z11-16:Ac had a flatter track angle distribution with no distinct unimodality supporting the notion that overall their tracks were more tortuous than those of the control males (Fig. 2B). With the addition of 1% Z11-16:Ac to the blend the track angle distribution was not as sharply unimodal compared with the control males (Fig. 2C). Only two males made upwind flights that were long enough to be used for track angle analysis in this latter category; neither male continued upwind flight to locate the source. Males that did respond in these plumes often made only very brief upwind excursions, frequently failing to enter the camera's field of view.

Single-pulse experiment

From the point-source experimentation we included a series of treatments for further investigation at our finest level of behavioral resolution, the response by males to the interception of a single filament of pheromone. Males responded to the clean air following truncation of the pheromone plume by entering into crosswind casting flight, never making any upwind movement. This was accompanied by a decay in the time period between reversals (Table 1). Tracks from two of these males are illustrated in Fig. 3A. Males in all three of the single-filament treatment groups responded similarly to the clean air after plume truncation by entering into casting flight. However, those males intercepting a single pulse of normal pheromone surged upwind towards the source (Fig. 3B). In contrast, the surges in response to filaments tainted with either 0.1 or 1% Z11-16:Ac (Fig. 3C, D) did not appear qualitatively the same as those in response to the control blend (Fig. 3B), with the males not making as much upwind progress in response to these pulses before returning to casting flight.

Males that responded to truncation of the pheromone plume did so by turning their track angle more across the wind-line (Fig. 4A–D). Prior to the clean air, during flight in the 5 s^{-1} pulsed plume, the average track angle value was about 30°. Following pheromone OFF, the track angle values increased and showed a significant change at 0.3 s, which was fully sustained at 0.5 s. Values remained significantly elevated for the remainder of the sampling time, where they reached 90° (0.9 s following OFF). During this time the resultant ground-

