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Effects of the structure and composition of pheromone plumes on the response of the male almond moth, Cadra cautella.

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EFFECTS OF THE STRUCTURE AND COMPOSITION OF PHEROMONE PLUMES ON THE RESPONSE OF THE MALE ALMOND MOTH, *Cadra cautella*

A Dissertation Presented

by

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DEDICATION

Aos meus queridos pais,
Inge and Ben-Hur Mafra,

Para a luz da minha vida,
Kim Li Spencer,

and to the
Josés Ripper

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ABSTRACT

EFFECTS OF THE STRUCTURE AND COMPOSITION OF PHEROMONE PLUMES ON THE RESPONSE OF THE MALE ALMOND MOTH, *Cadra cautella*

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The influence of the completeness of the blend and quantity of female produced pheromone on the response of male *Cadra cautella* (Lepidoptera: Phycitidae) was investigated. The threshold concentrations for initiation of pheromone-mediated behaviors are set solely by (Z,E)-9,12-Tetradecadienyl Acetate (the major component), but the presence of (Z)-9-Tetradecenyl Acetate (the minor component) at concentration levels above threshold increased the proportion of males engaging in intermediate and late in-the-sequence behaviors. The organization of male response to pheromone in *C. cautella* is in accordance with the component hypothesis, in that the dimensions of the active space were delimited by only part of the blend, and not by the whole blend acting as a unit, as stated by the blend hypothesis.

Investigation of the effects of blend and concentration of pheromone upwind flight orientation of *C. cautella* males demonstrated that males fly directly upwind not only to the blend that mimics the female gland extract, but also to an array of "wrong" or "sub-optimal" pheromone blends (i.e., incomplete blends and blends containing the "inhibitor" (Z,E)-9,12-tetradecadienol. Parameters of the flight of males along filamentous plumes of complete blends were indistinguishable from males flying along filamentous plumes of incomplete blends. Male flight is influenced by both the composition of the chemical blend and the structure of the pheromone plume: when the plume is wide and non-turbulent, *C. cautella* flew faster to incomplete blends than to complete blends.

The structure of the pheromone plume influences the flight pattern of *C. cautella* males flying to the complete blend at optimal dosages. Increase in plume size resulted in faster ground velocities, lower turning frequency, narrower turns, and reduced track angles. In short, increasing plume size results in faster and more direct upwind flight. Although changes in pheromone concentration had discernible effects on male upwind flight, concentration effects were smaller than the effects related to changes in plume shape. The internal structure among the plumes was manipulated to produce pulses of pheromone in turbulent plumes and no pulses in the homogeneous filament plume. The turbulent plumes had different mean pulse frequencies, mean pulse durations, and mean pulse sizes. Males flying to smaller and less turbulent plumes had tracks with counterturns dominating the flight pattern; males flying to larger and more turbulent plumes suppressed counterturning, which resulted in flight tracks straighter upwind.

When filamentous pheromone plumes were marked with smoke, in wind tunel situations, we were able to monitor *C. cautella* males changing their inflight maneuvers in response to encounters with pheromone plumes. *C. cautella* males turned more crosswind when contacting non-turbulent filamentous plumes, and turned more upwind when contacting turbulent pockets of smoke and pheromone in the same filamentous plume.

We explored two features determining the internal structure of the plume, the volume of "continuous" plumes, and and the interval between several pulse durations. Males fly faster and straighter to intermittent pheromone plumes consisting of large puffs pulsed at high frequency. When pheromone puffs were delivered at low frequencies, moths responded to individual pulses by "locking on" and flying upwind after contact. The basis of the in flight pheromone-mediated behavior might be the individual responses to single pulses. A modified version of Wright's olfactory guidance model that incorporates behavioral responses to single odor pulses best describes the different patterns of upwind flight tracks observed with the changes in plume structure tested.

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CHAPTER I

EFFECT OF PHEROMONE BLEND AND CONCENTRATION ON THE THRESHOLD AND ORGANIZATION OF MALE RESPONSE IN *Cadra cautella* (LEPIDOPTERA: PHYCITIDAE)

1.1 Introduction

An outstanding question in the study of behavioral responses of insects to their sex pheromone is the influence of each component of a pheromone blend in setting the thresholds for each stage of behavioral response. These thresholds in turn delimit the boundaries of the active space of each reaction. Two hypotheses have been proposed to explain the influence of threshold on the dimension of the active space (summarized by Linn *et al.* 1987; Linn and Roelofs 1989). The **component hypothesis** states that the earliest responses (e.g., activation & flight upwind or "locking on" to the pheromone plume) are based on the presence of one to perhaps several of the "major" components of a blend. Additional components participate in the elicitation of later behaviors, such as approach of the pheromone source, landing and courtship. The **blend hypothesis** contends that the entire composite of pheromone components has the lowest threshold for all behaviors and therefore the blend acts as an ensemble to mediate the initial to the final behaviors in a sequence of response.

A variant on this motif (particularly in moths) is the response being contingent upon a precise ratio of components: either the response is not evoked by unnatural ratios, or if response does occur, the natural ratio has the lowest threshold (Roelofs 1978), an organization of response consistent with the blend hypothesis. Alternatively, the earliest responses might be triggered by all components (the blend), but the threshold may not be sensitive to some deviation from the natural ratio of some or all of the components (Cardé & Charlton 1984). However, at higher concentrations the later behaviors would have the lowest threshold for the complete blend at the natural ratio.

We investigated the influence of the completeness and quantity of the pheromone blend on the response of the male almond moth, *Cadra cautella* (Walker) (Lepidoptera: Phycitidae). This stored product pest originated from the tropics and now has a cosmopolitan distribution (Levinson & Buchelos 1981). Three components are known from abdominal pheromone glands of *C. cautella* females: (*Z,E*)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), (Brady *et al.* 1971; Kuwahara *et al.* 1971a, 1971b), (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) (Brady 1973) and (*Z,E*)-9,12-tetradecadienol (Z9,E12-14:OH) (Kuwahara & Casida 1973, Read & Beevor 1976). The two acetates, but not the alcohol, were consistently present in airborne pheromone collections of calling females, in ratios similar to those reported for gland extractions (Coffelt *et al.* 1978; Barrer *et al.* 1987; Coffelt & Vick 1987; Shani 1990). In field trials Z9,E12-14:Ac alone was attractive and Z9,E12-14:OH and Z9-14:Ac were not attractive by themselves. When added to Z9,E12-14:Ac, the monounsaturated acetate increased trap catch, whereas the addition of the alcohol depressed trap catch (Read & Haines 1976). Read and Haines suggested that the alcohol could function as a "postcopulatory repellent." Coffelt and Vick (1987) concluded that, since the alcohol was not being released by females (before or after mating), it is was not part of the *C. cautella* pheromone.

Other phycitine species utilize pheromone components found in *C. cautella* females glands. The alcohol is a minor component of the pheromone of frequently sympatric phycitine *Plodia interpunctella* (Hübner) and *Anagastha kuehniella* (Zeller). Because this component inhibits courtship in *C. cautella* males, it was viewed as an important reproductive isolating mechanism among these species and *C. cautella* (Ganyard & Brady 1971; Grant & Brady 1975).

This study was undertaken to define the patterns of *C. cautella* male response in a wind tunnel to pheromone sources of varying completeness and concentration.

1.2 Material and Methods

1.2.1 Insects

The *C. cautella* colony was started in March 1989 from some 500 larvae and pupae from Kansas State University, Manhattan, Kansas, and was maintained as a continuous culture at a level of at least 400 mating pairs per week. The *C. cautella* were reared from eggs to larvae in 1 liter glass jars on an artificial diet consisting of 3 kg poultry laying mash, 2 kg rolled oats, 200 ml glycerin, 100 g Brewer's yeast. The rearing room was held at 25-27°C, 50-60% RH on a 16:8 L:D. Individuals were sexed at the last larval instar (when the males testes are visible). Females were held in the same room as the main colony. Males were reared from last larval instar to adult in a separate room inside environmental chambers with the same photoperiod, 70% RH and 25-26°C. Male pupae were held inside a 25 X 25 X 25 cm screened cage, where adults emerged. The pupae were transferred daily to new cages, leaving newly emerged males in the old cage. This procedure generated a constant supply of 1-day-old males.

Glands were excised from 2-day-old females during the first hour of the scotophase and extracted with 25 µl of redistilled hexane. The abdomen was squeezed to extrude the abdominal tip, the tip was cut off and immersed for 3 minutes in 25 µl hexane with 10 ng dodecyl acetate (12:Ac) as the internal standard. Optimal time of hexane extraction for *C. cautella* was determined to be as 3 minutes, when >90% of the pheromone present in the gland was extracted and the amount of contaminant was minimized compared to longer intervals.

Gland extracts were placed in 150 µl microvials and stored in 4 ml screw-cap vials with Teflon® liners; 200 µl of hexane was added to saturate the vial's internal atmosphere, thus avoiding evaporation of the sample. The extracts were stored at -20°C.

1.2.3 Chemicals

Chemicals were obtained from either Farchan Chemicals [Z9,E12-14:Ac, 97% pure; Z9-14:Ac, 99% pure; and Z9,E12-14:OH, 99% pure] or IOB [Z9,E12-14:Ac, 97% pure]. The diunsaturated acetate was purified to 99.9% on a silver nitrate/Florisil column with an increasing polarity gradient of isopropyl-ether and hexane. The purity of compounds was determined by capillary gas chromatographic analysis on a Supelco 30 m x 0.32 mm ID SP 2340 column held at 70°C for 4 min., programmed at 12 °C $min.^{-1}$ to 200°C, and held at 200°C for 10 minutes.

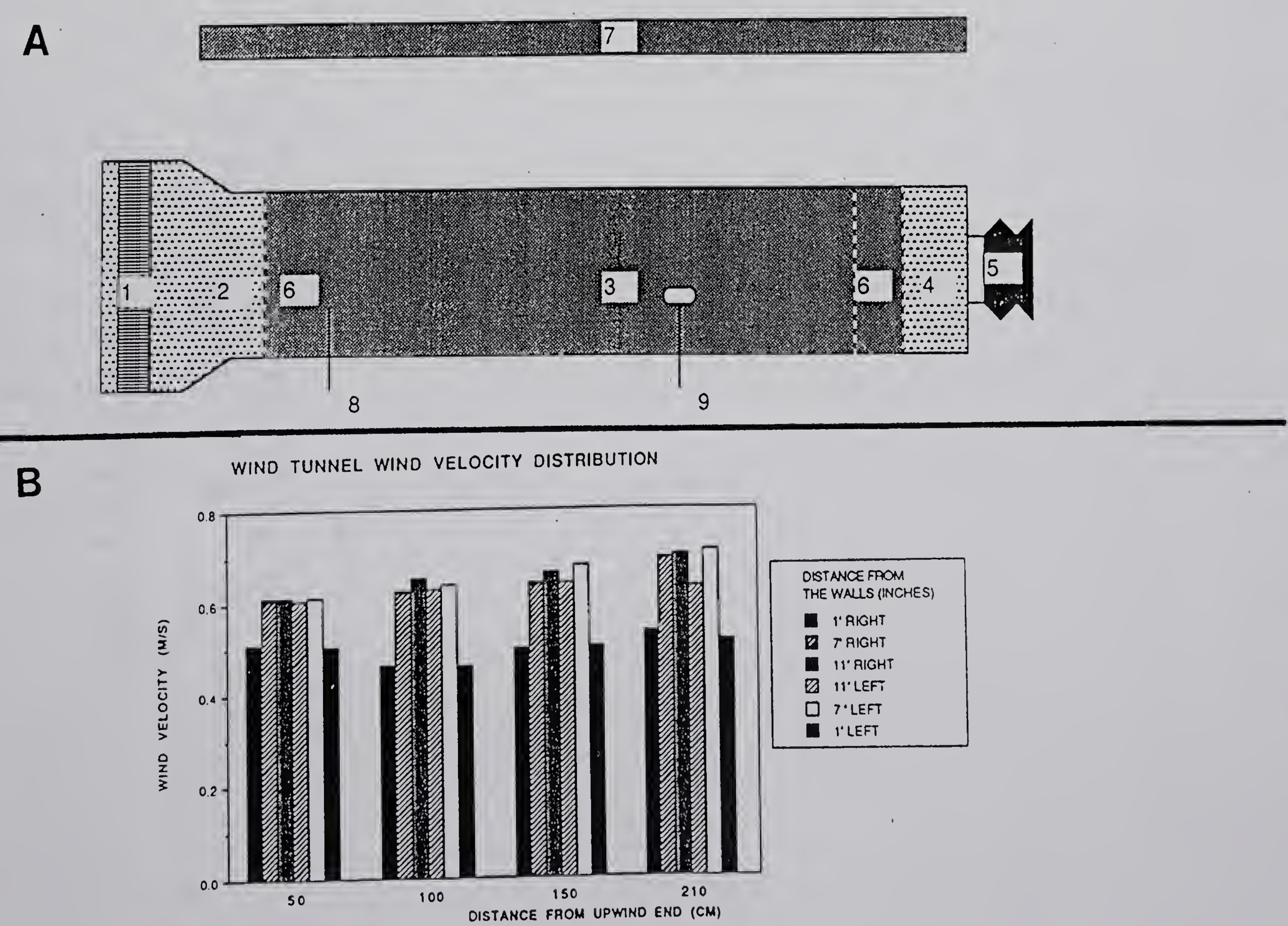
The synthetic pheromone components were formulated gravimetrically into solutions of 1µg μl^{-1}, and then volumetrically into the four mixtures. Since the Z9-14:Ac and the alcohol alone or in combination do not evoke male response unless accompanied by Z9,E12-14:Ac (Brady *et al.* 1976; *et ante*), all the treatments except a control contained Z9,E12-14:Ac. The ratio of the three components Z9,E12-14:Ac, Z9-14:Ac, and Z9,E12-14:OH in the female's gland was **5.67:1:1.25** and these proportions, respectively, were utilized in all blends of two or three components. Four treatments were tested: Z9,E12-14:Ac alone; Z9,E12-14:Ac plus Z9-14:Ac; Z9,E12-14:Ac plus Z9,E12-14:OH; and Z9,E12-14:Ac plus Z9-14:Ac plus Z9,E12-14:OH. The doses of pheromone tested ranged in decade steps from 450 fg to 450 µg of Z9,E12-14:Ac The filter paper odor source, a 0.7 cm diameter circle of Whatman #1 filter paper, was impregnated with 10 µl of the solution.

1.2.2 Wind Tunnel

The tunnel (Fig. 1) was constructed by bending a 3 mm thick clear sheet of Vivac®(2.5 x 1.8 m) lengthwise into a half cylinder 47 cm high. This was placed on top of a 5-mm-thick Plexiglas® floor (2.5 x 0.9 m) and was secured at right angles using aluminum corner (L) fixtures placed lengthwise. To increase the stability of the structure, metal bars were fastened along the long edges of the tunnel by screws passing through the aluminum fixtures, the Plexiglas® floor, and the bars themselves.

The upwind portion of the tunnel was attached to a 30 cm long tapered, rigid cardboard box which funneled incoming air into the body of the tunnel. Before entering the tapered section air passed through a 10 cm-thick Hexell® honeycomb aluminum layer, which reduced wind swirl and turbulence (Vogel 1983). A layer of fine polyester mesh covered the entrance into the plastic body of the tunnel. Turbulence caused by the "edge effect" (friction of the wind in contact with the wind tunnel's walls) was minimized by the tapering of the upwind box, which increased the air velocity along the sides of the tunnel. Air flow through the wind tunnel was laminar. This was confirmed visually using $TiCl_4$ "smoke" plumes and also by the low variance obtained from measurements of the wind speed in the tunnel using a hot-wire anemometer (Yokogawa, 2141). Air exiting the working section flowed through two layers of fine polyester mesh separated by 30 cm in a downwind box, which was attached to a 30 cm diameter exhaust pipe which pulled all air from the wind tunnel out of

Fig. 1. Schematic representation of the lateral view of the wind tunnel and histogram of distribution of wind velocity. **A)**. Schematic representation of the lateral view of the wind tunnel. **(1)**Hexell honeycomb aluminum layer, **(2)** rigid cardboard box (lightly hatched on the left) with tapered section, **(3)** working section of the wind tunnel, **(4)**downwind box, **(5)** exhaust pipe, **(6)** layer of fine polyester mesh, **(7)** light box, **(8)** source release device, **(9)** male release cage device. **B)**. Distribution of wind velocities in the working section of the wind tunnel measured at a height of 23 cm from the wind tunnel floor at six positions perpendicular to the length of the tunnel (2.5, 18, and 28 cm from both the right and the left walls) at the distances of 50, 100, 150, and 210 cm from the upwind screen.


A
7
1
2
6
3
6
4
5
8
9
B
WIND TUNNEL WIND VELOCITY DISTRIBUTION
WIND VELOCITY (M/S)
0.0
0.2
0.4
0.6
0.8
50
100
150
210
DISTANCE FROM UPWIND END (CM)
DISTANCE FROM THE WALLS (INCHES)
1' RIGHT
7' RIGHT
11' RIGHT
11' LEFT
7' LEFT
1' LEFT

the building. Airflow was measured using the anemometer positioned at the center of the tunnel, and wind speed was set to 50 cm sec^{-1} using a voltage regulator to control the exhaust fan.

The odor-impregnated filter paper disk was held in a horizontal position (parallel to the floor) by a #1 insect pin attached to the top of a hollow copper tube (0.3 mm diameter) that could be slid through the floor, allowing for the regulation of the vertical position of the odor plume in the tunnel. The source release device was located 45 cm from the sides of the tunnel, and 10 cm from the upwind screen.

The "smoke" plume generated by pipetting $TiCl_4$ into the source release device's filter paper was narrow and unbroken filament: 0.5 cm wide by 0.2 cm thick 10 cm downwind from the source, and 0.8 wide by 0.2 cm thick at 100 cm, where the male release cage device was located. A point intercepting this plume was continuously engulfed by smoke. This plume will be referred as filamentous plume.

The male release cage device was located 1 m downwind from the source release device in a position established using the $TiCl_4$ plume. The male release cage device consisted of a cylindrical aluminum screen cage (4.5 cm diameter x 5 cm). One end was covered with the same screen and the other end was open. The ragged edge of the cut screen on the open end was removed because in pilot studies the roughness of the edge was found to be an important source of variation in the time necessary for the behavioral transition from activation to initiation of flight. The cages were positioned with the open side facing upwind. The cages were held in position by a rigid Teflon® tube that had one end inside the cage and the other connected to a hollow glass tube. The hollow glass tube passed through the wind tunnel floor and the other end outside the wind tunnel. This design allowed the introduction of moths from outside the tunnel directly inside the positioned release cage without disrupting the pheromone plume. The height of the release platform was regulated by sliding the glass tube through the floor. Pilot experiments demonstrated that, after the male initiated flight, it was necessary to remove the release cage from the pheromone plume to obtain consistent rates of upwind flight. This was accomplished by moving the cage to 5 cm above the floor as soon as the male initiated flight.

Moths were gently transferred from the emergence cages to the male release cage glass tube using an aspirator. After exposure to the pheromone plume, the screen cage and the Teflon® tube were replaced by clean ones (rinsed with acetone and then held for > 5 hr at 500°C in temperature oven).

The light source was a box at the top of the working section of the tunnel with five red and five white 25 watt incandescent light bulbs and a filter/diffuser made of one layer of white Styrofoam (0.5 cm thick). Light level was set at 5.5 lux by voltage regulator. Humidity was maintained at 75-85% and temperature at 25±1°C (mean ± sd).

The wind tunnel floor had four circular ports (15 cm diameter) located in the mid line of the tunnel at 30, 110, 160, and 210 cm from the upwind screen. The floor pattern was made up of 10 cm red acetate circles randomly arranged on the Plexiglas® floor (David 1982).

1.2.4 Bioassay Procedure

The test of the 29 treatments was randomly ordered over a period of 4 days, i.e. a block, when ten males were tested per treatment. The experiment consisted of 6 blocks of four days; a total of 60 males therefore, were tested per treatment. In a given day, two groups of males were tested during the first hour of their scotophase (the first group with lights off at 14:00 and the second at 16:00), for 7 to 8 treatments. There was no a priori selection of males, i.e., the test was every adult male's first exposure to female pheromone, and the data from all males tested was used for the final statistical analysis.

Adult emergence cages were placed at experimental conditions of light and relative humidity (as described) for at least 30 min. prior to testing. Moths were selected randomly from emergence cages, and transferred to the release platform positioned below the level of the pheromone plume, 15 cm above the wind tunnel floor. The pheromone source was positioned 35 cm above the floor. Observations using either $TiCl_4$ "smoke" or high pheromone concentrations at the source showed that the plume did contact the release cage. Each quiescent male was held in the screen cage for 20 sec. Following 20 sec of quiescence, the pheromone source was lowered 15 cm and the male behavior was recorded. Each male was observed for 2 min., unless he landed on the source or on the wind tunnel walls. Males that touched the pheromone source had their upwind track, with the video camera, and behavioral observations, with the event recorder, terminated, but their behavior in the source was video recorded for one additional minute. Males that landed elsewhere had the observation of their behavior terminated as soon as they touched a surface other than the odor source platform.

After the male initiated flight, the release platform was lowered to 5 cm above the floor, removing it from the position where it intercepted the pheromone plume. This way the pheromone plume was more uniform downwind from the release platform. It also allowed males that locked onto the plume to proceed flying upwind without encountering the release platform.

An event recorder program for a computer (Tandy model 100) (Zanen *et al.* 1989) was used to record continuously the sequence and duration (in whole sec) of all male behaviors (Fig. 2). The sequence and duration of the following mutually exclusive behaviors at the platform and during upwind progression were monitored:

QUIESCENT (Q): no perceptible movement of the body or body parts;

WALKING (W) walking on the release platform without wing fanning;

WING FANNING AND WALKING (WFW): walking on the release platform while wing fanning;

WING FANNING (WF): wing fanning while stationary;

FLIGHT INITIATION (FI): time at which the male flies off of release platform;

RANDOM FLIGHT (RF): non-oriented flight, i.e., the male does not fly upwind along the pheromone plume;

CROSSWIND FLIGHT (CW): flight across the pheromone plume in wide zigzags without upwind progress;

ORIENTED FLIGHT (OF): zigzag flight upwind along the pheromone plume;

ZIGZAG (ZZ): a narrow (< 15 cm. wide) stationary zigzag flight across the pheromone plume (narrower than "crosswind flight")

LANDING ON THE SOURCE (LS): landing on the pheromone dispenser. This behavior terminates video and event recording of upwind flight for this male, and marks the beginning of recording of his behavior at the source platform for an additional minute;

LANDING ELSEWHERE (L): landing outside the pheromone plume, i.e., wind tunnel walls or floor. This behavior terminates video and event recording for this male.

Male upwind flight was video recorded through the tunnel floor,

Fig. 2. Example of the record of behavioral data and extraction of parameters used to measure behavior: **A)**.The event recorder generates a behavioral strings with two lines for each male. The first line contains the code of behavior performed followed by its duration in whole seconds. The second line (shaded) has the information about treatment (TRT), date (m.d), code for the record (MOTH), time of day (TIME), temperature of wind in the working section of the wind tunnel (TEMP), and an overall classification of the male's performance (CLASS). **B)**. The information contained in the first line of the behavioral string was extracted in three different ways: binary record for moths that performed (1) or not (0) the specified behavior (FREQUENCY), proportion of the total recorded time (for that moth) that the moth spend performing the specified behavior (PTT), and latency for the first occurrence of the specified behavior (LATENCY).

A. BEHAVIORAL STRINGS

MALE #1
Q 3; WFW 2; FI 1; RF 6; CW 8; OF 2; ZZ 8; OF 8; ZZ 8; OF 1; LS 1.

TRT: 25 DATE 10:04 MOTH:001 TIME: 15:43 TEMP: 25 CLASS: LANDED

MALE #2
Q 4; WFW 2; W 3; WFW 3; W 0; WFW 2; FI 1; RF 12; ZZ 8; L 0.

TRT: 25 DATE 10:04 MOTH:002 TIME: 15:46 TEMP: 25 CLASS: FAILED

MALE #3
Q 120.

TRT: 25 DATE 10:04 MOTH:003 TIME: 15:50 TEMP: 25 CLASS:FAILED

B. BEHAVIORAL DATA FROM THE STRINGS

	FREQUENCY			PROPORTION TOTAL TIME			LATENCY		
BEHAVIOR	MALE # 1	MALE # 2	MALE # 3	MALE # 1	MALE # 2	MALE # 3	MALE # 1	MALE # 2	MALE # 3
Q	1	1	1	0.055	0.148	1.000	0	0	0
W	1	1	0	0.000	0.111	0.000	120	6	120
WFW	0	1	0	0.037	0.259	0.000	3	4	120
WF	1	0	0	0.000	0.000	0.000	120	120	120
FI	0	1	0	0.018	0.037	0.000	5	12	120
RF	1	1	0	0.111	0.444	0.000	6	15	120
CW	1	0	0	0.148	0.000	0.000	14	120	120
OF	1	0	0	0.278	0.000	0.000	20	120	120
ZZ	1	0	0	0.333	0.000	0.000	22	120	120
LS	1	0	0	0.018	0.000	0.000	53	120	120
L	1	1	0	0.000	0.000	0.000	120	27	120
TOTAL	9	6	1	0.998	1.000	1.000			

using a Sony RSC 1050 rotary-shutter video camera connected to a SLO 340 video recorder in a field of view of 80 x 90 cm ending 15 cm from the odor source platform. Close flight approach to the pheromone source was recorded from the side using a second camera and video recorder. This recorder was equipped with audio capacity and verbal observations for all males flying within 20 cm of the odor source supplemented the video record. For all males landing on the source, subsequent behaviors were characterized using the event recorder. The video was replayed, and after the male first touched the source, the duration and sequence of the following behaviors were recorded over the course of one minute:

LANDING: landing on the odor release device (filter paper, pin or copper tube);

WING FANNING: wing fanning, walking, or hairpencil presentation performed at the odor release device;

SIT: male stationary with wings folded on the odor release device;

INITIATE FLIGHT: flight from odor-release device

AWAY FROM THE SOURCE: male was not in contact with the odor release device (state follows landing).

1.2.5 Data Analysis

Since these experiments were performed throughout a relatively long period of time (almost two months), it was necessary to test for the possible changes of male responsiveness due to variations in conditions or other independent variables which had not been accounted for (e.g., possible phenotypic variances of males due to rearing, variation in barometric pressure, etc.). The complete randomized block factorial experimental design allowed us to account for possible effects of both block and the interaction between blocks and treatments. In addition, the effect of each tested treatment on male responsiveness was accounted for using multiway factorial ANOVA models I to III (Zar 1974). There was a prominent block effect on all concentrations for both the proportion of time spent on the behavior and number of males performing the behavior. There was no interaction on the effect of treatment among the blocks on the frequency of males performing the behavior. Interaction among treatment and the blocks was clear for the proportion of time the males spent performing certain behaviors If the treatments were presented in a certain order (e.g., ascending concentration, selected treatments per day, etc.) instead of a random order, it would be virtually impossible to determine the error associated with these variables, reducing the power of the statistical analysis or leading to spurious results.

Each male was used once in the course of the experiment, and its behavior recorded in an event recorder, creating a record as in Fig. 2. These data were analyzed in three different ways. A binary output was obtained if the moth engaged or not in a particular behavior, the (1) frequency of a behavior. From the sequence and the duration of each behavior, two other measurements were made: the (2) latency from the male's introduction to the pheromone plume and the first expression of a particular behavior, and the (3) mean proportion of total time spent in a particular behavior (see Fig. 2).

1.2.5.1 Frequency Data

To detect and account for any changes in male responses which take place over the 2 months of experimentation, the data were tested for no interaction (on either the linear or the logit scale) using a CATMOD procedure (SAS 1989a, 1989b). The binary data were then analyzed in a two way design (block of days by pheromone treatment) with 10 subjects per cell.

1.2.5.2 Proportion of the Total Time

Levene's test using absolute residuals was used to detect equality of variance among the cells. The response to the treatments was analyzed for each concentration in a two way design (block of days by pheromone treatment). When variances were not equal, a weight (Weight=1/sample variance of the cell) was added to the ANOVA model. Since not every moth performed each behavior, it created an impasse: should the moth have the "missing behaviors" scored as of duration zero sec, or they should be treated as missing data? The statistical analysis was performed for both sets of data, the data with missing behaviors and the data with the score of zero sec for the missing behaviors. The data with missing behaviors did not allow to account for the interaction block by treatment due to the unequal numbers of subjects per cell. On the data with the score of zero, every moth had a value associated for each one of the 11 behavioral classifications, i.e., a score of zero seconds was given for the behaviors that the moth did not perform. The equal number of subjects per cell allowed the test for interaction of this set of data. For each behavioral class, comparisons among the treatments at each concentration were done using a table of contrasts in CATMOD. A small value (0.01 sec) was added to the zero values to run the CATMOD procedures.

1.2.5.3 Latency of Behavior

The latencies of the eleven behaviors that could precede source contact were determined. If a male did not performed a given behavior, it received a score of 120 sec. for the specific behavior (Fig. 2). The mean of latency of a behavior was analyzed on a two way design (block by treatment) for each concentration. Levene tests of homoscedasticity were run. Where the assumption of equal variances was not met, weighted two way ANOVA procedures were used (W=1/cell variance).

1.3 Results

1.3.1 Pheromone Titters and Ratios

The mean titters of pheromone found in the female gland extractions (n=30) of our laboratory population were 4.3 ng (SD=2.85) of

Fig. 3. Frequency of C. cautella males performing a specified behavior (**a-j**) (y axis is the number of males, total n=60) responding to four blends at seven concentration (x axis, concentrations increases in decade steps from **1** ,450 fg, to **7** ,450 ng, of **ZE**). Where **ZE** is Z9,E12-14:Ac alone; **ZE+Z** is Z9,E12-14:Ac plus Z9-14:Ac; **ZE+OH** is Z9,E12-14:Ac plus Z9,E12-14:OH; and **ZE+Z+OH** is Z9,E12-14:Ac plus Z9-14:Ac.plus Z9,E12-14:OH.

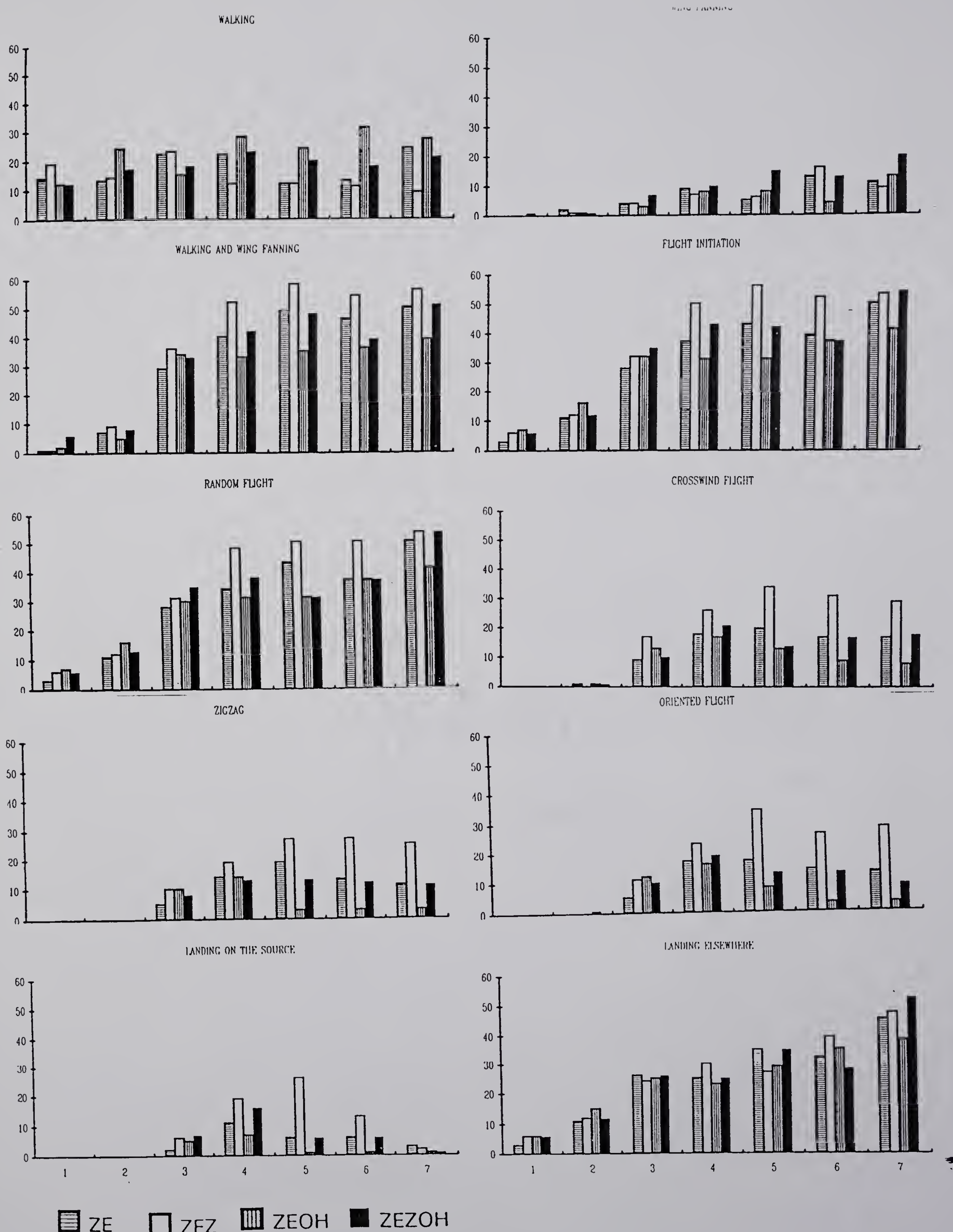

WALKING
WALKING AND WING FANNING
FLIGHT INITIATION
RANDOM FLIGHT
CROSSWIND FLIGHT
ZIGZAG
ORIENTED FLIGHT
LANDING ON THE SOURCE
LANDING ELSEWHERE
0
10
20
30
40
50
60
1
2
3
4
5
6
7
ZE
ZEZ
ZEOH
ZEZOH

Fig. 4. Mean of the proportion of total time that *C. cautella* males spent performing a specified behavior (y axis is the proportion of the total time where 1 is the maximum possible value) (n=60) responding to four blends at seven concentration. Details as per Fig. 3.

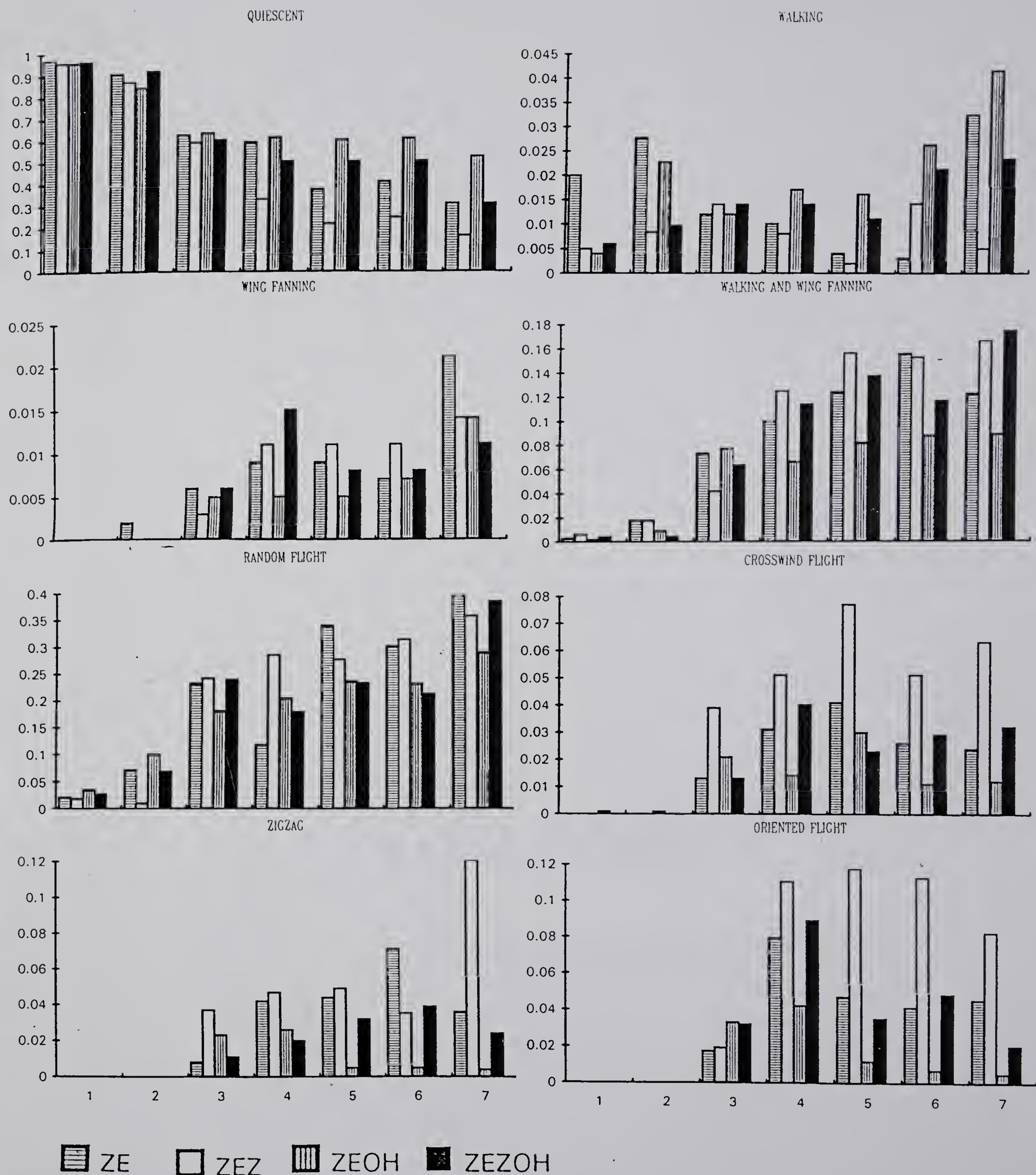
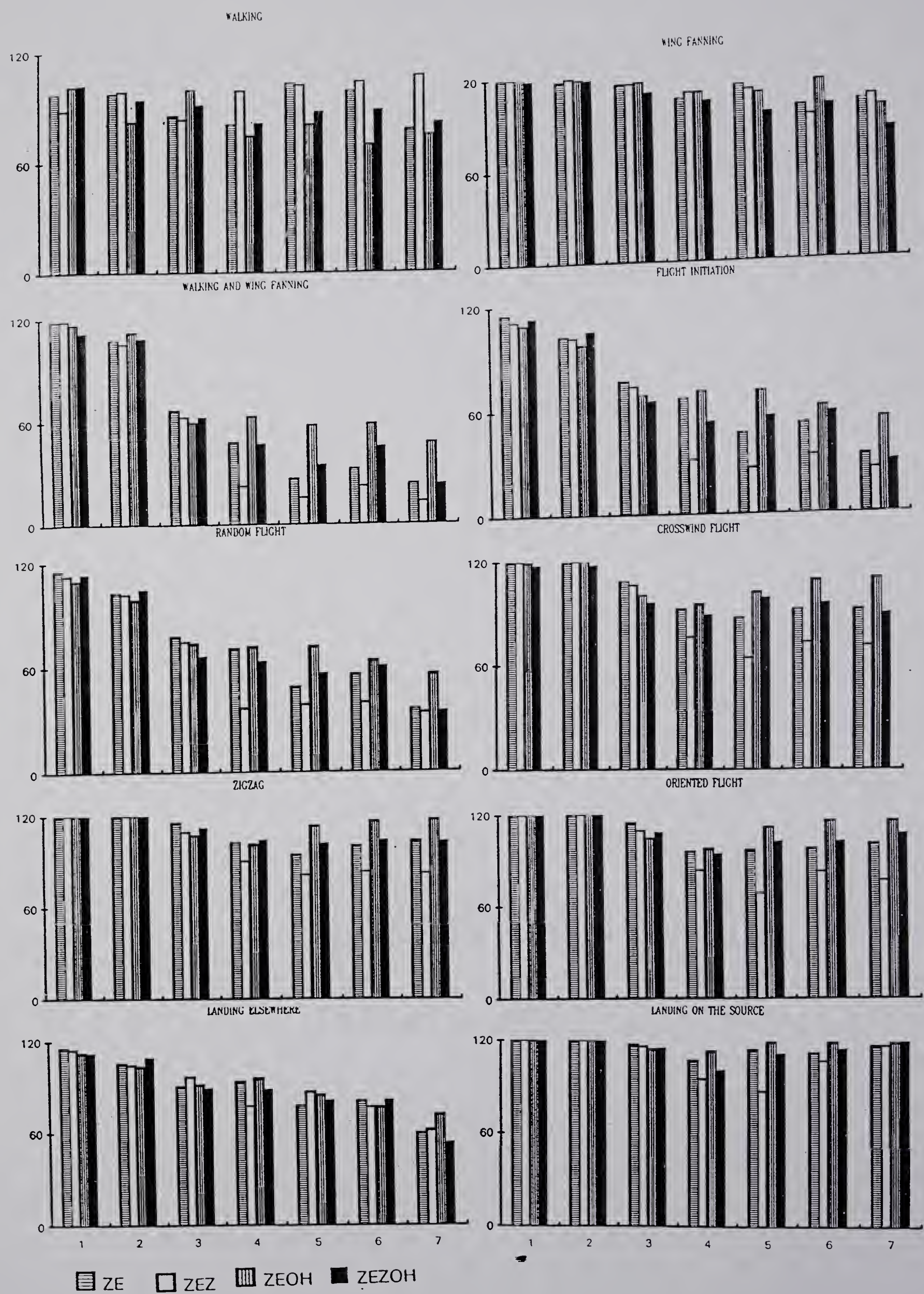

QUIESCENT
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WALKING
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
WING FANNING
0.025
0.02
0.015
0.01
0.005
0
WALKING AND WING FANNING
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
RANDOM FLIGHT
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CROSSWIND FLIGHT
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
ZIGZAG
0.12
0.1
0.08
0.06
0.04
0.02
0
1
2
3
4
5
6
7
ORIENTED FLIGHT
0.12
0.1
0.08
0.06
0.04
0.02
0
1
2
3
4
5
6
7
ZE
ZEZ
ZEOH
ZEZOH

Fig. 5. Mean latency from exposure of *C. cautella* males to the pheromone plume to the first performance of a specified behavior (y axis is the mean time in seconds, where 120 s is the maximum possible value) (n=60,). Males were tested to sources of four blends at seven concentration. Details as per Fig. 3.


WALKING
WING FANNING
WALKING AND WING FANNING
FLIGHT INITIATION
RANDOM FLIGHT
CROSSWIND FLIGHT
ZIGZAG
ORIENTED FLIGHT
LANDING ELSEWHERE
LANDING ON THE SOURCE
120
60
0
1 2 3 4 5 6 7
ZE
ZEZ
ZEOH
ZEZOH

Z9,E12-14:Ac, 0.8 ng (SD=1.50) of Z9-14:Ac, and 1.0 ng (SD=1.24) of Z9,E12-14:OH, giving a ratio of 5.67:1.00:1.25. The blends used on this study were based on these means. The ranges observed for each component were: 0.8 to 10.91 ng for Z9,E12-14:Ac; 0.0 to 5.1 ng for Z9-14:Ac; and 0.1 to 5.1 ng for Z9,E12-14:OH.

1.3.2 Male Response

The numbers of males performing each behavior at each blend and concentration are given in Fig. 3. The mean proportions of the total time that males spent performing each behavioral sequence are presented in Fig. 4. The latencies of behaviors are shown in Fig. 5.

The effectiveness of incomplete and complete blends of pheromone, namely Z9,E12-14:Ac alone and Z9,E12-14:Ac plus Z9-14:Ac, on activating quiescent males was the same at low concentrations (no difference:$P>0.05$, LSD, SAS). At some concentrations Z9,E12-14:Ac alone appears even more apt to evoke certain early behaviors: the proportion walking at concentrations 4, 6, and 7 (Fig. 3) and the time spent on walking and wing fanning and at crosswind flight at concentration 3 (Fig. 4B and 4C). Males spent the same proportion of time quiescent at concentrations near threshold for both the complete and incomplete blends (Fig. 4A). The threshold concentration for the expression of the complete sequence of behaviors (from quiescence to landing on the pheromone source) was 45 pg. of Z9,E12-14:Ac (Concentration 3). Of males that were initially activated at this concentration, the same proportion took off and initiated flight for treatments with and without Z9-14:Ac. However, the proportion of males making a transition from random flight to crosswind flight was reduced by about 50% in the absence of Z9-14:Ac (different, P=0.0466, LSD, SAS). The same trend can be seen at higher concentrations, although it was not statistically significant. For example, the trend for the facilitation of the transition from random flight to crosswind flight in the presence of Z9-14:Ac was present at concentration 5, but it was not large enough to be significant at a 95% of confidence level (no difference, P=0.099, LSD, SAS, at concentration 5). The presence of Z9-14:Ac elevated the proportion of males making the transition from oriented flight to landing on the source at concentration 5 (different, P=0.0016, LSD, SAS).

Addition of the alcohol (Z9,E12-14:OH) to Z9,E12-14:Ac reduced the proportion of males responding to this component of the pheromone. At most concentrations of treatments with both the Z9-14:Ac and the alcohol added to Z9,E12-14:Ac (Fig. 3), responses were elicited in the same proportion of males as to Z9,E12-14:Ac alone. These similarities suggest that the alcohol "counterbalances" the addition of Z9-14:Ac to Z9,E12-14:Ac.

After landing on the source, the male performs a courtship consisted of wing fanning, walking, hairpencil display, and protrusion of the abdomen, among other behaviors (see Phelan and Baker 1990 for a description of the behavioral sequence). This sequence of behaviors can be repeated a number of times and it was jointly classified as **contact with the source**. The male in contact with the source can either **initiate**

flight and go **away from the source**, or just **sit** on the source, folding his wings and remaining still for some period of time. When the source contained the incomplete blend, males spent a higher proportion of time away from the source than in contact with the source (proportion away/contact=6.9, n=11) than when the source contained the complete blend (proportion away/contact=1.9, n=19) at concentration 4 (P<0.05, LSD, SAS). The proportion away/contact is statistically the same for both the complete and incomplete blends at higher concentrations (no statistical differences, P>0.05, LSD, SAS), although the mean is usually slightly higher for the incomplete blend.

1.4 Discussion

The earliest behaviors performed by a quiescent male engulfed by pheromone plume are to walk, wing fan, or walk and wing fan, and then initiate flight. *C. cautella* males almost always leave the release cage in an ascending flight (ca. 20 cm above the release platform), thereby loosing contact with the pheromone plume, then drift downwind while losing altitude, finally fly randomly or crosswind and then upwind ("locking on" to the plume) from a position 30-50 cm downwind from where they initiated flight. This initial loop may hinder a male re-locating a narrow, filamentous pheromone plume, and results in a transition of behaviors from random flight to oriented upwind flight with very little success at a low concentration of pheromone. For example, at the threshold Concentration 3, at which about half of the males walked, wing fanned and initiated flight (Fig. 3D), only about 20% of these males were able to relocate the pheromone plume and engage in crosswind (Fig. 3D) and oriented flight (Fig. 3F).

In this experiment the pheromone solutions were tested using a point source platform that generated filamentous plumes. These filamentous plumes, due to their constant size and unbroken structure, provide stimulation of similar intensity independent where, in relation to the source, males intercept the plume. Mafra-Neto & Cardé (Chapter III) reported that the overall percentage of *C. cautella* males responding to pheromone was significantly lower when pheromone was presented in filamentous pheromone plumes than when it was presented in turbulent plumes. The percentage of males landing on sources, for example, increased from ca. 25% to ca. 80% following a change in plume structure, from narrow, filamentous plume to a wide, turbulent plume (Chapter III, Fig. 18).

Flight initiation was used to demarcate the behavioral sequence into early behaviors (quiescence to take off) and intermediate behaviors (taking off to landing on the odor source). Landing was used to demarcate the late behaviors (landing on the odor source and source-associated behaviors). The lowest concentration causing males to perform the early, intermediate, and late behaviors consistently was the third concentration (45 pg. of Z9,E12-14:Ac) of all four blends tested (Fig. 3). Therefore, Concentration 3 is here referred to as the threshold concentration for the expression of the complete behavioral sequence.

Males have the same or an increased rate of early behavioral response to any of the three non-female-released blends (containing the alcohol or missing the minor acetate) below or at the threshold concentrations as they do to female-released (complete) blend (Fig. 3 A-D). The proportion of males walking and wing fanning to the three component blend was higher than to either Z9,E12-14:Ac alone or the Z9,E12-14:Ac plus Z9-14:Ac blend at the lowest concentration (450 fg) ($P<0.05$). For walking, the Z9,E12-14:Ac and the alcohol blend was higher than both Z9,E12-14:Ac alone ($P=0.009$) and the Z9,E12-14:Ac and Z9-14:Ac component ($P=0.025$) at the second lowest concentration (4.5 pg.). The fact that the same proportion of *C. cautella* males performed early behaviors and spent the same proportion of time on them, independent of the blend to which they were being exposed, suggests that at low concentrations male response is based on Z9,E12-14:Ac alone.

Intermediate and late behaviors in the sequence are sensitive to the presence of Z9-14:Ac. At the same concentrations, flying males can distinguish between sources containing only the major component from those containing a second component in the blend (Fig. 3G). The frequency of males taking off and flying is the same for all blends (Fig. 3D), but fewer males perform crosswind flight after re-contacting the plume when the plume contains only the Z9,E12-14:Ac ($P=0.048$). The transition from random flight to oriented flight is more apt to occur ($P>0.05$) in males exposed to the two component blends than to Z9,E12-14:Ac alone or to the three component blend: 40% of the males do the transition for the Z9,E12-14:Ac/OH, 35% for the Z9,E12-14:Ac/Z9-14:Ac, 28% for the Z9,E12-14:Ac/OH/Z9-14:Ac, and 17% for the Z9,E12-14:Ac/OH. Statistically significant differences in the proportion of the total time spent in specific behaviors are shown for crosswind casting before locking on the plume (ZE/Z-14:Ac > ZE-14:Ac P<0.05) (Fig. 4F) and the zigzagging when the male follows the plume to the source (ZE/Z-14:Ac > ZE-14:Ac, P<0.05) (Fig. 4). Both behaviors (crosswind flight and zigzagging) are associated with male upwind flight to non-optimal blends, or concentrations in other studies on flight behavior (Willis & Baker 1988; Kuenen & Baker 1983). The numbers of *C. cautella* males initiating flight are the same for all blends at the threshold level.

At concentrations above the threshold (concentration 4 or 0.045 ng and above) the presence of the Z9-14:Ac shortens the latency of the first behaviors, and increases the likelihood of a successful transition from quiescence to the next behavior (Fig. 5). The highest proportion of males landing on the source was for the plume with the complete blend (the two acetates) ten times less concentrated than the mean of quantities found in the female glands for our population.

The highest proportion of males landing on incomplete blend and the blends containing the alcohol was observed for plumes generated by sources 100 times less concentrated than the mean female gland (FE) (Concentration 4), or 10 times higher than the threshold concentration (Concentration 6, Fig. 3I). This indicates that males distinguish more precisely between blend compositions when there is a source concentration approximately ten times higher than the threshold concentration. The highest proportion of males landing on the complete female blend were observed for plumes generated by sources 10 times less concentrated than the mean titter from the female's gland (Concentration 5, Fig. 3I).

A major difficulty in applying either the component or the blend hypothesis to the communication system of a given species is the selection of a behavior or a cluster of behaviors that are diagnostic of the "initial" response. In the wind tunnel milieu, the first reactions of a quiescent male to the introduction of the pheromone may include antennal movement, walking, and wing fanning (collectively termed "activation"). These behaviors may be followed by initiation of flight, non-oriented flight, and oriented flight along the plume or "locking-on". The wind tunnel bioassay, therefore, mimics the circumstances of a quiescent male in the field being enveloped by a pheromone plume.

The value of the threshold activation responses of quiescent males as reliable precursors of later behaviors, especially displacement toward the pheromone source, rests on several issues. First, what is the transition probability of an activated male (at the lowest threshold evoking these reactions) preceding to locking-on? If the probability of such transitions from the activation responses to locking on is not significant, then at the lowest thresholds evoking the activation responses but not the later behaviors are of little value in understanding how these behaviors are organized by either component or blend.

Second, do the thresholds for the transition to locking-on differ substantially between the behavioral states of in-flight scanning males and males that have initiated flight in the plume following activation by pheromone? In natural setting the non-pheromone-mediated behaviors that precede locking on to a plume may be quite different than those categorized above as "activation". In the field a male may be flying (in-flight scanning) prior to contact with the plume. Indeed, the presumption is that such scanning is the *usual* way in which moths (and other insects) intercept odor plumes (e.g., Cardé & Charlton 1985; Elkinton & Cardé 1983, Sabelis & Schippers 1984; Dusenberry 1989).

The transition from in-flight scanning to locking on is not observed in the wind tunnel, simply because flying males that do not orient to the wind quickly end up on the other side of a wind tunnel. The unstated assumption, however, is that the behavioral thresholds for transitions to locking on from in flight scanning in the field and from activation following quiescence in the laboratory wind tunnel are equivalent. In at least one moth species, *Lymantria dispar*, the thresholds for activation are lower than fro continued upwind orientation (Cardé & Hagaman 1983, Hagaman & Cardé 1984). This species (typical of several families of Lepidoptera) does not feed as an adult and its preflight behavior (thoracic warming by wing fanning for more than 1 min.) may be, therefore, unlike of the other species so far used to validate the blend hypothesis (see Linn & Roelofs 1987, 1990).

In conclusion, in *C. cautella* males the threshold concentrations for initiation of pheromone behaviors are set solely by Z9,E12-14:Ac (the major component), but the presence of Z9-14:Ac (the minor component) at concentration levels above threshold increases the proportion of males engaging in intermediate and late behaviors. Thus in the bases of thresholds the organization of the initial male responses to pheromone in *C. cautella* conforms with the component hypothesis. The transitions from early to some intermediate behaviors, however, was influenced by the presence of Z9-14:Ac, perhaps indicating that neither the component nor the blend hypotheses are adequate to understand the organization of these reactions.

CHAPTER II

INFLUENCE OF SEX PHEROMONE BLEND AND CONCENTRATION ON THE UPWIND FLIGHT OF *Cadra cautella* MALES IN FILAMENTOUS AND WIDE PLUMES

The mechanisms modulating male pheromone-mediated flight are still a matter of debate (Cardé 1986; Preiss & Kramer 1986a, 1986b; Kennedy 1986; Baker 1989). The model which has been most accepted to explain the mechanisms involved in the location of a pheromone source evokes two mechanisms: a positive **optomotor anemotaxis** (Kennedy & Marsh 1974; Kuenen & Baker 1982a) and a **central nervous system (CNS) counterturn generator**. Both mechanisms are triggered by in-flight contact with a pheromone plume. Optomotor anemotaxis is regulated by the feedback of a changing visual environment caused by wind-induced drift that provides cues for the flying insect to polarize its flight maneuvers and to displace upwind. This mechanism is responsible for maintaining a constant angular velocity of image motion across a male insect's retinal surface (Cardé & Hagaman 1979, Kuenen & Baker 1982a, but see below). This constant velocity is achieved by keeping flight altitude (Preiss & Kramer 1983), ground speed, angles for turning into the wind, and course steering at constant preferred values (reviewed by Kennedy 1983). The CNS counterturn generator causes the male to turn back and forth across the wind, in a regular fashion which is temporally consistent for most moths studied (Baker *et al.* 1984; Kuenen & Baker 1982b).

It has been shown that in pheromone-mediated flight, males of several moth species maintain ground speed, course angles and turn-counterturn intervals at constant levels when the extrinsic environment is manipulated (Marsh *et al.* 1978; Kuenen & Baker 1982a; Willis & Cardé 1990, Charlton *et al.* 1993). Only *Grapholita molesta* and *C. cautella* have been shown to change the rhythm of counterturning. This change in rhythm is mediated by changes in pheromone concentration for *G. molesta* (Kuenen & Baker 1982b) and by changes in plume shape for *Cadra cautella* (Chapter III).

Another model which explains the zigzagging upwind flight tracks of male is Preiss & Kramer's (1986) which we will refer to here as the flight **imprecision model**. The primary hypothesis of this model is that males try to fly directly upwind. Preiss and Kramer argue that the typical zigzagging flight tracks of male moths flying to pheromone are simply a consequence of the male's inability to fly straight upwind, and not the result of a CNS counterturning program. When moths steer course angles other than 0° (due upwind), they drift away from the wind line. This deviation from course is magnified in the male's transverse retinal image flow which triggers a proportional turn back toward 0°. The data presented in support of this model is the unimodal distribution of course angles in tracks of tethered gypsy moths tested in a flight simulator with moving visual patterns to simulate wind-induced drift. This hypothesis was further corroborated by the unimodal distribution of course angles obtained from tracks generated by a computer simulation model of moth flight using the parameters of their hypothesis (Preiss & Kramer 1986a). The imprecision model is simpler than the optomotor anemotaxis/counterturning model described above. It uses only the optomotor anemotaxis to explain the flight tracks of moths. The concepts of internal counterturning, an internally-set, anemotactically-steered track angles, and internally-set ground speed are not invoked. The validity of the imprecision model was called into question primarily because the moths used were tethered. Tethering restricts movement in all three planes of rotation (David 1986, David & Kennedy 1987). Tethering also introduces a mechanoreceptive input that is not present for free-flying moths; this might allow the moths to control their steering and velocity (David 1986, David & Kennedy 1987). Free-flying gypsy moths submitted to actual wind (not only visual cues) were recently shown to behave quite differently than predicted under the imprecision model. The regularity of the zigzag of their tracks was best explained by an internal counterturning mechanism (Willis & Cardé 1990).

Witzgall and Arn (1990 a,b) recently proposed a variation on the imprecision model. This variant will be referred to here as the **chemical imprecision hypothesis**. According to this hypothesis, counterturning, and the zigzagging flight that results, is an experimental artifact which is generated by synthetic pheromone blends, and not by natural pheromone sources, such as gland extracts or calling females. As experimental evidence, they show that *Lobesia botrana* males zigzag toward synthetic blends and fly straight upwind toward calling females. The frequency distribution of flight angles in their report reflected these two contrasting forms of flight. Flight toward calling females generated unimodal distributions, flights toward synthetic sources produced bimodal distributions of track and course angles. They suggested that the imprecision in male flight postulated by Preiss and Kramer (1986), was generated by the synthetic "nature" of pheromone components used in experiments, they question, therefore, the validity of the experiments with synthetic compounds that led to the hypotheses about an internal counterturning program. They concluded that "directness" of flight is a powerful and reliable diagnostic test for completeness of pheromone blends. The chemical imprecision hypothesis predicts that synthetic blends accurately mimicking the composition and concentration of the airborne blend released by the female should trigger direct flights with unimodal distribution of both course and track angles, whereas incomplete or "wrong" blends should trigger zigzagging flight tracks, with characteristic bimodal distribution of course angles and track angles.

Here we report the results of experiments designed to test the effect of blend and concentration on the upwind flight of males responding to pheromone. The insect used was the almond moth, *Cadra cautella* (Walker) (Lepidoptera: Phycitidae). The identity of the complete blend of long distance sex pheromone of the almond moth, *C. cautella* is well established (Mayer & McLaughlin 1990). Three components are found in the abdominal pheromone glands of *C. cautella* females: (*Z,E*)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac), the major component (Brady *et* *al.* 1971; Kuwahara *et al.* 1971a,b); (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) (Brady 1973); and (*Z,E*)-9,12-tetradecadienol (Z9,E12-14:OH) (Kuwahara & Casida 1973; Read & Beevor 1976). The two acetates (but not the alcohol) are consistently present in airborne pheromone collections from calling females (Coffelt *et al.* 1978; Barrer et al. 1987; Shani 1990) in the same ratio as they are found in gland extractions (Coffelt & Vick 1987). Field trials determined that Z9,E12-14:Ac alone was attractive, and was, therefore, the "major" component. Although Z9,E12-14:OH and Z9-14:Ac did not have any effect by themselves, when Z9-14:Ac was added to the major component, it had a "synergistic" effect and when Z9,E12-14:OH was added to the major component, it had an "inhibitory" effect on levels of trap capture (Read & Haines 1976). Similar trends were observed in wind tunnel experiments for late-in-the-sequence behaviors (sensu Chapter I). Since there is considerable variability in the proportion of pheromone components contained in the blend among geographically isolated populations of *C. cautella* (Barrer et al. 1987), the proportion used in this experiment was the proportion determined from pheromone gland extractions from females in our laboratory colony: Z9,E12-14:Ac to Z9-14:Ac at 4.5 to 1.

Although the alcohol is not part of *C. cautella* pheromone, it is a pheromone component of the frequently sympatric phycitines *Plodia interpunctella* (Hübner) and *Anagastha kuehniella* (Zeller) (Mayer & McLaughlin 1990). The alcohol appears to enhance the reproductive barriers among these species and *C. cautella*: *C. cautella* males pre-exposed to pheromone blends containing alcohol had their subsequent response to conspecific calling females drastically reduced (Grant & Brady 1975).

In this study we compare upwind flight of *C. cautella* males to plumes of pheromone blends mimicking the female blend and to plumes of intraspecific or incomplete pheromone blends across a wide range of concentrations.

2.2. Material and Methods

2.2.1 Insects

Our *C. cautella* colony has been continuously maintained at the University of Massachusetts since March 1989. It was started from greater than five hundred larvae and pupae from Kansas State University, Manhattan, Kansas, and the population has never been lower than 400 mating pairs. For this experiment the insects were reared from eggs to larvae in one quart Mason jars. The diet was made in batches using 3 kg poultry laying mash, 2 kg rolled oats, 100 mg Brewer's yeast and 200 ml glycerin. The rearing room was kept at 25-27°C on a 16-8 hr light-dark cycle and at 50-60% relative humidity. Individuals were sexed at migrant stage (last larval instar) when the male testes are easily seen. Males were reared from last larval instar to adult in an environmental chamber under a 16-8 hr light-dark cycle, 70% relative humidity and 25-26 °C held in separate room. Adult males emerged inside 25 X 25 X 25 cm screened cages. The pupae were transferred daily to new cages leaving only newly emerged males in the old cages. This procedure generated a continuous supply of 1-day-old males that were used for behavioral assays during the first two hours of their first dark period as adults.

2.2.2 Chemicals

Chemicals were obtained from Farchan and IOB. The combined diunsaturated acetate was purified to 99.97% by separation on a silver nitrate/Florisil column with an increasing polarity gradient of isopropyl-ether and hexane. The purity of compounds was determined by capillary gas chromatographic analysis using a 30m x 0.32 mm ID/SP 2340 column operated at 70 °C for 4 minutes, then temperature programmed at 12 °C per minute to 200 °C, and maintained at the final temperature for 10 minutes.

The stock solutions reflect the mean proportions found on individual gland extractions from females from our colony (Chapter I). Since the Z9-14:Ac and the alcohol alone or in combination do not show any biological activity unless accompanied by Z9,E12-14:Ac (Brady *et al.* 1976; and pilot studies), all treatments contained Z9,E12-14:Ac. The mixtures were Z9,E12-14:Ac alone (incomplete blend or ZE), Z9,E12-14:Ac and Z9-14:Ac (the complete blend or ZEZ), Z9,E12-14:Ac and Z9,E12-14:OH (ZEOH), and Z9,E12-14:Ac plus Z9,E12-14:OH and Z9-14:Ac (ZEZOH). The synthetic pheromone components were formulated gravimetrically into solutions of 1µg per µl, then volumetrically into the four blends (Z,E-9,12-14:Ac 11.5 : Z9-14:Ac 1.0 : Z,E-9,12-14:OH 1.8) which were serially diluted to seven concentrations ranging from concentration 1 of 4.5 fg per µl, to concentration 7 of 45 ng per µl of the Z9,E12-14:Ac.

2.2.3 Wind Tunnel

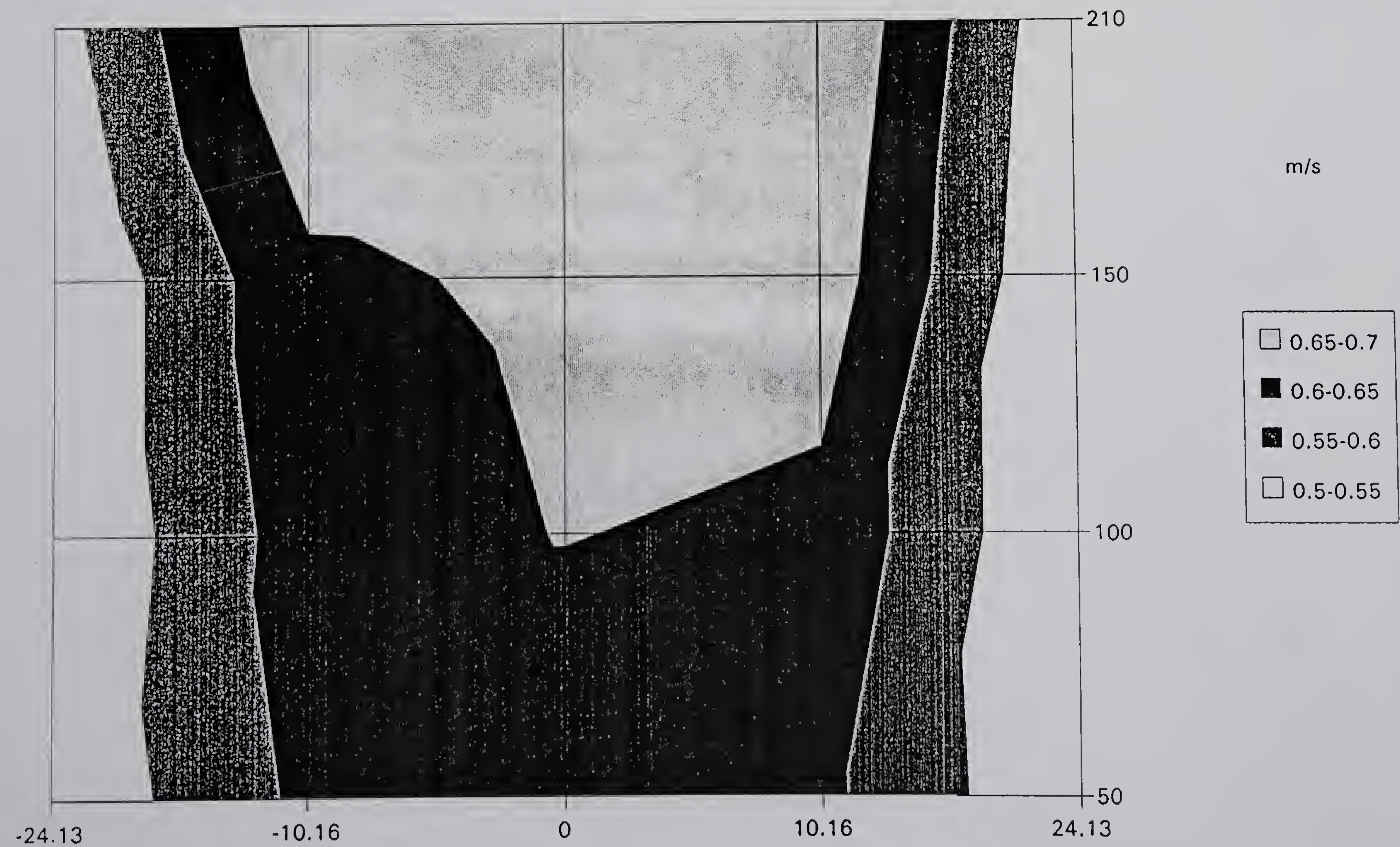
The wind tunnel used is described in detail elsewhere (Chapter I). In brief, it is a 2.5 m long semi-cylinder of Plexiglas® and Vivac®, suspended 130 cm off the ground. Each end is covered by a mesh screen. The upwind screen sits between the body of the wind tunnel and the upwind air laminator. Two downwind screens separate the working section of the tunnel from the exhaust system. Airflow through the wind tunnel was laminar. This was confirmed visually using $TiCl_4$ smoke plumes, and also by the low variance obtained from repeated measurements of wind speed at predetermined points in the tunnel using a hot-wire anemometer (Yokogawa model 2141) (Fig. 6). Before each experimental session the wind velocity was set at 45 cm sec^{-1} using the anemometer and a voltage regulator to control the exhaust fan.

Light was provided by a light box at the top of the working section of the tunnel with five red and five white 25 watt incandescent light bulbs and a filter/diffuser made of one layer of white Styrofoam (0.5 cm thick). Light conditions were adjusted with a voltage regulator to 5.5 lux, and relative humidity ranged from 75-85%.

Red acetate circles randomly arranged on the Plexiglas® floor

Fig. 6. Map of the distribution of wind velocities on the working section of the wind tunnel at the level of the central axis of the plumes (20 cm above the wind tunnel floor). The Y axis is the distance from the upwind screen in cm, the x axis is the distance in cm from the center of the wind tunnel (center=0), and the different shades of gray represent the different wind velocities.

WIND TUNNEL VELOCITIES



provided non-directional optomotor cues (David 1982). Since the same type of red filter was placed over the lens of the video camera used for filming, the circles were almost completely transparent in the resulting video image, facilitating the analysis of the video image(Section 2.2.5).

2.2.3.1 Male Release Device

The male release device was located 1 m downwind from the odor source. It was in a position where it intercepted a smoke plume generated by the point source platform (Section 2.2.3.2). Males were released from a cylindrical aluminum screen cage (4.5 cm diameter x 5 cm). One end of the cylinder was covered with the same screen and the other end was open. The cage was positioned with the open side facing upwind. The cages were held in position by a rigid Teflon® tube that had one end opening inside the cage and the other connected to a hollow glass tube. The hollow glass tube passed through the wind tunnel floor and opened outside the wind tunnel. This design allowed for the introduction of moths from outside the tunnel to inside of the release cage without disrupting the pheromone plume. The height of the release platform was regulated by sliding the glass tube through the floor.

Moths randomly selected were transferred from the emergence cages to the glass tube of the male release device using an aspirator. After every exposure to the pheromone plume, the screen cage and the Teflon® tube were replaced by clean ones (as in Chapter I).

2.2.3.2 Point Odor Source

The point odor source was a disk of filter paper (Whatman #1) 0.7 cm in diameter. It was held in a horizontal position, parallel to the floor, by an insect pin (# 1). The pin was attached to one end of a hollow copper tube (3 mm diameter) that slid easily through a hole in the floor of the wind tunnel. This allowed for regulation of the vertical position of the odor plume in the tunnel. The filter paper disk was impregnated with 10 µl of the solution being tested. This paper disk was replaced every 10 minutes to ensure a constant release rate throughout the experimental session. The odor source was positioned 45 cm from either side of the tunnel, and 10 cm from the upwind screen.

2.2.3.3 Wide Odor Source

The wide source platform had the insect pin replaced by a "**Y**"-shaped wire structure. The two upper arms of this structure were 20 cm apart, positioned at the same height. A piece of dental floss (Johnson & Johnson, fine, unwaxed) was tied to the end of each arm, resulting in a horizontal straight line, parallel to the floor and perpendicular to the wind line. The central lower arm of this **Y** structure was attached to the end of the 3 mm diameter hollow copper tube (as in 2.2.3.2). The dental floss was evenly impregnated with 20 µl of the solution being tested and was replaced every 10 minutes to ensure a constant release rate throughout the experimental session.

2.2.4 Bioassay Procedure

Adult emergence cages were placed at experimental conditions of light and relative humidity (as in 2.2.3.1) at least 30 minutes prior to testing. The pheromone source was positioned 35 cm above the floor. Moths were transferred to the release platform positioned underneath the pheromone plume, 20 cm above the wind tunnel floor. Observations using either $TiCl_4$ "smoke" or high pheromone concentrations at the source platform verified that at this position the plume did not contact the release cage. Each quiescent male was held in the screen cage for 20 seconds. At the end of 20 seconds of quiescence, the pheromone source was lowered 15 cm and male behavior was observed. With flight initiation the release platform was lowered to 5 cm above the wind tunnel floor; in this position it no longer intercepted the pheromone plume and the pheromone plume was kept uniform throughout the entire working section of the wind tunnel. Lowering the release platform also allowed males that locked onto the plume downwind from the release platform to proceed flying upwind without encountering the release platform. Each male was observed for a maximum of 2 minutes. Males that flew upwind had their upwind track recorded (Section 2.2.5). Males that touched any surface after taking off had their upwind track recording terminated.

2.2.4.1 Blend and Concentration

Males were tested using plumes generated by the point source odor platform impregnated with one of the four blends at seven concentrations, ranging from 45 fg to 450 fg, or 0.0001 FE to 100 FE, or using hexane as a control. A total of 29 treatments was tested (Section 2.2.2). A complete random factorial design was used to schedule the testing sequence of the 29 treatments. The 29 treatments were tested over a four day block. During each block a total of ten males were tested for each treatment. Each day two groups of males were tested during the first hour of their scotophase. The first group had a dark period beginning at 14:00 hours and the second group had a dark period beginning at 16:00 hours; this allowed for the testing of 7 to 8 treatments per day. A total of 6 test blocks were run, resulting in a total of 60 males tested per treatment. There was no *a priori* selection of males, i.e., the test was every adult male's first exposure to pheromone. The flight tracks of males that landed on the source were transcribed and analyzed. All others were discarded.

2.2.4.2 Complete and Incomplete Blends

To contrast the effect of completeness of blend on flight tracks, individual males were tested using one female equivalent (concentration 5) of the complete blend (Z9,E12-14:Ac plus Z9-14:Ac) and the incomplete blend (Z9,E12-14:Ac alone). Two different source platforms, a point odor source platform (Section 2.2.3.2) and a wide odor source platform (Section 2.2.3.3), were used to test for effects of plume structure on male upwind flight. A total of twenty-five tracks was obtained for each treatment.

2.2.5 Data Analysis

Males that flew upwind and located the odor source had their upwind flight track video recorded from below in a two-dimensional view using a Sony RSC 1050 rotary-shutter video camera with a 8.5 mm wide-angle lens. The field of view at the level of plume altitude (20 cm above the wind tunnel floor) yielded a 80 x 90 cm rectangular area that extended from 15 cm to 105 cm downwind from the plume source. Flight tracks of individual moths were transferred to a Sony SVM-1010 motion analyzer, and played back frame-by-frame through a 41 cm Panasonic WV-5470 black-and-white video monitor. Two points of reference on the wind tunnel floor and the moth location every 1/30th of a second were transcribed onto transparent acetate. The X and Y coordinates of moth position in a two dimensional plane were obtained using a digitizer pad (Apple Graphics Tablet). Ground speed, track angle and net velocity every 1/30th sec were computed using Basic Programs (Charlton *et al.* 1993). Course angles, drift angles, and airspeed were obtained using the triangle of velocities method (Marsh *et al.* 1978) and the transverse and longitudinal components of visual flow (T and L) were calculated using Ludlow's (1984) and David's (1986) method. The inter-reversal distance, turn frequency and the inter-reversal times were calculated directly from the track. A turn was defined as a change in course angle that would result in a vector that crossed to the opposite side of the wind line (left/right). The mean of the flight parameter for each individual was analyzed using GLM, two way Anova, and two sample t-tests (SAS and Excel procedures).

2.3. Results

2.3.1 Blend and Concentration

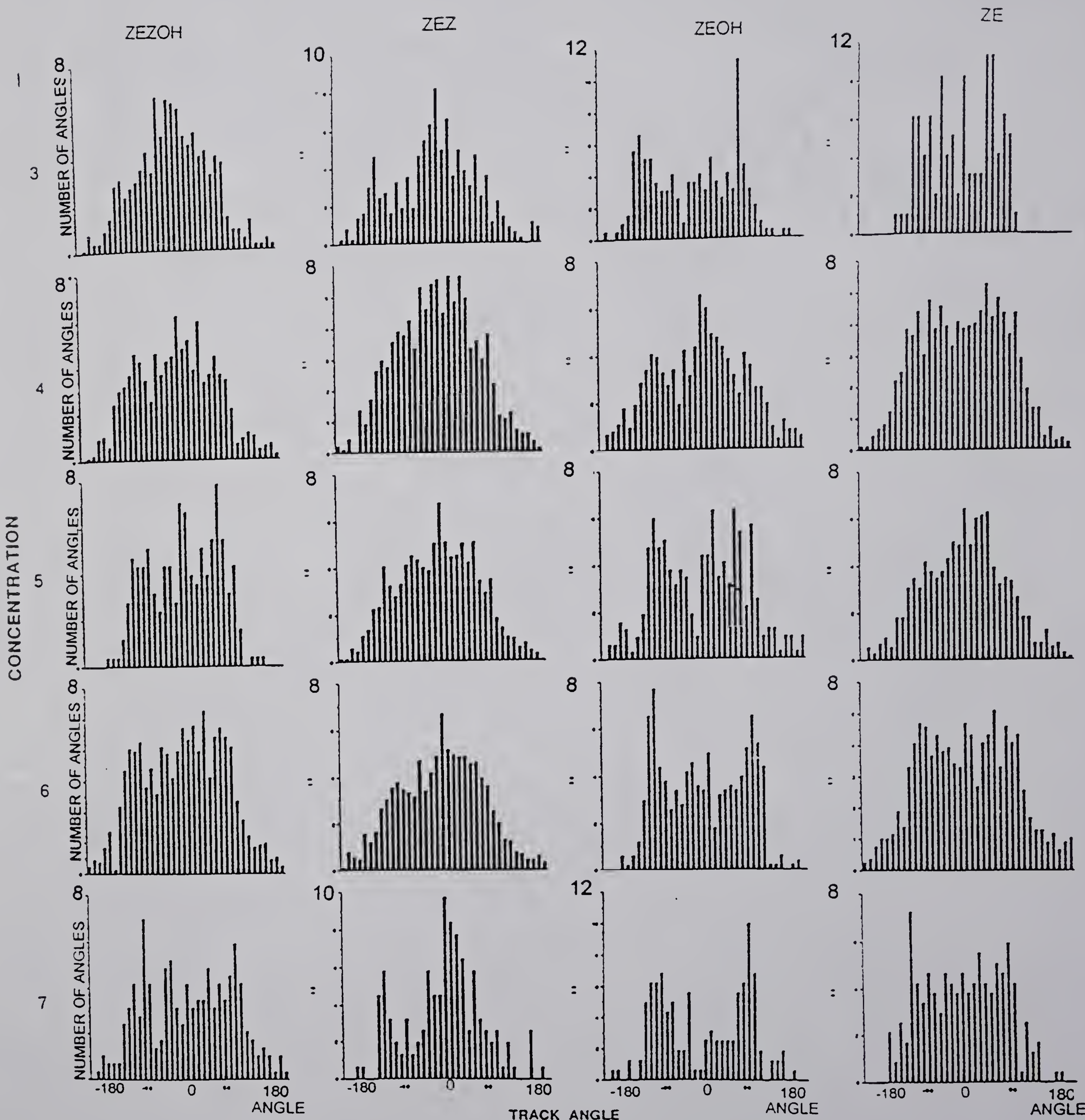
Different proportions of *C. cautella* males perform late-in-the-sequence behaviors when exposed to sources of different blends and concentrations (Chapter I), so that an unequal number of flight tracks were obtained, therefore a descriptive statistical analysis was performed for each of the treatments tested in the "blend and concentration" experiment. Since the effects of day and of blocks on the determination of male behavior was not significant for any of the measured parameters of flight, the days and blocks effects were dropped from the statistical analysis.

Unimodality of flight angle distribution can usually be correlated with straightness of flight path (e.g., Witzgal & Arn 1990, but see Chapter IV). For every pheromone blend tested, at least one concentration produced unimodal distributions of the flight angles measured (track, drift and course angles). This indicates that modality of the distribution of the flight angles depends not only on the blend of pheromone but also on the concentration of the blend being tested.

Concentration 3 (0.01 female equivalent or FE) was the lowest concentration at which flight tracks were obtained. At this concentration the tracks of males flying to blends ZEZOH and ZEZ show a clear

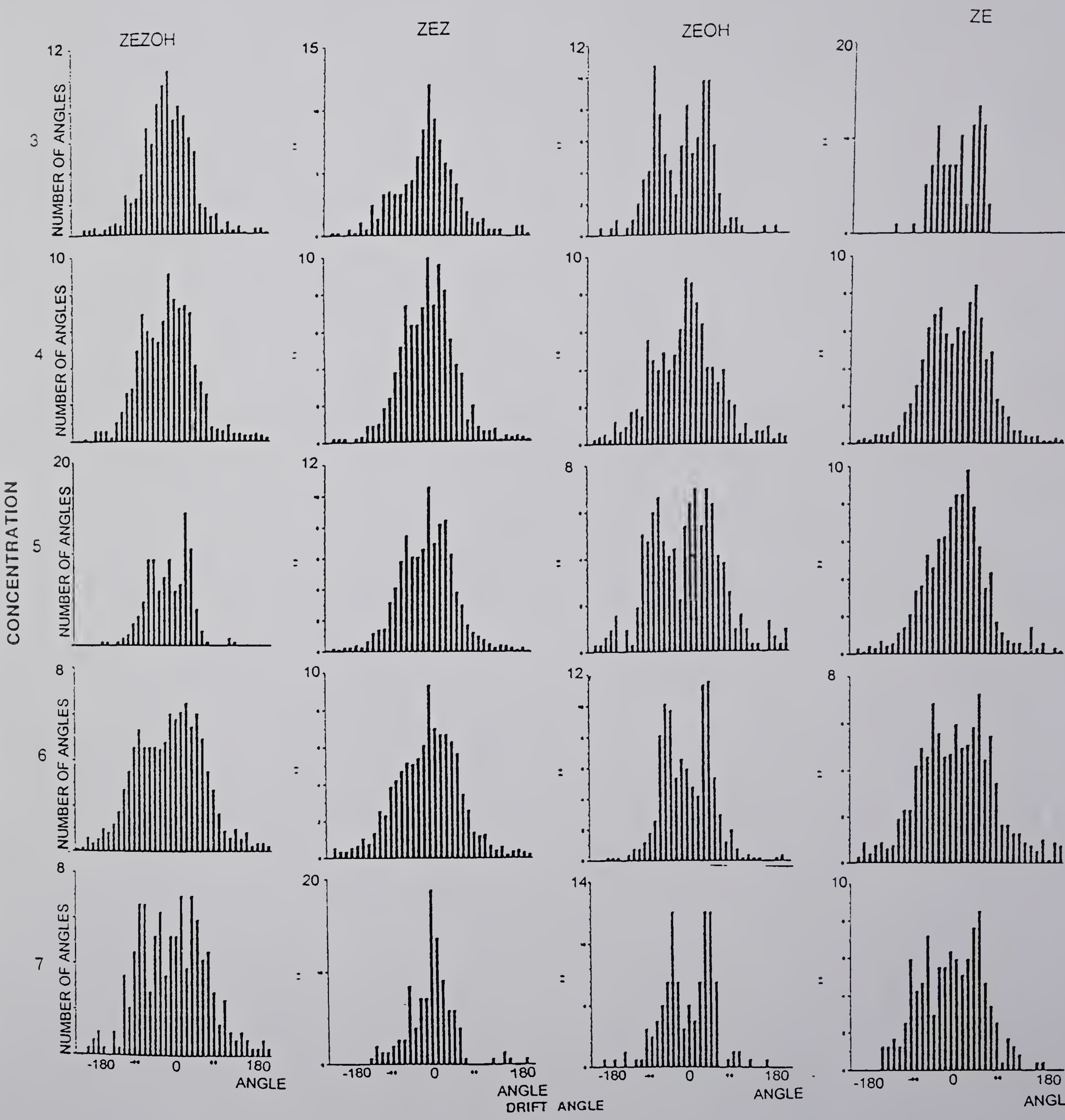
Fig. 7. Frequency distribution histograms of flight angles steered by *C. cautella* males flying toward plumes of four different blends at different concentrations. **A.** frequency distribution histogram of track angles. **B.** frequency distribution histogram of drift angles. **C.** frequency distribution histogram of course angles. Angles were sampled every 30th of a second.

A.



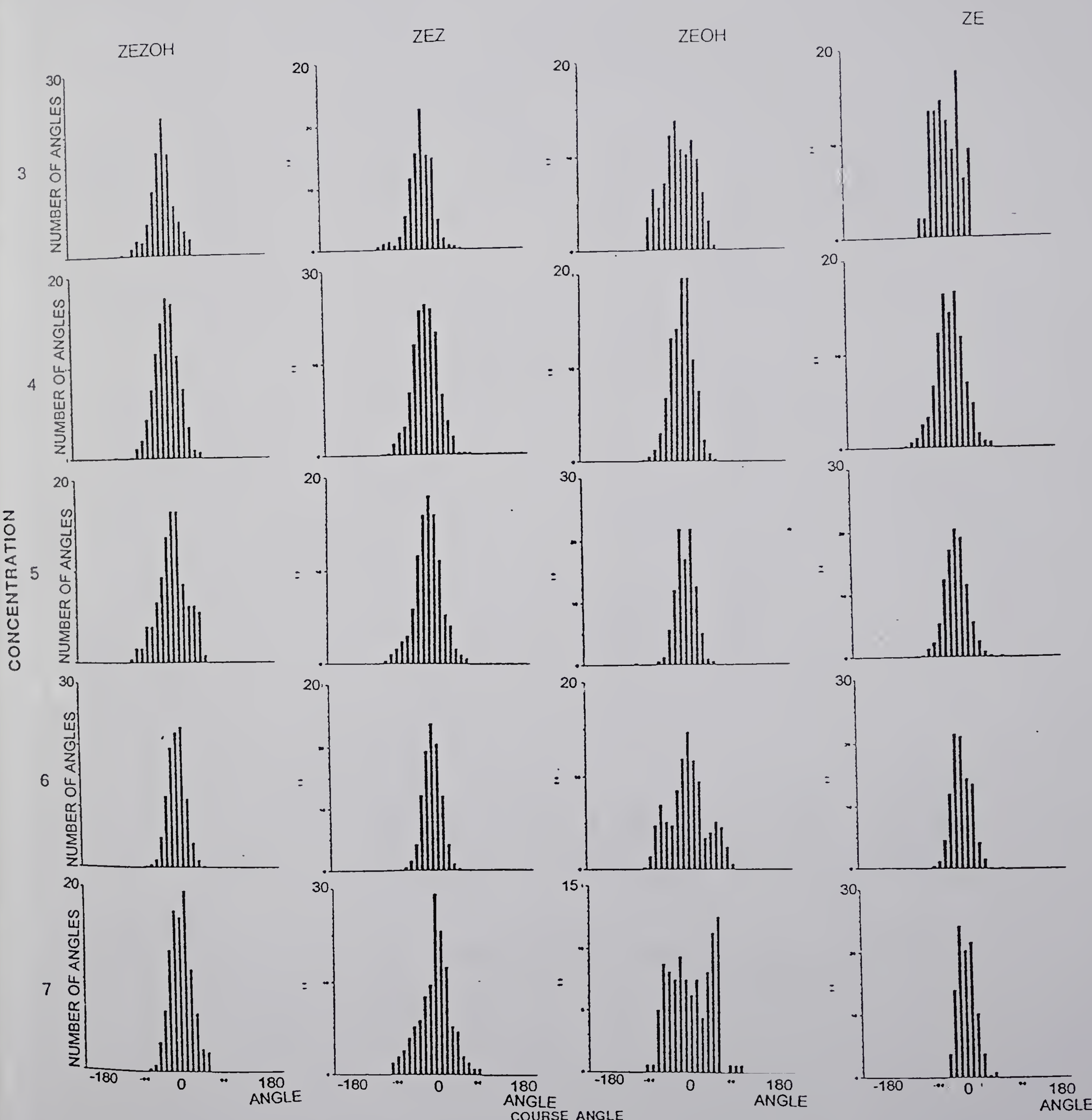
Continued next page.

B.



Continued next page.

C.


ZEZOH
ZEZ
ZEOH
ZE
CONCENTRATION
3
4
5
6
7
NUMBER OF ANGLES
-180
0
180
ANGLE
COURSE ANGLE

unimodal distribution of the track angles (Fig. 7a), whereas the tracks toward ZEOH and to ZE, however, show trends toward bimodality. At concentration 4 (0.45 ng, 0.1 FE) tracks to ZEZOH begin to show a trend toward bimodality similar to the bimodal distribution of angles steered by males exposed to ZEOH sources. At this concentration (concentration 4), the distribution of the track angles to the incomplete blend ZE and the complete blend are unimodal. At concentration 5 (4.5 ng, 1 FE), ZEOH and ZEZOH show clear bimodal distributions, whereas the tracks toward the complete (ZEZ) and the incomplete (ZE) blends continue to show unimodal distribution. When the concentration is increased another decade step to concentration 6 (45 ng, 10 FE), the upwind flight tracks to all blends show some sign of bimodality in the distribution of flight angles. At this concentration ZEZ has the distribution that most closely resembles a unimodal distribution, whereas ZEOH has a more bimodal distribution of the track angles. At a higher concentration (concentration 7, or 450 ng) the trend toward bimodality increases, with ZEOH showing the strongest signs of bimodality.

The distribution of the drift angles (Fig. 7b) shows the same general trends as the distribution of the track angles. At concentration 3, ZEZ and ZEZOH show unimodality of the distribution of drift angles. The histogram of the distribution of the drift angles shows a tendency toward bimodality for ZE, whereas the drift angle distribution is clearly bimodal for ZEOH. At concentration 4 (0.45 ng) all the distributions of drift angles tend toward unimodality. At concentration 5, ZE and ZEZ have clearly unimodal distributions, ZEZOH shows a distribution with a trend toward bimodality, while ZEOH shows clear bimodal distribution of the drift angles. At concentration 6, the distribution of drift angles is bimodal for ZEOH, while it is unimodal for the complete blend ZEZ, the incomplete blend ZE, and to ZEZOH. Fewer males land on the source at the highest concentration, but the drift angle distribution of these tracks shows a trend toward unimodality for ZE and ZEZ plumes, while for ZEZOH and ZEOH it tends toward bimodality.