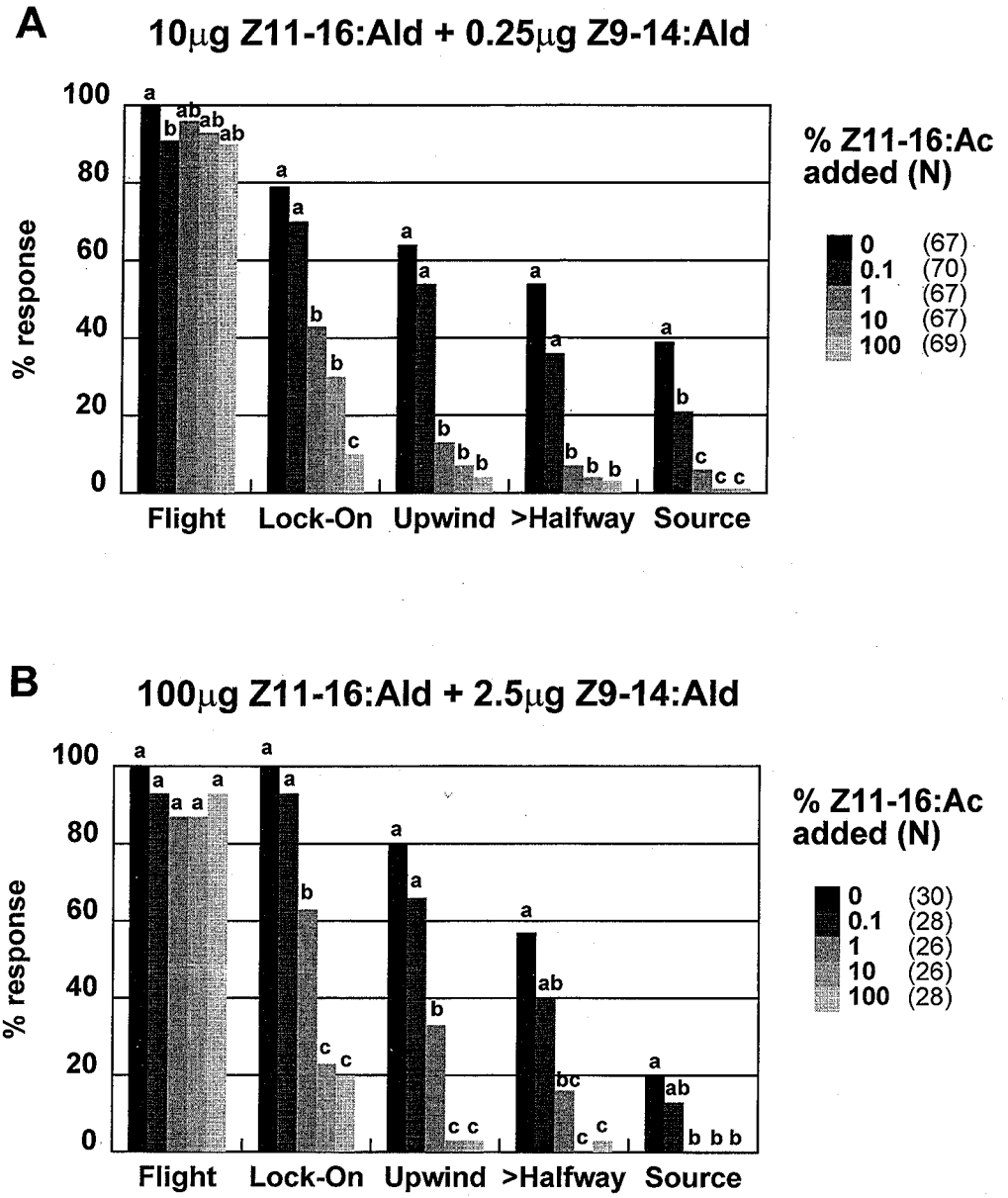

Fig. 1A–B Percent responses (*y*-axis) falling into various behavioral categories of male *H. virescens* in response to point-source plumes dosed with either (**A**) 10-μg or (**B**) 100-μg of Z11-16:Ald (+ 0.25-μg or 2.5-μg of Z9-14:Ald, respectively) and containing varying amounts of Z11-16:Ac (0–100%). Almost all males take flight (*Flight*) in response to the inversion of the small flight cage but pheromone-mediated behaviors of attempting to lock-on to the pheromone plume above the take-off platform (*Lock-On*), upwind flight (*Upwind*), upwind flight greater than halfway to the source (*> Halfway*), and source location (*Source*) are reduced by the addition of even as little as 0.1% Z11-16:Ac. The presence of 1% Z11-16:Ac or more results in complete inhibition of source location (**B**). The trends are similar for the two different dosages. *Bars* in the same behavioral category with no letters in common are significantly different according to an adjusted χ^2 2 × 2 test of independence ($P < 0.05$)

speed (Fig. 4D) changed little. Prior to OFF, the groundspeed values were about 40–50 cm s^{-1}. There were no consistent, sustained changes in groundspeed to accompany the alteration in track angle values. The behavioral changes that manifested themselves as the alteration in track angle seem to be entirely due to changes in the direction that the males steered (course angle, Fig. 4A), because following OFF there was no concomitant change in the airspeed (Fig. 4C) although there does appear to be a slow overall decline. The course angles became significantly greater and remained so, 0.5 s following OFF.

The other aspect of flight that was altered following truncation of the plume was the rate of the reversals from left to right across the wind-line (counterturning) (Table 1). The third and fourth reversals following the track leg where the male had the last possibility of contacting a filament were significantly longer than the one immediately preceeding the OFF leg. Consequently, changes in counterturning tempo took at least 0.5 s to manifest themselves following OFF. This result indicates that the anemotactic and counterturning systems re-