All histograms for course angle distribution show clear unimodal distributions (Fig. 7C), with a single exception: ZEZOH at concentration 7 shows bimodal distribution. This reinforces the conclusion that modality of flight track angles in *C. cautella* does not necessarily reflect the completeness of the pheromone blend being used.

The track angles were relatively constant (range 60° to 68°) for ZEZ, ZE, ZEZOH, and ZEOH for all concentrations tested (Fig. 8).

The relationships among the mean drift angles steered toward the four different blends vary depending on the concentrations (Fig. 8). At concentration 3 tracks toward the ZE blend show an drift angle of 38°. This was the smallest drift angle observed among all blend at this concentration. Treatment ZEZ has the lowest drift value at concentration 4, while ZEZOH has the smallest values at concentration 5, ZEOH has the smallest values at concentration 6, and treatment ZEZ has the smallest values at concentration 7 (Fig. 8).

Males maintained mean airspeed and mean ground speed at constant values for all blends from concentration 3 to concentration 5

Fig. 8. Mean values for the angular parameters of flight of *C. cautella* males toward plumes of four different blends at different concentrations. **A.** plot of mean values of angles steered by males flying toward ZE sources; **B.** plot of mean values of angles steered by males flying toward ZEZ sources; **C.** plot of mean values of angles steered by males flying toward ZEOH sources; and **D.** plot of mean values of angles steered by males flying toward ZEZOH sources. Filled diamonds (◆) represent mean values for track angles, open squares (□) represent mean values for drift angles, and filled squares (■) represent mean values for course angles.

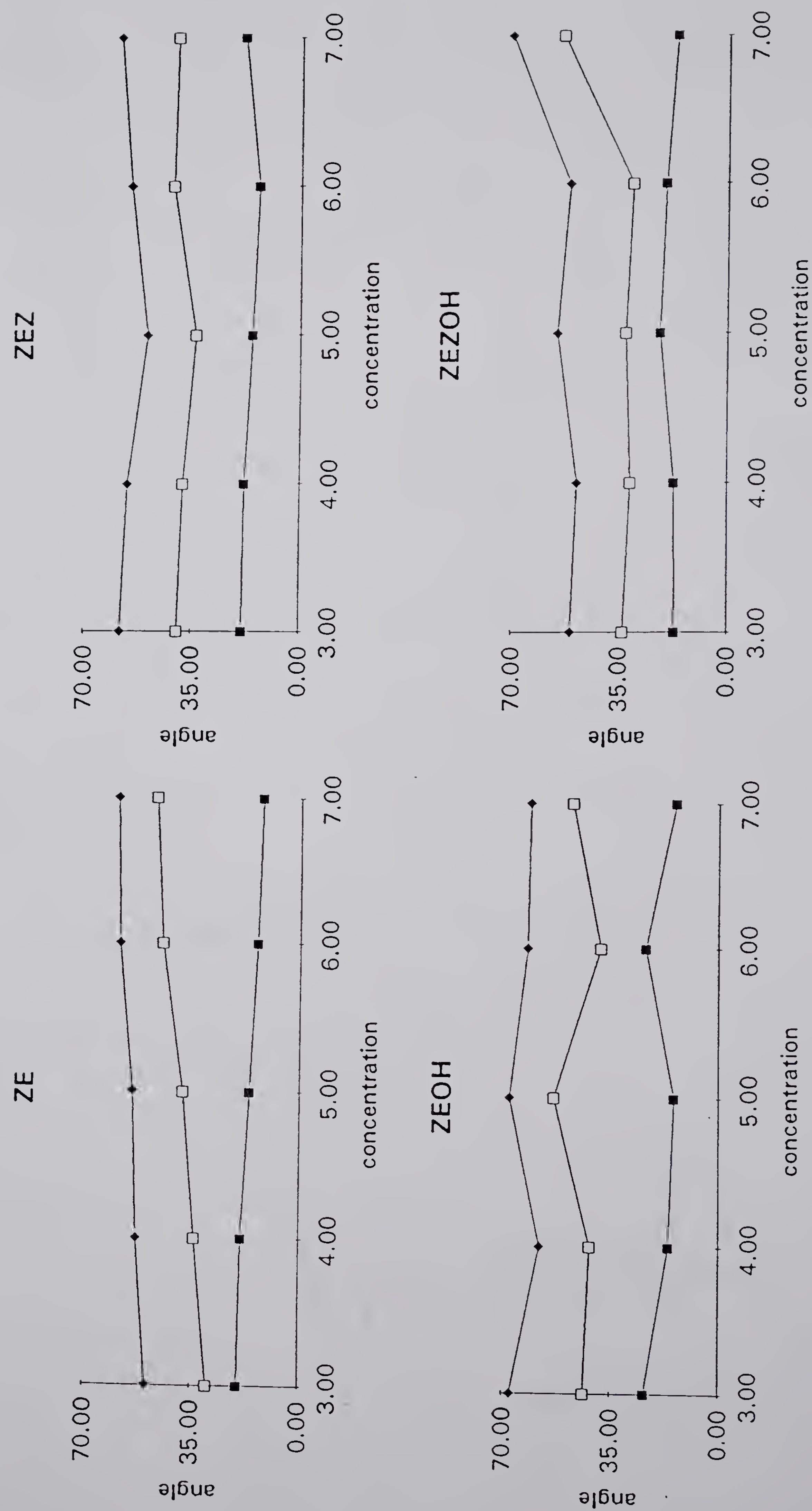
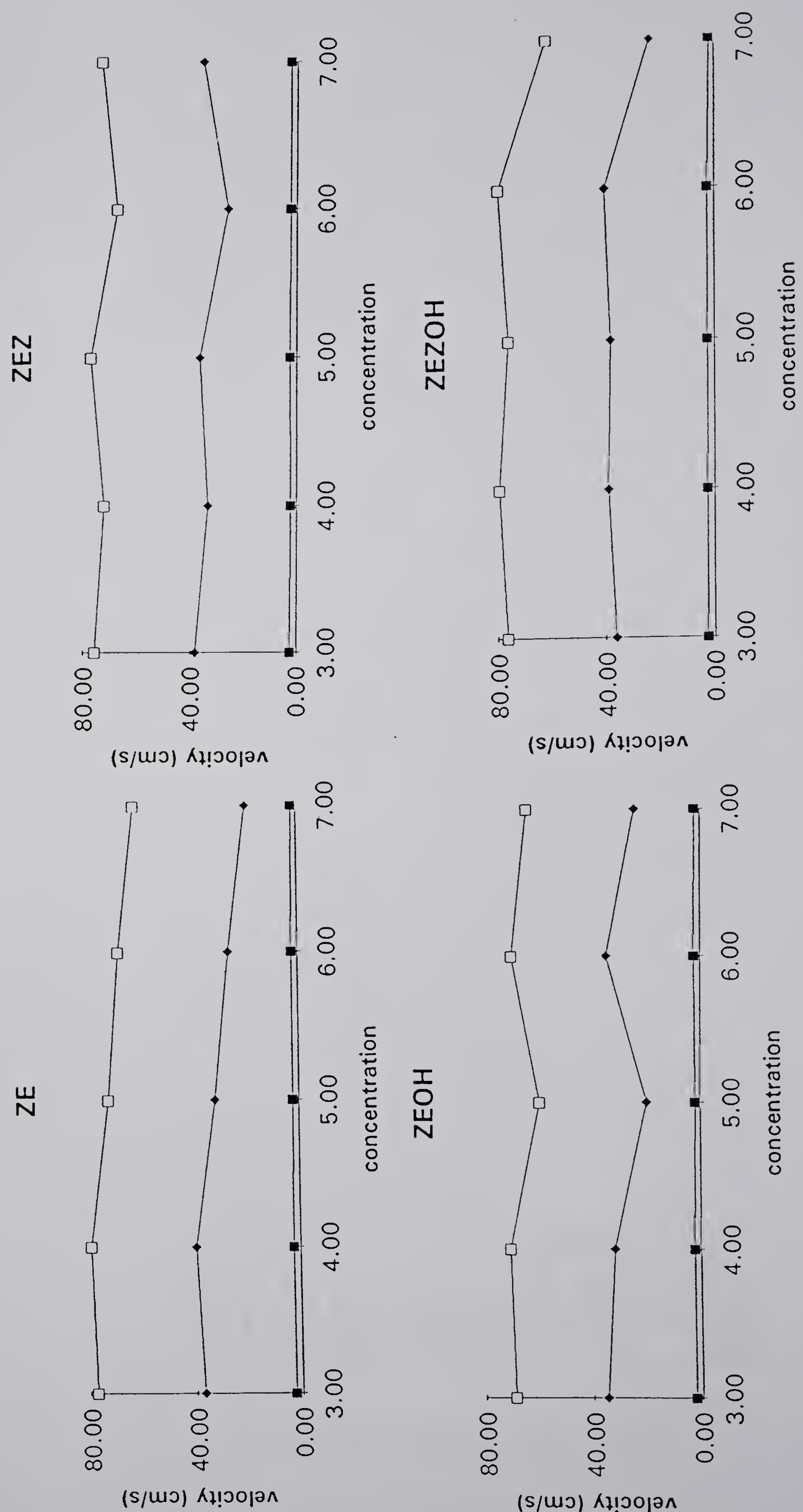

ZE
ZEZ
ZEOH
ZEZOH
angle
concentration
70.00
35.00
0.00
3.00
4.00
5.00
6.00
7.00

Fig. 9. Plots of the mean values of velocity parameters of the flight of *C. cautella* males flying toward sources of four different blends at different concentrations. **A.** graphs of the mean values of flight velocities for males flying to blend ZE; **B.** graphs of the mean values of flight velocities for the males flying to blend ZEZ; **C.** graphs of the mean values of flight velocities for the males flying to blend ZEOH; **D.** graphs of the mean values of flight velocities for the males flying to blend ZEZOH. Filled diamonds (◆) represent the mean ground velocity, open squares (□) represent the mean airspeed, and filled squares (■) represent the mean vector traveled.


ZE
ZEZ
ZEOH
ZEZOH
velocity (cm/s)
80.00
40.00
0.00
concentration
3.00
4.00
5.00
6.00
7.00

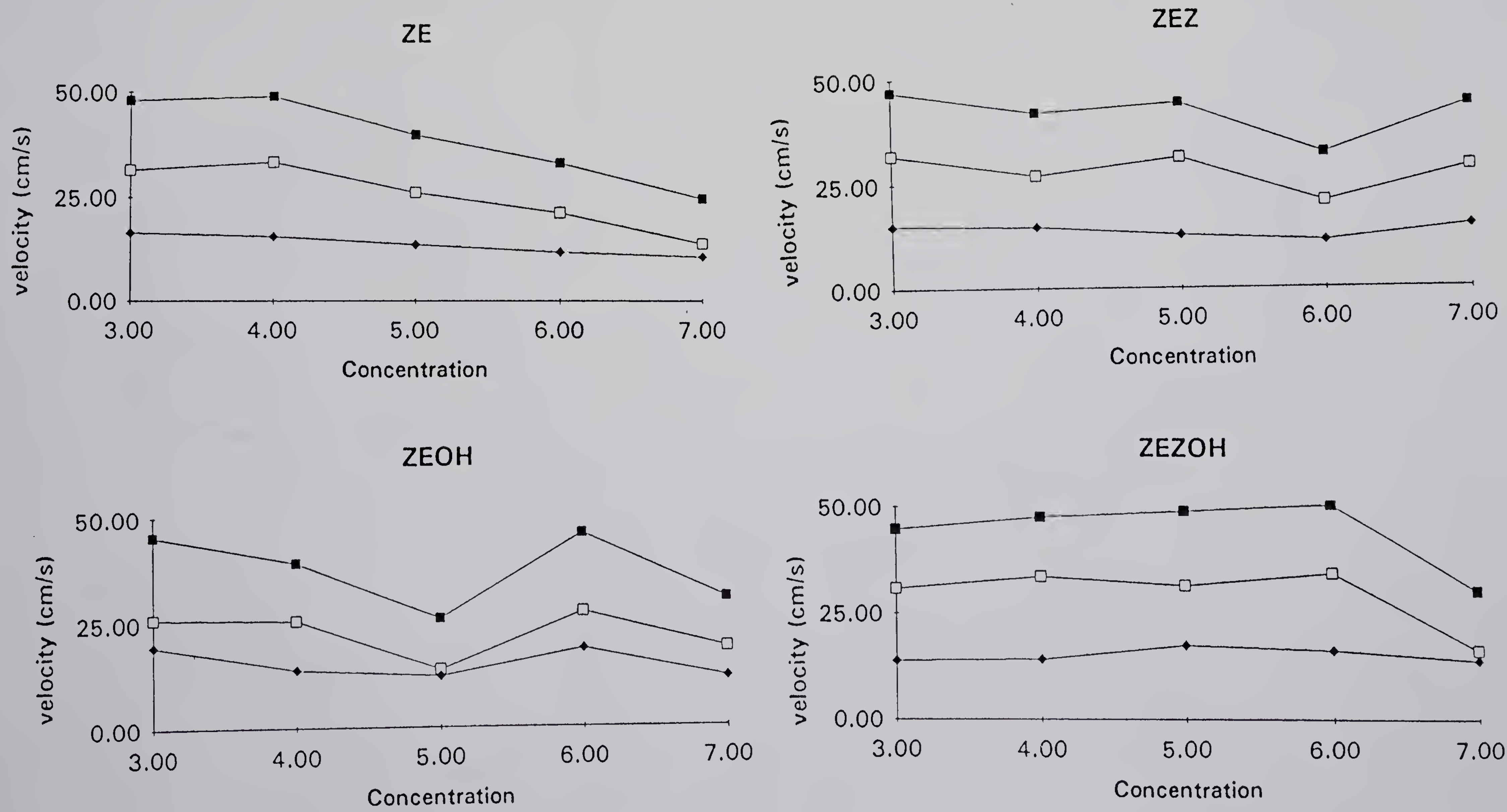
(Fig. 9), but an overall trend toward a decrease in flight speed was observed with an increase in concentration. This is in accordance with observations reported for several other moth species (Willis & Baker 1988, Charlton *et al.* 1993).

Several hypotheses postulate a predetermined relationship among the components of the visual flow for flying males. David (1986) suggested that the relation T+L (or $\sqrt{(T^2+L^2)}$) are kept constant, and the imprecision model of Preiss & Kramer (1986a) suggests that moths control their airspeed by a feedback mechanism from the L component, and the course angles by the feedback from T: minimizing T and maintaining L at a low positive values. Although *C. cautella* males maintained L at a constant level when concentrations changed, there is no clear indication that *C. cautella* males maintain the postulated parameters T and T+L of the visual flow at any constant value when concentrations are systematically changed for all blends tested (Fig. 10).

2.3.2 Plume Structure and Completeness of Blend

To investigate the effects of completeness of blend and plume width on male upwind flight, twenty five flight tracks toward filamentous plumes (point source) and wide plumes (wide source) of complete and incomplete blends at the concentration of one female equivalent were obtained. The trends detected in the blend and concentration experiment were strengthened by the results of the experiments contrasting flight to complete and incomplete blends.

Fig. 10. Plots of the mean values of parameters of visual flow of *C. cautella* males flying toward sources of four blends at different concentrations. **A.** graphs of components of the visual flow for males flying to ZE; **B.** graphs of components of the visual flow for males flying to ZEZ; **C.** graphs of components of the visual flow for males flying to ZEOH; **D.** graphs of components of the visual flow for males flying to ZEZOH. Filled diamonds (◆) represent the mean of the longitudinal component of the visual flow, open squares (□) represent the mean of the transverse component of the visual flow, and filled squares (■) represent the mean of the interaction between the longitudinal and the transverse components of the visual flow (L+T).


ZE
ZEZ
ZEOH
ZEZOH
velocity (cm/s)
50.00
25.00
0.00
3.00
4.00
5.00
6.00
7.00
Concentration

2.3.2.1 Point source

The smoke plume generated by pipetting $TiCl_4$ onto the point source platform was a continuous homogeneous plume with no evidence of turbulent growth while traversing the working area of the wind tunnel in a 45 cm sec^{-1} wind. Frame-by-frame analysis of the resulting video image demonstrates that a stationary point intercepting the plume would be constantly embedded in the plume. The dimensions of the filamentous smoke plume were roughly 0.8 x 0.1 cm.

C. cautella males fly similarly to plumes of complete and incomplete blends of pheromone. The inter-reversal distances of the tracks of males flying to both treatments were always larger than the boundaries of the point source plume (Fig. 11 a, b).

A crosswind zigzagging pattern, typical of male moths responding to pheromone (Kennedy 1986; Willis & Cardé 1990), was present in all tracks of males flying to the filamentous plume, independent of blend (e.g., Fig. 11).

The means of the components of visual flow, L and T, are similar for both complete and incomplete pheromone blends ($P>0.5$, LSD, GLM SAS) (Fig. 12a). The L/T ratio is also not significantly changed when chemically different treatments are compared. This indicates that there is no measurable "imprecision of flight" being triggered by an incomplete pheromone blend.

Fig. 11. Representative flight tracks of *C. cautella* males flying to non-turbulent plumes generated by two different sources structures containing either complete or incomplete blend of pheromone. A. Flight track toward a point source releasing an incomplete blend (ZE); B. Flight track toward the point source releasing a complete blend (ZEZ); C. Flight track toward the wide source releasing an incomplete blend (ZE); D. Flight track toward the wide source releasing a complete blend (ZEZ). Where, for each track, the open arrow on the left indicates the direction of flight, the filled arrow on the right indicates wind direction, the distance between the two asterisks is 65 cm, and the vertical lines on the right of tracks C and D, represent the position of the wide source platform. Each point of the track represents the sequential position of the male at intervals of 1/30th of a second.

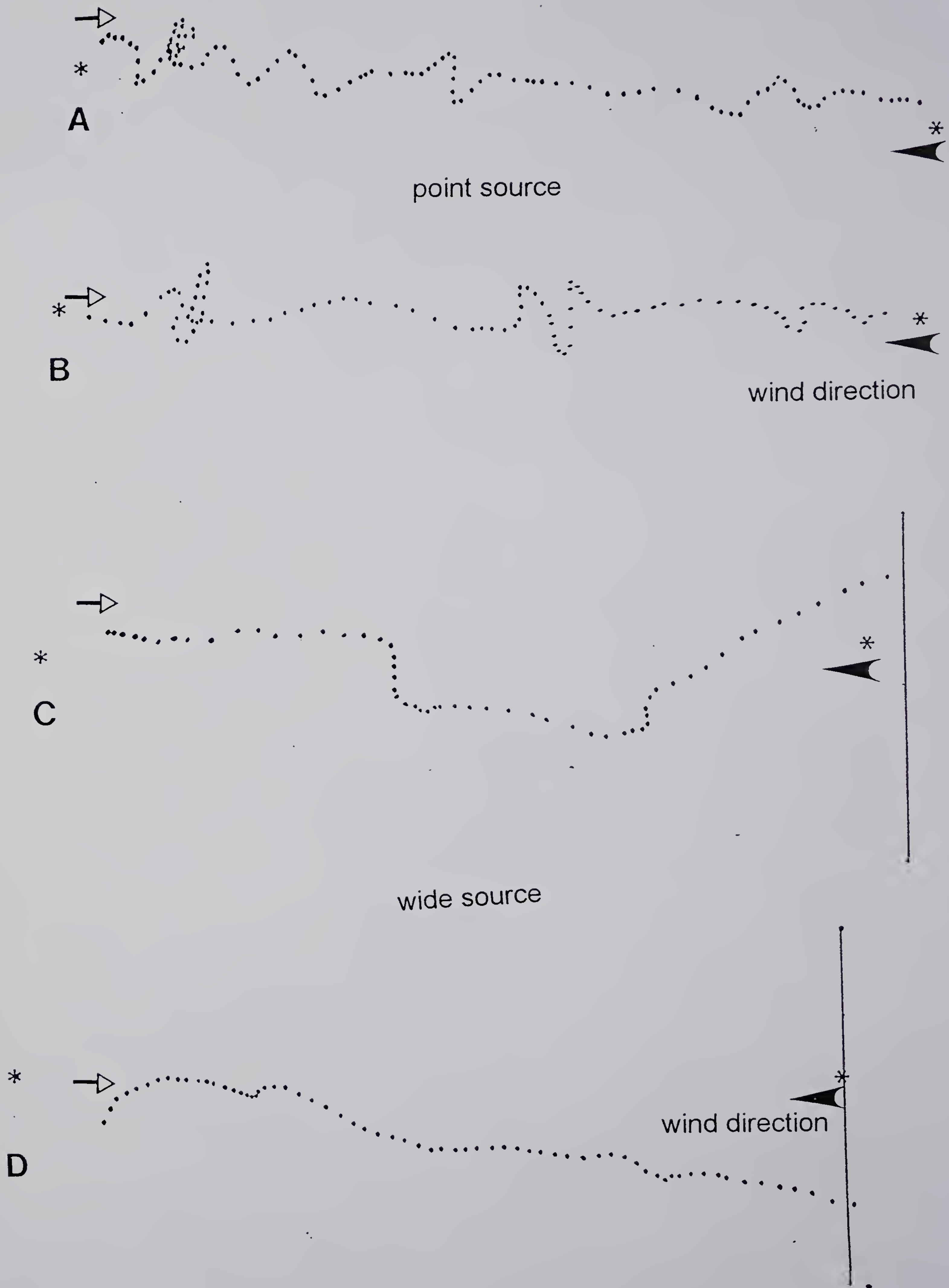

A
point source
B
wind direction
C
wide source
D
wind direction

Fig. 12. Parameters of flight of *C. cautella* males flying toward a point source of complete blend (ZEZ) or incomplete blend (ZE). **A.** components of the image flow, where filled bars (■) represent the longitudinal (L) component of the visual flow, open bars (□) represent the transverse (T) component of visual flow, and hatched bars ([▧]) represent the interaction between T+L; **B.** parameters of velocity of flight, where the filled bars (■) represent the mean ground velocity, the open bars (□) represent the mean vector traveled, and hatched bars ([▧]) represent the mean airspeed; **C.** angular parameters of the flight, where filled bars (■) represent mean drift angles, open bars (□) represent the mean track angles, and hatched bars ([▧]) represent the mean course angles.

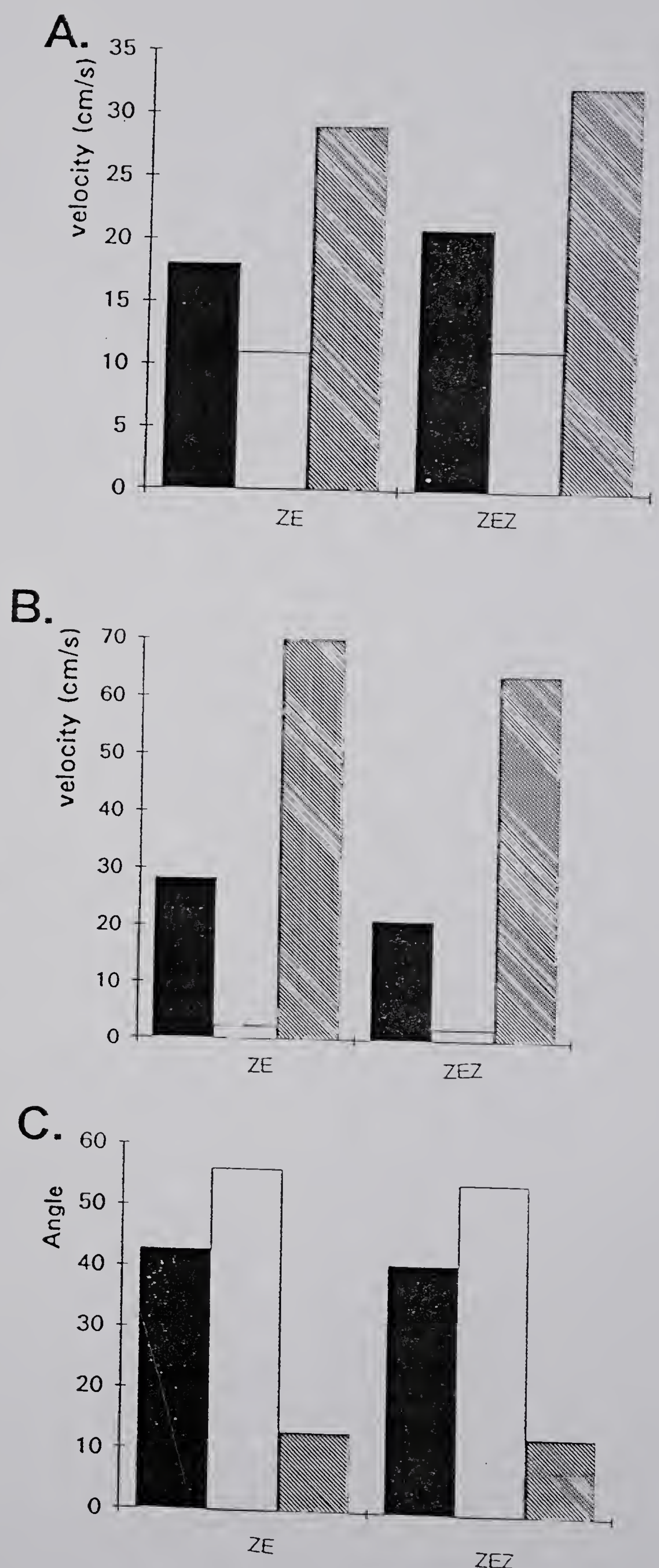
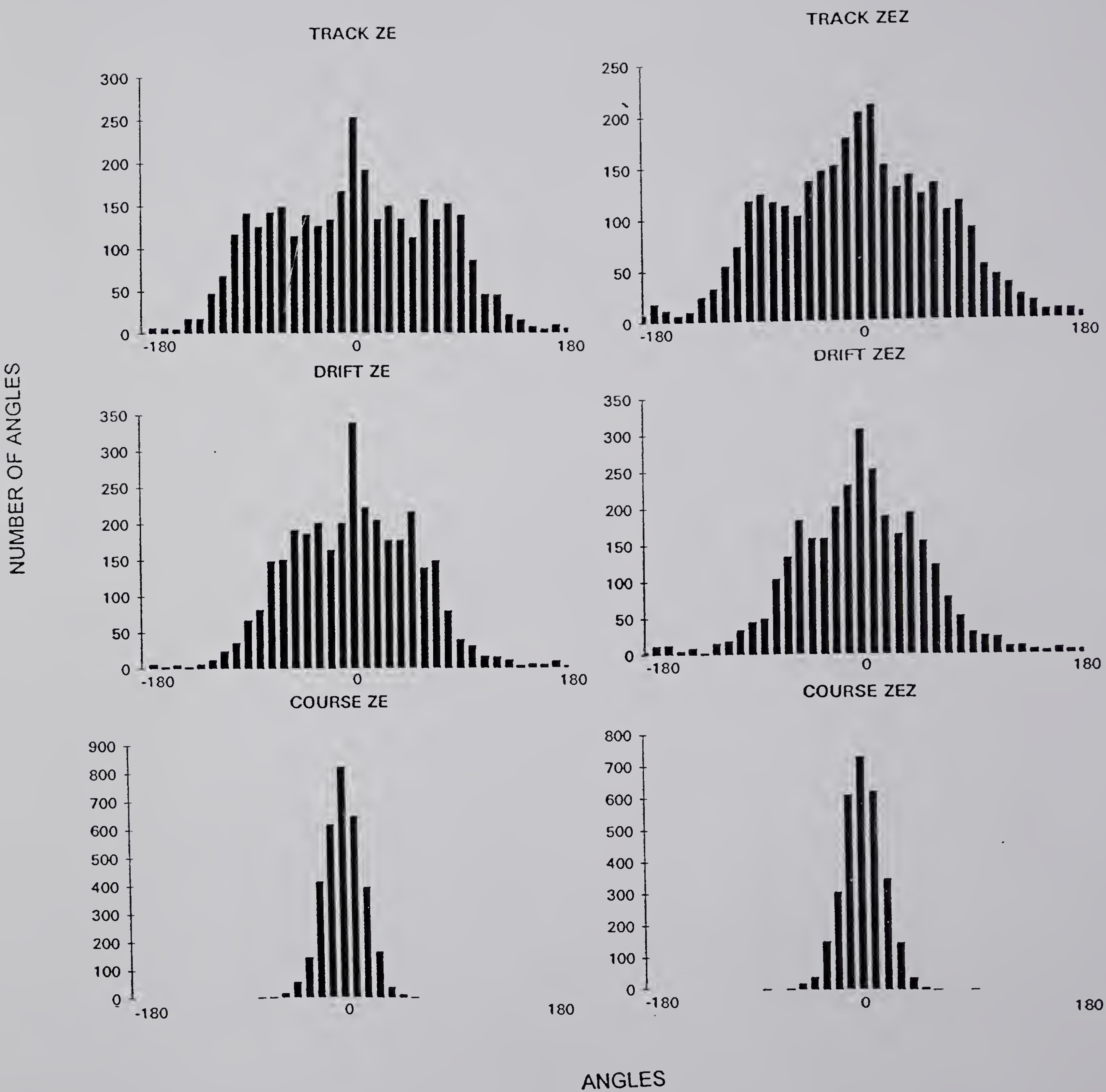

A.
velocity (cm/s)
35
30
25
20
15
10
5
0
ZE
ZEZ
B.
velocity (cm/s)
70
60
50
40
30
20
10
0
ZE
ZEZ
C.
Angle
60
50
40
30
20
10
0
ZE
ZEZ

Fig. 13. Frequency distribution histograms of the angles steered by *C. cautella* males flying toward point source plumes with either the complete or the incomplete blend of pheromone (sampled every 30th of a second). **A.** histogram of mean track angles of flights toward the incomplete blend (ZE). **B.** histogram of mean track angles of flights toward the complete blend (ZEZ). **C.** histogram of mean drift angles of flights toward the incomplete blend (ZE). **D.** histogram of mean drift angles of flights toward the complete blend (ZEZ). **E.** histogram of mean course angles of flights toward the incomplete blend (ZE). **F.** histogram of mean course angles of flights toward the complete blend (ZEZ).



TRACK ZE
TRACK ZEZ
DRIFT ZE
DRIFT ZEZ
COURSE ZE
COURSE ZEZ
NUMBER OF ANGLES
ANGLES
-180
0
180

The flights of *C. cautella* males toward complete and incomplete blends sources were indistinguishable. The effect of blend on airspeed and ground velocity is statistically insignificant (P>0.05, LSD, GLM SAS) (Fig. 12b). Although the means of the values of track angles, drift angles, and course angles are slightly larger for the complete blend (males steered and drifted slightly more crosswind when flying to the complete blend than to the incomplete blend) these differences were not statistically significant (P>0.05, LSD, GLM SAS) (Fig. 12c). Frequency histograms for track angles, course angles, and drift angles show a trend for the mode and the mean to approach 0° (Fig. 13). This reinforces the previous suggestion that unimodality of the distribution of these flight parameters is not a diagnostic test for the completeness of the blend in *C. cautella*. Flight track analysis by itself is not as reliable a technique to diagnose completeness of blend as the behavioral sequence analysis used in Chapter I.

2.3.2.2 Wide source

The smoke plume generated by pipetting $TiCl_4$ onto the dental floss of the wide source release device was a wide continuous homogeneous plume (ca. 20.0 cm x 0.2 cm) which is best described as a sheet of smoke or odor. There was no evidence of turbulent diffusion while this sheet traversed the working section of the wind tunnel in a 45 cm sec^{-1} wind. Frame-by-frame analysis of the resulting video image demonstrates that a stationary point intersecting this plume would be continuously engulfed by the plume.

Although there are no statistical differences in flight tracks to point sources with the complete or the incomplete blends, some differences emerge when these blends are presented in wide plumes.

Inter-reversal distances in tracks of males flying to the sheet plumes were always narrower than the plume boundaries in the horizontal plane (Fig. 11c and 11d). The means of the inter-reversal angles and distances of flight tracks from the sheet plumes do not differ from the ones to the filamentous plumes. The inter-reversal angles steered toward the complete blend are not statistically different from those steered toward the incomplete blends (ZEZ=154° and ZE=151°) ($P>0.05$).

The mean values of the track angles for both the complete and incomplete blends is centered around 50° ($P>0.05$, LSD, GLM SAS) (Fig. 14). The distribution of the frequency histogram for the track angles of males flying to the wide source is unimodal for both the complete and the incomplete blends, with distribution centered around 0° (Fig. 15). The mean value of the course angles for the incomplete blend ZE blend was 15°, two degrees larger than the mean course angle for the complete ZEZ blend which was 13° (different, $P=0.051$, LSD, GLM SAS) (Fig. 14). Frequency distribution histograms of the course angles for both treatments are unimodal with a median around 0° (Fig. 15). The mean of the drift angles is the same for both treatments (ZE=34°, ZEZ=38°, $P>0.05$, LSD, GLM SAS). The frequency distribution histogram for the drift angles is **unimodal** with a mode of 0°, whether or not the blend is

Fig. 14. Parameters of flight for *C. cautella* males flying to wide sources with either the complete or the incomplete blend. **A.** parameters of velocity of flight, where filled bars (■) represent the mean ground velocity, open bars (□) represent the mean vector traveled, and hatched bars (▧) represent the mean airspeed; **B**. angular parameters of flight, where filled bars (■) represent the mean drift angles, open bars (□) represent the mean track angles, and hatched bars (▧) represent the mean course angles; **C.** components of image flow, where filled bars (■) represent the longitudinal component of visual flow, open bars (□) represent the transverse component of visual flow, and hatched bars (▧) represent the interaction between T+L.

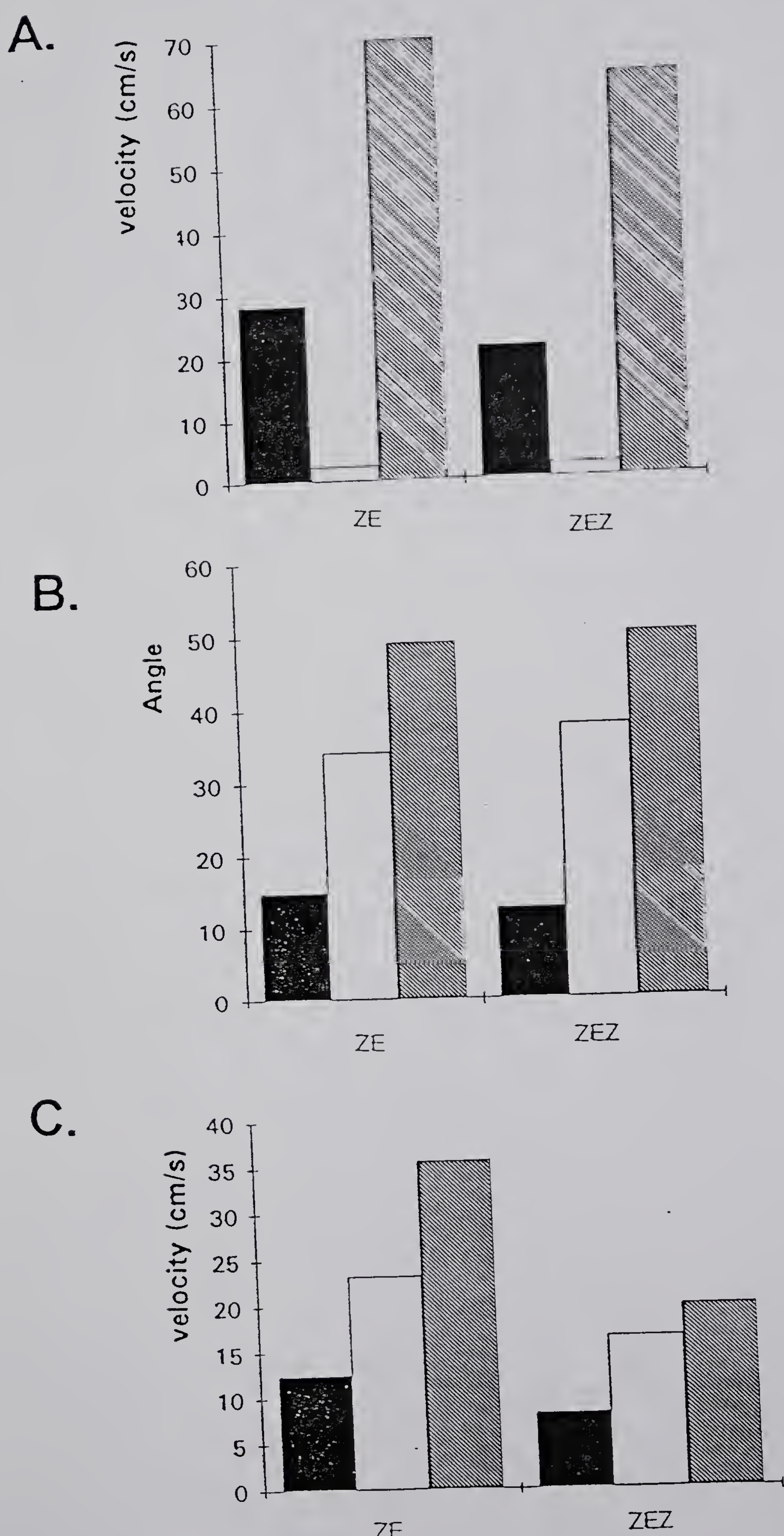
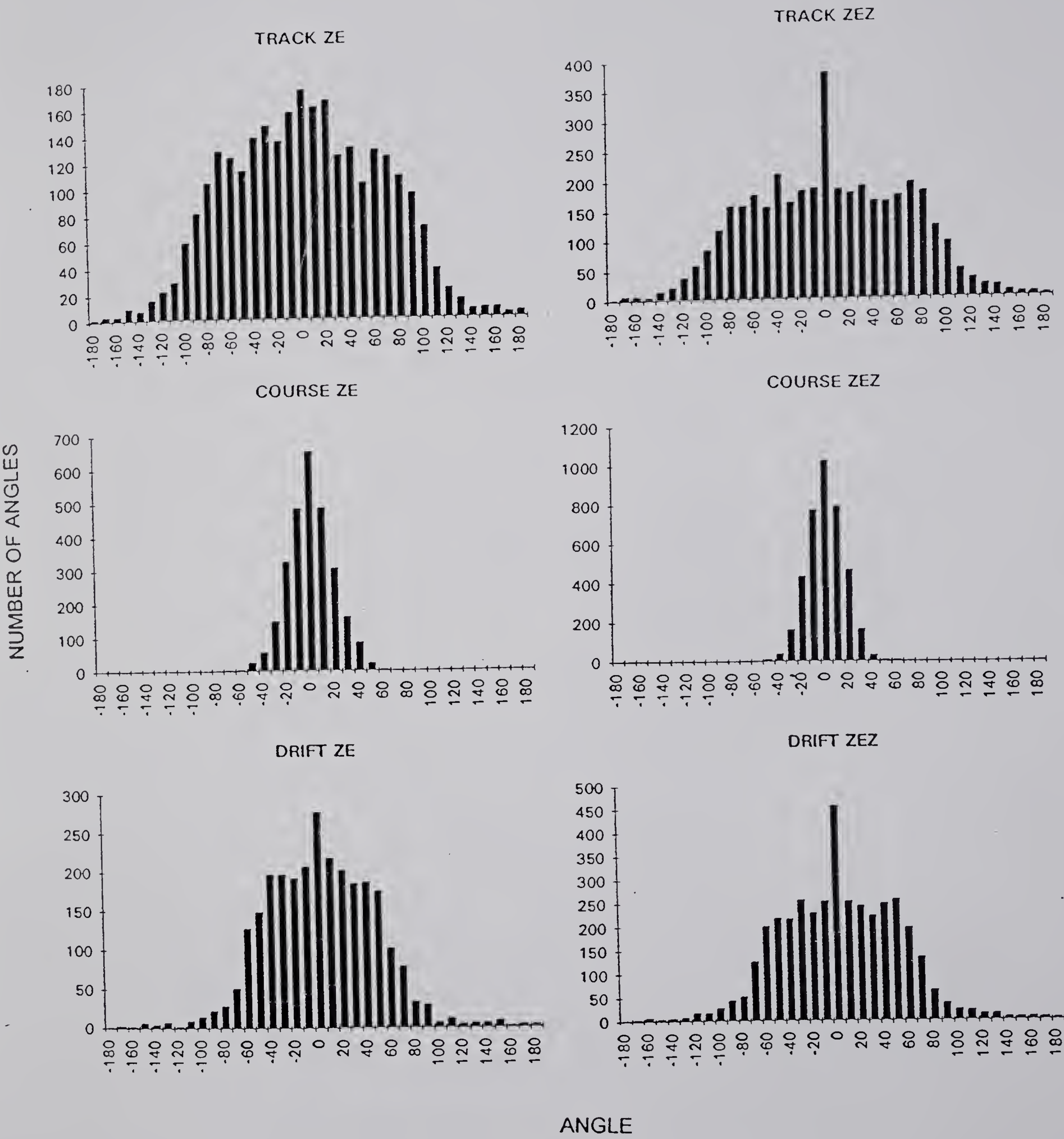

A.
velocity (cm/s)
70
60
50
40
30
20
10
0
ZE
ZEZ
B.
Angle
60
50
40
30
20
10
0
ZE
ZEZ
C.
velocity (cm/s)
40
35
30
25
20
15
10
5
0
ZE
ZEZ

Fig. 15. Frequency distribution histograms of the flight angles steered by *C. cautella* males flying toward wide source plumes with either complete or the incomplete blend of pheromone (sampled every 30th of a second). **A.** frequency distribution histogram of track angles steered toward incomplete blend (ZE). **B.** frequency distribution histogram of track angles steered toward complete blend (ZEZ). **C.** frequency distribution histogram of course angles steered toward incomplete blend (ZE). **D.** frequency distribution histogram of course angles steered toward complete blend (ZEZ). **E.** frequency distribution histogram of angles drifted when flying toward incomplete blend (ZE). **F.** frequency distribution histogram of angles drifted when flying toward complete blend (ZEZ). Angles were sampled every 30th of a second


TRACK ZE
TRACK ZEZ
COURSE ZE
COURSE ZEZ
DRIFT ZE
DRIFT ZEZ
NUMBER OF ANGLES
ANGLE

complete (Fig. 15). The consistently unimodal distribution of flight angles, independent of blend, suggests that unimodality of distribution of these flight parameters is not a good measure of completeness of pheromone blend. The similarity in the drift angles between the two treatments implies that males have similar lateral slip, independent of blend. This similarity also indicates that no additional imprecision (sensu Witzgall & Arn 1990) occurs when males fly to incomplete pheromone blends.

C. cautella males flew faster to the incomplete blend (ZE) than to the complete blend (ZEZ) (Fig. 14). The mean airspeed was 70 cm sec^{-1} for the incomplete blend ZE and 64 cm sec^{-1} for the complete blend (different, P=0.0002, t-test, Excel). Mean ground velocity was 28 cm sec^{-1} for the incomplete blend and 21 cm sec^{-1} for the complete blend (different, P=0.0001, t-test, Excel). According to the chemical imprecision model, flight tracks toward the incomplete blend should be slower than flights toward the complete blend.

Since flight velocity differs between complete and incomplete blends while flight track angles remain constant, the parameters of visual flow, which are derived from ground velocity and angular orientation (track angle), should also differ. Both the transverse (ZE=12.44 cm sec^{-1} , and ZEZ=8.14 cm sec^{-1}) and the longitudinal (ZE=23.16 cm sec^{-1} , and ZEZ=16.41 cm sec^{-1}) components of the visual flow change with completeness of the blend (changes are significant at P=0.003 for transverse, and at P=0.0001 for longitudinal, t-test, Excel) (Fig. 14). This difference in the transverse (T) image flow is associated with the velocity that males displace laterally when zigzagging. Although males flying to the incomplete blend displace faster crosswind than those flying to complete blend, they also displace faster upwind, to the extent that the ratio L/T is maintained the same for both blends (T/L=2.0 $\pm$0.1) (no difference, P>0.05, t-test, Excel).

2.4. Discussion

C. cautella males have a decreased ability to perform the-late-in-the-sequence behaviors (e.g., upwind flight, land on the source) when exposed to plumes of incomplete blends or of blends containing the alcohol (Chapter I). This resulted in different proportions of males landing on the various sources (Chapter I). Because flight track data were collected only for males who landed on a source, the number of tracks was generally low for the blend and concentration experiments. However, some trends became evident from the analysis of these flight tracks. Virtually straight upwind flight, reflected by unimodal distribution (mode=0°) of the flight angles, was observed for each blend tested, although the concentration at which this form of flight occurs varied from blend to blend. All but one treatment showed unimodal distribution of course angles. Unimodal frequency distribution of track angle is evident in the flight tracks of males exposed to concentrations ranging from 0.045 ng to 45 ng of blends ZEZ and ZEZOH. Unimodal frequency distribution is seen from concentrations of 0.45 ng to 45 ng for sources containing treatment ZE, and at a concentration of 0.45 ng for treatment ZEOH. This indicates that the "nature" of the chemical stimulus, i.e., the interaction between blend and concentration, is a determining factor in the flight maneuvers of males following the plume. The flight tracks toward a specific blend can generate unimodal *or* bimodal angle frequency distributions depending on the concentration of the stimulus to which the male was exposed.