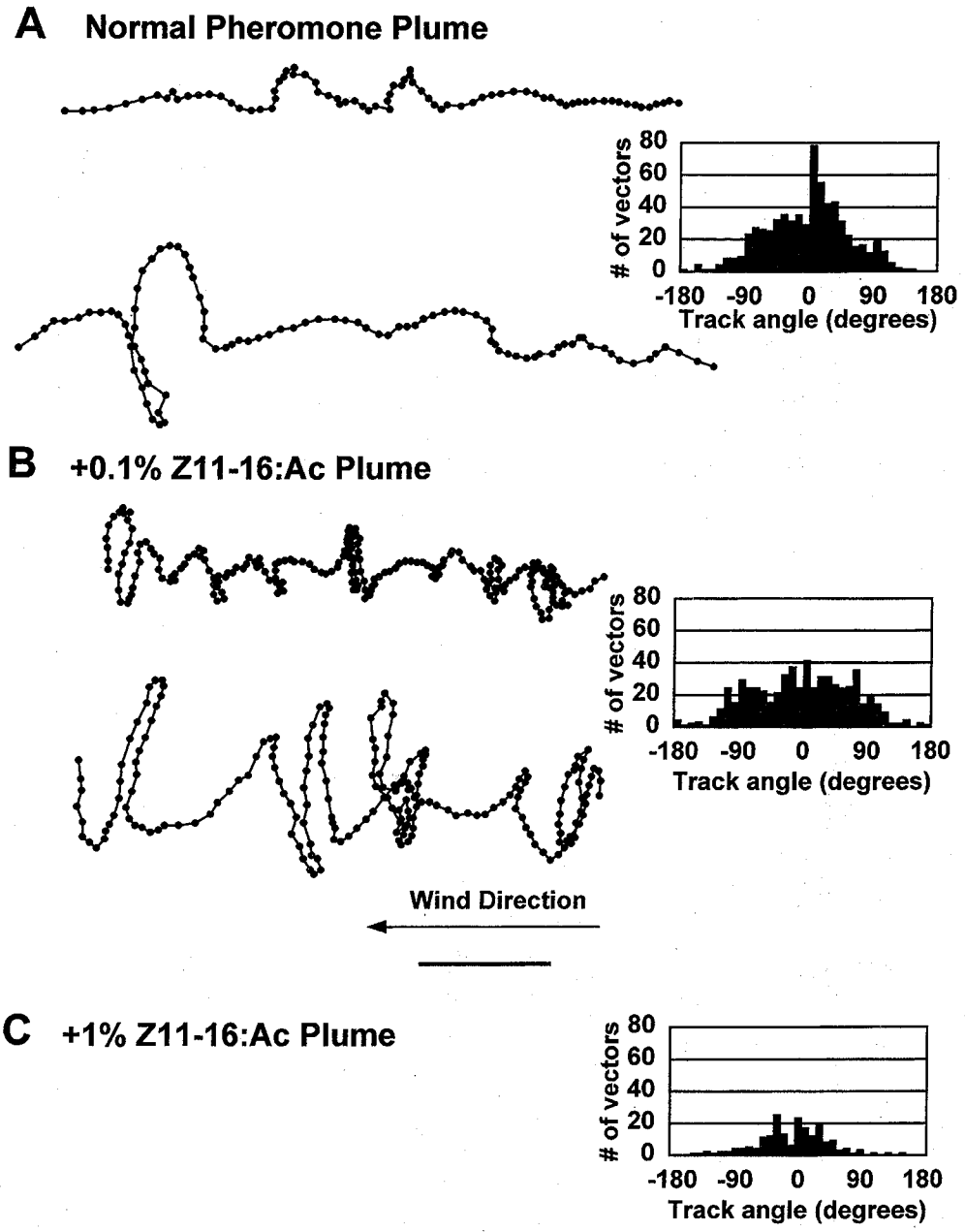

Fig. 2A–C Examples of complete flight tracks (source subsequently located by male) through the video camera's field of view in response to a control blend (100-μg Z11-16:Ald + 2.5-μg Z9-14:Ald) containing either 0% Z11-16:Ac (**A**) or 0.1% Z11-16:Ac (**B**). Tracks to plumes containing 0.1% Z11-16:Ac were typically more tortuous (**B**) than those of males responding to a control blend (**A**). Scale bar is equivalent to 20 cm. Inset in each treatment category is a plot of track angle vector distribution for all complete flights to the source. There is a clear unimodal distribution for a normal pheromone blend ($N = 6$ males, 653 vectors) suggesting more direct upwind flight compared with blends tainted with 0.1% Z11-16:Ac ($N = 4$ males, 599 vectors), as evidenced by the more even non-unimodal distribution. Blends containing 1% Z11-16:Ac elicited nearly complete flights in only two males (neither male subsequently located the source) and the distribution of their track angle vectors during the upwind portion of the flight track (**C**) appears to be non-unimodal ($N = 2$ males, 196 vectors). Track angles were grouped into 10° bins from −180° to +180°

sponded to the loss of pheromone with approximately the same time course.

When males intercepted a pulse of the binary blend (ON), they responded by making an upwind movement towards the source (Fig. 3B). This was revealed by the sustained, significant decrease in the track angles to more upwind beginning 0.3 s following ON and lasting for 0.3 s before slowly reverting to casting (90°) flight by 0.9 s following ON. The course angles steered by the males reflected this change in the values for resultant track angle. Males headed more upwind with a significant decrease in course angle 0.2 s following ON, which lasted for 0.4 s (Fig. 5C). This was accompanied by a change in airspeed that was shorter than the steering maneuver (Fig. 6C), lasting only for 0.2 s. However, the surge in airspeed was coincident with the turn into the

Table 1 Counterturn tempo (as measured by track leg duration in seconds) for males responding to truncation of the pheromone plume (OFF). Track legs prior to the one on which the male encountered the last possible filament of the pulsed plume are indicated as negative (−1 to −4). The tempo of counterturning for track legs, including and following OFF were compared against the track leg immediately prior (i.e., −1). Track legs of significantly greater tempo are indicated (*). Significance was determined by a one-tailed Wilcoxon matched-pairs rank test at $P < 0.02$. Tempos marked^{NT} were not tested. $N = 10$ in all categories

Track leg	−4	−3	−2	−1	OFF	1	2	3	4
Counterturn tempo	0.28^{NT}	0.27^{NT}	0.22^{NT}	0.23	0.23	0.3	0.28	0.35*	0.33*
(±MAD)	0.05	0.07	0.05	0.07	0.03	0.03	0.05	0.08	0.05

wind made by the males. There was no change in groundspeed during the upwind surge, and this was common to all experimental groups, whether there was an overt, significant response or not.

In contrast, males made no apparent changes in orientation or counterturning frequency when they intercepted a pulse containing no pheromone (blank pulse). Resultant track angles continued to be oriented at 90° with respect to the wind-line indicating that the males remained in casting flight (Fig. 5B). Not surprisingly, the underlying anemotactic behaviors of alterations in course angle (Fig. 5A) and airspeed (Fig. 6A) by these males also remained relatively constant, there being no significant differences between the 0.1 s immediately prior to the arrival of the pulse and any of the following 0.1-s intervals. Groundspeeds of these males fluctuated more, but there were still no significant differences or consistent trends (Fig. 6B).

The reasons why the surges seemed to be compacted and contorted in response to filaments containing either 0.1 or 1% Z11-16:Ac were revealed by the behavioral analyses. With 0.1% Z11-16:Ac in the filament blend, males made a significant upwind change in their track occurring with a 0.1-s latency, half the latency of normal filament males. However, the duration of the more upwind track angle was less than that of the control males responding to a filament lacking the acetate, only being sustained for 0.2 s compared with 0.3 s in those males responding to the binary-blend filament (cf. Fig. 5F, D). These males also quickly reverted back to casting flight, 0.6 s following ON (Fig. 5F) compared with 0.9 s in