Increasing pheromone concentration resulted in overall lower flight velocities, independent of whether the parameter being tested was airspeed, ground velocity, or net lateral displacement velocity (XT). This is in accordance with observations made using other moths including *Pectinophora gossypiella* (Farkas *et al.* 1974), *Choristoneura fumiferana* (Sanders et *al.* 1981), *G. molesta* (Kuenen & Baker 1982b), and *L. dispar* (Charlton at *al.* 1993). With regard to the net upwind velocity, the situation varies with completeness of pheromone blend. *C. cautella* males flying to incomplete blend reduce their net upwind velocity with an increase in concentration, as seen for other moths, but those males flying to plumes of the complete blend maintain their upwind velocity at a preferred level at all gradations of concentration.

That the course angle and the track angle frequency distributions are unimodal for both the complete and incomplete blends throughout a broad range of concentrations was a somewhat unexpected result. This indicates that *C. cautella* males exhibit a reasonably straight upwind flight within this range. The flight tracks analyzed demonstrated a flight pattern that was more direct than might have been expected based on data previously reported for other moths (Kennedy *et al.* 1981; Kuenen &

Baker 1983; Baker *et al.* 1984; David & Kennedy 1987, Willis & Baker 1987) and such direct flight was observed over a broader blend and concentration range than might have been expected from the other data on moths flying straight upwind (Witzgall & Arn 1990).

Unimodal distribution of course and track angles has been reported for two moth species flying to natural blends at a single wind velocity: *Amyelois transitela* (navel orangeworm) males flying to female gland extracts (Haynes & Baker 1989) and *L. botrana* flying to calling females (Witzgall & Arn 1990). A unimodal distribution of flight angles was considered so exceptional that Witzgall and Arn (1990) postulated that unimodality of distribution was an exclusive characteristic of male upwind flight toward natural sources, and it was an "intrinsic nature" of synthetic blends that did not precisely match the natural pheromone to generate bimodal distribution of these flight track angles.

In addition to an overall reduction in airspeed in response to systematic increases in concentration, *C. cautella* males change the flight angles steered dependent on the blend being tested. Males flying toward the complete blend maintained constant track angles, with a slight increase in the course angles steered as concentration increased. When flying to the incomplete blend, males steered increasingly smaller course angles, which resulted in higher values for both track and drift angles, when concentration increased. This suggests that *C. cautella* males adopt different headings when flying toward complete as opposed to incomplete blends. At low concentrations the heading is more directly upwind for males flying to sources with the complete blend, while the reverse is true for the incomplete blend: a more directly upwind heading is observed at higher concentrations.

The addition of the alcohol to Z9,E12-14:Ac reduces the proportion of males responding to this component of the pheromone (Chapter I). The addition of the alcohol increased the transverse component of the visual flow in the flight tracks when present at concentrations above one female equivalent. This effect of the alcohol was virtually eliminated by the addition of Z9-14:Ac to the mixture. Although the addition of Z9-14:Ac to the incomplete blend significantly increased the proportion of males landing on the odor source (Chapter I), it did not change the way that the males perform their flight maneuvers while approaching the source.

Our data in *C. cautella* does not corroborate Witzgall and Arn's hypothesis that a zigzag path is generated by the incompleteness of pheromone blend. Using *C. cautella* we demonstrated that unimodal distribution of flight angle frequency can be obtained from tracks of males flying toward odor sources generated by synthetic components. The unimodal distribution of these flight angles was not restricted to the blend and concentration mostly closely mimicking calling females; it was also observed in flight tracks toward sources of various concentrations of the complete blend, the incomplete blend, and even toward sources containing the alcohol Z9,E12-14:OH, a component that is not part of the *C. cautella* long distance sex pheromone (Coffelt *et al.* 1978), and which has an inhibitory effect on the male pheromone-related behaviors (Read &

Haines 1976; Grant & Brady 1975, Chapter I). We conclude that, at least for *C. cautella,* unimodality of flight angle distribution is not diagnostic of whether a given pheromone blend is complete.

A more prominent zigzag pattern is present in flight tracks toward the sources containing Z9,E12-14:Ac and the alcohol Z9,E12-14:OH at high concentrations. This is in accordance with the chemical imprecision model. It is worth noting, however, that the elaborate technique of measurement of flight parameters is able to detect differences only among nonspecific blends at high concentrations, and not among complete and incomplete blends. Less elaborate techniques, such as simply scoring the frequency of males landing on the source, can readily detect such differences at concentrations above threshold (sensu Chapter I).

Our results also do not support the assumption that the relationship T+L or $\sqrt{(T^2+L^2)}$ (David 1986) are kept constant control their course angles by feedback from T (minimizing T and maintaining L at a low positive value) (Preiss & Kramer 1986a). This suggests that *C. cautella* males are either not regulating their flight by a visual flow feedback, or that they are maintaining a different relationship of visual parameters than suggested by David or by Preiss & Kramer. An alternative is that males maintain some unknown but constant relationship of the visual flow parameters not only by regulating their flight angle and velocity, but also by performing compensatory movements of their body parts. For example the moth may perform compensatory turns of its head to regulate the retinal velocity of the image flow, or it may perform body movements that we normally do not account for in flight track analysis for free-flying insects, e.g., pitch, roll, and yawn. Another alternative is that the relationship between T and L is different from that previously proposed: in these experiments we found that *C. cautella* males increase both the L and T components of retinal image flow when flying faster upwind, but the ratio (L/T) is kept relatively constant.

Comparing the parameters of flight tracks of males following point source plumes containing either complete or incomplete blend of pheromone at a concentration of 1 female equivalent (4.5 ng) yields no statistically significant differences at the 95% confidence level. This result is in accordance with the trends observed in the blend and concentration experiment; it is also an indication that the trends obtained in that experiment will remain valid when larger numbers of flight tracks are obtained and analyzed.

The form of the pheromone plume has an effect on upwind flight tracks and the perception of pheromone blend. When the plume is wide and thin, males steer the same flight angles for both blends, with the same unimodal distribution as when they fly to a point source. Unlike the males flying to the point source, however, these males fly faster in the wide plume with incomplete blend than to the wide plume with complete blend. These results contradict the chemical imprecision model (Witzgall & Arn 1990), and imply that the structure of the odor plume is another factor which may have to be addressed in general hypotheses regarding the role of the chemical stimulus on odor-mediated upwind flights.

Males flying to the turbulent pheromone plumes commonly used in wind tunnel experiments are crossing pulses of pheromone of different sizes and concentrations at variable frequencies, depending on where the plume is being intercepted with respect to the odor source. The turbulent odor plume arrives downstream as pulses of varying strength and temporal patterning (Murlis & Jones 1981; Murlis 1986). In turbulent plumes some of the odor pulses travel long distances before being diluted by turbulent eddies; in others the odor-laden air will be mixed with clean air soon after it leaves the source (Murlis *et al.* 1990). Aylor *et al.* (1976) argue that the threshold for an odor-induced behavior will not be defined by the overall mean concentration of a turbulent plume as calculated by time average models. The threshold will, instead, be defined by the peak concentration of the more concentrated pulses.

In experiments designed to detect the effects of varying blend and concentration of odors, it is important that the males being tested always receive the same chemical stimulus when crossing the odor plume, independent of the sequence of behavior they are performing, or their distance from the odor source platform. The structure of the plumes presented to *C. cautella* males in these experiments was substantially different from the ones described in other wind tunnel studies. The plume normally used in wind tunnel experiments is broad, with its expansion primarily due to turbulent growth (Murlis 1986). Such plumes are the result of lack of perfect laminarity in air flow which is usually associated with the air swirl of "blowing" wind tunnels, with turbulence generated by the use of large odor sources (e.g., rubber septa), or with the intentional addition of "turbulence generators" to the source (Chapter III).

The filamentous plume and the wide plume (plumes dominated by molecular diffusion growth instead of turbulent growth) were chosen for this experiment because they provided a uniform and predictable odor stream throughout the working section of the wind tunnel. Uniformity and predictability of plumes are desirable characteristics when studying the effect of the chemical signal on the male pheromone-related behavior and its upwind flight. A male, when contacting these non-turbulent pheromone plumes would receive a chemical stimulus of similar intensity and size whether the plume was intercepted close to the source or further downwind. One problem with the use of point source plumes is the lower proportion of males capable of relocating the plume after taking off when compared with larger plumes (Chapter III). It is interesting that males flew similarly to the wide plume and the point source plume. Males that had lost the wide plumes had a better chance of recontacting it by moving vertically close to the center of the working section of the wind tunnel, than the ones that lost the filamentous plumes.

Mafra-Neto and Cardé (**Chapter I**) demonstrated that the presence of the Z9-14:Ac in the odor source is important for *C. cautella* males to perform the entire behavioral sequence when the stimulus presented was above that threshold. The analysis of the flight tracks of males who successfully located the odor source indicates that *C. cautella* males fly differently to the four blends tested, and that, for a given blend, the form of flight changes drastically when moving within a range of concentrations. All blends generated a unimodal distribution of flight angles at least at one concentration, whereas bimodal distribution of the same angles was obtained at other concentrations. This indicates that straight upwind flight can be obtained under several different sets of conditions, and it does not necessarily reflect the presence of an optimal blend. This point is illustrated by the statistically indistinguishable flight tracks obtained from males flying to plumes generated by sources containing the complete blend and by sources containing the incomplete blend at concentration of one female equivalent; these results suggest that *C. cautella* male flight patterns are not dependent on the completeness of pheromone blend.

Behavior change dependent on completeness of blend is reflected in the differential of the proportion of males performing late-in-the-sequence pheromone-mediated behaviors, and the latency of performance (**Chapter I**), and not in on the divergence of parameters of the flight track analysis (see concentration 4 in Fig. 7).

2.5. Conclusions

Using *C. cautella* we show that: (1) males fly directly upwind to synthetic blends of pheromone; (2) males fly directly upwind not only to the synthetic blend that mimics the female gland extract, but also to "wrong" or "non-optimal" pheromone blends (i.e., incomplete blends and blends containing the "inhibitor" Z9,E12-14:OH); (3) a clear bimodal distribution of track and course angles from male flight track analysis was present only when males flew to plumes with very high concentrations of blends containing the Z9,E12-14:OH (the inhibitor), and that track and course angle distributions for all other blends and concentrations tended toward a unimodal distribution; (4) the distribution of angles of tracks of males flying toward plumes of complete and incomplete blends are very similar and the distribution is unimodal, independent of whether the plume is generated by a point source or a wide source; (5) directness of flight and unimodality of course and track angles are poor diagnostic tests for completeness of blend when compared to frequency and latency of performance of late-in-the-sequence behaviors, and (6) plume structure effects male response to pheromone.

CHAPTER III

INFLUENCE OF PLUME STRUCTURE AND PHEROMONE CONCENTRATION ON THE UPWIND FLIGHT OF *Cadra cautella* MALES

3.1. Introduction

Most studies of odor-mediated flight orientation behavior in insects have used the upwind flight of male moths to a source of a female pheromone as a model (Kennedy 1986; Baker 1988). These studies have been done in laboratory wind tunnels where males released downwind fly upwind to a pheromone source, following the odor plume generated by that source.

The most accepted model developed to explain the mechanisms involved in the location of a pheromone source by a male insect invokes two mechanisms (Baker 1989): a positive **optomotor anemotaxis** (Kennedy & Marsh 1974; Kuenen & Baker 1982) and a **central nervous system (CNS) turn generator**, both of which are triggered by in-flight contact with the pheromone plume. The first mechanism, optomotor anemotaxis, is regulated by feedback from the changing visual environment caused by wind-induced drift; this feedback provides polarity to the flight maneuvers, resulting in upwind displacement. Optomotor anemotaxis is responsible for maintaining a constant angular velocity of image motion across the male's retinal surface (Marsh et *al.* 1978, Cardé & Hagaman 1979; Kennedy 1951, Kuenen & Baker 1982). Males are able to control the image by maintaining flight altitude (Preiss & Kramer 1983), ground velocity, angles for turning into the wind, and course steering at constant preferred values. The second mechanism, a CNS counterturn generator causes the male to turn back and forth across the wind in a temporally regular fashion (Baker *et al.* 1984; Kuenen & Baker 1982).

It has been shown that in pheromone-mediated flight, insects maintain ground velocity, course angles and counterturn intervals at constant levels when the extrinsic environment is changed (Marsh *et al.* 1978; Kuenen & Baker 1982; Willis & Cardé 1990, Charlton et al. 1993). The only moth demonstrated to change the rhythm of counterturning is *Grapholita molesta*, in this moth it appears that counterturning rhythm is modulated by changes in pheromone concentration (Kuenen & Baker 1982).

Pheromone concentration affects the output of pre-flight and in-flight pheromone-related behaviors of *C. cautella* males (**Chapter I**). At high concentrations the likelihood of a male to become arrested in-flight is higher.

In this report we demonstrate that the structure of a pheromone plume influences the flight pattern of males. An increase in plume size results in higher ground velocities, lower frequency and amplitude of turns, and smaller track angles: these changes result in a faster and more direct upwind flight. We observed that males fly in and out of narrow plumes, and within the boundaries of wider plumes.

3.2. Material and Methods

3.2.1. Insects

Our *C. cautella* colony has been maintained in continuous culture at the University of Massachusetts since March 1989. It was started from ca. five hundred larvae and pupae from Kansas State University, Manhattan, Kansas, and the population has never been lower than 400 mating pairs. For this experiment the insects were reared from eggs to larvae in one quart Mason jars. The diet was made using 3 kg poultry laying mash, 2 kg rolled oats, 100 mg Brewer's yeast and 200 ml glycerin. The rearing room was kept at 25-27°C on a 16-8 hr light-dark cycle and at 50-60% relative humidity. Individuals were sexed at migrant stage (last larval instar) when the male testes are easily seen. Males were reared from last larval instar to adult in an environmental chamber under a 16-8 hr light-dark cycle, 70% relative humidity and 25-26°C, held in a separate room. Male pupae emerged inside 25 X 25 X 25 cm screen cages. The pupae were transferred daily to new cages leaving only newly emerged males in the old cages. This procedure generated a continuous supply of 1-day-old males which were used for behavioral assays during their first dark period.

3.2.2. Wind Tunnel

The wind tunnel used is described in detail elsewhere (**Chapter I**). In brief, it is a 2.5 m long semi-cylinder of Plexiglas® and Vivac®, suspended 130 cm off the ground. Each end is covered by a layer of fine polyester mesh screen. The upwind screen sits between the body of the wind tunnel and the upwind air laminator. Two downwind screens separate the working section of the tunnel from the exhaust system. Airflow through the wind tunnel was laminar. This was confirmed visually using $TiCl_4$ "smoke" plumes, and also by the low variance obtained from repeated measurements of the wind velocity at the same predetermined point in the tunnel using a hot-wire anemometer (Yokogawa model 2141). Airflow was measured using the hot-wire anemometer positioned at the center of the tunnel, and a wind velocity of 45 cm sec^{-1} was set using a voltage regulator to control the exhaust fan.

3.2.2.1. Odor Source

The odor source was a disk of filter paper (Whatman #1) 0.7 cm in diameter (Fig. 16). It was held in a horizontal position, parallel to the floor, by an insect pin (# 1). The pin was attached to one end of a hollow copper tube (3 mm diam) that slid easily through a hole in the floor of the wind tunnel. This allowed for regulation of the vertical position of the odor plume in the tunnel. The odor source was located 45 cm from either side of the tunnel, and 10 cm from the upwind screen.

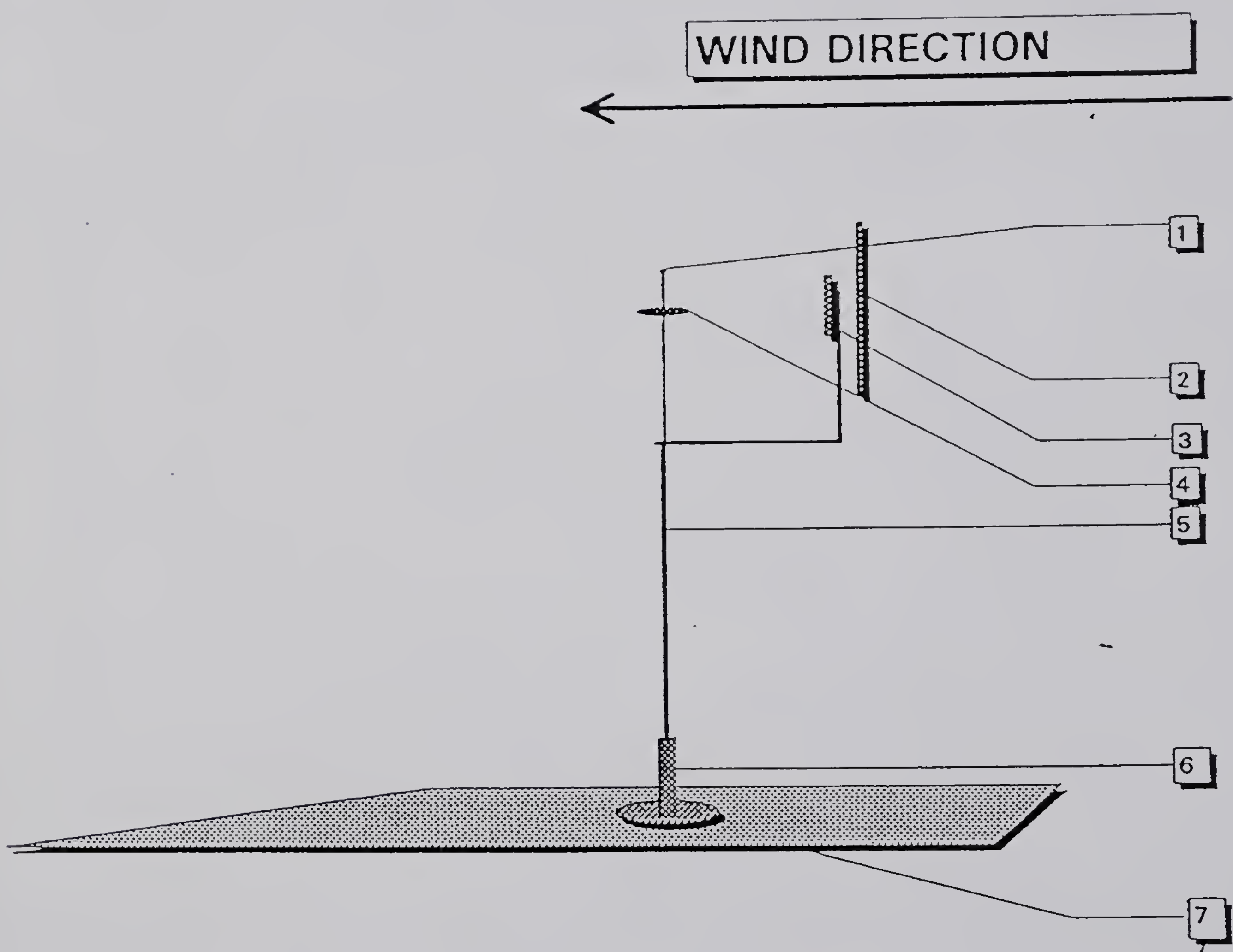
Synthetic pheromone components were formulated gravimetrically into solutions of 1μg μl^{-1}, and then volumetrically into the binary blend (*Z,E*)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac) and (*Z*)-9-tetradecenyl acetate, (Z9-14:Ac) (11.5:1). This solution was serially diluted to three concentrations of Z9,E12-14:Ac: 0.0045 ng μl^{-1} (concentration 11), 0.045 ng μl^{-1} (concentration 12), and 0.45 ng μl^{-1} (concentration 13).

A filter paper disk was impregnated with 10 μl of test solution. The dose of pheromone in the filter paper was 0.045 ng for concentration 11, 0.45 ng for concentration 12, and 4.5 ng for concentration 13. The paper disk of the odor source was replaced by a fresh one every 10 minutes to ensure a relatively constant odor source.

The addition of wind deflectors positioned 4 cm upwind of the filter paper (Fig. 16) resulted in three distinctive plumes. The narrow plume generated by the release platform without any deflector will be referred to as the **filamentous plume**. The plumes generated using a 1 x 1 cm deflector made of acetate, and a 3 x 3 cm deflector made of acetate will be referred to as the **narrow turbulent plume** and the **wide turbulent plume,** respectively.

The structure of the plumes was evaluated using frame-by-frame analysis (as in flight track analysis below) of horizontal and vertical high-contrast video images of smoke plumes. "Smoke" plumes were

Fig. 16. Odor source platform for the plume size manipulation. Where: **1**. insect pin, **2.** large (3 cm^2) diffuser (not in place), **3.** small (1 cm^2) diffuser supported in place by a thin wire attached to (5), **4.** filter paper disk impregnated with the solution to be tested, **5.** copper tube (3 mm), **6.** Teflon® tube connected to the wind tunnel floor, **7.** wind tunnel floor.


WIND DIRECTION
1
2
3
4
5
6
7

generated by pipetting $TiCl_4$ onto the filter paper serving as odor source. A high intensity directional light from a fiber optic illuminator (Dolan Industries, Inc. Model 190) was aimed at the center of the longitudinal axis of the smoke plume. This plume was then filmed against a black background. The resultant video image was analyzed frame by frame, and the sizes of 100 pulses per treatment were measured.

3.2.2.2. Male Release Device

The male release device was located 1 m downwind from the odor source. The device was in a position where it would intercept the **filamentous** smoke plume. Males were released from a cylindrical aluminum screen cage (4.5 cm diameter x 5 cm). One end of the cylinder was covered with the same screen and the other end was open. Cages were positioned with the open side facing upwind, and held in position by a rigid Teflon® tube that had one end opening inside the cage and the other connected to a hollow glass tube. The hollow glass tube passed through the wind tunnel floor and opened outside of the wind tunnel. This design allowed for the introduction of moths from outside of the tunnel to inside of the release cage without disrupting the pheromone plume. The height of the release platform was regulated by sliding the glass tube through the wind tunnel floor.

Randomly selected moths were transferred from emergence cages to the glass tube of the male release device using an aspirator. After every exposure to the pheromone plume, the screen cage and the Teflon® tube were replaced by clean ones (as in **Chapter I**).

A light box containing five red and five white 25-watt incandescent light bulbs and a filter/diffuser made of one layer of white Styrofoam (0.5 cm thick) was placed above the working section of the tunnel. Light conditions were adjusted with a voltage regulator to 5.5 lux, and relative humidity ranged from 75 to 85%.

Red acetate circles randomly arranged on the Plexiglas® floor provided non-directional optomotor cues (David 1982). Since the same type of red filter was placed over the lens of the video camera used for filming, the dots were almost completely transparent in the resulting video image.

3.2.3. Bioassay Procedure

We performed two different bioassays. The first, was a study of the simultaneous use of a pheromone plume by two males. These observations led to the second bioassay in which we studied the effects of plume shape and concentration on male flight.

3.2.3.1. Simultaneous Flight to a Single Plume

Two males were released simultaneously in the wind tunnel to a filamentous plume at concentration 12. The insect pin of the odor source device had two filter paper disks: one to release the odor plume and another placed 2 mm above the first which released $TiCl_4$ smoke. When both filter papers were impregnated with $TiCl_4$, a continuous and homogeneous smoke plume resulted. Twenty centimeters downwind from the source, the plume was 5 mm in height and 10 mm in width; 150

cm downwind from the source, it expanded to approximately 7 mm in height and 15 mm in width. Because the accumulation of oxidized $TiCl_4$ at the source device altered the structure of the smoke plume, the $TiCl_4$ filter paper was changed before accumulation of the oxidized material formed crystals which interfered with a consistent plume structure.

Male upwind flight was recorded in the horizontal plane from below (as in section 3.2.3.2). Male flight and instantaneous plume structure were evaluated using frame-by-frame video analysis. These male/plume interactions were evaluated in fifteen pairs of video-recorded flight tracks.

3.2.3.2. Plume Shape and Concentration

Three different concentrations of the same synthetic pheromone blend and three different pheromone plume sizes were studied. Using a complete factorial design (3 concentrations x 3 plume sizes), nine treatments were generated and randomly assigned to an order for testing. Twenty *C. cautella* male flight tracks were obtained from each of these nine treatments. A total of 180 flight tracks was obtained and analyzed.

Adult emergence cages were placed under the experimental conditions described above for at least 30 minutes prior to testing. Moths were selected randomly from emergence cages and transferred to the release platform positioned underneath the pheromone plume. The release cage was positioned 15 cm above the wind tunnel floor and the source platform was positioned 35 cm above the wind tunnel floor. Using this set-up, none of the three pheromone plumes tested came into contact with the release cage. This was confirmed using visual techniques and behavioral tests: $TiCl_4$ smoke at the source, and high pheromone concentrations at the source with quiescent *C. cautella* males at the release platform. The pheromone source was then lowered 20 cm to 15 cm above the wind tunnel floor, and the cage holding the male was turned so that the open end of the cage faced upwind. Lowering of the pheromone source marked the beginning of each bioassay. When the male initiated flight, the release platform was lowered to 5 cm above the wind tunnel floor, effectively removing it from the position where it intercepted the pheromone plume. The pheromone plume was thus kept uniform downwind from the release platform. This maneuver also allowed males that locked onto the plume downwind from the initial position of release to proceed flying upwind without encountering the platform.

3.2.4. Data Analysis

Males had their upwind flight tracks video-recorded from below in a two-dimensional view using a Sony RSC 1050 rotary-shutter video camera with a 8.5 mm wide-angle lens, connected to a Sony SLO 340 video recorder. Measuring the field of view at the level of the smoke/pheromone plumes (15 cm above the wind tunnel floor) yielded a 80 cm x 90 cm rectangular area which extended from 15 cm downwind from the plume source to 105 cm downwind from the source.

Only upwind flight tracks of males that contacted the pheromone source were analyzed. Flight tracks of individual moths were transferred to a Sony SVM-1010 motion analyzer, and played back frame-by-frame through a 41 cm Panasonic WV-5470 black-and-white video monitor. Two points of reference on the wind tunnel floor, and the moth location in every other frame (each 1/30th of a sec) were transcribed onto transparent acetate. The X and Y coordinates of the moth location in a two dimensional plane were obtained using a digitizer pad (Apple Graphics Tablet), and analyzed with Quick Basic programs for ground velocity, track angle, and net velocity (Charlton *et al.* 1993). Course angles, drift angles, and airspeed were obtained using the triangle of velocities method (Marsh *et al.* 1978). Inter-reversal distance, turn frequency, and the inter-reversal time were calculated directly from the track. The definitions of the parameters of flight are as in Charlton et. al. (1993). The data were analyzed using two way Anova (SAS) and two sample t-tests (LSD-SAS and Excel).

3.3. Results

3.3.1. Simultaneous Flight in a Single Filamentous Plume

Frame-by-frame analysis of the tracks generated by two *C. cautella* males flying simultaneously in the same filamentous plume shows that males flying across the smoke plume disturb the homogeneous structure of the plume. Bursts of smoke (and pheromone) occur at a semi-regular frequency which is determined by the encounters of the male with the plume.

When two *C. cautella* males fly upwind simultaneously along the same filamentous pheromone plume, one of the males will fly very differently than when flying alone. This change in flight pattern is dependent on the male's position in the plume relative to the other male. The male in the upwind position flies in the same manner as individually tested males flying to an undisturbed pheromone plume. This male crosses the pheromone plume in a continued zigzagging fashion, making slow upwind progress. Every time the male crosses the plume, the filamentous plume structure is disrupted briefly. Such disruption creates a burst of pheromone which is a sudden expansion of the single filament of odor. The second male begins its upwind flight with the usual zigzagging flight behavior. When the second male encounters a burst of pheromone created by the upwind male, he changes from a regular, counterturning flight pattern to an almost straight upwind flight. This direct upwind flight is maintained until the second male encounters the first male. If the second male passes the first male, his flight pattern changes back to regular turns and counterturns. If the two males contact each other, usually one (or both) of the males performs a large loop out of the pheromone plume and then drifts downwind.

Visualization of the structure of the plume using smoke techniques demonstrates that the second male flew a zigzag path when the narrow pheromone plume was intact. However, if the male encountered bursts of pheromone generated by another male flying across the plume upwind, he surged upwind toward the odor source (Fig. 17). This pattern was consistent for all such "interactive tracks" analyzed. The change in flight described suggests that pheromone plume structure is an important factor in the modulation of the pheromone-mediated upwind flight of *C. cautella* males.

3.3.2. Plume Shape and Concentration

3.3.2.1. Plume Structure

The smoke plume generated by pipetting $TiCl_4$ on the smallest source (a filter paper disk <1 mm thick x 7 mm diameter) was a continuous, homogeneous plume with no evidence of turbulent growth while traversing the working area of the wind tunnel in a 45 cm sec^{-1} wind. This observation is in congruence with estimates of the effective molecular growth stage being within a range of several meters for a plume generated by a 1 mm source in light winds (Miksad & Kittredge 1979). Frame-by-frame analysis of the resulting video image demonstrated that a stationary point intercepting the plume would be in constant contact with the plume. The dimensions of the smoke plume 10 cm downwind from the release platform were ca. 0.8 cm in width x 0.1 cm in height; 150 cm downwind from the release platform, plume dimensions were ca. 1.0 cm in width x 0.2 cm in height.

The 1 cm^2 and 3 cm^2 deflectors generated plumes with a structure determined primarily by turbulence. Turbulence generated by the

Fig. 17. Representative flight track of *C. cautella* males flying upwind on a non-turbulent filamentous plume of pheromone mixed with a visual marker. Each point represents the position of the moth at intervals of 1/30th of a second. The small arrows show where the male entered in contact with the mixed plume. The letters associated with the arrows indicate if the plume the insect is encountering is homogeneous (H) or disturbed (D). The shaded area represents the time-average position of the pheromone and smoke plume.

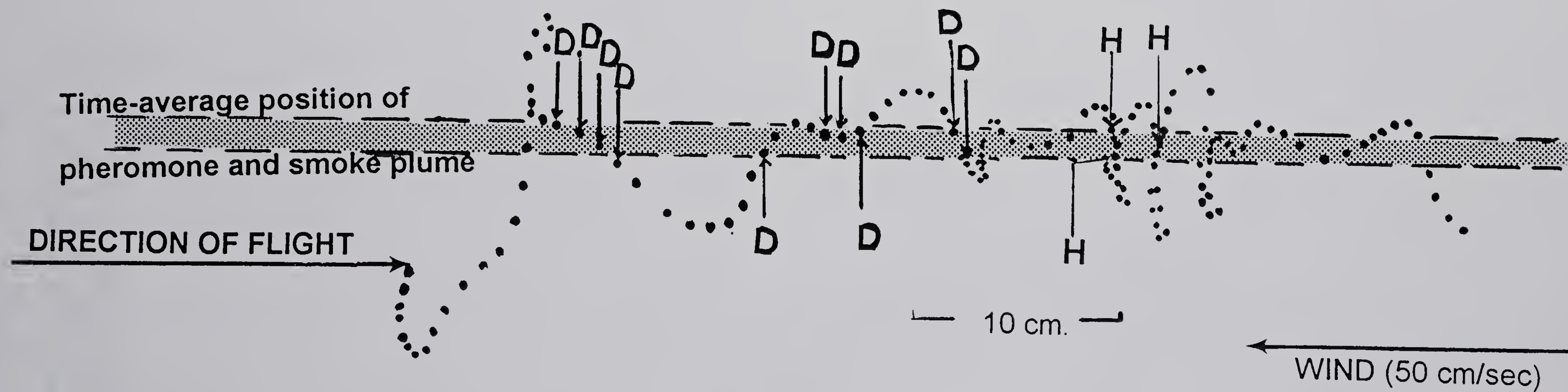

Time-average position of
pheromone and smoke plume
DIRECTION OF FLIGHT
D
H
10 cm.
WIND (50 cm/sec)

Table 1. Counterturning tempo (in seconds) for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations.

	concentration								
	11			12			13		
	mean	std		mean	std		mean	std	
Filamentous	0.20	0.020	**D**	0.20	0.021	**D**	0.21	0.039	**D**
Narrow Turb.	0.27	0.072	**BC**	0.24	0.065	**C**	0.24	0.043	**C**
Wide Turb.	0.30	0.063	**B**	0.38	0.105	**A**	0.36	0.005	**A**

Based on twenty moths tested for each combination of plume size and concentration. Means having no letters in common are significantly different (α=0.05, LSD comparisons, SAS). Where Turb. is turbulent plume, std is standard deviation.

deflectors resulted in plumes composed of bursts of smoke intercalated with clean air. Video analysis of the structure of these turbulent smoke plumes showed that a stationary point positioned in the center of the plume, 150 cm downwind from the source platform, was intermittently surrounded by puffs of smoke and clean air. Bursts occurred at a regular frequency which was characteristic of each deflector size. The 1 cm^2 deflector generated puffs of pheromone with a mean **intraburst duration** of 0.07 $\pm$ 0.04 sec (x ± CI), every 0.19 $\pm$ 0.06 sec The interval of clean air between bursts, the mean **interburst duration,** was 0.11 $\pm$ 0.01 sec. The 3 cm^2 deflector generated smoke puffs with a mean **intraburst duration** of 0.17 $\pm$ 0.04 sec every 0.25 $\pm$ 0.04 sec. The mean **interburst duration** for this deflector was 0.08 $\pm$ 0.01 sec.

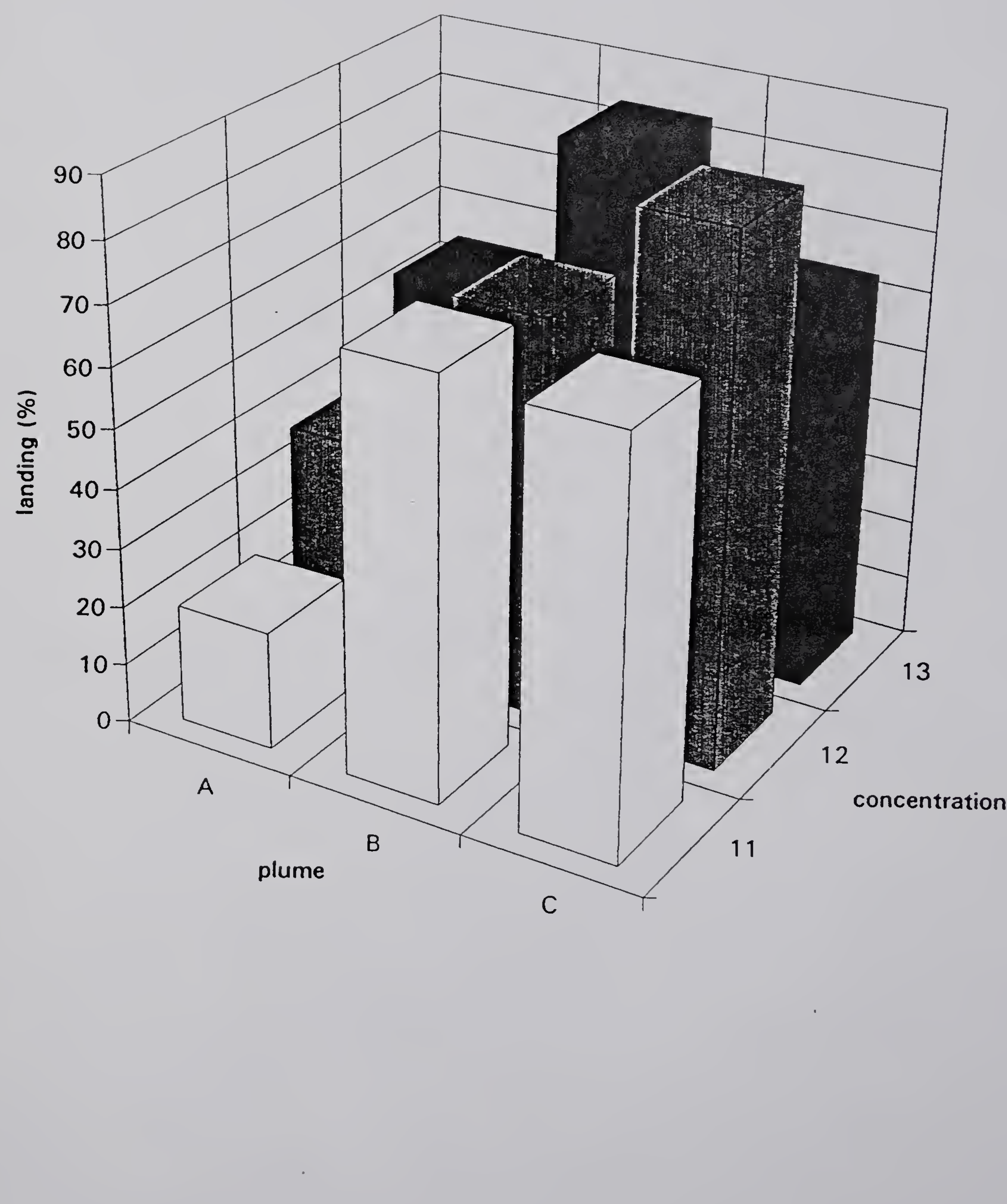
3.3.2.2. Flight Tracks

Because *C. cautella* males responded differently to each combination of plume structure and odor concentration, the proportion of males that flew upwind and landed on the source varied with treatment. A variable number of males were tested for each treatment in order to obtain 20 flight tracks (Fig. 18).

Since the effect of day or block is not significant determining the flight behavior of *C. cautella* males, days and blocks were dropped from statistical analysis of the flight track.

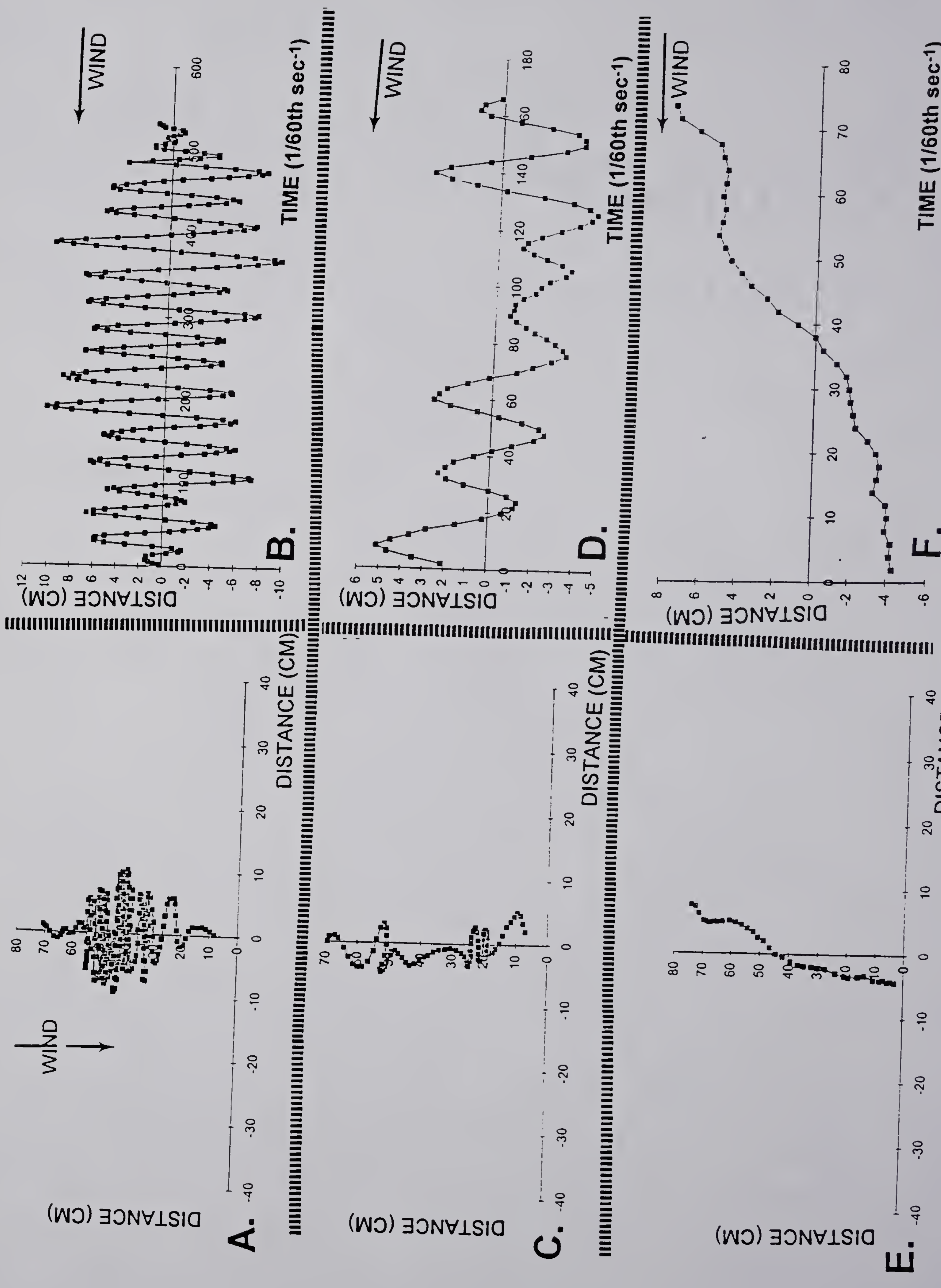
The lateral extent of the zigzagging tracks of males flying to the filamentous plume always exceeded the pheromone plume boundaries

Fig. 18. Percentage of *C. cautella* males landing on sources containing three concentrations of pheromone (11 is 0.045 ng, 12 is 0.45 ng, and 13 is 4.5 ng) presented at three different plume sizes (A is filamentous plume, B is narrow turbulent plume, and C is wide turbulent plume). A total of 414 males were tested in order to obtain 20 tracks per treatment (104 males tested for treatment 11A, 59 males tested for treatment 12A, 45 males tested for treatment 13A, 36 males tested for treatment 11B, 38 males tested for treatment 12B, 29 males tested for treatment 13B, 38 males tested for treatment 11C, 28 males tested for treatment 12C, and 37 males tested for treatment 13A).



90
80
70
60
50
40
30
20
10
0
landing (%)
A
B
C
plume
11
12
13
concentration

Fig. 19. Representative flight tracks of *C. cautella* males flying upwind to three plumes of different size at three concentrations. Where: **filamentous plume** (**A** and **B**), **narrow turbulent plume** (**C** and **D**), and **wide turbulent plume** (**E** and **F**) at concentration 11. The trends seen for concentration 11 (**A** to **G**) are similar to those of tracks of males flying to concentrations 12 (**H**) and 13 (**I**). Flight tracks showing both lateral displacement (x=due crosswind, in cm.) and longitudinal displacement (y=due upwind, in cm.) are shown in **A**, **B**, and **C.** The dots represent the position of the moth every 30th of a second. Graphs **D**, **E**, and **F** show the lateral displacement in cm. (ordinate) plotted over time (abscissa). Scales differ among graphs D, E and F in order to accommodate the maximum values of lateral displacement of each track. Note the rhythmic zigzagging for the **filamentous** on graph **D** and for the narrow turbulent plume, on graph **E**, contrasting with the virtual lack of zigzags and tempo for the **wide turbulent**, on graph **F**.



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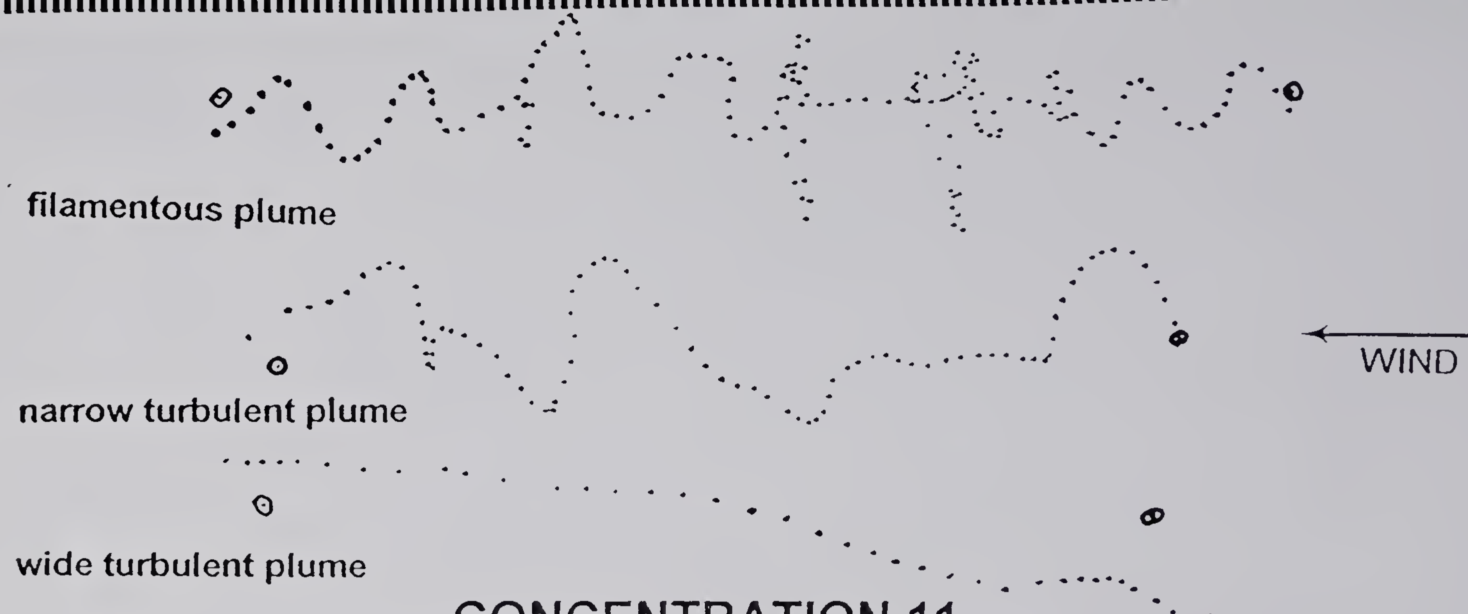
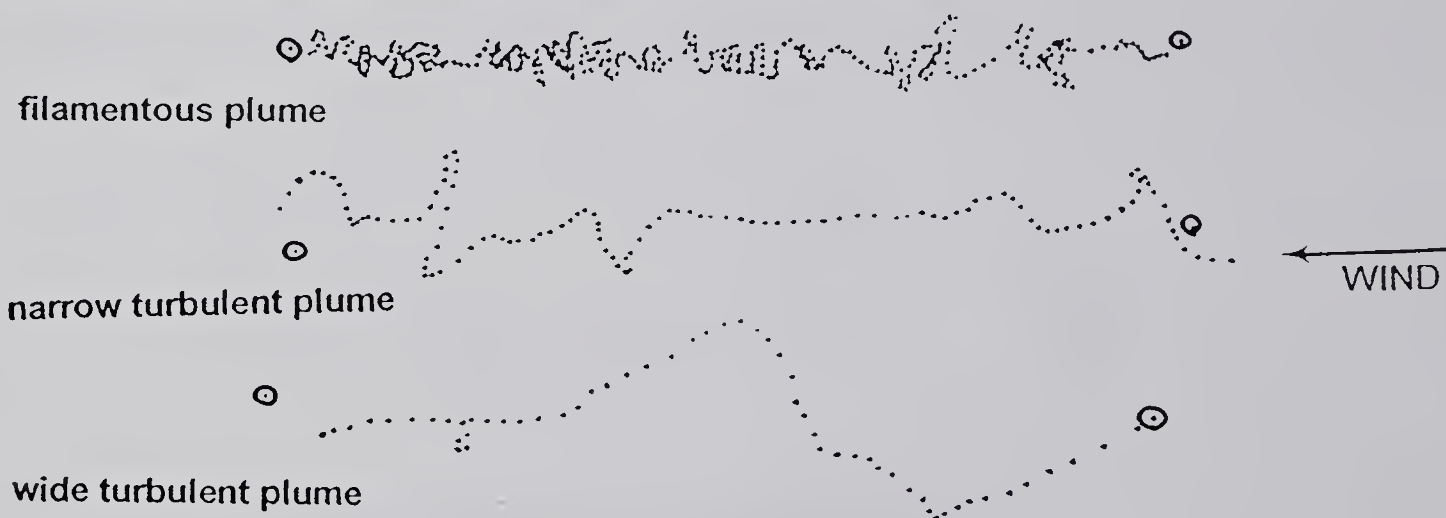
G.



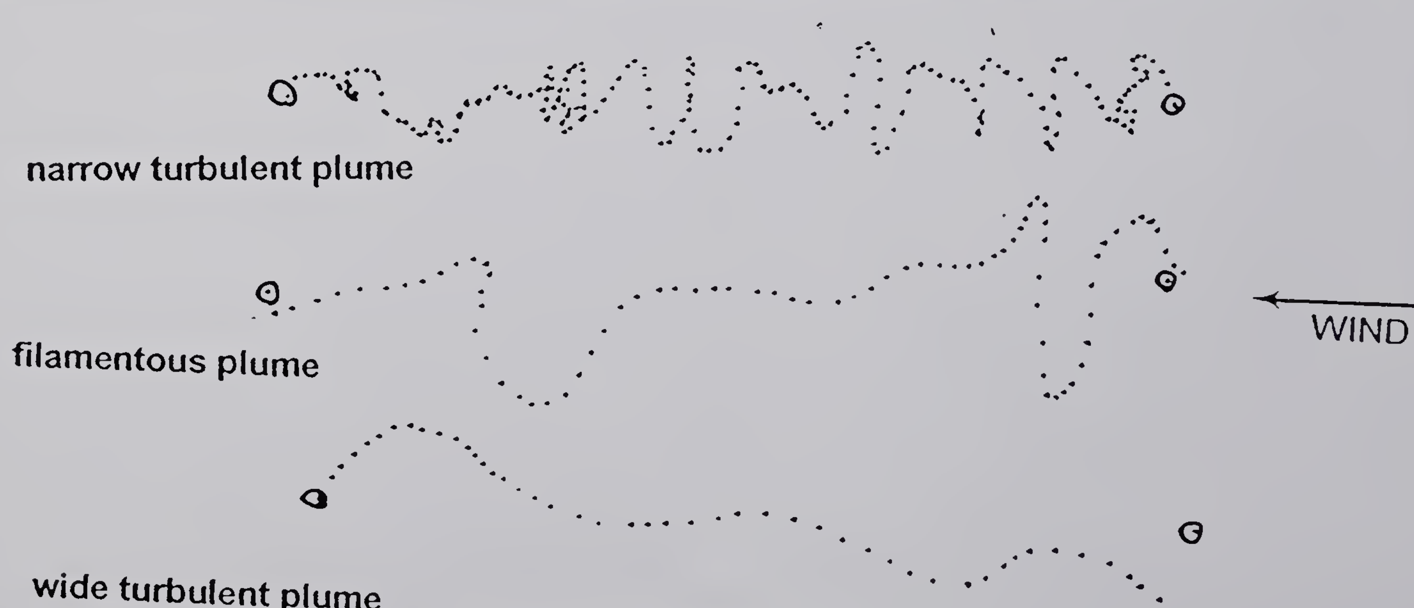
CONCENTRATION 11

H.



CONCENTRATION 12

I.



CONCENTRATION 13

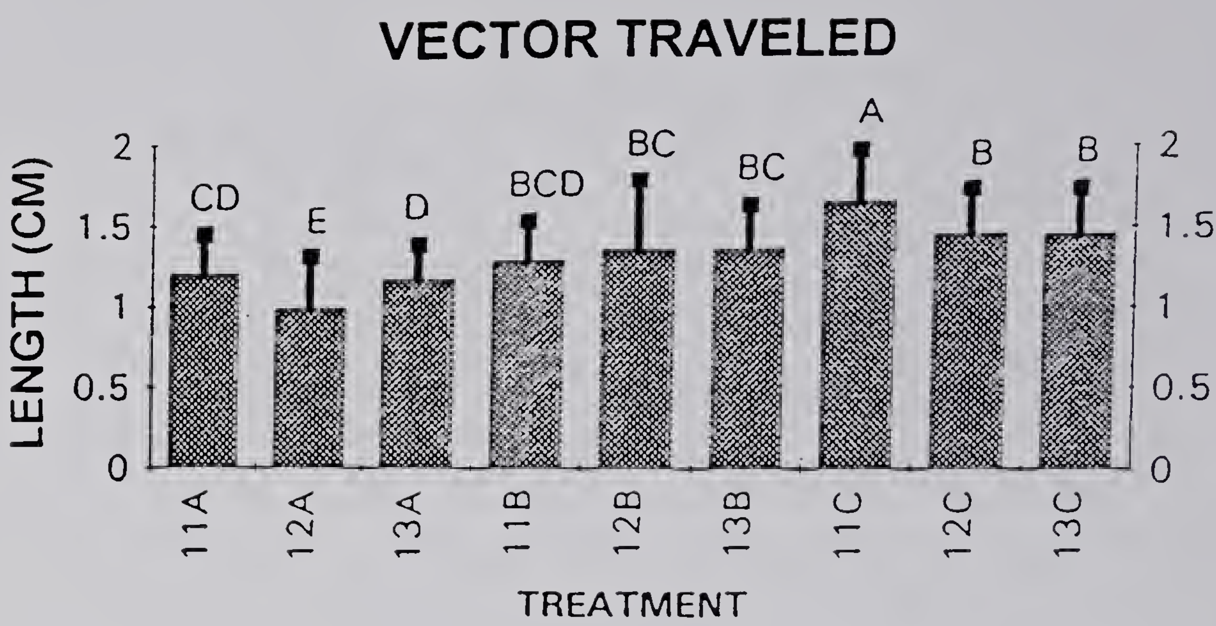
distances of the tracks of males flying to the wide turbulent plume were, (Fig. 19a). Inter-reversal distances of the tracks of males flying to the narrow turbulent plume were on average larger than the plume boundaries. However, individual turns were observed both inside and outside of the boundaries of this plume. (Fig. 19c). The inter-reversal distances of males flying to wide turbulent plumes was on average, within the plume boundaries (Fig. 19e).

The crosswind zigzagging pattern typical of male moths responding to pheromone (Kennedy 1986; Willis & Cardé 1990), is observed in all tracks of males flying to the filamentous plume (e.g., Fig. 19). Although some males turned irregularly when flying in the narrow turbulent plume, the zigzag pattern is observed in most of these tracks (Fig. 19). Fewer flight tracks of males flying in the wide turbulent plume show the crosswind zigzagging pattern of flight. In some of the flight tracks where the wide turbulent plume is used, the suppression of the self-steering zigzag is so effective that it is difficult to discern turns and correlated counterturns in the track (Figs. 19e and 19f).

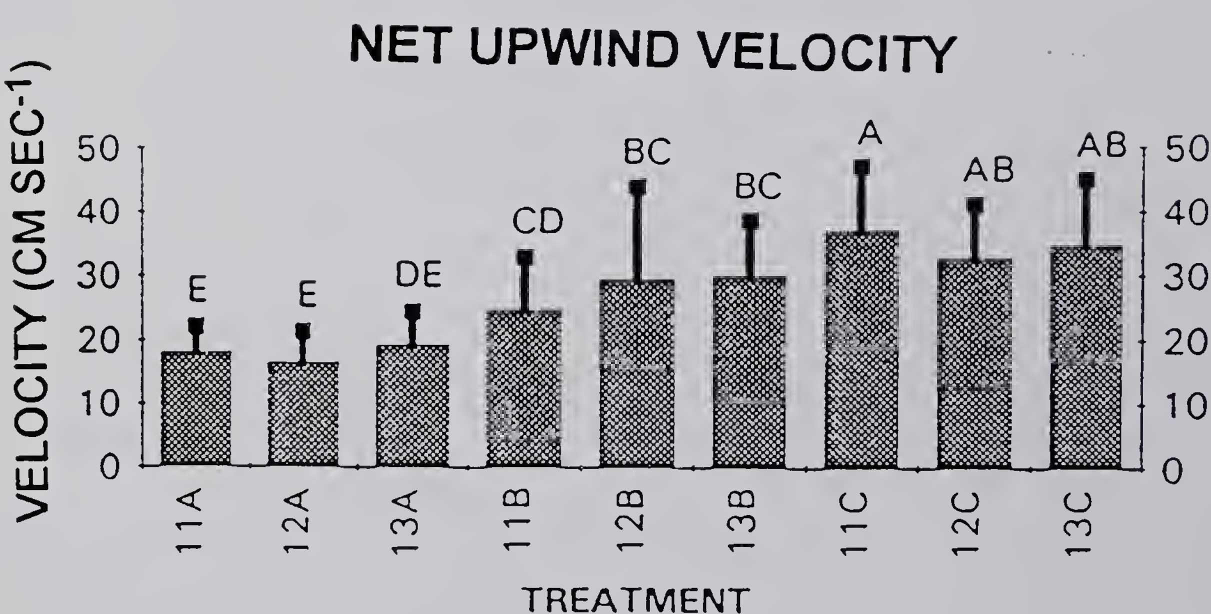
Moths flying to the wide turbulent plume at the lowest concentration tested (concentration 11, 0.045 ng) traveled longer vectors per unit of time measured (1/30th sec) than moths flying to all other treatments (Fig. 20a). The shortest vectors traveled per unit time were observed for moths flying in the filamentous plume at concentration 13 (4.5 ng) (Fig. 20a). Measurement of net upwind velocity followed a similar trend: males flying

Fig. 20. Parameters of velocity of flight tracks of *C. cautella* males flying to nine different treatments. Three plume sizes at three concentrations of pheromone where 11 is concentration 0.45 ng, 12 is concentration 4.5 ng, 13 is concentration 45 ng, A is plume filamentous, B is narrow turbulent plume, and C is plume wide turbulent. **A**. histogram of mean values for vector traveled for the nine treatments. The wide bars represent mean values of vector traveled for the 20 tracks, the narrow bars represent one standard deviation above the mean. Bars without letters in common are statistically different at $\alpha=0.05$ level. **B.** histogram of mean values for net upwind velocity for the nine treatments. **C.** histogram of mean values for net crosswind velocity for the nine treatments. **D.** histogram of mean values for airspeed for the nine treatments. **E.** histogram of mean values for ground velocity for the nine treatments.

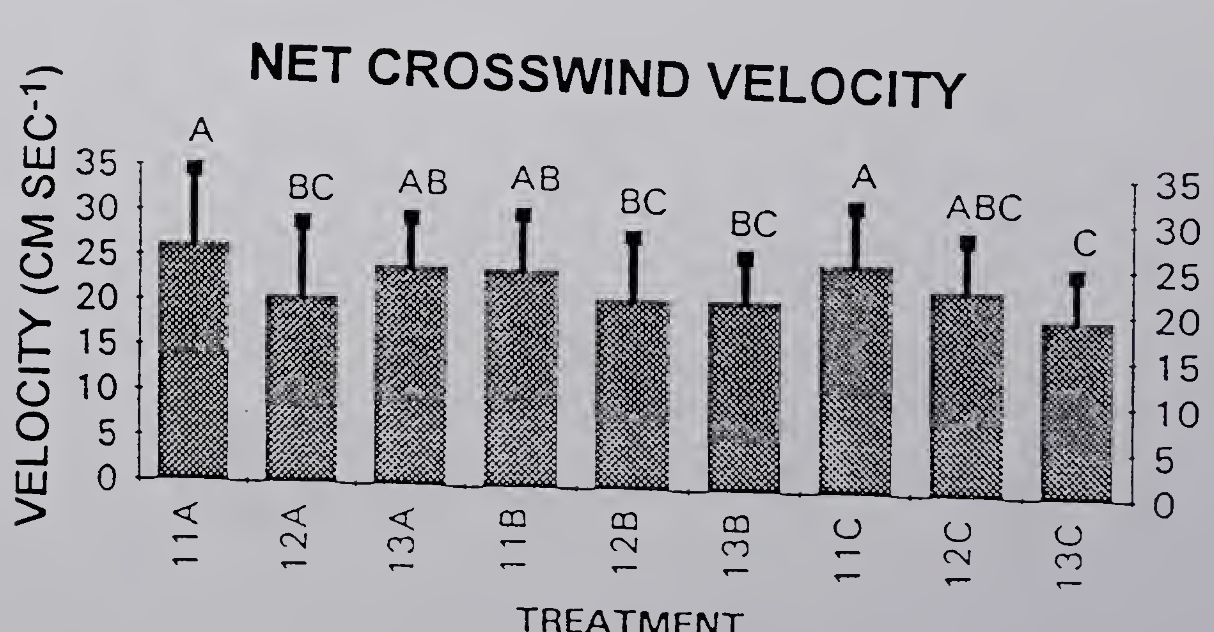
A.



B.



C.



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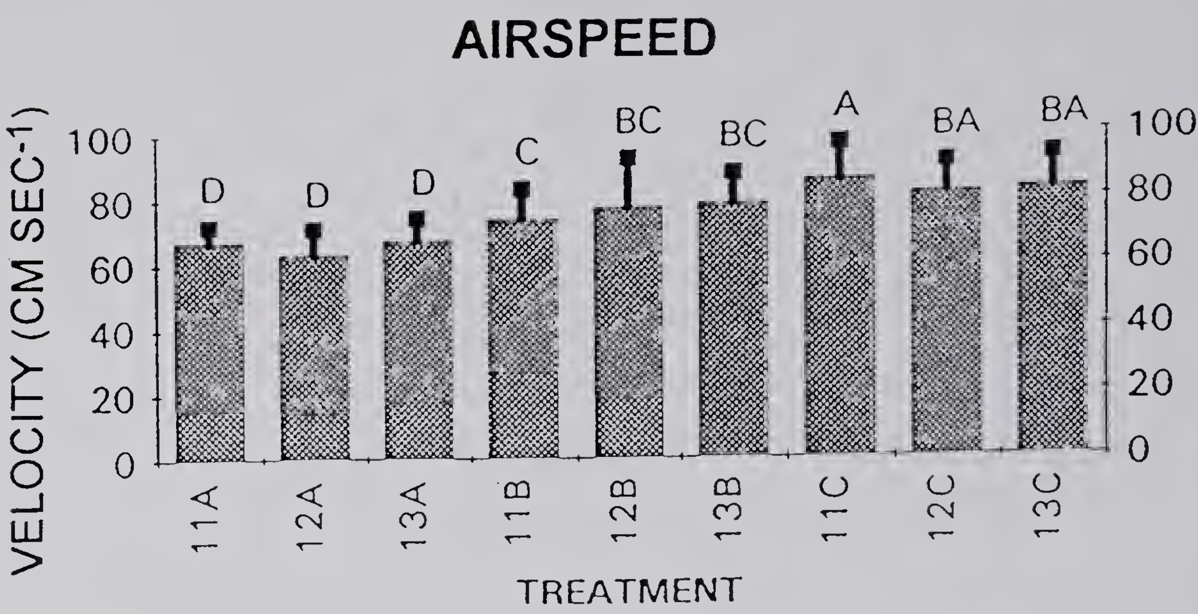
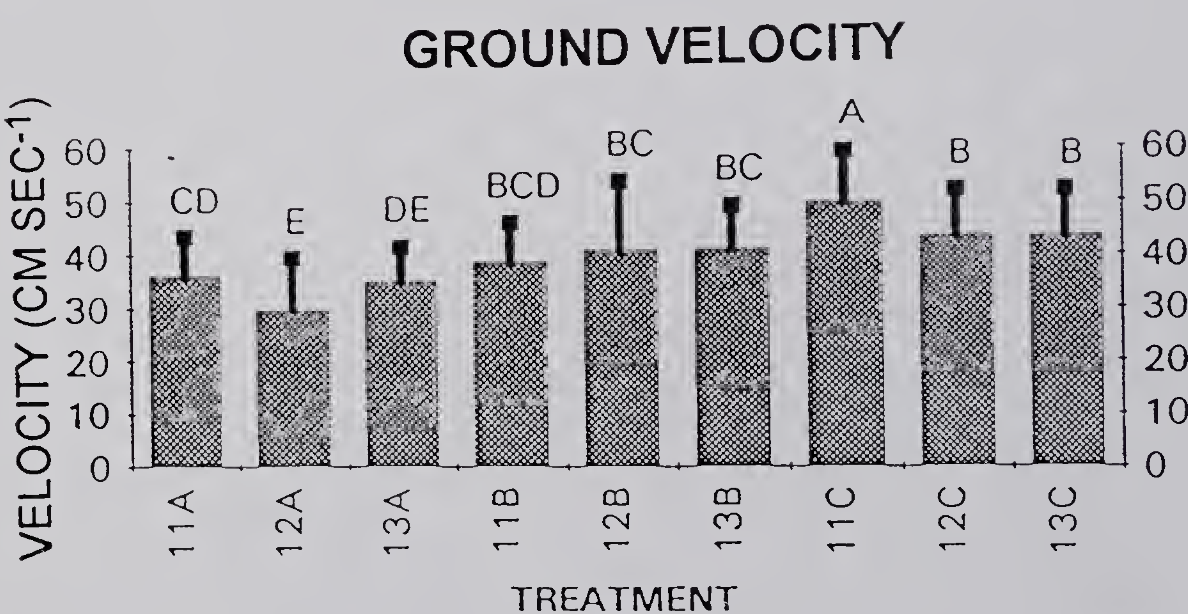
D.


AIRSPEED
VELOCITY (CM SEC-1)
100
80
60
40
20
0
D
D
D
C
BC
BC
A
BA
BA
11A
12A
13A
11B
12B
13B
11C
12C
13C
TREATMENT

E.


GROUND VELOCITY
VELOCITY (CM SEC-1)
60
50
40
30
20
10
0
CD
E
DE
BCD
BC
BC
A
B
B
11A
12A
13A
11B
12B
13B
11C
12C
13C
TREATMENT

in the wide turbulent plume flew significantly faster upwind than males flying in the filamentous plume (P<0.05).

In general, concentration of pheromone has more effect on crosswind velocity than on net upwind velocity: males flying to the lowest concentration tested (0.045 ng) have faster crosswind velocities than males flying to other treatments, although these differences are not always statistically significant (Fig. 20c).

Although changes in odor source concentration had discernible effects on flight parameters like "length of vector traveled," net upwind velocity, and crosswind velocity, no statistical trends were observed (Figs. 20a and 20c).

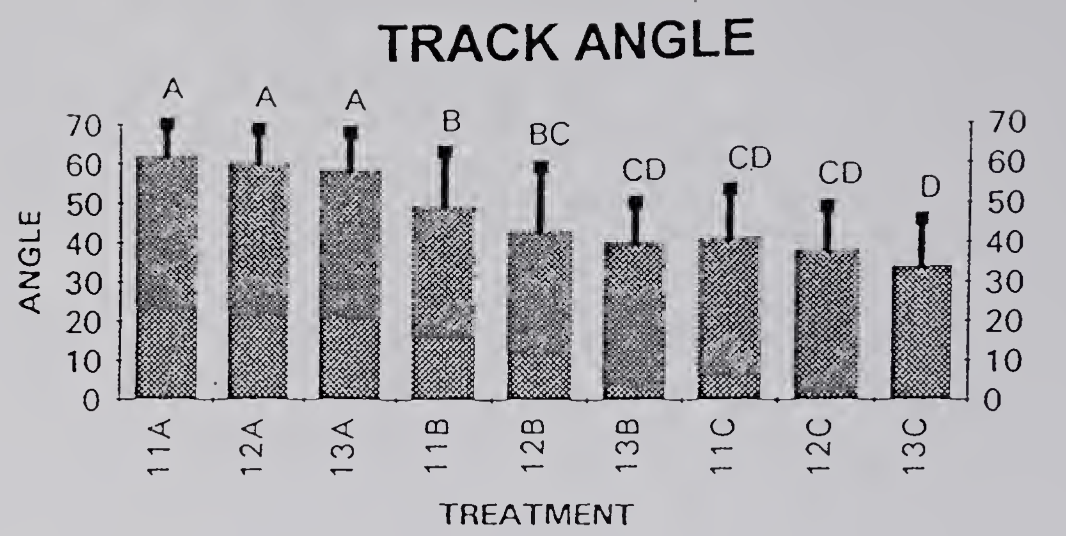
Longer vectors traveled and the faster net upwind velocity resulted in significantly faster airspeeds for males flying toward the turbulent plumes than for males flying to the homogeneous filamentous plume (P<0.012) (Fig. 20b).

Ground velocity generally tends to increase with plume size as does airspeed (Figs. 20d and 20e). The mean ground velocity of males flying to wide turbulent plumes is significantly faster than the mean ground velocity of males flying to the filamentous plume, independent of concentration (P<0.003). The mean ground velocity was lowest for moths flying to filamentous plumes at concentration 12 and highest for moths flying to a wide turbulent plume at concentration 11 (P<0.0001). The difference was a factor of 1.8. The greatest increase in ground velocity at a fixed concentration was observed using concentration 12. The ground velocity of male flight in the filamentous plume was 29 cm sec^{-1}, and the ground velocity using the wide turbulent plume was 45.5 cm sec^{-1}. These velocities differ by a factor of 1.6. The effects of concentration on flight velocity interact with the effects of plume size on flight velocity; this makes the inferences about the effects of concentration valid only when referring to one type of plume structure(Fig. 20).

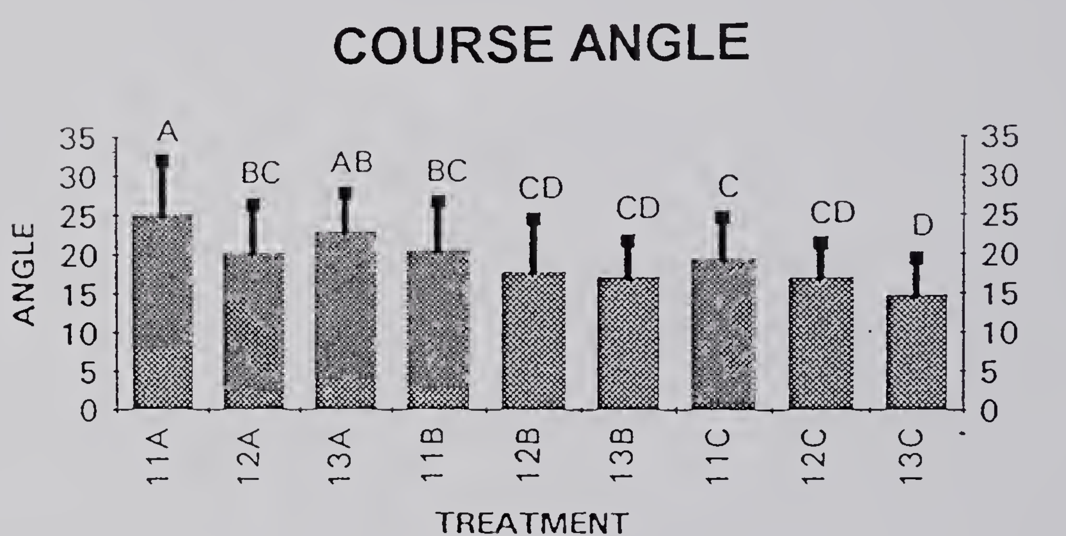
The mean of the values of track angles decreases with plume size (Fig. 21). For the filamentous plume, the mean of the means of individual moths of the values of the track angles at three concentrations are significantly different from the mean of the means of the values of the track angles at the same three concentrations for the turbulent plumes ($P<0.017$, when filamentous plume compared with narrow turbulent plume). For the filamentous plume, the mean is centered around 60° for the three pheromone concentrations. Increasing plume size to the narrow turbulent plume results in a overall reduction of the mean to 44.5° for the values of the track angles for all three concentrations tested. With this plume, differences among the means of the values of the track angles for the three concentrations increase: concentration 13 has the lowest mean (43°) and concentration 11 has the highest mean value (57°) ($P<0.0147$). Using the wide turbulent plume, the relative relationships between the concentrations are maintained, but the differences in the mean track angles steered as a result of changing concentration fade. Concentration 11 still has the highest values associated with the track angles, and concentration 13 has the lowest mean value for the same parameter

Fig. 21. Mean values for the angular parameters of flight of *C. cautella* males flying to nine different treatments. **A.** histogram for mean values of track angles. **B.** histogram for mean values of course angles. **C.** histogram for mean values of drift angles. **D.** histogram for mean values of interleg angles. Details as per Fig. 20.

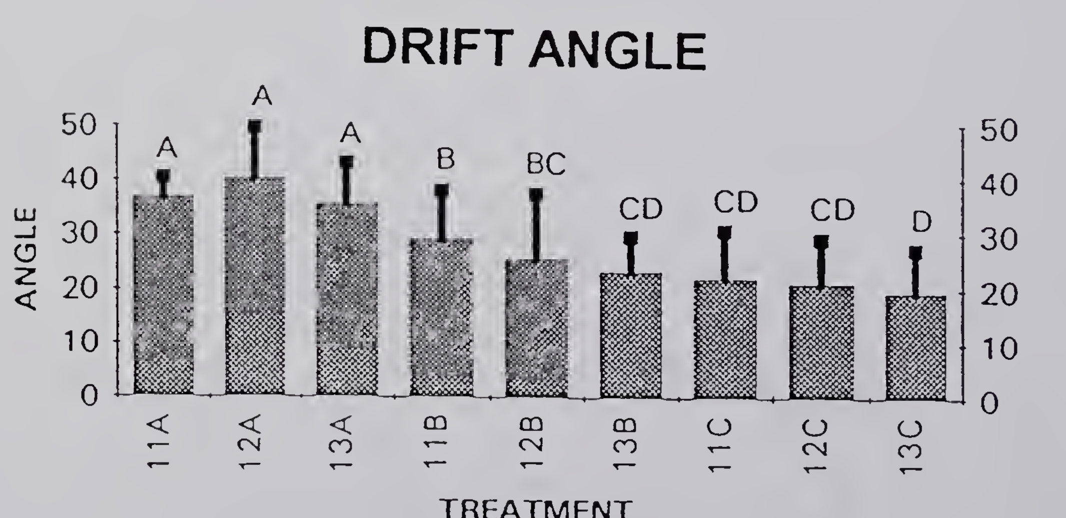
A.


TRACK ANGLE
ANGLE
A A A B BC CD CD CD D
0 10 20 30 40 50 60 70
11A 12A 13A 11B 12B 13B 11C 12C 13C
TREATMENT

B.


COURSE ANGLE
ANGLE
A BC AB BC CD CD C CD D
0 5 10 15 20 25 30 35
11A 12A 13A 11B 12B 13B 11C 12C 13C
TREATMENT

C.


DRIFT ANGLE
ANGLE
A A A B BC CD CD CD D
0 10 20 30 40 50
11A 12A 13A 11B 12B 13B 11C 12C 13C
TREATMENT

D.

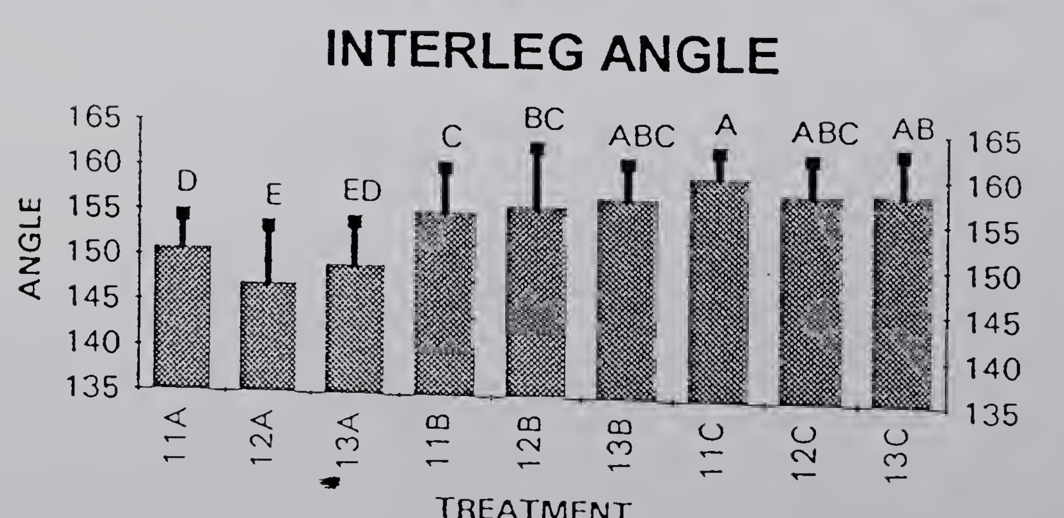
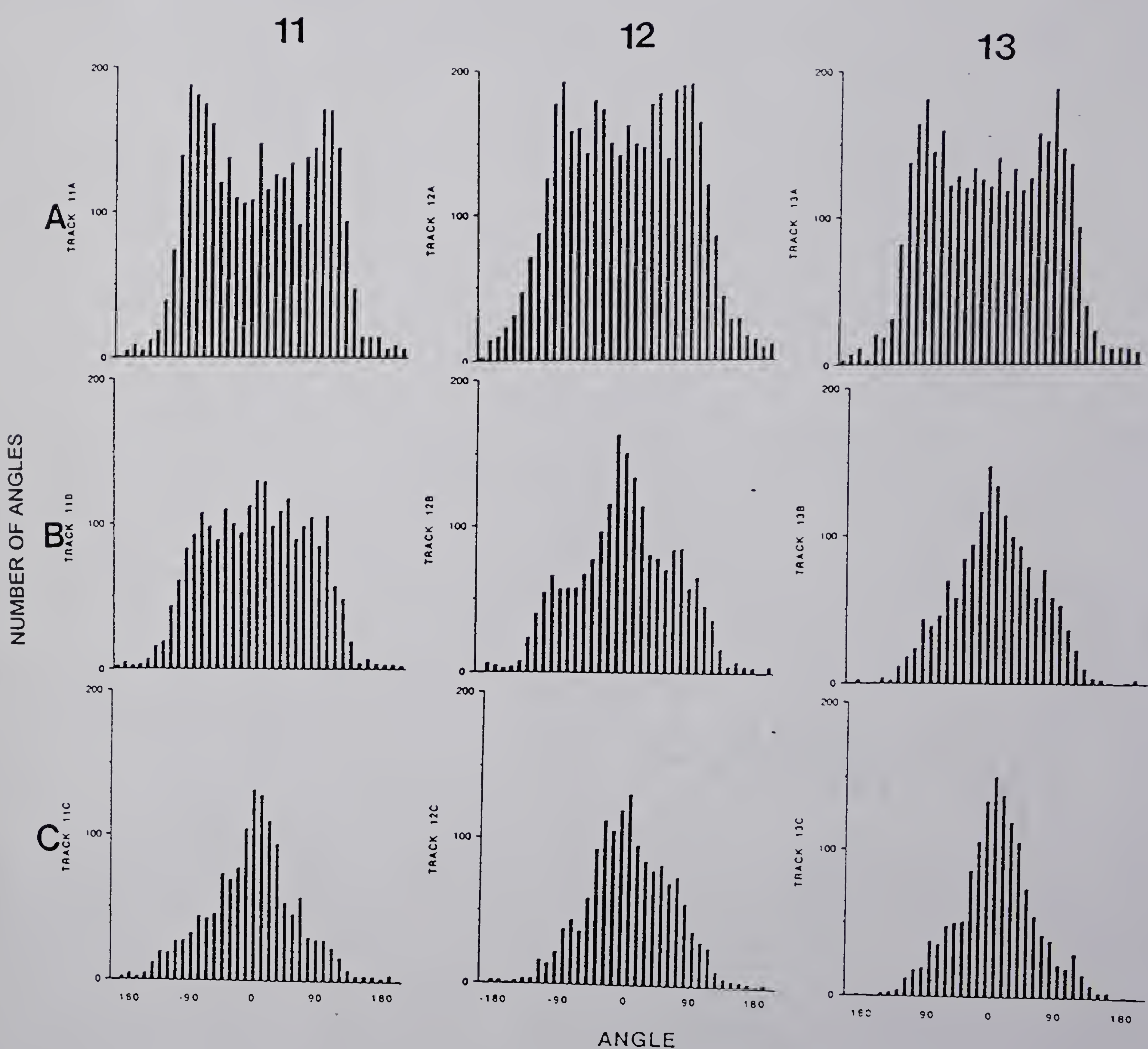

INTERLEG ANGLE
ANGLE
D E ED C BC ABC A ABC AB
135 140 145 150 155 160 165
11A 12A 13A 11B 12B 13B 11C 12C 13C
TREATMENT

Fig. 22. Frequency distribution histograms of flight track angles steered by *C. cautella* males flying toward plumes of different shapes (A is filament plume, B is narrow turbulent plume, and C is wide turbulent plume) and concentrations (concentration 11, concentration 12, and concentration 13). Frequency distribution histogram track angles. **B.** Frequency distribution histogram for course angles. **C.** Frequency distribution histogram for drift angles. The angles were sampled every 1/30th of a second.

A.



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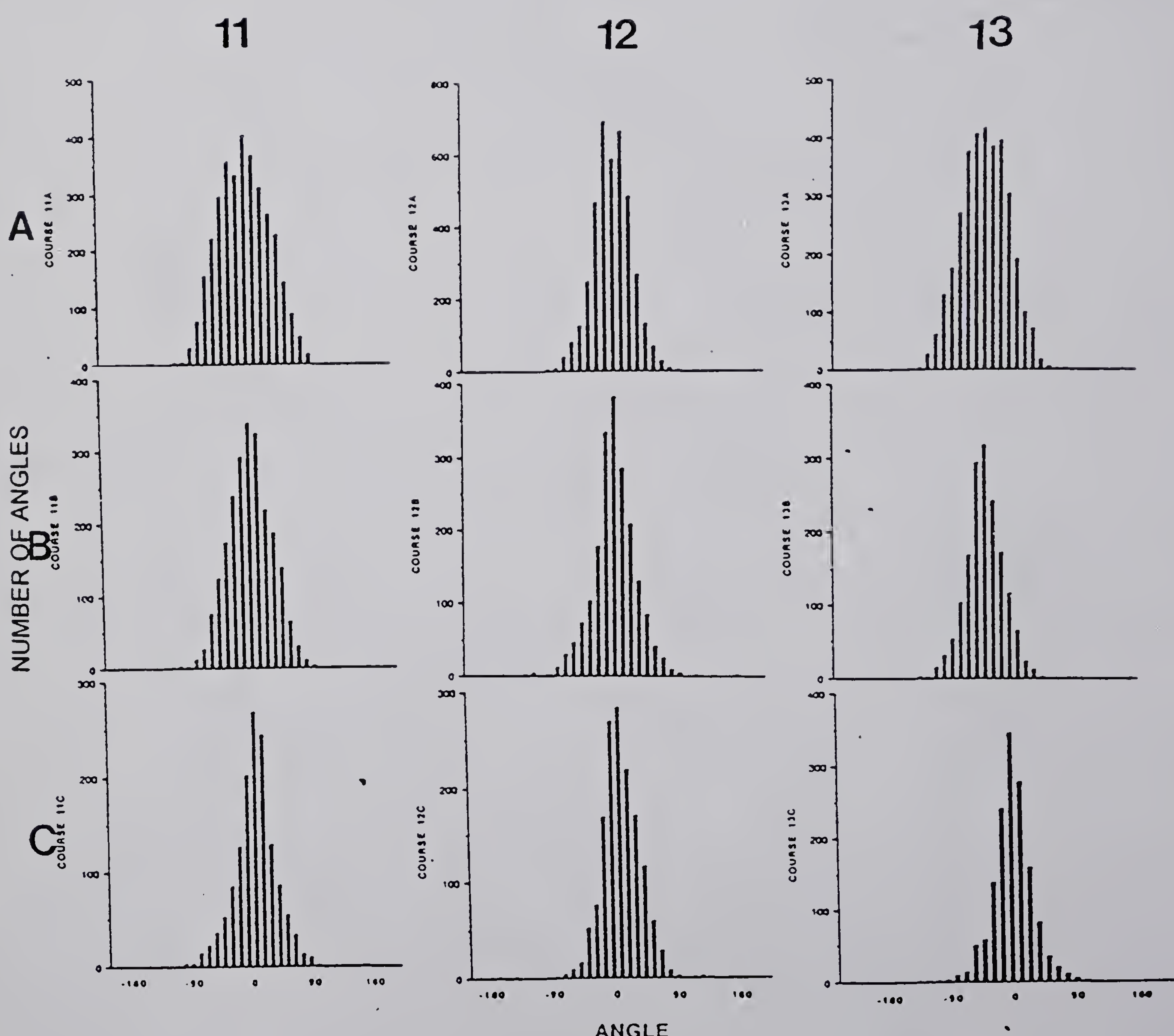
B.



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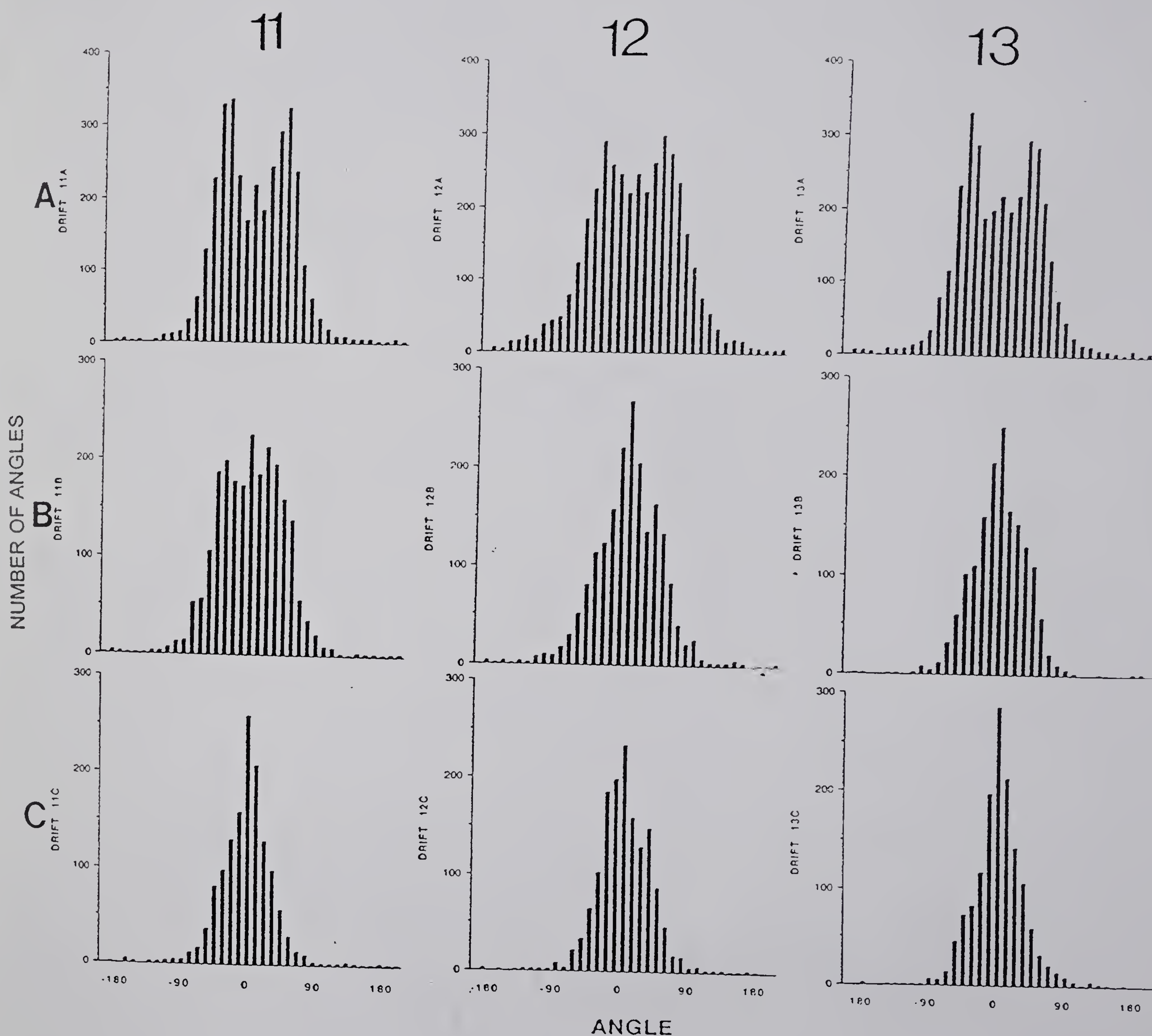
C.


11
12
13
A
B
C
NUMBER OF ANGLES
DRIFT 11A
DRIFT 12A
DRIFT 13A
DRIFT 12B
DRIFT 13B
DRIFT 11C
DRIFT 12C
DRIFT 13C
-180
-90
0
90
180
ANGLE

(P=0.571). The overall mean of the means of track angles steered by males using this turbulent plume for all three concentrations tested was 38.0º.

The bimodal distribution of the frequency histogram for the track angles of males flying to filamentous plumes (Fig. 22a) illustrates the crosswind counterturning characteristic of male moth upwind flight (Willis & Cardé 1990). The two modes of the frequency distribution of the track angles of males flying to the filamentous plume are clustered around ± 90° (the crosswind direction), independent of concentration; the mean of the track angles was clustered around 0° (due upwind). With increasing plume size there is a tendency of the mode and the mean to approach the same value. The unimodal distribution of the track angles of males flying to **narrow turbulent** and wide turbulent plumes represents a more direct upwind flight with angles distributed around 0° (Fig. 22a). The directness of flight to turbulent plumes can usually be correlated with the dispersion of track angles around 0°. Frequency distribution histograms showing the lowest variance in distribution (e.g., the wide turbulent plume at all concentrations), correspond to treatments where most of the flight tracks were directly upwind.