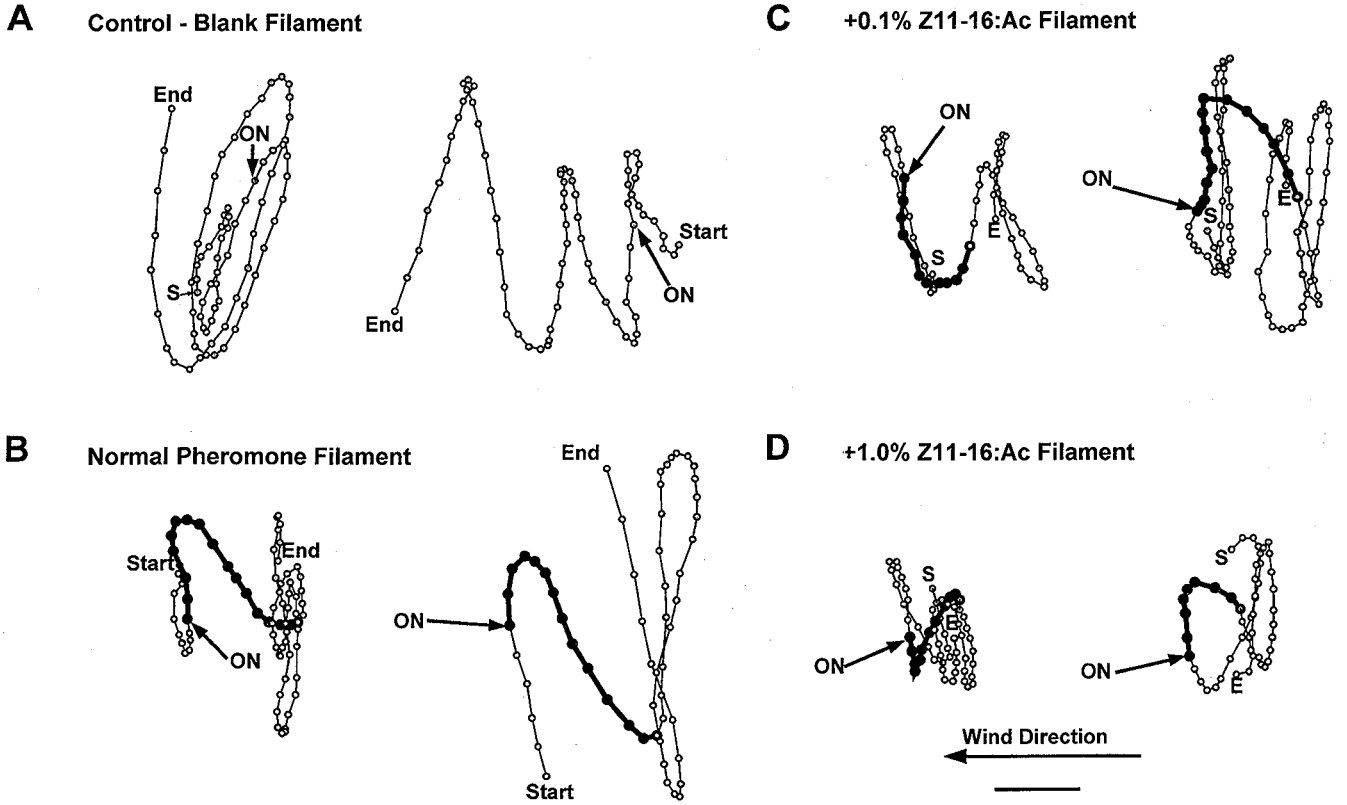

Fig. 3A–D Males did not make upwind movements during casting flight in clean air following the truncation of the plume in response to a control, blank pulse (**A**). In (**B**) males responded to interception of a normal pheromone filament by making a toward-source upwind movement. Males, casting prior to *ON* (*grey circles, thin line*) exhibited a short latency followed by an upwind movement (latency and surge indicated by *bold circles and line*, surge track angles < 60°) which lapsed back to casting (as defined by track angles > 60°, indicated by return to *grey circles and thin line*) between 0.3 and 0.4 s later. Following single pulse interception, the presence of 0.1 or 1% Z11-16:Ac in the filament appeared to diminish the upwind portion of the surge (**C** and **D**, respectively). Scale bar is equivalent to 10 cm

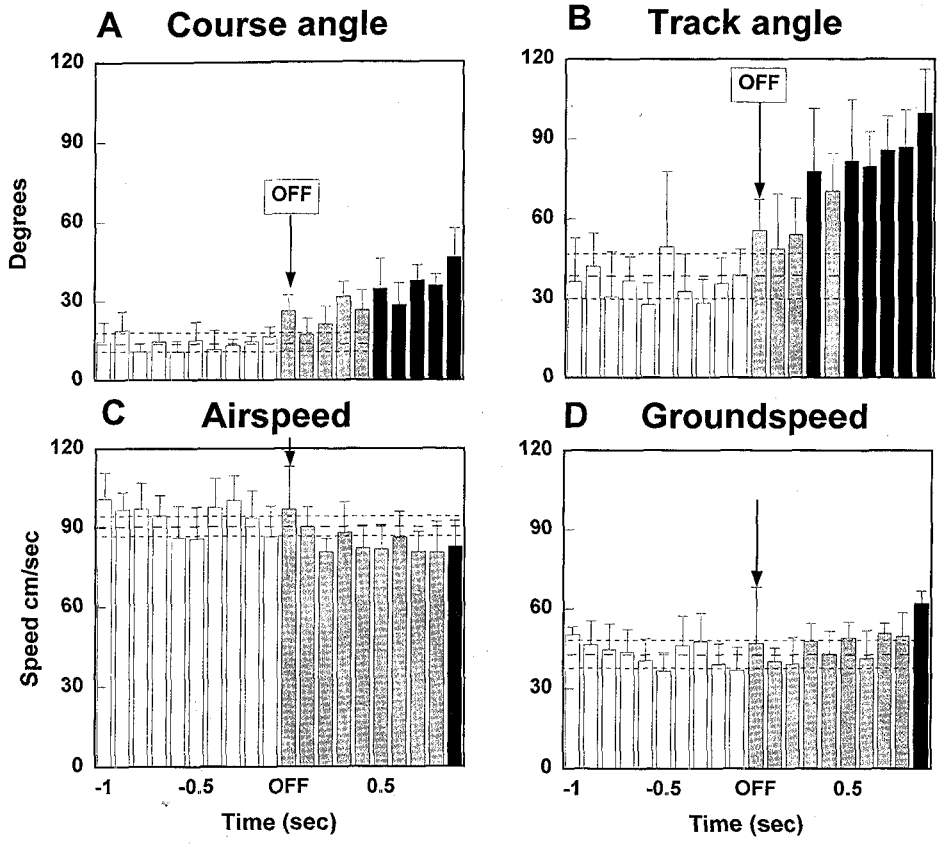

Fig. 4A–D Triangle of velocities analysis of behavior of males responding to their envelopment in clean air following plume truncation ($N = 10$). The *broad dashed line* represents the median value for all males for 1 s prior to *OFF* (*arrowed bar*) and the *fine dashed lines* represent the MAD about the median. Each 0.1-s bar from *OFF* to the end of the record is compared with this median value, but bars prior to *OFF* are not (*unshaded bars*). *Black* but not *grey bars* are significantly different from the median value ($P < 0.01$), indicating a significant change in behavior from that established prior to *OFF*

males responding to the control, binary-blend filament (Fig. 5D). Groundspeed remained at its previous level (Fig. 6F) and the change that occurred in track angle was largely attributable to a significant increase in airspeed (Fig. 6E). The anemotactic change in airspeed in males responding to acetate-tainted filaments was not accompanied by significant changes in the course angles steered by the responding males.

A ten-fold increase in the dosage of Z11-16:Ac resulted in an apparent further diminution of the behavioral responses (Fig. 3D). Even though it was clear that individual males made an upwind movement in response to interception of a filament, the responses were so reduced that when viewed in combination there was no significant difference between variables following odor contact compared with those just before arrival of the filament. Track and course angles did become oriented more upwind after odor contact but no significant differences existed between any 0.1-s interval following ON and the median value for 0.3 s immediately prior to ON (Fig. 5G, H). Track angles returned to full casting (at 90°) within 0.6 s following interception of the tainted filament. Airspeeds did increase somewhat (Fig. 6G) but they were not sustained, nor did they approach the same high levels that were seen in the untainted filament treatment (Fig. 6C). Groundspeed, as with the other treatments, remained unchanged after contact with the filament (Fig. 6H).

Counterturning tempo also was affected by the quality of the odor filament that males intercepted (Table 2). Following contact with a normal filament, the two inter-reversal intervals following ON were significantly shorter than during the casting that occurred prior to ON, indicating that exposure to a good-quality filament increased the counterturning frequency of the male for more than 0.6 s (Table 2). In contrast, filaments containing 0.1% Z11-16:Ac resulted in only one reversal following ON being of significantly shorter duration than during casting, and further contamination with 1% Z11-16:Ac resulted in no significant increase in counterturning tempo for the three track legs following ON (Table 2).

Discussion

The addition of Z11-16:Ac to a two component blend of the sex pheromone of *H. virescens* resulted in a reduction in the proportion of upwind flights and source location (Fig. 1). Even amounts as low as 0.1% Z11-16:Ac relative to Z11-16:Ald, the major component of the blend, diminished the ability of males to lock-on, fly upwind, and locate the source. At 1% Z11-16:Ac male responsiveness was more markedly diminished indicating that males were highly sensitive to blends containing this previously undocumented antagonistic compound.