The mean of the values for the course angles is slightly larger for the filamentous plume and tends to decrease as size and concentration of the plume increase (Fig. 21b). The largest difference between the means of the values of course angles is observed between treatments 11A and

13C; the mean for treatment 11A was 25.15 $\pm$6.8° ($x \pm CI$) and the mean for treatment 13C was 14.81 $\pm$4.7 ($P<0.0001$).

Frequency distribution histograms of the course angles for all treatments is unimodally distributed around 0° (due upwind) (Fig. 22b). As plume size increases, the standard deviation associated with the mean course angle decreases, reflecting the fact that moths steer their course angles more precisely due upwind.

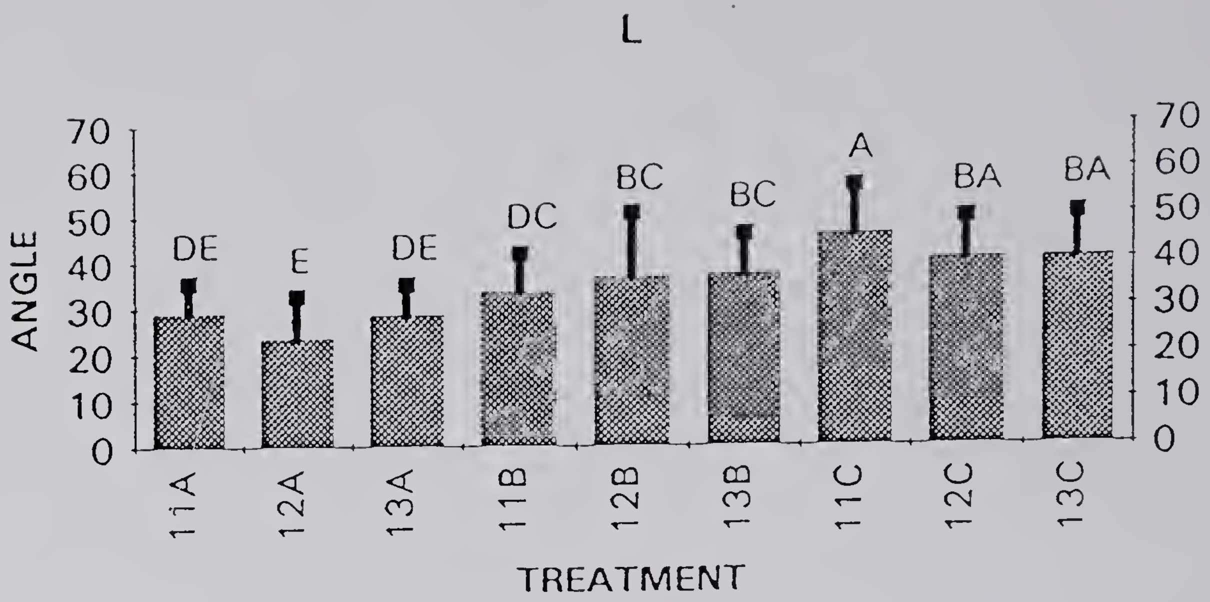
The values of the drift angles of males flying toward filamentous plumes is significantly different from the drift angle observed in males flying to the turbulent plumes ($P<0.015$)(Fig. 21c).

The mean interleg angles of flight tracks toward the filamentous plume are consistently significantly different from the tracks obtained from males flying toward the turbulent plumes ($P<0.0001$)(Fig. 21d). Males flying to the turbulent plumes fly straighter upwind, with interleg angles approaching 160°.

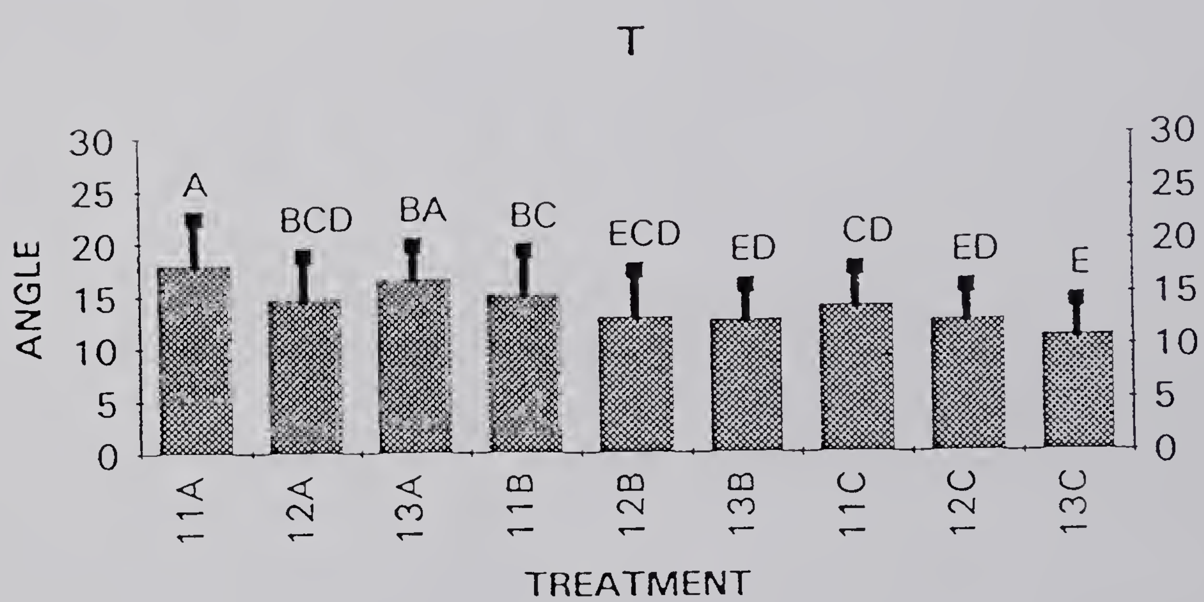
While the velocity of the longitudinal component (L) of visual flow increases significantly with plume size (Fig. 23a), the velocity of the transverse component (T) of visual flow decreases significantly (Fig. 23b). Several of the statistically significant differences observed at the level of individual components (T and L) fade when the relationship T+L is considered (David 1986). This suggests that the *C. cautella* males have a feedback mechanism for the control of the overall values of T+L; however, this compensatory mechanism is not perfect (Fig. 23c).

Fig. 23. Component of the image flow of *C. cautella* males flying toward plumes of different shapes (A is filament plume, B is narrow turbulent plume, and C is wide turbulent plume) and concentrations (concentration 11, concentration 12, and concentration 13). **A.** histogram for values of the longitudinal (L) component of image flow. **B.** histogram for values of the transverse (T)component of the image flow. **C.** histogram for values of the interaction T+L. Details as per Fig. 20.

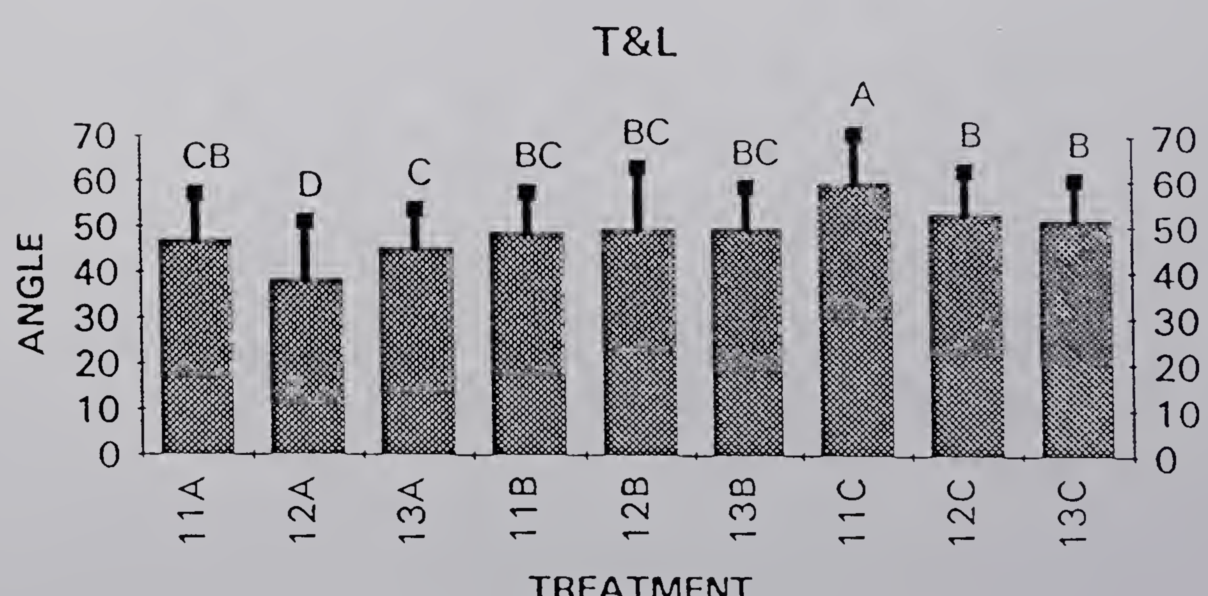
A.


L
ANGLE
TREATMENT
11A
12A
13A
11B
12B
13B
11C
12C
13C
DE
E
DE
DC
BC
BC
A
BA
BA
0
10
20
30
40
50
60
70

B.


T
ANGLE
TREATMENT
11A
12A
13A
11B
12B
13B
11C
12C
13C
A
BCD
BA
BC
ECD
ED
CD
ED
E
0
5
10
15
20
25
30

C.


T&L
ANGLE
TREATMENT
11A
12A
13A
11B
12B
13B
11C
12C
13C
CB
D
C
BC
BC
BC
A
B
B
0
10
20
30
40
50
60
70

The counterturn pattern exhibited by moths flying to the filamentous plume is very constant within a treatment (Fig. 19a and 19b). This regularity implies the existence of an internal counterturning generator. It is also evident that *C. cautella* males do change the rhythm of counterturning depending on treatment (Table 1, Fig 19). The only other moth demonstrated to change the rhythm of counterturning is *G. molesta* (Kuenen & Baker 1982). The counterturning rhythm of male *G. molesta* is modulated by pheromone concentration: the higher the concentration the faster the males counterturn. Concentration changes significantly the counterturning rhythm of *C. cautella* only when the pheromone plume is wide turbulent (Table 1). The structure of the pheromone plume has a much stronger effect on counterturning rhythm of *C. cautella* males than the concentration of the stimulus (Table 1): increase in plume size reduced the frequency of counterturns. The reduction in frequency of counterturn is so strong, that in some flight tracks there is no evidence of self-steering counterturning (e.g., Fig.19).

3.4. Discussion

Several studies have confirmed the idea that intermittence of the pheromone signal is important for male insect flight orientation (Kennedy *et al.* 1981; Willis & Baker 1984; Baker *et al.* 1985). Based on studies of several moth species, upwind progress toward a pheromone source is maintained only when flying to intermittent plumes (Baker & Haynes 1989; Baker *et al.* 1989). Moths do not fly consistently upwind in homogeneous clouds of pheromone, but they sustain upwind flight when a plume from a point source is superimposed on the homogeneous cloud (Kennedy *et al.* 1981; Willis & Baker 1984; Baker *et al.* 1985), or when the cloud is pulsed (Baker *et al.* 1985). Both of these modifications introduce intermittency into the plume structure.

Males of two moths species were demonstrated to sustain upwind flight to experimentally manipulated pulsed plumes (Cardé et al. 1987, Vickers and Baker 1992). *L. dispar* males fly similarly to continuous plumes and to slowly pulsed plumes (pulse intervals of $\geq$0.5 sec) (Cardé et al. 1987). Although an overall low proportion of *Heliothis virescens* males flew upwind to pulsed plumes, the highest proportion of males flew upwind to a plume with the second highest frequency tested: 44% of the males flew upwind toward a plume of 4 pulses per second (Vickers and Baker 1992).

Most male gypsy moth counterturns are executed within the time-averaged plume's boundaries (e.g., Willis & Cardé 1990; Charlton *et al.* 1993), although at low plume concentrations some counterturns occur following an excursion beyond the plume's edge (Charlton *et al.* 1993). The fact that *C. cautella* males fly to homogeneous filamentous plumes where pheromone is mixed with a visual tracer from a source allowed us to confirm that males were flying in and out of the plume. Although not every turn and counterturn necessarily translated into plume contact, males intercepted the pheromone plume at semi-regular intervals (Fig. 17). This suggests that males create an intermittent stimulus by zigzagging in and out of the pheromone plume. A similar effect has been reported for other moths (Kennedy *et al.* 1981; Willis & Baker 1984), in which males were maintained upwind progress while flying in and out of the edge of pheromone cloud located on one side of a wind tunnel (Kennedy *et al.* 1981; Willis & Baker 1984).

A male flying straight upwind in a heterogeneous plume receives intermittent signals from the antennae, caused by sequential encounters with bursts of pheromone and packets of clean air. The intermittency of signal generated by this plume structure may resemble the intermittent signal generated by the counterturning effect. If the difference in stimulation between high concentration and low concentration phasing (usually generated by the self steering counterturn) is maintained through another process (such as intermittence of the signal due to plume intermittence), the receptor/CNS motor feedback generating the self-steering counterturns may be turned off, allowing the male to fly straight and directly upwind. This may be the mechanism at work for the males flying straight upwind in turbulent pheromone plumes. If intermittency of the signal is the parameter modulating directness of upwind flight, then one should be able to elicit zigzagging or straight flights by modulating the frequency of pulses of pheromone.

Experiments with other moth species have shown that males respond to increasingly high pheromone concentrations by flying slower and steering progressively smaller course angles than they do at low concentrations, i.e., they fly more due upwind, and drift angles increase at high concentrations (Kuenen & Baker 1982; Charlton *et al.* 1993).

Airspeed and ground velocity of *C. cautella* males flying to higher pheromone concentrations are dependent on plume size (Figs. 20d and 20e), a trend toward steering smaller course angles is evident when plume concentration increases while plume structure is maintained (Figs. 21b and 22b).

One of the confounding effects of increasing the size of the pheromone plume is the dispersion of pheromone molecules over a wider area; this dispersion reduces pheromone concentration within the plume. *C. cautella* responded to increasing plume sizes by flying faster, as would be expected, given the reduction of pheromone concentration associated with the increase in plume size. However, this concentration effect does not explain why males consistently steer course angles more directly upwind, and exhibit smaller drift angles when flying to large plume sizes compared to small plume sizes (Fig. 21b and 22b). We conclude that pheromone plume structure affects the way males steer their course angles upwind and drift downwind.

The structure of turbulent odor plumes changes as the distance from the source increases. Changes in the internal structure of pheromone plumes can be detected both physically (using visual markers), behaviorally (using wing-fanning bioassays, Charlton *et al.* 1993), and physiologically (using EAGs, Baker & Haynes 1989) even at short distances from the pheromone source, in a low turbulence, laminar-flow wind tunnel (Charlton *et al.* 1993).

In light winds, a plume is generated as a dense filament, that is broken apart by small scale air turbulence, first in small packets, which will be subsequently expanded and subdivided as the plume moves away from the source (Murlis & Jones 1981, Murlis 1986, Murlis *et al.* 1990, Murlis *et al.* 1992). In natural environments, a filamentous plume may meander around the large eddies of turbulence for several meters (depending on wind turbulence and wind velocity) before the filament is expanded laterally and broken by small scale air turbulence. The laminarity of the airflow in wind tunnels suppresses the meandering movement of plumes, generating plumes with an artificially straight linear central axis. Although plumes as straight as the ones generated in wind tunnels are extremely rare in natural environments, the internal structure these wind tunnel generated plumes is comparable to the internal structure of plumes occurring in natural environments The plumes that we used in this experiment represent three phases of the described process of turbulent growth of pheromone plumes (Murlis & Jones 1981, Murlis 1986, Murlis *et al.* 1990, Murlis *et al.* 1992). The filamentous plume, the smallest size tested, represents the structure of the plume close to the odor source, where diffusion can still influence the plume structure (Aylor *et al.* 1976). The narrow turbulent plume, an intermediate size, represents the plume encountered at an intermediate distance from the source, where turbulence starts to disrupt the plume. Finally, the wide turbulent plume, the largest plume tested, represents the turbulent plume that males encounter farther away from the source. The internal structure of the three plumes tested, as defined by temporal and spatial parameters, changed systematically with plume size. These changes parallel the systematic changes that plumes undergo in field conditions when these parameters are measured at increasing distances from a source (Murlis 1986; Murlis *et al.* 1990). The data presented here indicate that fine-scale modifications of plume structure modify flight output in *C. cautella*. Males of another moth species can resolve pheromone stimulus presented at a frequency up to 10 Hz (Christensen & Hildebrand; 1989). We might expect, therefore, that males can perceive changes in aspects of the fine-scale structure of the pheromone plume. Because the internal structure of plumes changes with distance from the source, decoding of the temporal information contained in the fine-scale spatial distribution of the stimuli within the pheromone plume could give information about the distance to the pheromone source. It is conceivable that the information about the distance from the source could influence the "giving up" times in the case of the male's loss of the pheromone plume (Willis *et al.* 1991) following a shift in wind direction (Elkinton *et al.* 1987). The fine structure of the pheromone plume could also give information about the direction of the wind (Wright 1958).

The flight characteristics of *C. cautella* males in the three plume shapes in a wind tunnel, appear similar to those described by Willis *et al.* (1991) for tracks of gypsy moth males flying at varying distances from the source in a forest. The general trend of parameters such as mean of track and course angles, the track angle, course angle, and drift angle distribution histograms, the interturn duration (or frequency), and interreversal distance of the tracks of male gypsy moths flying 2.5, 10, and 20 m downwind from the source is similar to the trends we found for the same parameters for C. cautella males flying to the **filamentous**, **narrow turbulent**, and **wide turbulent plumes**, respectively. Although Willis et al. did not control for the effects of concentration on male gypsy moth flight, the similarities in the flight patterns observed in both studies suggest that, indeed, both *L. dispar* and *C. cautella* males are able to perceive differences in the fine-scale structure of pheromone plumes, and that these differences are a factor influencing upwind flight (although differences of concentration of the stimulus cannot be isolated on the case of *L. dispar*).

Further experimentation on male upwind flight using visual markers and pheromone plumes are needed to describe the exact moment that a flying male makes antennal contact with the odor plume, and correlate this contact with a behavioral response. Our experiment with males flying to mixed plumes of smoke and pheromone suggest that this response occurs immediately upon contact with pheromone (e.g., Fig. 17). When the plume was condensed into one filament, male instantaneous response to pheromone contact was a crosswind turn and a reduction of flight velocity. When the male encountered a large burst of pheromone, the response was a surge upwind of ca. 0.30 sec, i.e. the male increases its flight velocity and turns more toward upwind. These observations suggest that the process of upwind flight of males to pheromone sources can be explained by the instantaneous changes in the airborne male behavior as result of single interactions of that male with the pheromone pulse/plume.

CHAPTER IV

EFFECT OF THE INTERNAL STRUCTURE OF PHEROMONE PLUMES: PULSE FREQUENCY MODULATES ACTIVATION AND UPWIND FLIGHT OF *Cadra cautella* MALES

4.1. Introduction

The mechanisms modulating male pheromone-mediated flight are still a matter of debate (Cardé 1986; Preiss & Kramer 1986a; Kennedy 1986; Baker 1989). The model which has been most accepted to explain the mechanisms involved in the location of a pheromone source evokes two mechanisms: a positive **optomotor anemotaxis** (Kennedy & Marsh 1974; Kuenen & Baker 1982a) and a central nervous system (**CNS**) **counterturn generator**. Both mechanisms are triggered by in-flight contact with a pheromone plume. Optomotor anemotaxis is regulated by the feedback of a changing visual environment caused by wind-induced drift that provides cues for the flying insect to polarize its flight maneuvers and to displace upwind. This mechanism is responsible for maintaining a constant angular velocity of image motion across a male insect's retinal surface (Cardé & Hagaman 1979, Kuenen & Baker 1982a, but see below). This constant velocity is achieved by keeping flight altitude (Preiss & Kramer 1983), ground velocity, angles for turning into the wind, and course steering at constant preferred values (reviewed by Kennedy 1983). The CNS counterturn generator causes the male to turn back and forth across the wind, in a regular fashion which is temporally consistent for most moths studied (Baker *et al.* 1984; Charlton *et al.* 1993; Kuenen & Baker 1982b).

It has been shown that in pheromone-mediated flight males of several moth species maintain ground velocity, course angles and turn-counterturn intervals at constant levels when the extrinsic environment is manipulated (Marsh *et al.* 1978; Kuenen & Baker 1982a; Willis & Cardé 1990, Charlton *et al.* 1993). Only *Grapholita molesta* and *C. cautella* have been shown to change the rhythm of counterturning. Changes in rhythmicity are mediated by changes in pheromone concentration for *G. molesta* (Kuenen & Baker 1982b) and by changes in plume shape for *Cadra cautella* (Chapter III).

An alternative model to explain the zigzag upwind flight tracks of male is Preiss & Kramer's (1986), which we will refer to here as the **flight imprecision model**. The primary hypothesis of this model is that males attempt to fly directly upwind. Preiss & Kramer argue that the typical zigzag tracks of male moths flying to pheromone is simply a reflection of the male's inability to fly straight upwind, and not the result of a CNS counterturning program. When moths steer course angles other than 0º (due upwind) they drift away from the wind line. This deviation from course is reflected in the male's transverse retinal image flow which triggers a proportional turn back toward 0º. The data presented in support of this model are the unimodal distribution of course angles in tracks of tethered gypsy moths tested in a flight simulator with moving visual patterns to simulate wind. The hypothesis was further corroborated by the unimodal distribution of course angles obtained from tracks generated by a computer simulation model of moth flight using the parameters of their hypothesis (Preiss & Kramer 1986a). The imprecision model is simpler than the optomotor anemotaxis/counterturning model described above. It uses only the optomotor anemotaxis mechanism to explain the flight tracks of moths. The concepts of internal counterturning, an internally-set, anemotactically-steered track angles, and internally set ground velocity are not invoked. The validity of the imprecision model was called into question primarily because the moths used were tethered. Tethering restricts movement in all three planes of rotation (David 1986, David & Kennedy 1987). Tethering also introduces a mechanoreceptive input that is not present for free-flying moths; this mechanoreceptive input might allow the moths to control their steering and velocity (David 1986, David & Kennedy 1987). The temporal regularity of the zigzag of free flying gypsy moths in wind is consistent with an internal counterturning mechanism (Willis & Cardé 1990).

Turbulent plumes are packets of pheromone separated by clean air (Wright 1958, Jones & Murlis 1980), and moths are physiologically capable of perceiving individual pheromone pulses even at high pulse frequencies (up to 10 Hz for *Manduca sexta*, Christensen & Hildebrand 1988). The internal structure of the odor plume could be an important factor in the perception of the chemical signal by the insect, and influence how males navigate upwind toward a pheromone source. Pheromone plume intermittency was demonstrated to be necessary to elicit sustained upwind flights for several moth species (Willis & Baker 1984; Kennedy *et* *al.* 1980, 1981). Recently Mafra-Neto & Cardé reported that change in the pheromone plume structure alters both the output of the optomotor anemotaxis and the counterturn generator of *C. cautella* males (Chapter III). Males flying to **turbulent plumes** suppress the counterturn program and thereby increase the longitudinal velocity of the image motion on the retinal surface. Males flying to a **homogeneous filament plume** maintain the tempo of the counterturning program and maintain their net upwind velocity at constant low levels over three log steps concentrations of pheromone. Mafra-Neto & Cardé suggested that the input from the internal structure of the plume was an important factor modulating male pheromone-mediated upwind flight. The consequences of a systematic manipulation of the components of the internal structure of pheromone plumes on male upwind flight tracks has not been explained.

There are several alternative or complementary models of male upwind flight that incorporate elements of the internal structure of the pheromone plume into the counterturning and optomotor anemotaxis model. Among them is Wright's model (1958), and Baker's model (1990).

Wright's model: After describing the instantaneous intermittent structure of turbulent smoke plumes, Wright (1958) suggested a mechanism of olfactory guidance for flying insects that were capable of detecting individual pulses of odor. In his model the tempo of a counterturning program was modulated by changes in chemosensory input. This input was measured by the insect as the frequency of the odor pulses it detected while flying in a turbulent odor plume. He predicted that when the insect experiences no signal (i.e., the pheromone signal is either homogeneous or absent) the male's flight pattern consists of "a series of rather long zigzag paths." This pattern has since been termed casting. Wright's prediction was first corroborated by the work of Kennedy *et al.* (1980, 1981) and Willis & Baker (1984) with experiments using clouds and corridors of pheromone. When entering into homogeneous pheromone clouds, males first flew upwind, but when pheromone contact continued they started casting, and eventually abandoned the pheromone oriented flight. If the cloud was pulsed, or if it was presented as a corridor with an edge of clean air, males were able to resume upwind progress (Kennedy *et al.* 1980, 1981; Willis & Baker 1984).

Wright also predicted that when an insect entered a pheromone plume, the tendency to turn would be inhibited as long as the interval between pulses tended to decrease. Turn inhibition would result in a "fixed flight path" straight toward the source. If the interval between pulses tended to increase, it "could release the inhibition on the tendency to turn," causing "the insect to abandon its fixed flight path and make a series of short, violent zigzags until it once more locates a path in which - the pulse interval tends to decrease." This mechanism could explain how insects flying to turbulent plumes may assess their heading direction toward the source using only information contained in the plume itself. Wright's model incorporates internally driven counterturning tempo, and does not rule out optomotor anemotaxis, but presents an additional mechanism to the use of optomotor anemotaxis for the male to determine wind direction.

Baker's model: Baker's (1990) model incorporates features of Wright's model with optomotor anemotaxis/counterturn mode (reviewed by Arbas et al. 1993). It proposes that the set point of the optomotor steering control system is determined moment to moment by contact with filaments of either pheromone or clean air. Contact with an odor filament causes immediate changes in steering angles toward 0°, loss of odor causes the set point for steering to change to 90°. This behavior results in a flight track with zigzags across the wind line. In this model, ground velocity is regulated by the instantaneous concentration of pheromone (Baker & Haynes 1987). When flying out of the pheromone plume into clean air, the male initiates casting flight with an increase in flight velocity and a decrease in tempo. When the male recontacts the pheromone plume he surges upwind, which results in progress toward the odor source before the next encounter with a large parcel of clean air (Baker 1990). At lower concentrations of pheromone, ground velocity will be higher but more perpendicular to the wind line; at higher concentration the ground velocity will be reduced, but the flight will be more directed upwind (Arbas et al. 1993).

Mafra-Neto & Cardé demonstrated that the structure of the pheromone plume influences the flight pattern of *C. cautella* males (**Chapter III**). An increase in plume size resulted in faster ground velocities, lower turning frequency, narrower turns, and reduced track angles. In short, increasing plume size results in faster and more direct upwind flight. Although changes in pheromone concentration had discernible effects on male upwind flight, the effects were smaller than the effects observed when plume shape was changed. Because Mafra-Neto & Cardé used diffusers to alter the size of the pheromone plumes, the manipulation of plume size was accompanied by changes in several other aspects of the internal structure of the plumes generated. It was not possible, therefore, to determine the importance of individual parameters of plume structure on the changes in male upwind flight. The difference in the internal structure among the plumes tested (Chapter III) was the presence of pulses of pheromone in the turbulent plumes and the absence of pulses in the homogeneous filament plume. The turbulent plumes had different mean pulse frequency, mean duration, and mean pulse size. Males flying to smaller and less turbulent plumes had tracks with counterturns dominating the flight pattern; males flying to larger and more turbulent plumes suppressed counterturning, which resulted in straighter upwind flight tracks. Male flight to each of the pheromone plumes differed dramatically; it might, therefore, be interesting to investigate the role of some of the plume structure parameters that were changed in that experiment. The most obvious changes observed in the pheromone plumes tested were the intermittency characteristics, more specifically changes in pulse dimension and pulsing frequency. These characteristics of plume structure will be studied here in more depth.

Wright's model is general, but it suggests an explanation of the overall patterns of interaction between plume structure and the flight tracks of *C. cautella* males observed in **Chapter III.** Baker's model is more explicit than Wright's model regarding the interactions between the instantaneous structure of the plume encountered and the behavior elicited. However, the instantaneous interactions predicted by Baker's model were not confirmed in our experiments with *C. cautella* males (**Chapter III**). When the pheromone plumes were marked with smoke we were able to monitor *C. cautella* males changing their instantaneous in-flight maneuvers in response to encounters with pheromone plumes (section 3.3.1). *C. cautella* males turned more crosswind when contacting undisturbed filamentous plumes, and turned more upwind when contacting the disturbed filamentous plume (i.e., when they contacted a large bursts in the plume). The monitoring of the instantaneous flight response of *C. cautella* to encounters with pheromone indicates that the relationship of concentration of the pulse/plume encountered and direction of turn is either the inverse that of predicted by Baker's model, or that these responses are dependent on other aspects of the internal structure of the plume which that model did not account for. Below we summarize the expected outcomes of male behavior in response to pulse interactions, based on our observations using marked plumes (Chapter III).

Our working hypothesis is that if the duration of the pheromone pulse is too long or if the pulse is too concentrated, the pulse will trigger the male to turn; if the pulse duration is just enough to contact the male antenna and disappear before the next encounter it will stimulate a straight upwind dash. If the next pulse is encountered before the end of this dash, continued upwind flight will be maintained. Thus, a sequence of these pulses results in a straight upwind flight track. For straight flight to occur, the male has to encounter the pheromone pulses within a certain time interval. The likelihood of encountering the plume is increased when the pheromone pulse has larger dimensions; this occurs in the field, when turbulence increases the dimensions of the odor pulses. If the dimensions of the plume are small the chances of the male intercepting that plume are lessened, in this case the male will begin casting shortly after losing contact with the plume/pulse. When contact with the pulse occurs again, the differential in pheromone concentration will trigger a turn. The turn may be more perpendicular to the wind direction as pheromone concentration increases. At "low" to "medium" pheromone concentrations, the turn will be more directly upwind resulting in net upwind displacement. If the differential of the background concentration and the concentration of pheromone pulse is more substantial, the turns will be more perpendicular to the wind line. In-flight-arrestment may occur if the pulse is repeated at a frequency higher than the limits for pulse determination.

This study was undertaken to define the influence of signal intermittency, and pulse frequency on the upwind flight of *C. cautella* males. Here we demonstrate that *C. cautella* males fly more directly upwind to certain pheromone pulse frequencies than to continuous plumes, and that pulse frequency influences other male pheromone-mediated behaviors from quiescence to location of the source. A modified version of Wright's olfactory guidance model that incorporates behavioral responses to single odor pulses best describes the different patterns of upwind flight tracks observed with the changes in plume structure that we tested.

4.2. Material and Methods

4.2.1. Insects

The *C. cautella* colony was started in March 1989 from some 500 larvae and pupae from Kansas State University, Manhattan, Kansas, and was maintained as a continuous culture at a level of at least 400 mating pairs per week. The *C. cautella* were reared from eggs to larvae in 1 liter glass jars on an artificial diet consisting of 3 kg poultry laying mash, 2 kg rolled oats, 200 ml glycerin, and 100 g Brewer's yeast. The rearing room was maintained at 25-27^{o}C, 50-60% RH on a 16:8 L:D. Individuals were sexed at the last larval instar when the males testes are visible. Female adults were kept in the same room as the main colony. Males were reared from last larval instar to adult in a separate room inside environmental chambers with the same photoperiod, 70% RH and 25-26^{o}C. Male pupae were kept in 25 X 25 X 25 cm screened cages where adults emerged. Male pupae were transferred daily to new cages, leaving newly emerged males in the old cage. This procedure generated a constant supply of 1-day-old males.

4.2.2. Chemicals

Chemicals were obtained from either Farchan Chemicals (Z9,E12-14:Ac, 97% pure; and Z9-14:Ac, 99% pure) or IOB (Z9,E12-14:Ac, 97% pure). The acetate, Z9,E12-14:Ac, from both sources was purified to 99.9% in a silver nitrate/Florisil column with an increasing polarity gradient of isopropyl-ether and hexane. The purity of compounds was determined by capillary gas chromatographic analysis on a Supelco 30 m x 0.32 mm ID SP 2340 column kept at 70^{o}C for 4 min., programmed at 12^{o}C min^{-1}. to 200 ^{o}C, and kept at 200^{o}C for 10 minutes.

Synthetic pheromone components were formulated gravimetrically into solutions of 1µg µl^{-1}, and then volumetrically into the mixture of Z9,E12-14:Ac and Z9-14:Ac at the ratio of 5.67:1.00. They were subsequently diluted to a concentration of 4.5 ng. A concentration of 4.5 ng is the optimal concentration for eliciting upwind flight of *C. cautella* males exposed to homogeneous filamentous plumes (Chapter III).

4.2.3. Wind Tunnel

The wind tunnel used is described elsewhere (Chapter I). Airflow through the wind tunnel was laminar. This was confirmed visually using $TiCl_4$ "smoke" plumes and also by the low variance obtained from repeated measurements of the wind velocity in the tunnel using a hot-wire anemometer (Yokogawa, 2141). Airflow was measured using a hot-wire anemometer positioned at the center of the tunnel, and a wind velocity of 40 cm sec^{-1} was set using a voltage regulator to control the exhaust fan.

4.2.4. Odor Delivery System

The pheromone plume was created using an air pulser (Stimulus Flow Controller SFC-2, Syntech) specifically designed to deliver air pulses of adjustable flow, duration, and repetition rate. The instrument delivers air pulses of size and duration determined by precision electronic mass flow controllers (F-201 C-FA-33-V, Bronkhorst Hi-Tech). The air is supplied to the pulser from compressed air tanks. The air flow in ml sec^{-1} is adjusted in the SFC-2 by potentiometers and monitored by digital LED indicators. The SFC-2 pulser delivers a continuous air flow to a complement output port. This continuous air flow is diverted to the pulse output port by solenoid valves controlled by an electronic timer and counter, resulting in a sequence of air pulses of identical volume, separated by similar intervals of clean air. The solenoid valves diverting the air flow were controlled manually, or automatically using internal SFC-2 programs or programs from a coupled computer.

Air flow from the pulse output port was connected to an odor delivery device located underneath the wind tunnel floor and 20 cm downwind from the upwind screen. The air delivered by the pulser enters a chamber (8 mm diam. x 4 mm depth) containing a filter paper disk (Whatman #1) of 0.7 cm diameter, impregnated with 10 µl of the pheromone solution. The pheromone laden air exits the odor chamber through a disposable micropipett (external diameter of 2 mm, internal diameter of 1.2 mm, 12.2 cm of length), that traverses the wind tunnel floor and opens inside the wind tunnel working section, 11 cm above the floor. The small diameter of the micropipett permitted the maintenance of low turbulence on the laminar airflow downwind from the odor source platform. The structure of individual pulses was therefore maintained throughout the entire working section of the wind tunnel.

To ensure constant pheromone concentration throughout the experimental sessions, the pheromone source (filter paper) was replaced every 10 minutes. To minimize contamination of the odor delivery system, the micropipett was substituted after testing 5 males, or after ten minutes, whichever came first.

4.2.5. Treatments

To determine the effect of pulse frequency on male upwind flight, moths were tested for four treatments using pulses of 0.1 sec duration delivered every 0.1 sec (i010), every 0.25 sec (i025), every 0.50 sec (i050), and every 1.50 sec (i150), and two controls (described below). Air flow was held constant at 5 ml sec^{-1}, resulting in pulses of 0.5 ml. The two controls consisted of continuous pheromone plumes with an air flow of 5.0 ml sec^{-1} (c50), and an air flow of 0.5 ml sec^{-1} (c05). The treatments were tested on a complete random order within each block (Chapter I, Chapter II, Chapter III).

The structure of the plumes was evaluated by frame-by-frame analysis (see section 3.2.4.) of horizontal and vertical high contrast video images of the smoke plumes. "Smoke" plumes were generated by pipetting $TiCl_4$ onto the filter paper serving as odor source. A high intensity directional light from a fiber optic illuminator (Dolan Industries, Model 190) was aimed along the longitudinal axis of the smoke plume. This plume was then videotaped against a black background. The resultant video image was analyzed frame by frame, and the size of 100 pulses per treatment were measured.

4.2.6. Bioassay Procedure

The male release cage device was located 1 m downwind from the source release device in a position established using the $TiCl_4$ plume. The male release cage device consisted of a cylindrical aluminum screen cage (4.5 cm diameter x 5 cm). One end was covered with the same screen and the other end was open. The cages were positioned with the open side facing upwind 35 cm above the wind tunnel floor. The cages were held in position by a rigid Teflon® tube that had one end inside the cage and the other connected to a hollow glass tube. The hollow glass tube passed through the wind tunnel floor and the other end opened outside the wind tunnel. This design allowed for the introduction of moths from outside of the tunnel directly into the release cage without disrupting the pheromone plume. The height of the release platform was regulated by sliding the glass tube through the floor. It was necessary to remove the release cage from the position where it intercepted the pheromone plume to get consistent rates of upwind flight (Chapter I). Moving the release cage down to 5 cm above the wind tunnel floor after the male initiated flight maximized the uniformity of pheromone plume downwind of the release platform. It also allowed males that locked onto the plume downwind from the point of release to proceed flying upwind without encountering the release platform.

Male adult emergence cages were placed at experimental conditions of light and relative humidity for at least 30 minutes prior to testing. Moths were randomly selected from emergence cages, and transferred to the release platform positioned below the level of the pheromone plume, 15 cm above the wind tunnel floor. Each quiescent male was held in the screen cage for 10 seconds. At the end of 10 seconds of quiescence, pheromone was introduced, and each male was observed for 2 min unless he landed on the source or on the wind tunnel walls.

As soon the male was placed inside the release platform, a video (flight track) and audio (verbal) record of his behavior was made. The sequence and duration (in whole sec) of the behaviors described by the verbal commands were transferred to a computer by means of an event recorder. The sequence and duration of the following mutually exclusive behaviors at the release platform and during upwind progression were recorded continuously:

QUIESCENT (Q): no perceptible movement of the body or body parts;

WING FANNING/WALKING (WFW): either wing fanning or walking on the release platform or both, i.e., walking while wing fanning;

FLIGHT INITIATION (FI): time at which the male flies off release platform;

RANDOM FLIGHT (RF): the male exhibits a non-oriented flight, i.e., a flight in which the male does not appear to fly upwind along the pheromone plume;

ORIENTED FLIGHT (OF): upwind flight (zigzag or straight) along the pheromone plume; it includes arrestment in-flight, when the male flies in the plume in stationary and narrow zigzag;

LOCATING THE SOURCE (LS): the male approaches a radius of 2 cm from the tip of the pheromone source, usually followed by hovering near or landing on the pheromone dispenser. This behavior terminates video and event recording for this male;

LANDING ELSEWHERE (L): landing outside the pheromone plume, contacting with wind tunnel structures other than the pheromone dispenser. This behavior terminates video and event recording for this male.

If a male did not take off he was tested for ability to fly. The male was removed from the release cage using an aspirator, and released in the air, about 30 cm above the floor. Males that did not fly were discarded; males that flew were scored as non-responders.

4.2.7. Data Analysis

4.2.7.1. Flight Track Analysis

Male upwind flight was recorded by video through the tunnel floor, using a Sony RSC 1050 rotary-shutter video camera connected to a SLO 340 video recorder. The field was view of 80 x 90 cm ending 15 cm from the odor source platform.

Flight tracks of individual moths were transferred to a Sony SVM-1010 motion analyzer, and played back frame-by-frame through a 41 cm Panasonic WV-5470 black-and-white video monitor. Two points of reference on the wind tunnel floor, and moth position in every second frame (every 1/30th of a sec) were transcribed onto transparent acetate. The X and Y coordinates of the moth position in a two dimensional plane were obtained using a digitizer pad (Apple Graphics Tablet), and analyzed with Quick Basic programs for ground velocity, track angle and net velocity (Charlton *et. al.* 1993). Course angles, drift angles, and airspeed were obtained using the triangle of velocities method (Marsh *et al.* 1978). Inter-reversal distance, turn frequency and inter-reversal time were calculated directly from the track, using Excel procedures. The definitions of the parameters of flight are the same as in Chapter II, and they are in accordance to the current nomenclature of flight parameters (e.g., Charlton et al 1993, Willis & Baker 1984; Willis & Cardé 1990). The means of the flight parameters measured for each moth were analyzed using GLM and ANOVA procedures of SAS, and two sample t-tests (SAS and Excel). The effect of days or blocks was dropped from the statistical analysis because they were not important (Chapter II, Chapter III).

4.2.7.2. Behavior Analysis

From the sequence and the duration of each behavior, two measurements were made: (1) the latency from the male's introduction to the pheromone plume to the first expression of a particular behavior, and

(2) the mean time spent in a particular behavior. Since most of the males performed the same sequence of 6 behaviors, the data were not processed for the male's behavioral sequence analysis or the categorical performance of specific behaviors as in Chapter I.

Latency : Every male had latency for seven behaviors scored. If a male did not perform a given behavior, he received a score of 120 sec for the specific behavior. The mean latency of a behavior was analyzed using a two way design (day by treatment). Levene tests of homoscedasticity were run. Where the assumption of equal variances was not met, weighted two way Anova procedures were used (Weight =1/cell variance) (Chapter I).

Time performing the behavior: Males were scored on their overall performance for locating the source or failing to locate the source. In addition, each male had the time associated with a specific behavior summed. For each overall performance class, a treatment-wise comparison of the time the males spent on a behavior was performed using Kruskal-Wallis (SAS). The treatments were contrasted using weighted Anovas (GLM procedures of SAS) (Chapter I).

4.3. Results

4.3.1. Plume Shape and Structure

The injection of pheromone laden air from the odor delivery apparatus at 90° relative to the wind direction disrupted the laminar air flow in the working section of the wind tunnel. The disruption, or turbulence, was proportional to the volume of air injected per unit of time, and it shaped the injected pulses of each treatment (Table 2).

The diameter of a pulse generated by injection of a volume of 5 ml sec^{-1} for 0.10 sec was 2.45±2.7 cm (mean±sd), with length ranging from 5.33±1.16 cm for treatment i010, to 6.67±1.20 cm for treatment i150 (Table 2). The lowest volume (c05) continuous-smoke plume had a cross section of 1.24±0.7x1.50±0.7 cm. The c05 plume was continuous, and no gaps were detected even on the edges of the plume. This plume was continuous for almost 10 sec, or 386.8 cm, and then discontinued for 0.33 sec, or 13.24 cm. The largest volume plume (c50) was more turbulent around the edges, and after a highly variable period (0.33±0.78 sec), that turbulence was strong enough to create air gaps (0.7±1.2 cm.) within the "continuous" smoke plume (Table 2).

The most rapidly pulsed plume (i010) had pulses of smoke of 5.33 cm in length isolated by 3.2 cm of air gaps. A small stationary object in the center of the plume would receive 0.08 sec of "clean" air between the 0.13 sec periods of exposure to the pulse. The plume that was pulsed most slowly (i150) had smoke pulses of 6.67 cm in length separated from each other by 58 cm of clean air. The same stationary object would experience 1.45 sec of clean air between the 0.17 sec periods of exposure to the pulse. The intermediate pulse frequency (i025) had pulses of 6.00 cm (0.15 sec) intercalated by 8.68 cm (0.217 sec) of clean air.

Table 2. Characteristics of the internal structure of the six smoke plumes tested (mean±standard deviation).