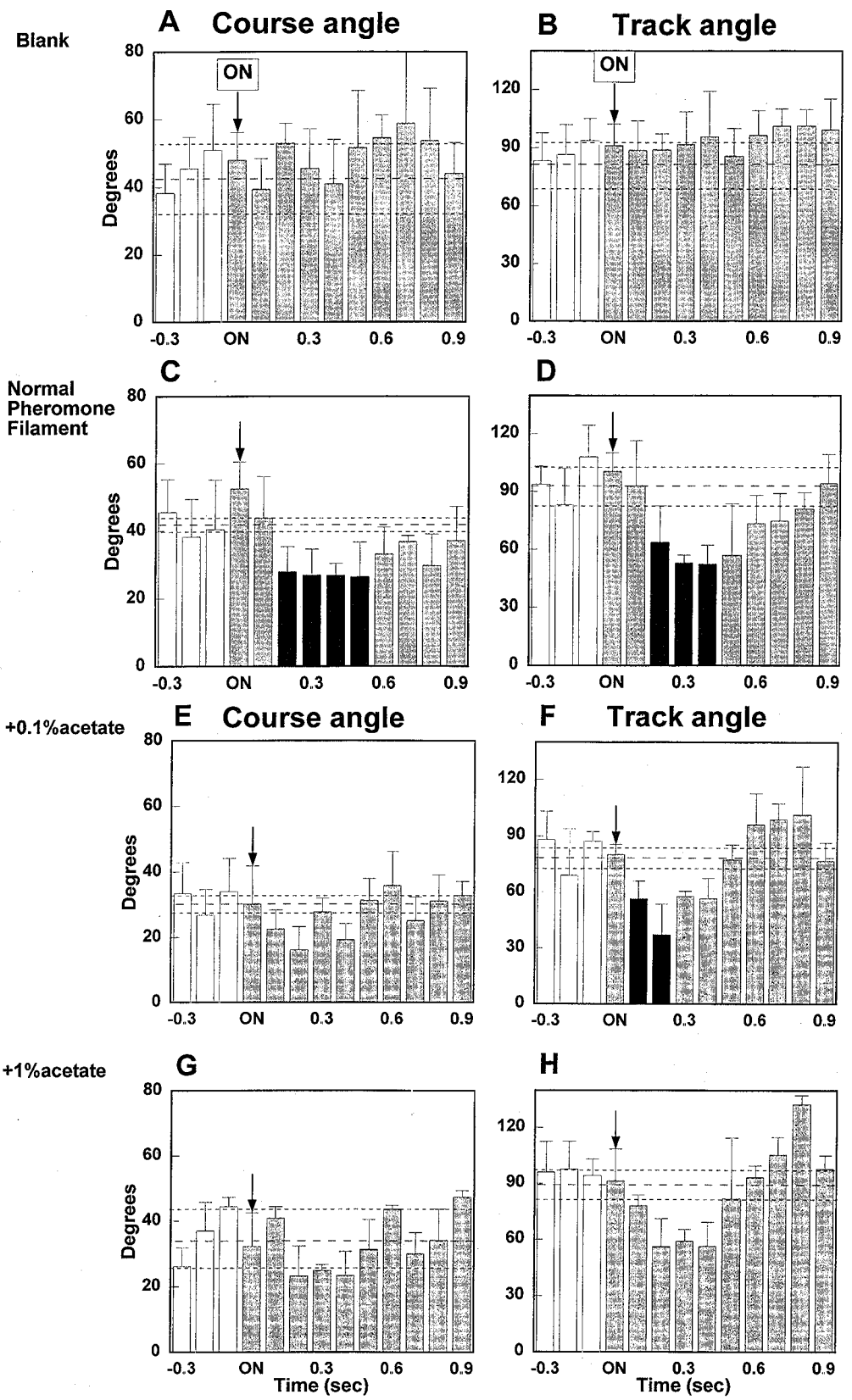

Fig. 5A–H Course and track angle variables for males exposed to blank filament ($N = 10$) and either normal blend filament ($N = 10$) or those containing either 0.1% Z11-16:Ac ($N = 10$) or 1% Z11-16:Ac ($N = 7$). A median value (*broad dashed line*) ± MAD (*fine dashed lines*) was calculated from the behavior of the males for 0.3 s prior to the odor event. *Bars* including and following *ON* (*arrowed bar*) were tested against this median value, *black* but not *grey bars* were significantly different at $P < 0.01$. Bar values prior to *ON* were not tested (*unshaded bars*) but are included to show the pattern of behavior. Course angles are plotted for each of the four treatments (**A**, **C**, **E**, and **G**). Similarly, track angles are represented by **B**, **D**, **F**, and **H**

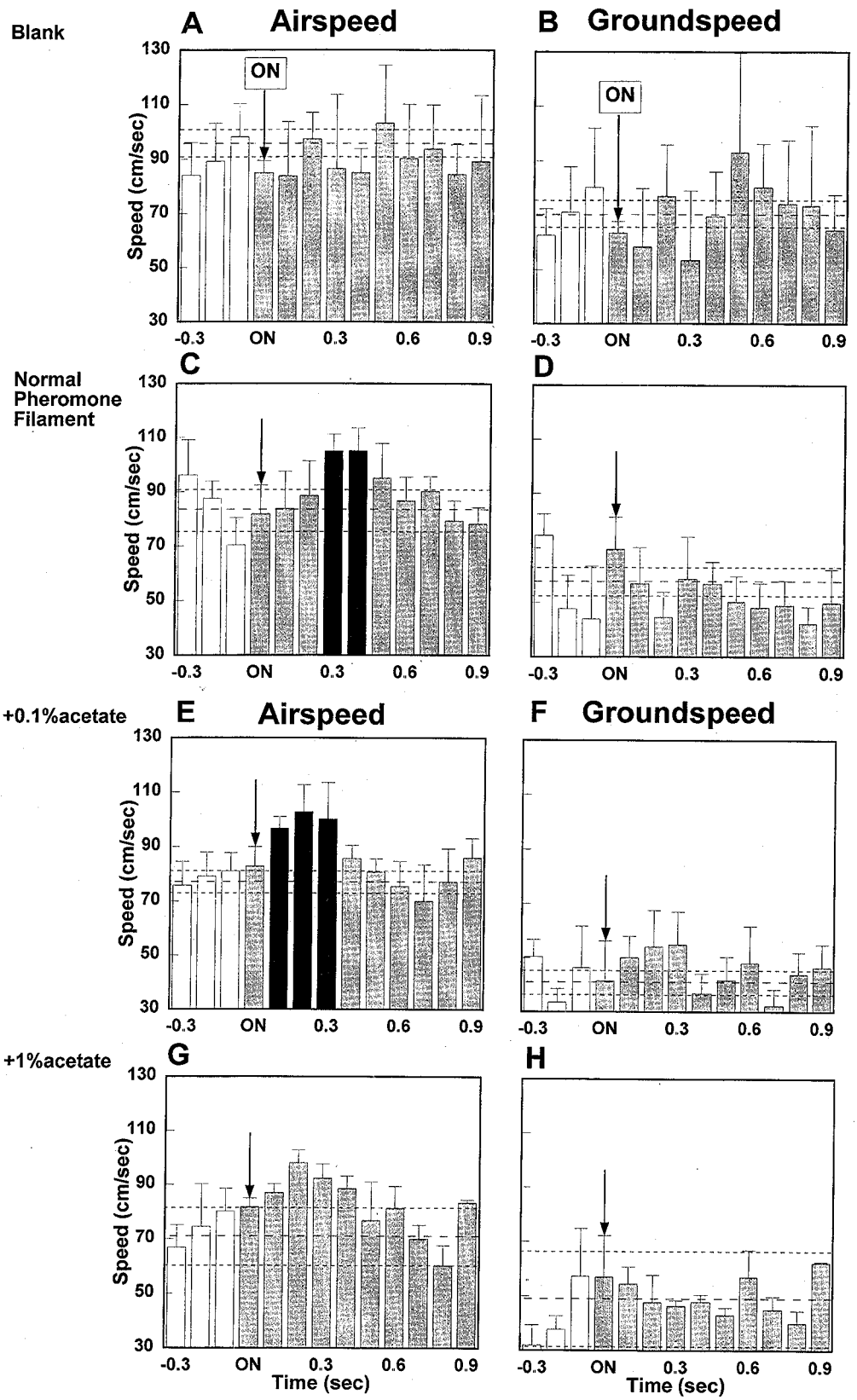

Fig. 6A–H Airspeed and groundspeed variables for the four experimental groups of males. Airspeeds are plotted for each of the four treatments (**A**, **C**, **E**, and **G**). Similarly, groundspeeds are represented by **B**, **D**, **F**, and **H**. Details as for Fig. 5

Table 2 Counterturn tempos (as measured by track leg duration in seconds) of males exposed to filaments of experimentally varied olfactory quality. Track legs prior to the one during which the pheromone filament was encountered (ON) are indicated as negative (−1 to −2). Median durations of track leg including and following ON were compared with the median duration of the track leg prior to ON (i.e., −1) for each treatment. Track legs that were significantly longer (blank treatment) or shorter (normal, +0.1%, +1%Z11-16:Ac) were determined by a one-tailed Wilcoxon matched pairs rank test at $P < 0.02$ and are indicated by *. Track legs prior to −1 were not tested as indicated by ^{NT}

Treatment	Track Leg					
	−2	−1	ON	1	2	3
Blank						
Counterturn tempo	0.32^{NT}	0.33	0.37	0.38^{*}	0.43^{*}	0.43^{*}
(±MAD)	0.03	0	0.05	0.05	0.07	0
N	6	10	10	10	9	7
Normal pheromone filament						
Counterturn tempo	0.4^{NT}	0.37	0.35	0.3^{*}	0.3^{*}	0.32
(±MAD)	0.07	0.07	0.02	0.03	0.03	0.02
N	8	10	10	10	9	7
+0.1% Z11-16:Ac						
Counterturn tempo	0.33^{NT}	0.37	0.32	0.3^{*}	0.27	0.33
(±MAD)	0.05	0.07	0.05	0.07	0.03	0.05
N	10	10	10	10	9	6
+1% Z11-16:Ac						
Counterturn tempo	0.37^{NT}	0.37	0.4	0.33	0.3	0.3
(±MAD)	0.1	0.07	0.13	0.03	0.03	0.05
N	4	7	7	7	7	6

Further experiments showed that the same small percentages of Z11-16:Ac that had diminished upwind flight in response to point-source plumes, but now placed into single filaments, stunted the upwind surges of those males that responded to contact with the filaments. Males did not make the same amount of upwind progress and returned to casting flight more quickly following interception of a Z11-16:Ac-tainted filament. Thus, our study indicates that the reduction of upwind flight in response to point-source plumes containing Z11-16:Ac is due to a reduction in the degree to which each upwind surge is sustained in response to each filament in the plume. Hence, the males spend more time in casting flight and less time surging toward the source.

Peripheral and central olfactory neurophysiology

Peripherally and centrally located chemoresponsive cells have been identified that are selective for the pheromone components and blends thereof in *H. virescens* males. Previous studies (Almaas and Mustaparta 1990, 1991) indicated that most receptor neurons housed in sensilla on the antenna were responsive to Z11-16:Ald or Z9-14:Ald. A third category responded to (*Z*)-11-hexadecenol (Z11-16:OH), a known disruptant of upwind flight (Shaver et al. 1982; Vetter and Baker 1983). Recently, Berg et al. (1995) concluded that those receptors previously classified as being specific for Z11-16:OH were in fact more sensitive to Z11-16:Ac and only secondarily did they respond to Z11-16:OH (Berg et al. 1995). As both of these compounds have now been shown to be antagonistic at the behavioral level (Shaver et al. 1982; Vetter and Baker 1983; Vickers and Baker, this study), we must conclude that it is this type of neuron that mediates the suppression of upwind flight when either of these two compounds is present.

Hansson et al. (1995) recently stained some of these three types of receptor neurons and showed, in three separate cases, that singly stained axons selective for Z11-16:Ac projected to a compartment of the MGC that was not innervated by receptors of any other type. This is the first known example of receptor neurons that are not responsive either to a pheromonal component or to an immediate metabolite thereof projecting to an area either in, or closely associated with, the MGC. In other species, receptor neurons responsive to compounds that are not a part of the pheromone blend have been identified but these compounds are thought to be the metabolite of the major component acted upon by esterases in the sensillar lymph. In *Agrotis segetum* receptor neurons responsive to (*Z*)-5-decenol (Z5-10:OH) are housed in the same sensilla as (*Z*)-5-decenyl acetate (Z5-10:Ac) cells (Hansson et al. 1992) and in *Trichoplusia ni* receptor neurons responsive to the major component (*Z*)-7-dodecenyl acetate are paired in the same sensillum type as those selective for (*Z*)-7-dodecenol (Z7-12:OH) (Todd et al. 1992), a known antagonist of upwind flight (Liu and Haynes 1992). In both of these species, two receptor neuron types project to distinct areas within the MGC (Hansson et al. 1992; Todd et al. 1995) and it is thought that their activity might indicate high concentrations of the major component, a scenario that might occur when other, sympatric species use similar compounds but at higher overall release rates.