	Smoke (seconds)	Clean Air (seconds)	Smoke Pulse Length (cm.)
i010	0.13±0.030	0.083±1.30	5.33± 1.16
i025	0.15±0.025	0.217±0.03	6.00± 1.00
i050	0.15±0.030	0.467±0.03	6.00± 1.20
i150	0.17±0.030	1.450±0.04	6.67± 1.20
c005	9.67±0.030	0.331±0.03	386.80± 1.20
c050	0.33±0.748	0.017±0.03	13.33±29.93

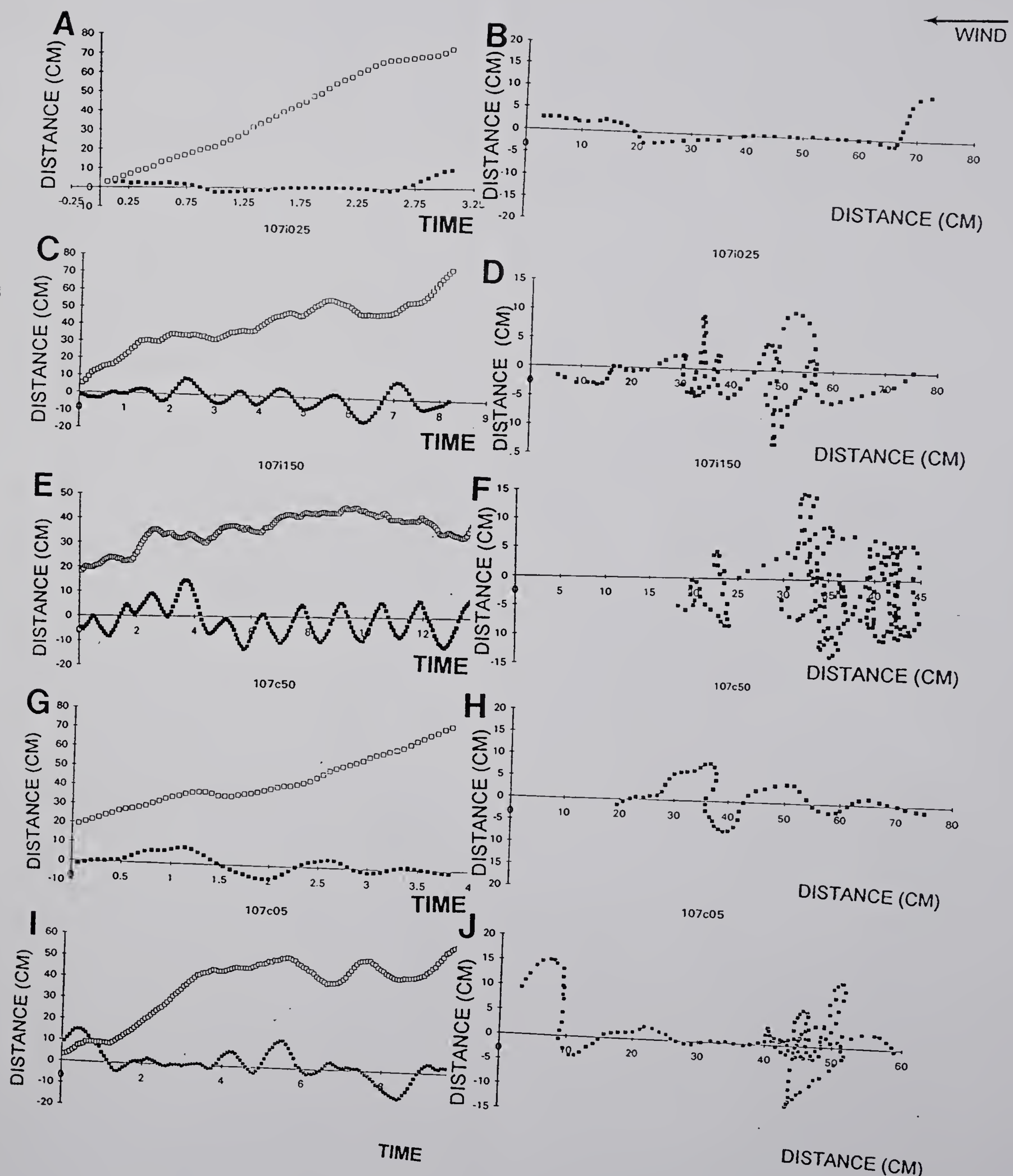
These treatments include the range of pulse frequencies of the turbulent plumes reported in Chapter III. The 0.11 sec interval between pulses observed for the narrow turbulent plume (Chapter III) is most similar to treatment i010 in this experiment. The 0.17 sec of interval between pulses observed for the **wide turbulent plume** is most similar to plume i025 in this experiment. The plume **single-filamentous** is most similar to plume c05.

4.3.2. Flight Track

The flight track of *C. cautella* males changes with the frequency of pulses (Fig. 24), with the general trend being toward straighter flight to higher frequency pulsed plumes. The trend is for males to fly straight toward i010 plumes (e.g., Fig. 24a and 24b), whereas the trend is for males to turn more across the wind line when flying to i150 plumes (e.g., Fig. 24e and 24f). The tracks of males flying toward plume i025 show less turning than tracks for i150, but more so than flight tracks to i010 (e.g., Fig. 24c and 24d).

For the continuous plumes, the larger the volume of pheromone-laden air injected, the straighter the male's flight. The flight tracks of males flying to the large continuous c50 plumes are the ones that most resemble those of males flying to i010. These tracks are relatively straight upwind, with very little zigzag (e.g., Fig. 24g and 24h). When the volume of the continuous plume is reduced (e.g., treatment c05), *C. cautella* males fly upwind zigzagging in and out of the plume (e.g., Fig. 24i and 24j).

Fig. 24. Representative flight tracks of *C. cautella* males flying to five different pheromone plumes. Graphs A and B are for **i010** with a pulse per 0.1 seconds, C and D are for **i025** with a pulse per 0.25 seconds, E and F are for **i150** with pulse per 1.5 seconds, G and H are for **c50** as continuous high volume, I and J are for **c05** as continuous low volume plume. On the graphs on the left (A, C, E, G, and I) the longitudinal (open squares) and transverse (filled squares) components of the flight are represented (X axis is time in seconds and the Y axis is distance traveled in cm.). The flight track are depicted on the graphs on the right (B, D, F, H, and J) (X axis is the transverse distance traveled and the Y axis is the longitudinal distance traveled, both in cm.). Note the slope of the longitudinal component in conjunction with the lower frequency of zigzag and the lack of tempo of the transverse component of the graph A, in contrast to the slope of the longitudinal component of the flight close to zero and a rigid tempo of the zigzags of the transverse component in graph E.


A
B
C
D
E
F
G
H
I
J
WIND
DISTANCE (CM)
TIME
107i025
107i150
107c50
107c05

At higher pulse frequencies, and greater volumes of continuous plumes, the suppression of counterturning is most effective (Fig. 24). This is in accordance with the finding that *C. cautella* males fly straighter upwind in large turbulent plumes (Chapter III).

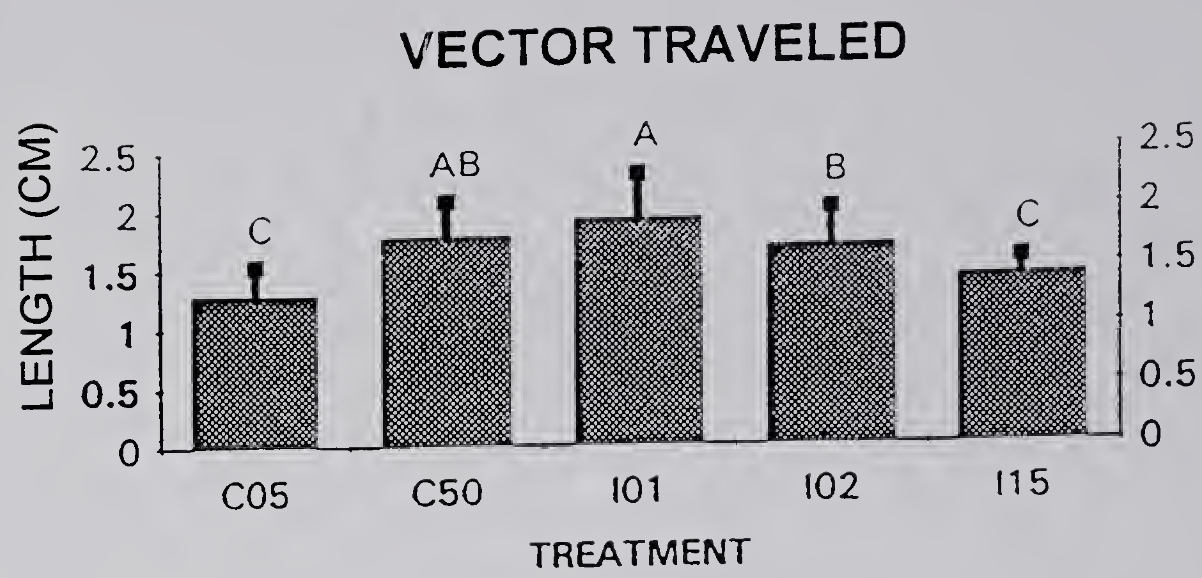
The mean length of vector traveled per interval sampled (0.033 s) increases with pulse frequency (i150 = 1.41±0.14 cm; i025 = 1.69± 0.31cm; and i010 = 1.93±0.37cm) and pulse diameter (c05 = 1.29± 0.24cm; and c50=1.77±0.29cm) (Fig. 25a).

Moths flying to treatment i150 show net lateral velocity (XT) significantly higher than the males flying to all other treatments (i150 = 34.3±4.38 cm sec-1; c50 = 29.9±8.80 cm sec-1, c05 = 29.1±6.09 cm sec-1, i010 = 28.5±7.62 cm sec-1, i025 = 27.6±7.95 cm sec^{-1}) (Fig. 25c).

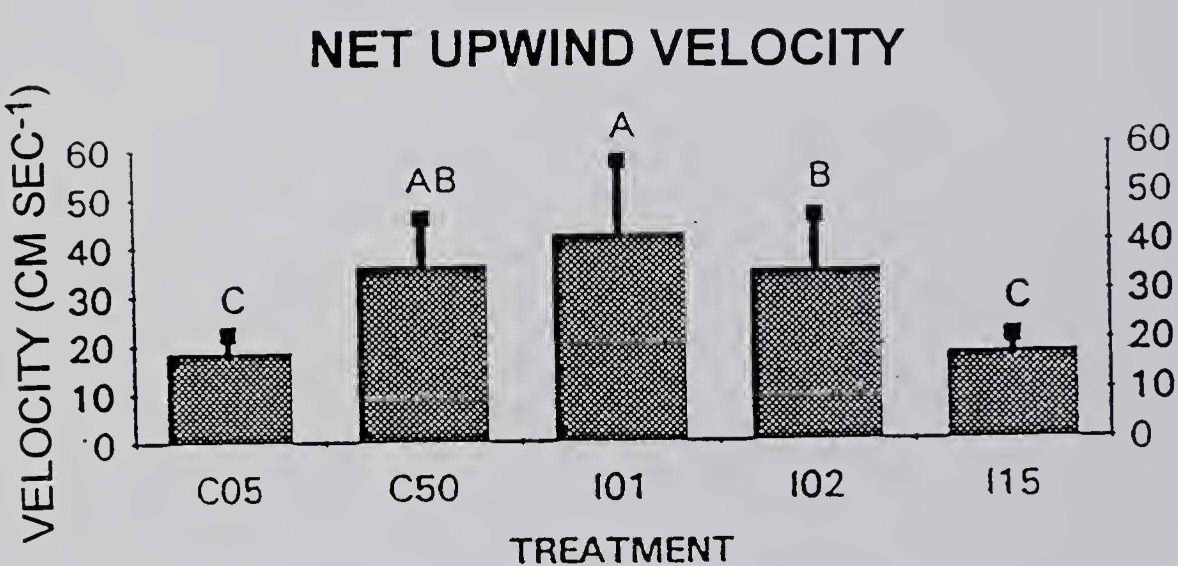
Although males flying to treatment i150 have values of the mean of vectors traveled larger than males flying to treatment c05, the net upwind velocity (YT) value is similarly low for both treatments (i150=17.9±3.10, c05= 18.5±3.57 cm sec^{-1}) (Fig. 25b). The vectors traveled by males submitted to treatment i150 have longer crosswind components than the vectors traveled by males flying to treatment c05. In addition, males fly faster crosswind (XT) to treatment i150, than to treatment c05 (Fig. 25c), resulting in similar low net upwind velocities (Fig. 25b). The upwind flight track of males flying toward i010 plumes contrasts with that for males flying to i150. Large vector size, an intermediate lateral velocity, and a flight more directly due upwind (smaller track angles) resulted in a faster net upwind velocity (YT) for treatment i010 than for any other treatment

Fig. 25. Parameters of velocity of flight tracks of *C. cautella* males flying to five different pheromone plumes. Treatments were i010, i025, i150, c05, and c50. **A.** Histogram of mean values of vector traveled for the five treatments. The wide bars represent mean values of vector traveled for the 20 tracks, the narrow bars represent one standard deviation above the mean. Bars without letters in common are statistically different at $\alpha=0.05$ level. **B.** Histogram of mean values for net upwind velocity for the five treatments. **C.** Histogram of mean values for net crosswind velocity for the five treatments. **D.** Histogram of mean values for airspeed for the five treatments. **E.** Histogram of mean values for ground velocity for the five treatments. Details as per Fig. 24.

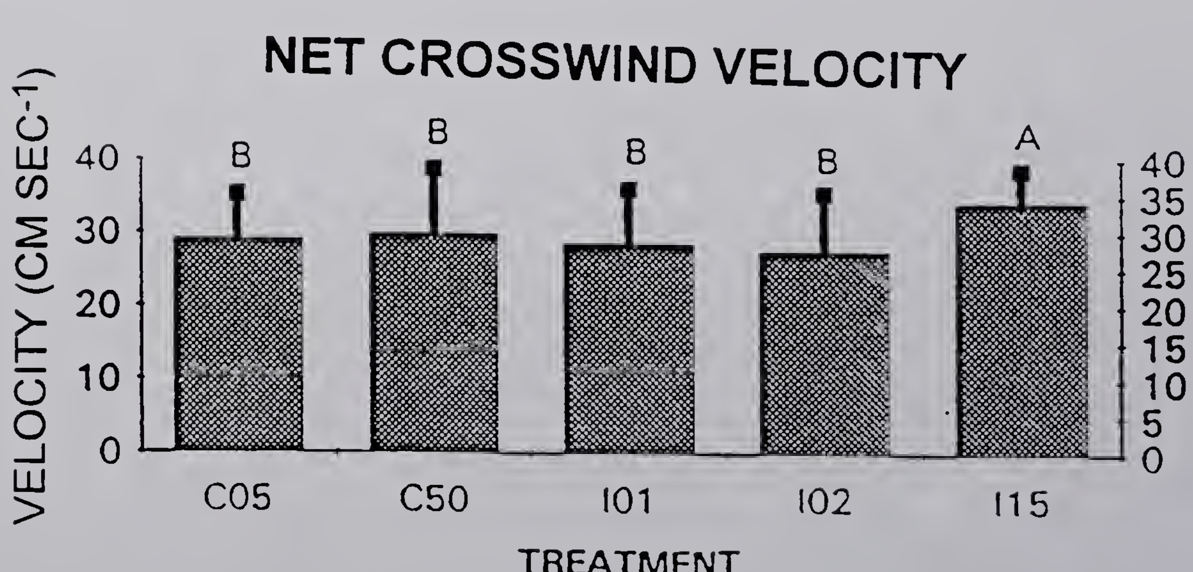
A.



B.

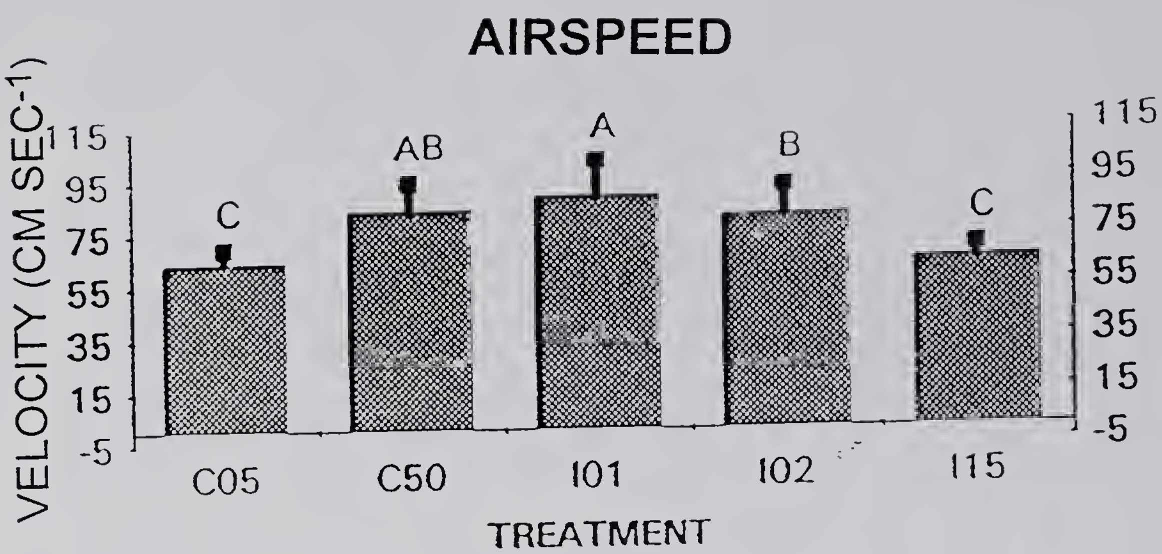


C.

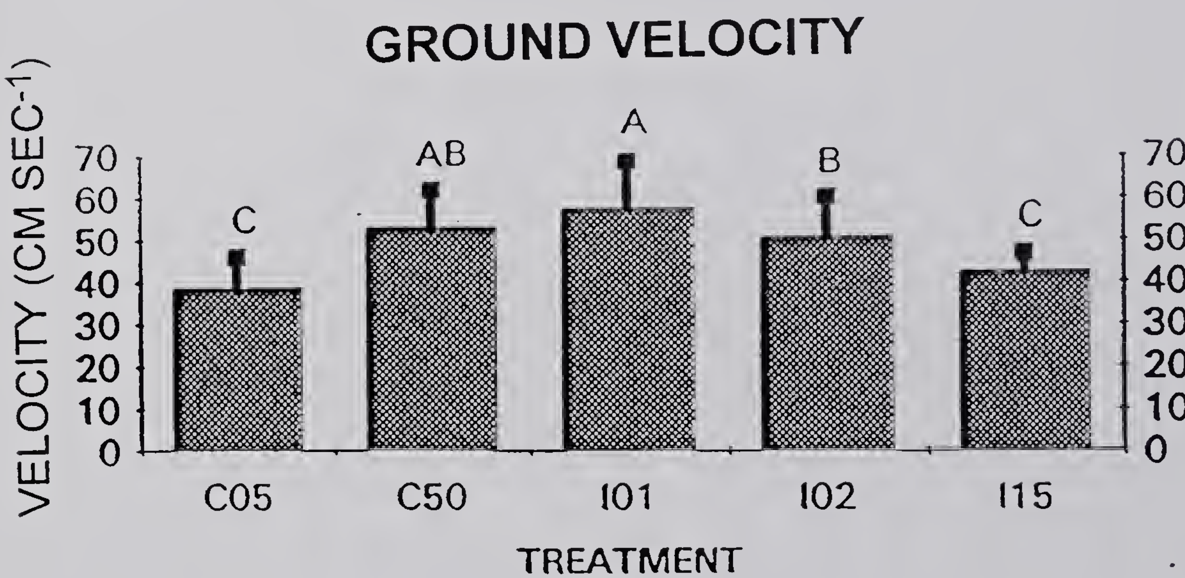


Continued next page.

D.


AIRSPEED
VELOCITY (CM SEC-1)
115
95
75
55
35
15
-5
C
AB
A
B
C
C05
C50
I01
I02
I15
TREATMENT

E.


GROUND VELOCITY
VELOCITY (CM SEC-1)
70
60
50
40
30
20
10
0
C
AB
A
B
C
C05
C50
I01
I02
I15
TREATMENT

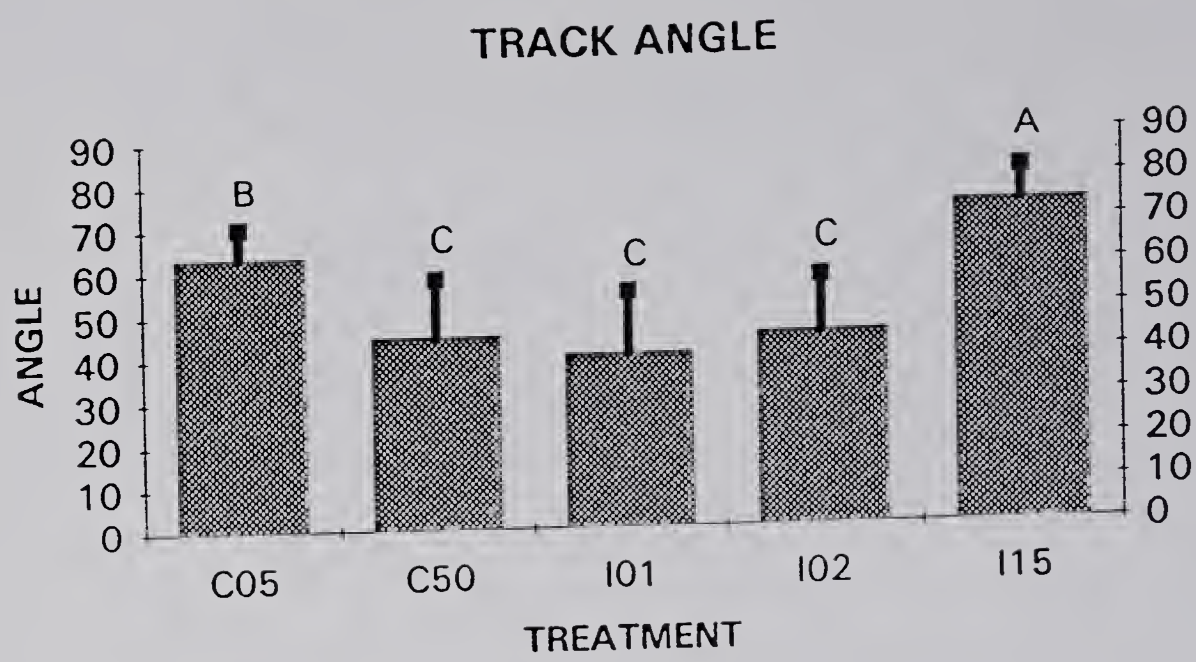
tested (Fig. 25b). The i010 mean net upwind velocity (YT) is 42 cm sec^{-1}, 60% faster than for i150 (17 cm sec^{-1}) (P=0.0001), 57 % faster than c05 (18 cm sec^{-1}) (P=0.0001), 20% faster than i025 (34 cm sec^{-1}) (P=0.0407), and 14% faster than the continuous plume c50 (36 cm sec^{-1}) (P=0.0782).

The same trend is seen for airspeed (Fig. 25d) and ground velocities (Fig. 25e). Males flying to c05 and i150 had lower airspeeds (c05=64.0 ±24.40 cm sec-1, i150=63.45±38.8 cm sec^{-1}) than those flying to c50, i25, and i010, (c50=80.0±47.00 cm sec^{-1}, i25=85.9±50.01 cm sec^{-1}, and i010=78.6±54.00 cm sec^{-1}) (P<0.05). The highest airspeeds were recorded for males flying to treatments i010 and c50. Males submitted to treatments c05 and i150 flew significantly slower ground velocities in comparison to males flying to the c50, i025 and i010 (P<0.05) (Fig. 25e). Males flying to the most rapidly pulsed plume (i010=58.0 ± 11.05 cm sec^{-1}) had a ground velocity on average 33% faster than males flying to a thin continuous plume (c05=38.8±7.24 cm sec^{-1}) (different, P=0.0001), and 27% faster than males flying to the slowest pulsed plume (i150=42.5 ±4.25 cm sec^{-1}) (P=0.0001). The ground velocity of males flying to plume i010 was 12% faster than the i025 (P=0.149). The ground velocity of males flying to c50 (53.24±8.67 cm sec^{-1}) was similar to the levels seen for i010 (P=0.984).

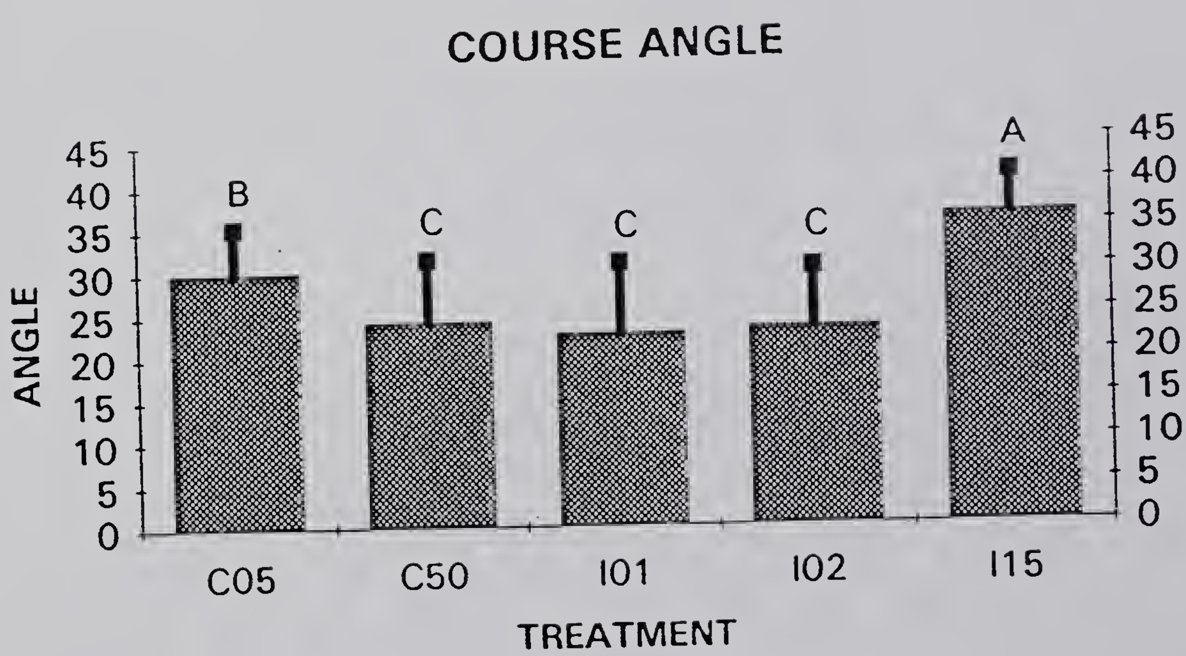
A rough measurement of straightness of upwind flight is the time spent by the male crossing the working area of the wind tunnel. Males flying to i150 spent, on average, four times more time to fly the working area than males flying to i010 (i150 = 8.52±2.59 sec, i010 = 2.09±0.75

Fig. 26. Mean values for the angular parameters of flight of *C. cautella* males flying to five different pheromone plumes. Treatments were i010, i025, i150, c05, and c50. **A.** Histogram for mean values of track angles. The bars represent mean values of vector traveled for the 20 tracks, and the narrow bar represents the standard deviation. Bars without letters in common are statistically different at the level of a=0.05. **B.** Histogram for mean values of course angles. **C.** Histogram for mean values of drift angles. Details as per Fig. 24

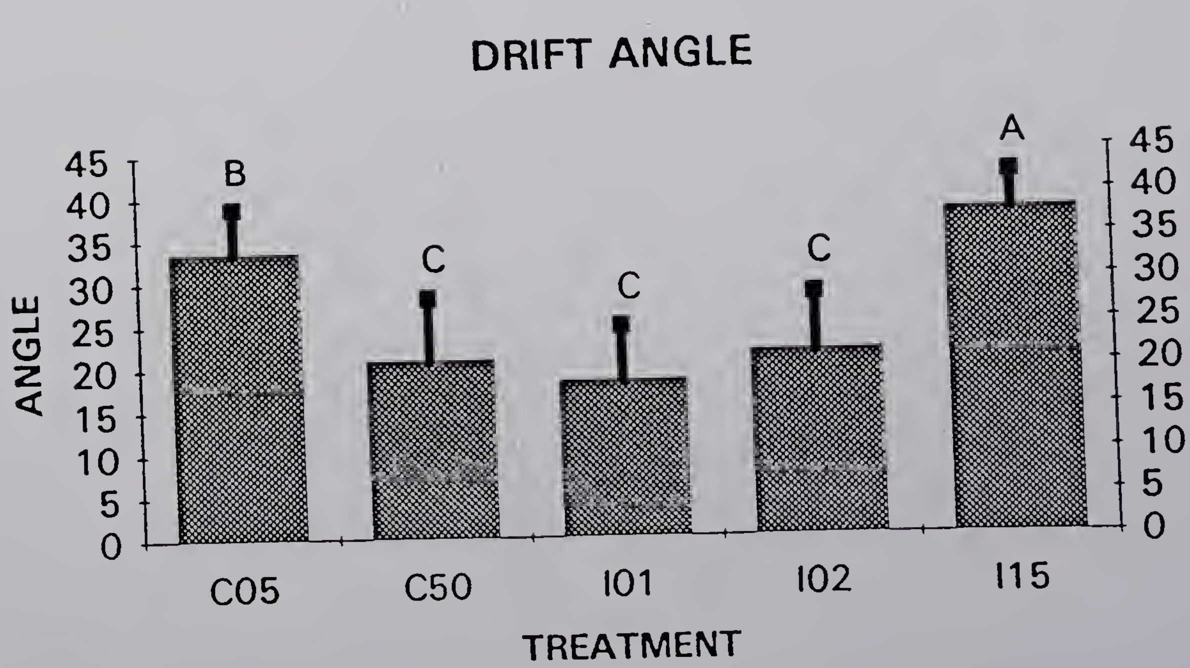
A.


TRACK ANGLE
ANGLE
TREATMENT
C05
C50
I01
I02
I15
B
C
C
C
A

B.


COURSE ANGLE
ANGLE
TREATMENT
C05
C50
I01
I02
I15
B
C
C
C
A

C.


DRIFT ANGLE
ANGLE
TREATMENT
C05
C50
I01
I02
I15
B
C
C
C
A

sec) and more than three times as much as males flying to i025 (i025=2.50 sec). Males flying to the continuous low-volume-plume (c05) spent on average more than twice as much time as males flying to the continuous high volume pheromone plume (c05 = 5.17±1.66 sec, c50 = 2.51±1.09 sec).

An overall reduction in the values of the three angular flight parameters (track angle, course angle, and drift angle) reflects a straighter flight due to the wind direction (Fig. 26). Males that spend significantly more time flying crosswind show larger values for at least one of these flight angles than those flying more directly upwind.

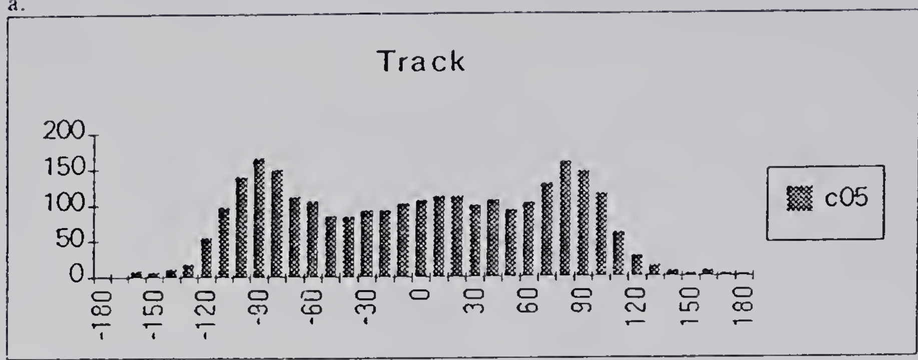
The mean values of the track angles increased with increasing interval between pulses (Fig. 26a). The treatment with the highest pulse frequency, i010 (pheromone pulses separated by only 0.1 second), resulted in the smallest value for mean track angles (i010=40.44±14.04), but was not statistically different from c50, and i025. The lowest pulse frequency, i0150 (pulses separated by 1.5 second), resulted in the highest values of mean track angles (i150=73.93±7.19) among all treatments ($P<0.05$). A similar trend is seen for changes in the volume of the continuous plumes. The smallest volume continuous plume had larger values of mean flight track angles (c05=63.50±6.59) than the largest volume continuous plume (c50= 44.82±13.09) ($P=0.0001$). Both trends were predicted by our modification of Wright's model (section 4.1), although there were no differences between the large continuous plume c50 (c50= 44.82±13.09) and the high frequency pulsed plumes i010 and i025 (i010=40.44±14.04, i025= 44.77±12.54).

Higher pulse frequencies resulted in smaller means of the value of course angle (i150= 36.10±4.69, i025= 23.19±6.97, i010=22.68±8.13) (Fig. 26b). The smallest volume continuous plume has greater values for course angles (c05=30.03±5.29) than the largest volume continuous plume (c50=24.08±7.29) (Fig. 26b) ($P=0.0028$). Similarly, the higher the pulse frequencies, the smaller the value of the means of the drift angles (i150=37.83±4.20, i025= 21.58±6.63, i010=18.25±6.35) (Fig. 26c). The tracks associated with the smallest volume continuous plume show greater values for the values of course angles (c05=33.47±5.20) than the largest volume continuous plume (c50=20.74±7.26) ($P<0.0001$) (Fig. 26c).

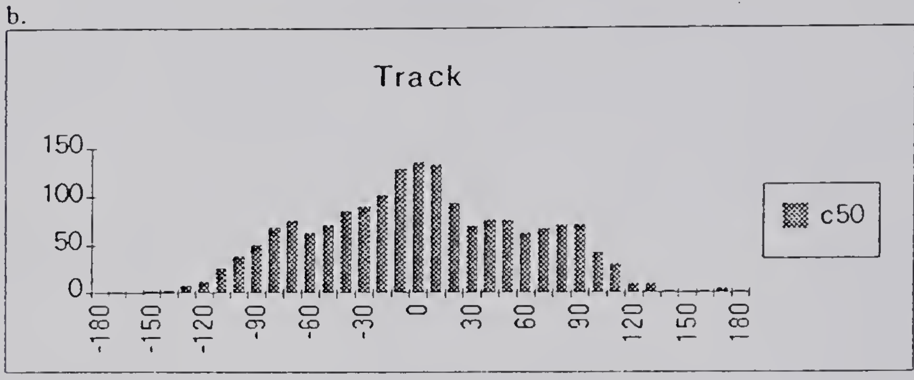
The distribution of flight angles for a particular treatment is usually demonstrated by pooling all the angles steered by each tested moth, and plotting the distribution of the angle values (e.g., Figs. 27, 28, and 29). The pooling of a different number of measurements from different individuals generates a complicated statistical problem. Moths that meander more, and thus spend more time in the working area, will contribute disproportionally more to the shape of the histogram, than moths that fly straight and quickly depart from the working area. The distribution histogram of the angles of pooled vectors of a particular treatment's flight tracks is only valid for suggesting general trends for the maneuvers of flight for that treatment. A track angle of 0° is directly upwind, 180° is directly downwind, and 90° is perpendicular to the wind line. The positive or negative value associated with the angle measurement indicates on which side of the longitudinal axis of the plume the maneuver is being performed: if positive it is on the right side, if negative on the left side, when facing upwind. The trend is for the distribution of the angles to have three peaks, one centered on 0° degrees (e.g., Figs. 27c, 28c, and 29c), and the other two usually spaced almost symmetrically from the center, on the negative and positive sides of axis of angle values (e.g., Figs. 27e, 28e, and 29e). In general the distribution of angles is either unimodal or bimodal. Unimodal distribution of angles usually has pronounced central peak, and two lateral peaks suppressed. The unimodal distribution of angles has class 0° (or a class close to it) as the mode. Unimodal distributions usually represent flights more directly upwind. Angles tightly clustered around 0° represent a more direct upwind flight. Bimodal distribution of angles is usually manifested by a decrease in the center peak, and an equal amplification of both lateral peaks (e.g., Figs. 27c, 28a, 29c, and 29c). A bimodal distribution of angles usually represents a flight track of males zigzagging back and forth across the wind line. Although straight flights cannot give rise to a bimodal distribution of the angles, certain zigzag flights patterns can result in unimodal distribution of angles: the moth may have a constant angular velocity (turning the same units of angles per time) on the left and on the right sides of the wind line, generating a sinusoid flight track. Although one must be cautious when drawing conclusions from the distribution histograms of pooled angles from all moths flying to one treatment, this form of representation is still valuable in describing general

Fig. 27. Frequency histogram distribution of the flight track angles steered by *C. cautella* males flying toward five different plumes. Where **a.** is the frequency histogram distribution for i010. Where the ordinate is the number of angles, and the abscissa is the angle steered; **b.** is the frequency histogram distribution for i025; **c.** is the frequency histogram distribution for i150; **d.** is the frequency histogram distribution for c05; and **f.** is the frequency histogram distribution for c50. The angles were sampled every 1/30th of a second. Details as per Fig. 24.

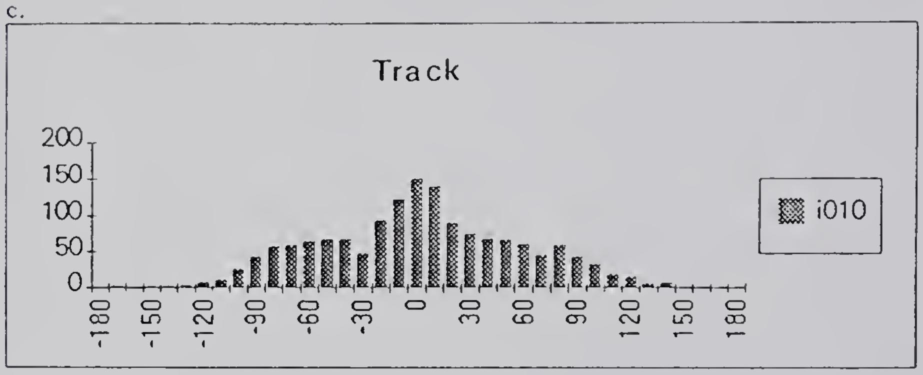
a.


Track
200
150
100
50
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
c05

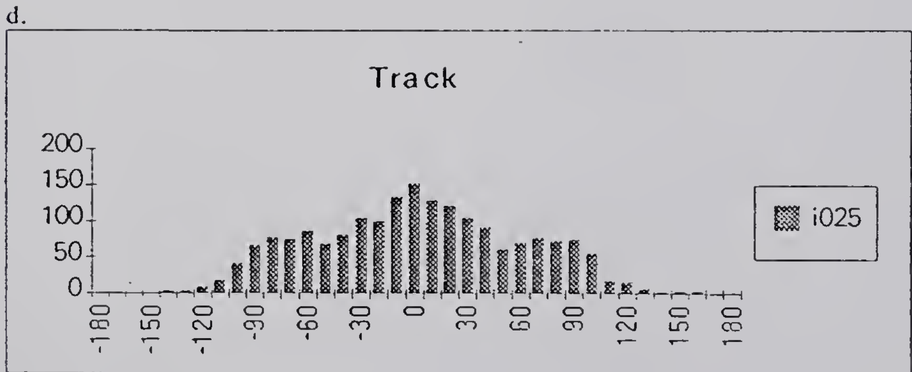
b.


Track
150
100
50
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
c50

c.


Track
200
150
100
50
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i010

d.


Track
200
150
100
50
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i025

e.

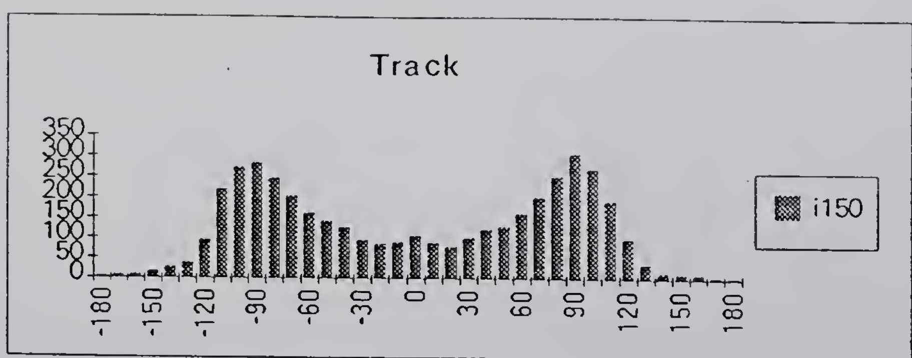
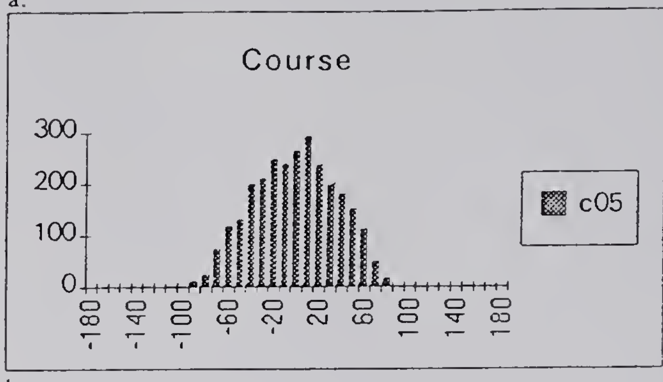
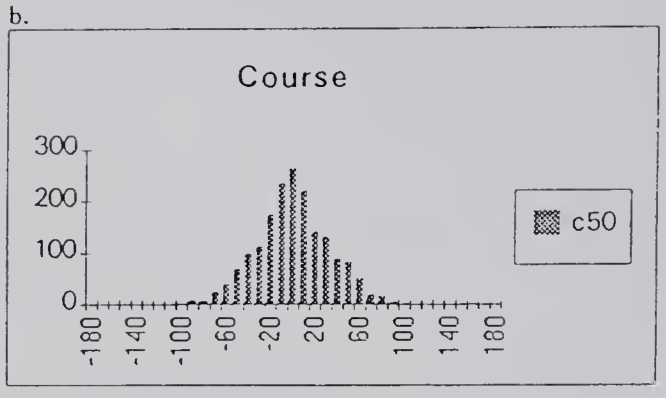

Track
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i150

Fig. 28. Frequency histogram distribution of the flight course angles steered by *C. cautella* males flying toward five different plumes. Where **a.** is the frequency histogram distribution for i010, the ordinate is the number of angles, and the abscissa is the angle steered; **b.** is the frequency histogram distribution for i025; **c.** is the frequency histogram distribution for i150; **d.** is the frequency histogram distribution for c05; and **f.** is the frequency histogram distribution for c50. The angles were sampled every 1/30th of a second. Details as per Fig. 24.

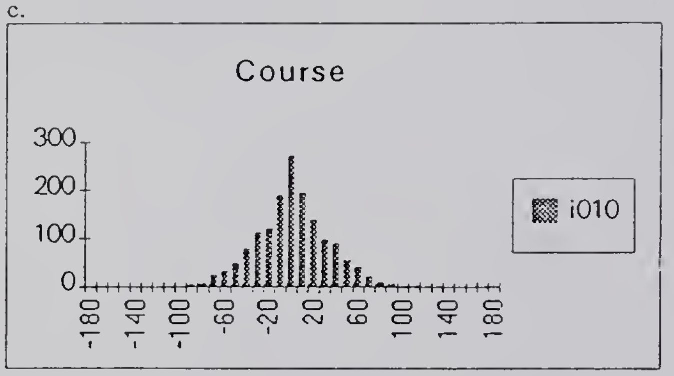
a.


Course
300
200
100
0
-180 -140 -100 -60 -20 20 60 100 140 180
c05

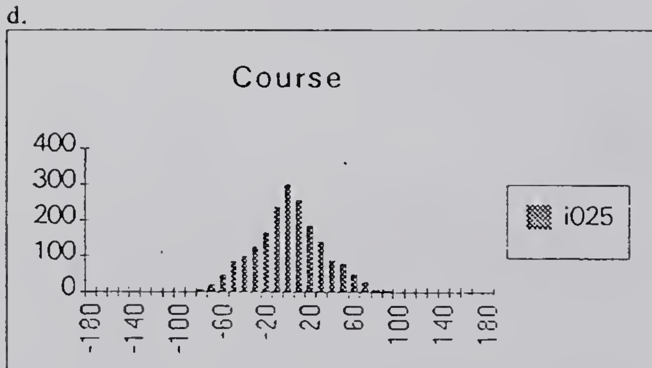
b.


Course
300
200
100
0
-180 -140 -100 -60 -20 20 60 100 140 180
c50

c.


Course
300
200
100
0
-180 -140 -100 -60 -20 20 60 100 140 180
i010

d.


Course
400
300
200
100
0
-180 -140 -100 -60 -20 20 60 100 140 180
i025

e.