In *H. virescens* males Z11-16:Ald is not degraded into Z11-16:Ac but rather the analogous carboxylic acid: (*Z*)-11-hexadecanoic acid (Tayasco J and Prestwich 1990a,b), and furthermore, the fact that these receptors are housed in different sensilla (Almaas and Mustaparta 1990, 1991;

Berg et al. 1995; Hansson et al. 1995) suggests that they may play a different role than the putative concentration detectors in *A. segetum* and *T. ni*. Their role would seem to be either to detect Z11-16:Ac in otherwise similar blends released by sympatric females of different species such as *H. subflexa* (Teal et al. 1981) or perhaps to detect the presence of other *H. virescens* males that emit Z11-16:Ac as part of a suite of compounds present in the hairpencil structures (Teal and Tumlinson 1989). The behavioral effect of Z11-16:Ac in influencing female *H. virescens* behavior has yet to be demonstrated.

The encounter between the moths' antenna(e) and a single filament will vary as a function of the moths' airspeed and course angle, and in a natural arena will also be affected by physical factors such as wind speed and turbulence. However, in the wind tunnel, under less variable physical conditions and short distances (< 1 m) from the point of origin, the encounter presumably lasts less than the 20-ms air pulse that created the filament. This brief encounter has long-lasting behavioral consequences. Receptor cells of *Antheraea polyphemus* are projected to reach their maximal firing rates 100 ms following odor contact and to finish responding after 300 ms (Rumbo and Kaissling 1989; Kaissling and Kramer 1990), approximately the time at which male *H. virescens* begin their behavioral response (Vickers 1992; Vickers and Baker 1994, 1996, this study). The male does not return to casting flight until 800–900 ms post-odor, and casting flight itself continues for many seconds longer even though the receptor cells are completely quiet. The entire sequence is modulated by the brief encounter between moth and odor, a feature incorporated by Baker (1990) in his model of pheromone-mediated upwind flight but not in the similar hypothesis of Kaissling and Kramer (1990).

Receptor cells and projection interneurons that reside within the antennal lobe are capable of following pulses delivered at high frequencies. Receptor cells on the antennae of the related species, *Helicoverpa zea* are known to be capable of following 20-ms pulses of pheromone delivered at up to 9.8 Hz (Almaas et al. 1991). Pheromone-specific projection interneurons in *Manduca sexta* also are able to follow pulses of odor delivered at up to 10 Hz (Christensen and Hildebrand 1988). In *H. virescens* interneurons have been physiologically characterized as being exclusively sensitive to Z11-16:Ac (Christensen et al. 1995). This type of neuron has not been morphologically identified but neurophysiological hallmarks indicate that they too convey information from the antennal lobe to higher centers in the brain. Other classes of projection interneuron represented in the antennal lobe include a rare class of blend cells that rely upon the presence of both essential pheromone components Z11-16:Ald and Z9-14:Ald to evoke a robust, long-lasting tonic response. The effect of the presence of Z11-16:Ac upon this type of interneuron and others is currently unknown. These different projection pathways from the antennal lobe may not perform complex integration of peripherally detected information but instead could simply report activity to higher centers in the brain for further processing in a relatively unadulterated form.

Behavioral effects of antagonistic compounds in other moth species

Analysis of flight tracks from males of other species responding to point-source plumes that have altered pheromone component ratios have revealed differences in flight track shape of *Grapholita molesta* (Willis and Baker 1988). Incomplete blends also had a profound effect upon flight track shape in *Ephestia cautella* (Quartey and Coaker 1993). In both studies males of each species progressed more slowly upwind toward the source. Perhaps the males do not surge as effectively upwind when off-ratio blends are presented, thus accounting for their slower and more tortuous upwind progression. Similarly, a calling female may elicit a fast, direct approach in a responding male compared with that evoked by a synthetic source (Witzgall and Arn 1990), the latter possibly lacking some of the necessary components at the correct ratio that would otherwise elicit optimal upwind surges and hence more direct upwind flight (Baker and Vickers 1996; Vickers and Baker 1996). The compensatory reactions of tethered moths also can be changed by the presence of a known attraction-inhibiting compound. Preiss and Kramer (1983) demonstrated with gypsy moths that (−)-disparlure presented in a racemic mixture with the attractant isomer (+)-disparlure, blocked the compensatory responses of altitude stabilization and flight speed that occurred in response to moving visual patterns when (+)-disparlure alone was present.

Responses of males of two further species to their respective pheromone blends also were shown to be affected by the presence of antagonists. In *T. ni*, addition of Z7-12:OH to the pheromone blend suppressed upwind flight and source location (Liu and Haynes 1992). Males steered more directly upwind and reduced their airspeed when Z7-12:OH was present in the blend resulting in slower net upwind progress in the plume (Liu and Haynes 1993). In *Coleophora laricella* Z5-10:Ac blended in as little as 0.001% of the attractive alcohol component Z5-10:OH resulted in a significant decrease in attraction, although the underlying anemotactic changes that may have accompanied this decrease were not reported (Witzgall and Priesner 1991).

The emerging picture of behavioral effects of altered blends is complemented by a growing understanding of receptor neuron and projection interneuron physiologies and morphologies. Where the disruptive effect of the presence of biologically relevant, attraction-inhibiting compounds occurs within the central nervous system will be the subject of future study.

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