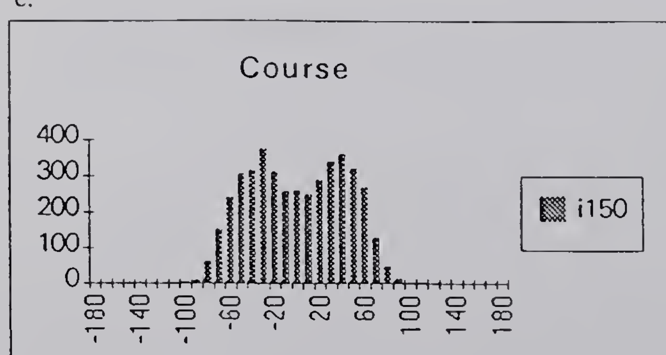
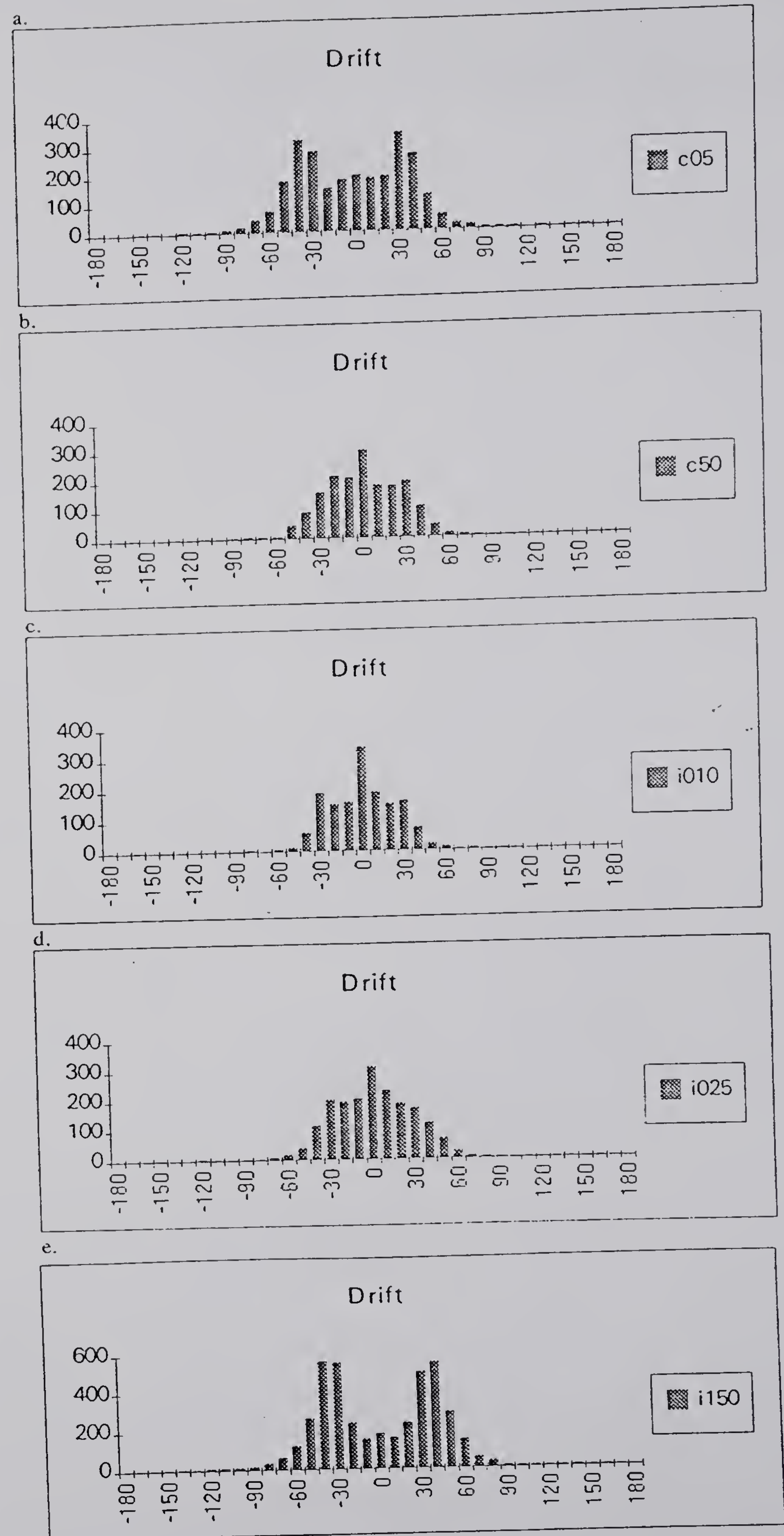

Course
400
300
200
100
0
-180 -140 -100 -60 -20 20 60 100 140 180
i150

Fig. 29. Frequency histogram distribution of the angles drifted by *C. cautella* males flying toward five different plumes. Where **a.** is the frequency histogram distribution for i010, the ordinate is the number of angles, and the abscissa is the angle steered; **b.** is the frequency histogram distribution for i025; **c.** is the frequency histogram distribution for i150; **d.** is the frequency histogram distribution for c05; and **f.** is the frequency histogram distribution for c50. The angles were sampled every 1/30th of a second. Details as per Fig. 24.


a.
Drift
400
300
200
100
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
c05
b.
Drift
400
300
200
100
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
c50
c.
Drift
400
300
200
100
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i010
d.
Drift
400
300
200
100
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i025
e.
Drift
600
400
200
0
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
i150

characteristics of flight to a given treatment. For the following description of the distribution histograms of class 10° of the flight angles, we will include in our definition of a **peak** only those classes representing at least 5% of the distributed angles.

The distribution histograms of the track angles for all treatments are seen in the Fig. 27. The distribution of the track angles for treatment i150 (Fig. 27e) is clearly bimodal, with a large percentage of angles clustered between -110° and -80° (23 % of the angles) on the negative side of the axis, and between 70° and 100° (24%) on the positive side. The center peak of mean 0° was strongly suppressed in this treatment i150. The flight tracks for treatment c05 (Fig. 27a) have similar distributions of track angles, which cluster between -100° and -80° (17%), and between 70° and 90° (16%). A center peak is evident in these tracks, although it is not as predominant as the lateral peaks. The three other treatments, c50, i010, and i025, show unimodal distribution of the track angles (Fig. 27b, 27c, and 27d), due mostly to the suppression of the lateral peaks. Treatment c50 (Fig. 27b) has a peak between -40° and 20° (43% of the track angles), and 0° is the mode (8% of the track angles). Treatment i010 (Fig. 27c) has 43% of the track angles clustered between -20° and 30°, and 0° is the mode representing 10% of the angles. Treatment i025 (Fig. 27d) has 50% of the track angles clustered between -30° and 40°, and 0° is the mode representing 8 % of the angles.

The distribution of course angles for all treatments is presented in Fig. 28. The overall trend of the distribution of course angles is toward unimodality, with angles clustered around 0^o. Treatment i150 is the only exception to this trend, showing clear bimodal distribution of the course angles (Fig. 28a). All classes from -50^o to 60^o contain at least 5% of the angles. This cluster holds 90% of the course angles for this treatment. It has two distinct modes, one at -30° containing 9% of the angles, and the second located at 40° containing 8% of the angles. The trend toward unimodality of course angle distribution holds for the other four treatments. Treatment c05 has the broadest distribution of course angles, with the cluster located between -50° and 50°, with class 10° as the mode containing 11% of the course angles. Treatment c50 has a peak between -40° and 40° with 81% of the course angles, and the mode at 0° represents 15% of the course angles (Fig. 28b). Treatment i010 has 83% of the track angles clustered between -40° and 40°, and the mode at 0° represents 17% of the angles (Fig. 28e and 28c). Treatment i010, has the tightest unimodal distribution of the course angles of all treatments. Treatment i025 has 78% of the course angles clustered between -40^o and 30^o, and the mode at 0^o represents 15% of the angles (Fig. 28d).

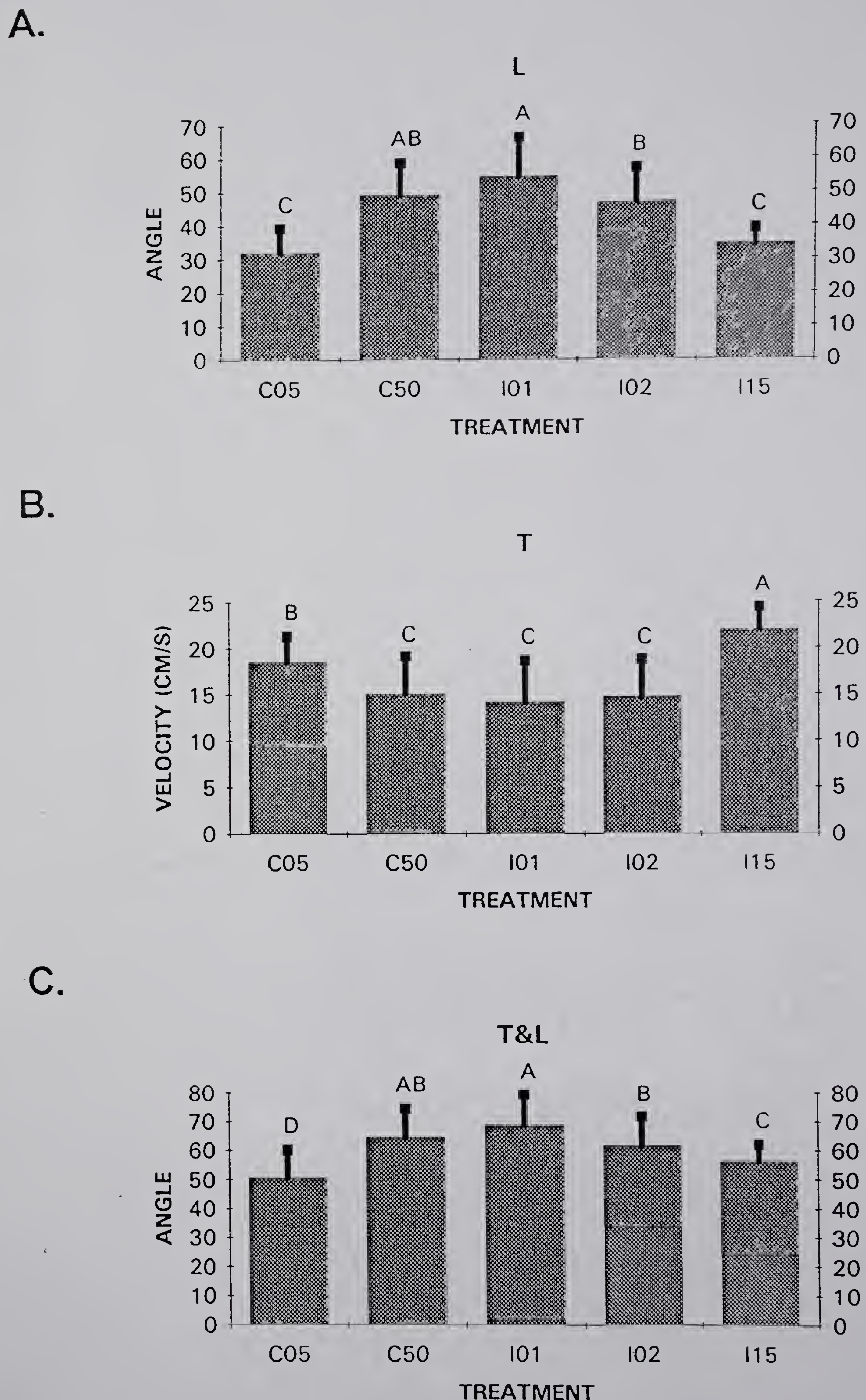
The distribution of drift angles for all treatments is presented in Fig. 29. The trend for the distribution of the drift angles is the same as described for the distribution of the track angles. Treatment i150 shows a strong bimodal distribution of drift angles, with one peak between -50^o and -20^o (37% of the angles) and the other between 20^o and 50^o (37% of the angles). The histogram for treatment c05 is also bimodal, but all classes from -50° to 50° contain at least 5% of angles. This cluster (from -50° to 50°) contain 88% of the angles and has two distinct modes, one at -40° containing 12% of the angles, and the second at 20°, representing 12% of the angles. The histogram of the distribution of drift angles is unimodal for the three other treatments. Treatment c50 (Fig. 29b) has a peak between -40° and 40° with 91% of the drift angles, and a mode at 0° representing 17% of the drift angles. Treatment i010 (Fig. 29c) has 91% of the track angles clustered between -30° and 40°, and mode 0° representing 22% of the angles. Treatment i010 has the tightest unimodal distribution of the drift angles of all treatments. Treatment i025 (Fig. 29d) has 90% of the drift angles clustered between -40° and 40°, and a mode at 0° representing 16% of the angles.

The counterturn/optomotor anemotaxis model suggests that, by optomotor feedback, moths maintain a constant relationship of two different components of the image flow field, the longitudinal (L) and the transverse (T) components, in order to maintain preferred flight parameters (Ludlow 1984; David 1986). David (1986) argues that the relationship between both parameters can be represented by the formula $\sqrt{(T^2+L^2)}$, but T+L is a good approximation. In this experiment we found that the mean values for the longitudinal component of the visual flow decreases with pulse frequency (i010=54 cm sec^{-1}, i025=47 cm sec^{-1}, and i150=34 cm sec^{-1}) and with plume size (c50=49 cm sec^{-1}, and c05=32 cm sec^{-1}) ($P \leq 0.0001$ for each pairwise comparison) (Fig. 30a). Males show some compensatory (inverse) relation of the transverse component of the visual flow (i010=14 cm sec^{-1}, i025=15 cm sec^{-1}, i150=21 cm sec^{-1}, c50=15 cm sec^{-1}, and c05=18 cm sec^{-1}) (Fig. 30b), but they do not maintain the relationship of T and L, i.e. $\sqrt{(T^2+L^2)}$, constant (Fig. 30c). The values for this relationship are: i010=56 cm sec-1, i025=49 cm sec^{-1}, i150=41 cm sec^{-1}, c50=52 cm sec^{-1}, and c05=37 cm sec^{-1}) (Fig. 30c). These results suggest that when one of the components of the image flow changes, *C. cautella* males make weak compensatory maneuvers, possibly as an attempt to adjust the other component of the image flow (David 1986). This optomotor feedback is not perfect and it is not enough to maintain constant any of the proposed relationships among T and L (Marsh *et al.* 1978, David 1986) when the plume structure is manipulated. This conclusion is consistent with that reached previously for *C. cautella* males flying to plumes of different shapes and concentrations (Chapter III) and to different blends and concentrations (Chapter I). It is also consistent with the conclusions of Preiss & Kramer (1986) using tethered flying gypsy moths and those of Willis & Cardé (1990) for free-flying gypsy moths tested in varying wind velocities.

4.3.3. Behavior

The internal structure of pheromone plumes influences several aspects of response in male *C. cautella*. The latency of the first occurrence of several behaviors and the time the male spends performing each behavior change with plume structure. In general, males exposed to rapidly-pulsed plumes, or large, non-pulsed plumes performed the sequence of behaviors more quickly than males exposed to slowly-pulsed plumes, or small non-pulsed plumes.

Fig. 30. Components of the image flow for *C. cautella* males flying to five different plumes (using the individual means). The treatments are i010, i025, i150, c05, and c50. **A.** Histogram of values of longitudinal (L) component of image flow. **B.** Histogram of values of transverse (T) component of image flow. **C.** Histogram of values of interaction T+L. Details as per Fig. 24.


A.
L
ANGLE
70
60
50
40
30
20
10
0
C
AB
A
B
C
C05
C50
I01
I02
I15
TREATMENT
B.
T
VELOCITY (CM/S)
25
20
15
10
5
0
B
C
C
C
A
C05
C50
I01
I02
I15
TREATMENT
C.
T&L
ANGLE
80
70
60
50
40
30
20
10
0
D
AB
A
B
C
C05
C50
I01
I02
I15
TREATMENT

Latency data : The latency for the male to perform several of the classified behaviors is a function of the pheromone plume to which they are being exposed (Table 3). The first behavior that a quiescent male will perform when exposed to pheromone is **wing fanning/walk** (Fig. 31a). Plume structure affects latency for this behavior, since males took longer to wing fan/walk when exposed to the filamentous continuous pheromone plume (c05=15.6±29.6 sec) than to any other treatment ($P<0.05$, c05 value is larger than any other treatment, pairwise comparisons LSD Anova, SAS). The latency for the low-volume continuous plume was greater than the latency observed for this behavior with the i150 plume (c05=12.6±22.7 sec). This value was also different from any of the other treatments ($P<0.05$, c05 value is larger than any other treatment, pairwise comparisons LSD Anova, SAS). Males flying in the i010, i025, i050, or c50 plumes, had the same latencies for wing fanning/walking (i010=i025=i050, $P\geq0.05$), but they were different from the first two treatments, i.e., c05 and i150 (c05=i50 > i010=i025=c50, $P<0.05$, LSD, GLM SAS). Males submitted to i150 have the same latency for **flight initiation** (Fig. 31b), **random flight** (Fig. 31c) and **oriented flight** (Fig. 31d) as the males exposed to c05 (no differences, $P\geq0.05$, LSD, GLM SAS), but the latency for these behaviors is different among these two treatments and all the other treatments (c50, i050, i025, and i010) (c05 and i150 are different than the rest, $P<0.05$, LSD, GLM SAS). Since few males **landed elsewhere** (Fig. 31e), the latency for this behavior is similar among all the treatments, and is, by our definition of latency, close to the predetermined maximum time of observation, 120 seconds. The

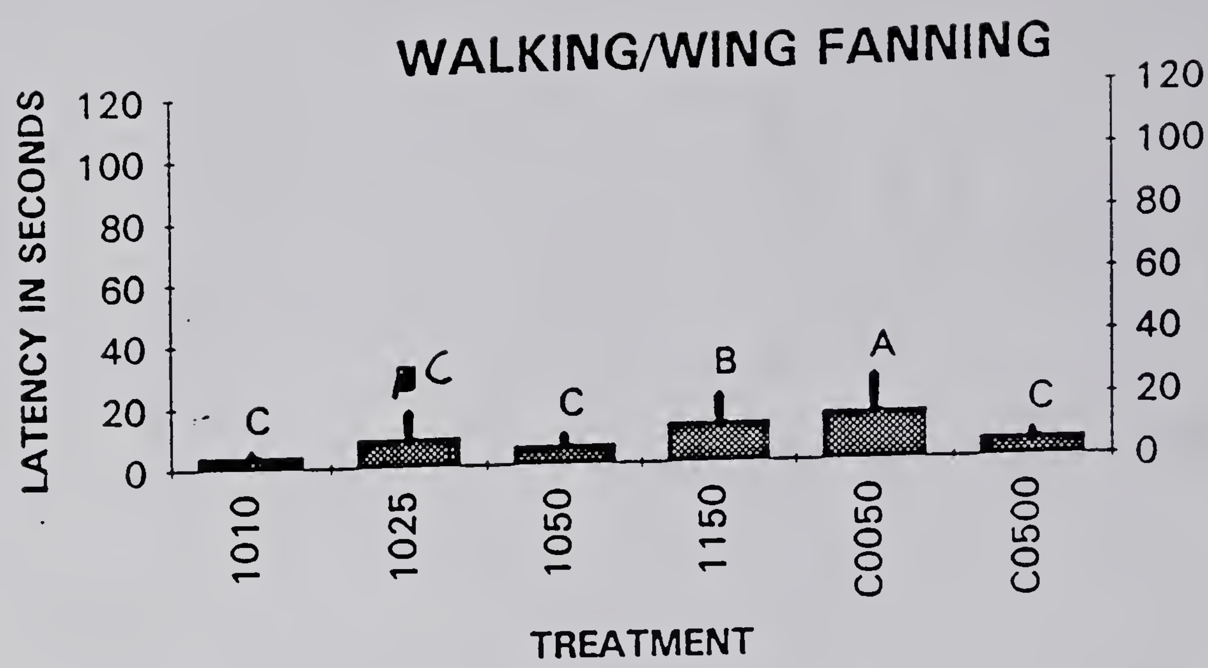
Table 3.: Mean latency (in seconds, mean±standard deviation) for the first occurence of behaviors of *C. cautella* males exposed to six different plume. Details as per fig. 24.

TRT	LANDING ELSEWHERE	FLIGHT INITIATION	RANDOM FLIGHT	UPWIND FLIGHT	WALKING WING FANNING	LANDING ON THE SOURCE
i010	120.00±0.00	10.12±8.93	10.76±9.03	17.72±23.18	3.96±1.71	19.52±9.79
i025	120.00±0.00	12.213±6.73	12.71±6.93	21.21±21.01	9.25±22.00	29.43±21.19
i050	116.15±19.62	11.85±7.48	16.69±22.38	19.38±22.04	6.27±6.72	36.77±21.58
i150	111.08±25.66	34.77±25.66	35.19±25.56	49.30±33.43	12.58±22.68	106.00±27.21
C050	118.53±6.42	30.00±32.24	30.84±31.69	44.74±41.00	15.63±29.63	69.42±35.35
C500	114.16±22.76	17.55±20.69	17.96±20.68	25.55±27.03	6.16±4.34	36.52±31.03

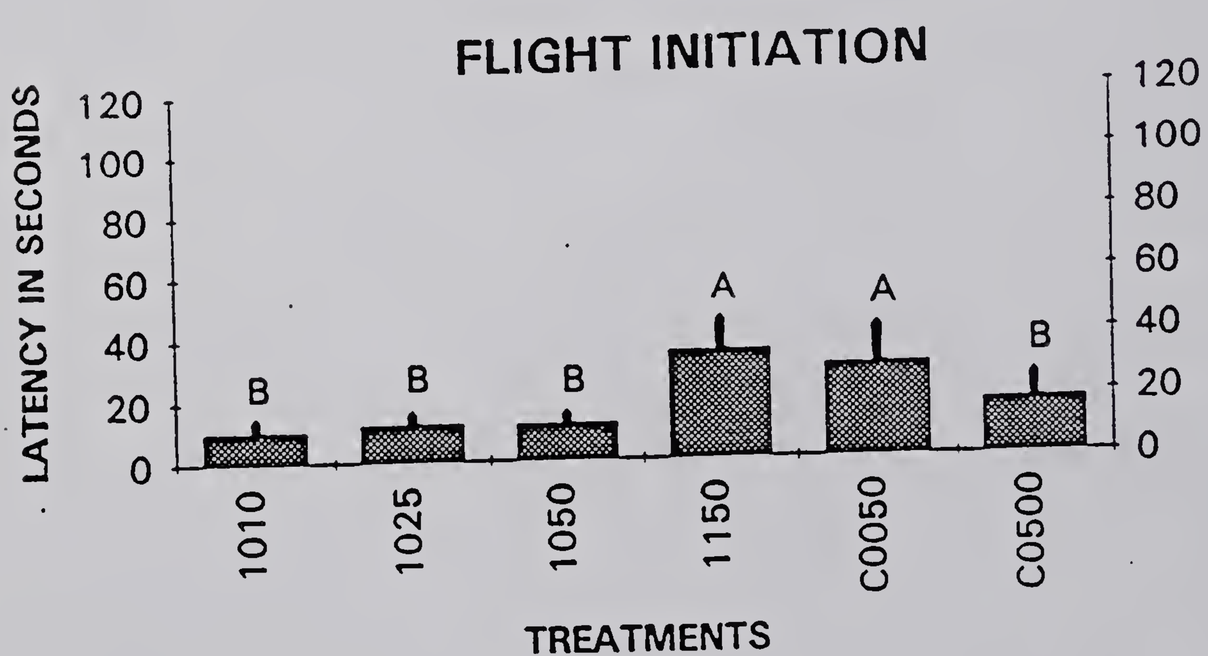
Based on 20 individual moths tested for each plume structure.

Fig. 31. Histogram of the mean latency in seconds for the occurrence of the behaviors of *C. cautella* males exposed to five different pheromone plumes. The treatments are i010, i025, i150, c05, and c50. **A.** Latency for wing fanning/walking. The bars represent the mean values for the 20 moths tested per treatment, and the narrow bar represents the standard deviation. Bars without letters in common are statistically different at α=0.05 level. **B.** Latency for flight initiation. **C.** Latency for random flight. **D.** Latency for oriented upwind flight. **E.** Latency for landing elsewhere. **F.** Latency for landing on the source. Details as per Fig. 24.

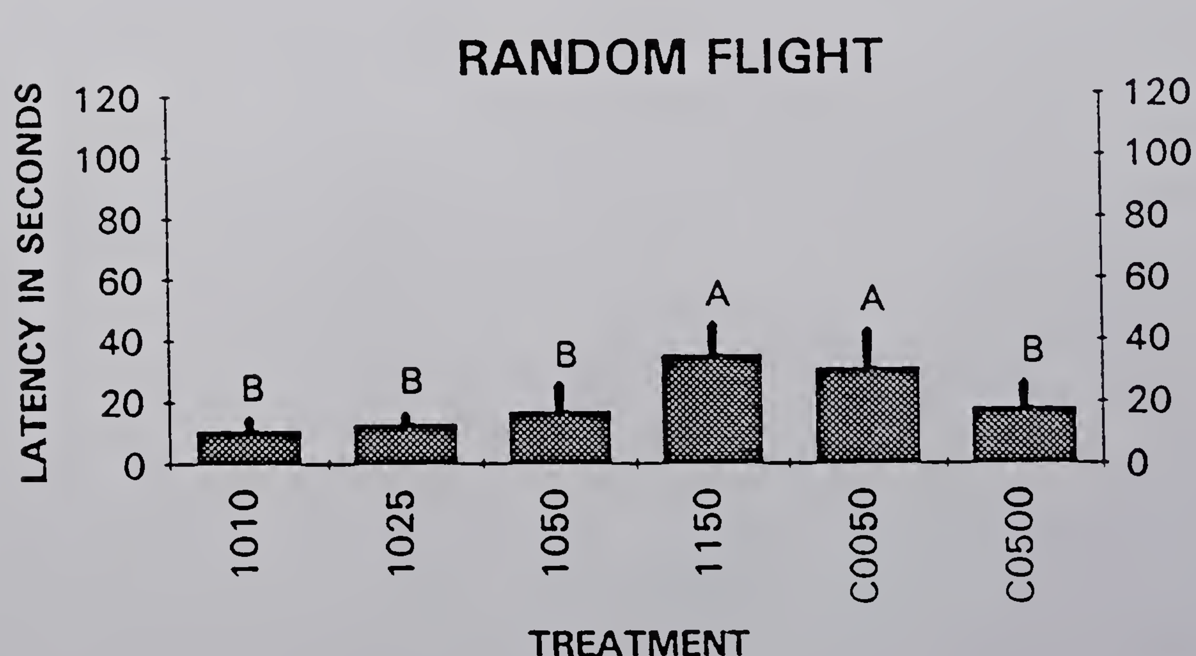
A.



B.

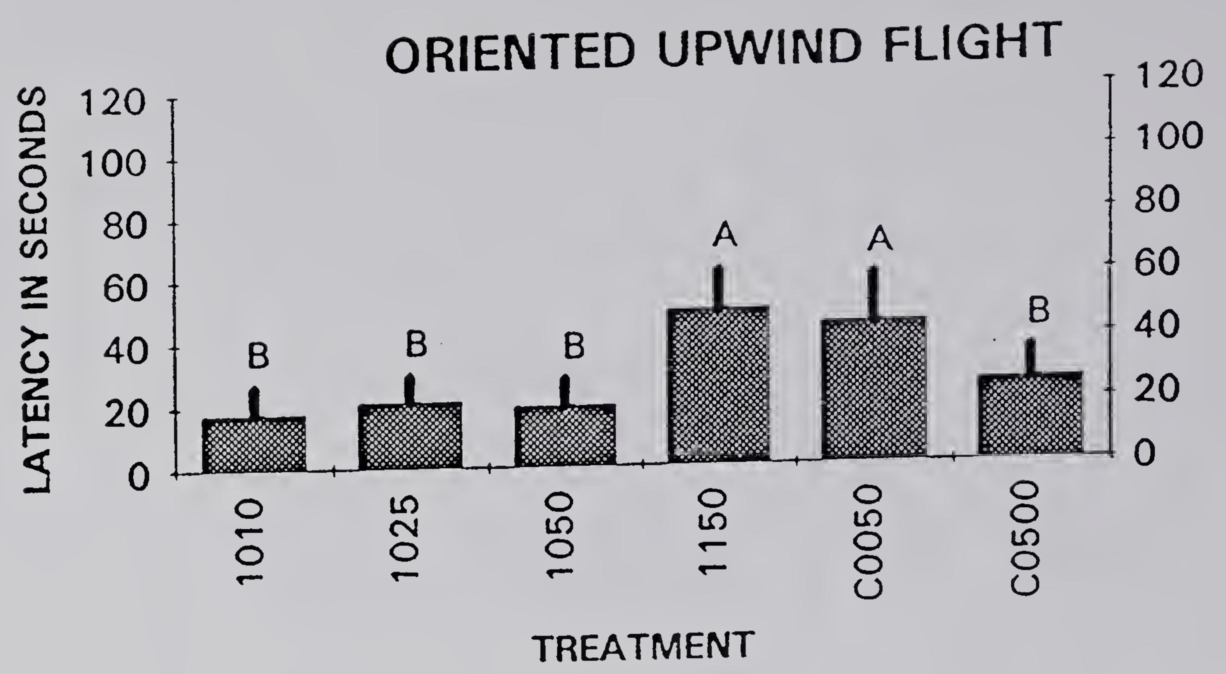


C.

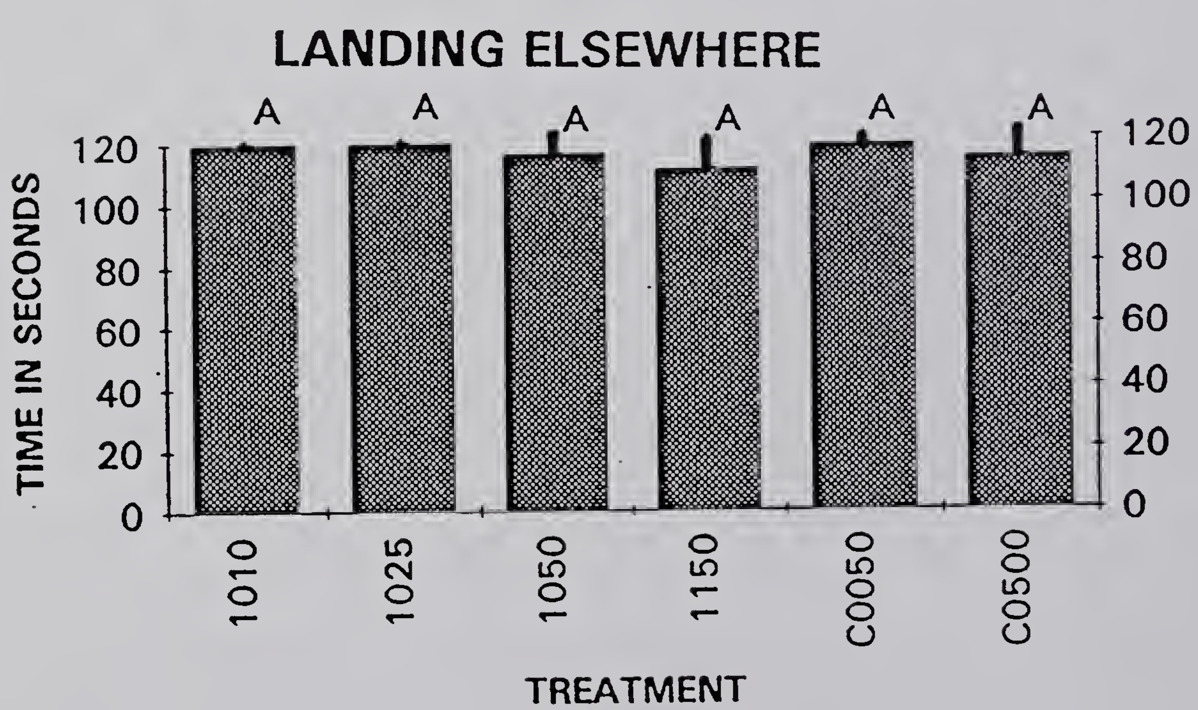


Continued next page.

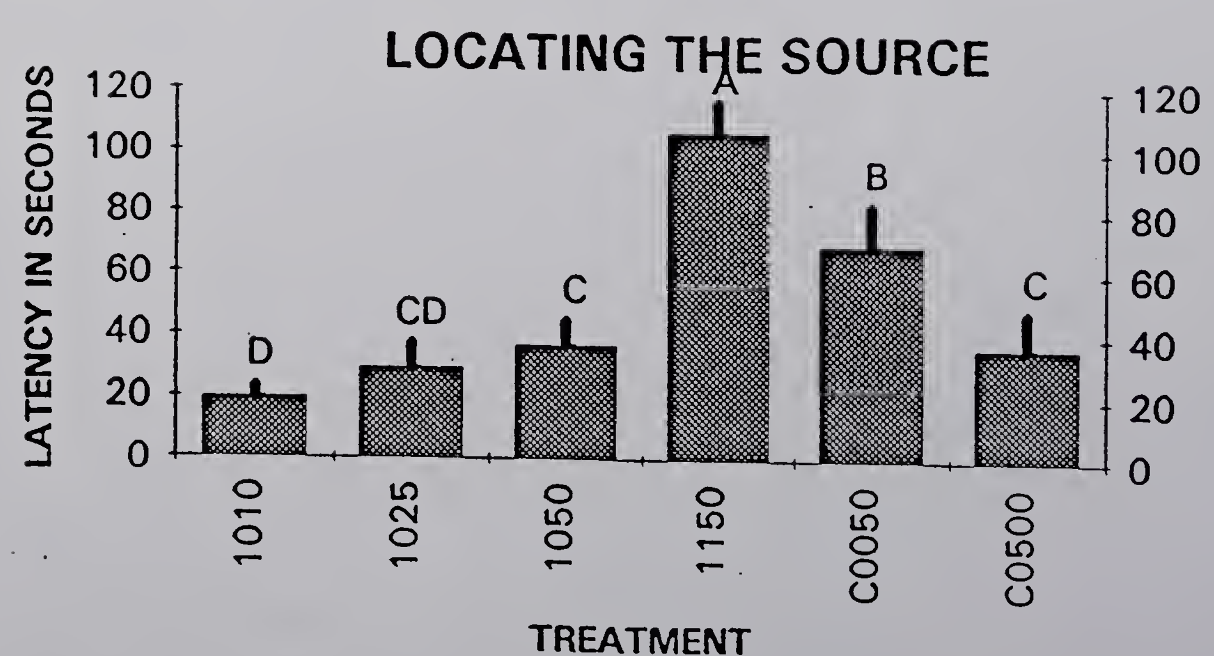
D.


ORIENTED UPWIND FLIGHT
LATENCY IN SECONDS
120
100
80
60
40
20
0
B
B
B
A
A
B
1010
1025
1050
1150
C0050
C0500
TREATMENT

E.


LANDING ELSEWHERE
TIME IN SECONDS
120
100
80
60
40
20
0
A
A
A
A
A
A
1010
1025
1050
1150
C0050
C0500
TREATMENT

F.


LOCATING THE SOURCE
LATENCY IN SECONDS
120
100
80
60
40
20
0
D
CD
C
A
B
C
1010
1025
1050
1150
C0050
C0500
TREATMENT

latency for the last behavior in the sequence of a successful flight, **landing on the source** (Fig. 31f), can be divided into four groups. It took longer (P<0.05, LSD, GLM SAS) for males flying to i150 to locate the source (106.0±27.2 sec) than under any other treatment. The second largest value observed was for treatment c05 (69.4±35.4 sec), which is significantly different from all other treatments (P<0.05, LSD, GLM SAS). The latency for landing on the source for treatments c05 (36.5±31.0 sec), i050 (36.8±21.6 sec). Males exposed to the treatment i010 were most "efficient" in locating the source, with the shortest latency to locate the source among all treatments, 19.5±9.8 sec (significantly less time than all treatments, P<0.05, with exception of i025 P>0.05, LSD, GLM SAS).

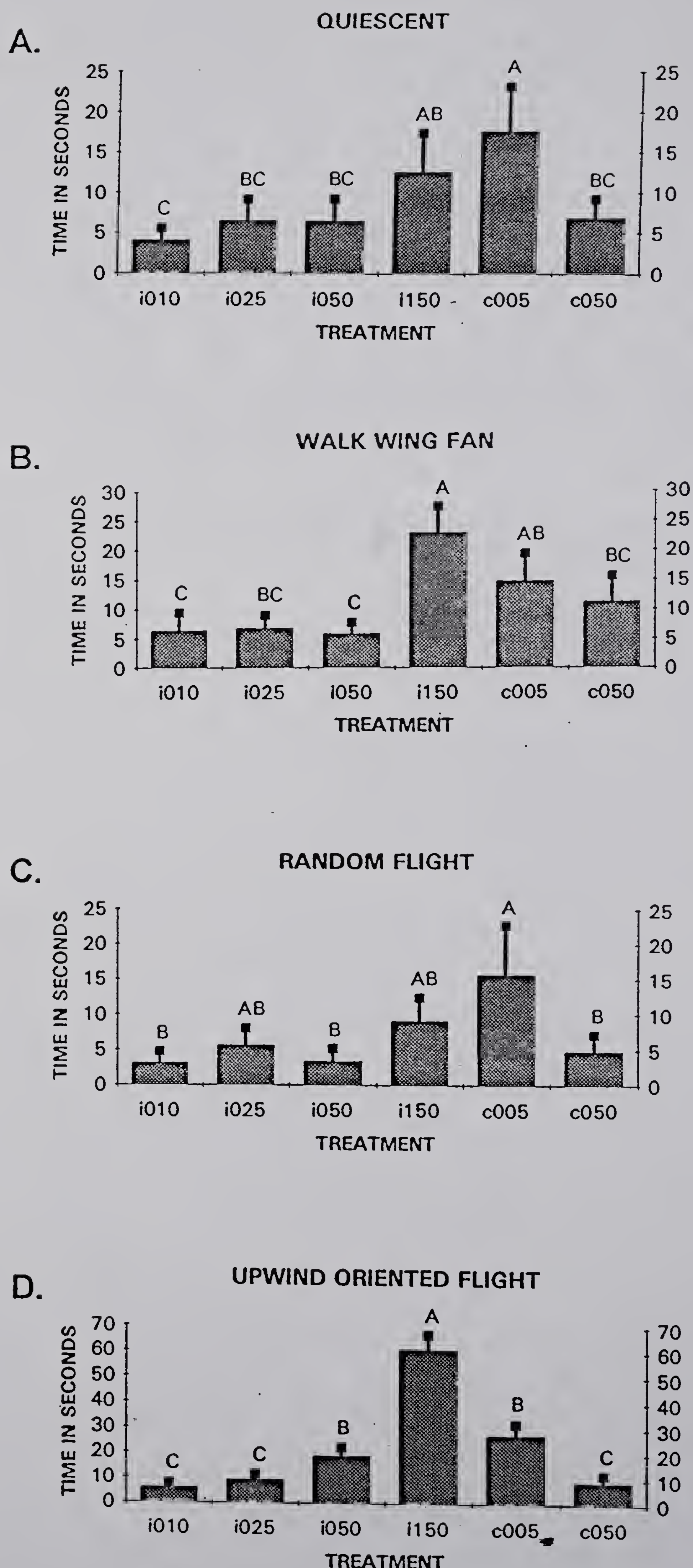
Mean time spent on each behavior. Males remained **quiescent** for shorter periods when presented with plumes of a high pulse frequency or the high volume continuous plume (Table 4) (Fig. 32a). Among the pulsed plumes, the shortest mean time spent on quiescent behavior was for the males exposed to the i010 plume (4.12 sec), followed by treatment i025 (6.57 sec), i050 (6.52 sec), and i150 (12.65 sec). The thin continuous plume c05 had the highest values for quiescence (17.72 sec), being comparable only to i150. Males exposed to the larger continuous plume c50 spent less time quiescent than the ones exposed to c05 (P<0.05, LSD GLM, SAS), but the same time as the males exposed to the pulsed plumes (P<0.05, LSD GLM, SAS).

Table 4. Mean time spent performing behaviors (in seconds, mean±standard deviation) for *C. cautella* males exposed to plumes of different structures.

TRT	QUI		RF		UF		WFW	
i010	4.12±1.92	c	3.16± 2.41	b	6.00± 3.00	c	6.42± 9.16	c
i025	6.57±6.50	bc	5.65± 5.56	ab	9.20± 5.01	c	6.84± 4.69	bc
i050	6.52±7.36	bc	3.45± 2.95	b	18.83±11.15	b	5.87± 3.45	c
i150	12.65±22.66	ab	9.12±10.71	ab	60.86±35.37	a	23.00±19.23	a
c005	17.72±30.49	a	15.70±49.37	a	27.56±18.10	c	14.76±20.36	ab
c050	6.90±5.44	bc	4.86± 5.28	b	9.03± 7.99	b	11.27±18.34	bc

Based on 20 moths tested for each plume structure. Means in the same column having no letters in common are significantly different ($P<0.05$), ANOVA with LSD option (SAS). Where **QUI** is quiescent, **RF** is random flight, **UF** is upwind flight, and **WFW** is walking and wing fanning

Fig. 32. Histogram of mean time *C. cautella* males spent on behaviors when exposed to five different pheromone plumes. The treatments are i010, i025, i150, c05, and c50. **A.** Time spent quiescent. The wide bars represent the mean values for the 20 moths tested per treatment, and the narrow bars represent standard deviations. Bars without letters in common are statistically different at α=0.05 level. **B.** Time spent wing fanning/walking. **C.** Time spent random flying. **D.** Time spent oriented flying. Details as per Fig. 24.


A.
QUIESCENT
TIME IN SECONDS
TREATMENT
i010 i025 i050 i150 c005 c050
C BC BC AB A BC
B.
WALK WING FAN
TIME IN SECONDS
TREATMENT
i010 i025 i050 i150 c005 c050
C BC C A AB BC
C.
RANDOM FLIGHT
TIME IN SECONDS
TREATMENT
i010 i025 i050 i150 c005 c050
B AB B AB A B
D.
UPWIND ORIENTED FLIGHT
TIME IN SECONDS
TREATMENT
i010 i025 i050 i150 c005 c050
C C B A B C

The time spent **wing fanning/walking** was longer for males exposed to the i150 (23.00 sec) and c05 (14.76 sec) plumes (Fig. 32b). Moths spent the same time wing fanning/walking when exposed to plumes c50 (11.27 sec), and i025 (6.84 sec). The shortest time spent on this behavior were for the pulsed plumes i010 (6.42 sec), and i050 (5.87 sec).

The males that initiated flight and engaged in **random flight** spent more time in this behavior if the plume was a continuous thin plume c05 (15.70 sec), and less time if the plume was either the pulsed i010 (3.16 sec), i050 (3.45 sec), or the large continuous c50 (4.86 sec) (Fig. 32c). Males exposed to plumes i150 and i025 spent an intermediate time performing random flight (i150=9.12, and i025=5.65 sec).

The time spent in **upwind flight** was significantly longer for males exposed to treatment i150 (60.86 sec) than to any other treatment (Fig. 32d). Males exposed to i050 and c05 spent the same time (P>0.05, LSD GLM, SAS) flying upwind. The lowest mean time spent flying upwind was for males tested using plume i010 (6.0 sec), although it is not significantly different (P>0.05, LSD GLM, SAS) from i025 (9.2 sec), or c50 (9.03 sec).

4.4. Discussion

Several studies have confirmed Wright's (1958) notion that intermittence of the pheromone signal is important to orientation (Kennedy *et al.* 1981; Willis & Baker 1984; Baker *et al.*; 1985, Vickers & Baker 1992). For several moth species, upwind progress toward a pheromone source is maintained only when flying in intermittent plumes

(Baker & Haynes 1989; Baker *et al.* 1989). Males of several moth species do not consistently sustain upwind flight in homogeneous clouds of pheromone, but they do so in pulsed ones (Baker *et al.* 1985). Some moths create their own signal intermittency. *C. cautella* males fly in and out of narrow filamentous homogeneous plume boundaries, but fly within the boundaries of wide turbulent plumes (Chapter III). Although not every turn and counterturn necessarily translated into plume contact, *C. cautella* males intercepted the filament plume at semi-regular intervals creating intermittence of the stimulus when zigzagging in and out of the pheromone plume. A similar effect has been reported for other moths where a plume from a point source is superimposed onto a continuous pheromone cloud (Kennedy *et al.* 1981; Willis & Baker 1984; Baker *et al.* 1985), or where males were able to resume upwind progress by flying in and out of a pheromone cloud that was present on only one side of the wind tunnel (Kennedy *et al.* 1981; Willis & Baker 1984).

There are several suggestions that the structure of odor plumes contains information that airborne males could decode (e.g., Wright. 1959, Conner et al. 1980, Chapter III). Wright (1958) suggested that turbulent plumes with a constant frequency of pulses would allow a male transecting the plume to resolve the direction of the wind by increasing (upwind) or decreasing (downwind) of the frequency of pulse interception, provided the male can resolve the intermittent signal. In turbulent, dispersed plumes, the temporal pattern of the pheromone plume could also contain information about the distance from the source (Wright 1958, Cardé et al. 1984). Conner et al. (1980) suggested that the temporal pattern of the internal structure of the pheromone plume could contain information important in species recognition, since females of several moth species pulse their glands while calling. Mafra-Neto and Cardé (Chapter III) observed that when males fly upwind in a pheromone plume, they disrupt the structure of the plume. It is conceivable that males flying downwind, in the modified pheromone plume could obtain information about the presence of other males flying in the plume upwind.

Although intermittency of signal was proven necessary for moths to sustain upwind flight (Kennedy et al. 1980, 1981, Willis & Baker 1984), so far the manipulation of the internal structure of pheromone plumes by the use of pulsers has not evoked "optimal" flight and transitions of pheromone-related behaviors in male moths (Cardé *et al.* 1984, Vickers and Baker 1992).

Cardé *et al.* (1984) introduced the concept of "apparency" between continuous and pulsed plumes: the temporal pattern of signal produced by pulsed plumes alters the rate of signal-to-noise comparisons transmitted by the sensory receptors, if the interpulse interval is sufficient to allow recovery of the receptor from stimulation. Additional comparisons provided by a pulsed pheromone message could diminish the threshold of behaviors and render a pulsed plume more apparent than a continuous one. The apparency hypothesis was tested by exposing quiescent *L. dispar* males to pheromone pulses of 0.5, 2 or 5 sec of duration, followed by a two-fold time interval of clean air, or to continuous pheromone plumes (Cardé *et al.* 1984). The moths responded similarly both in the proportions and latencies of wing fanning, and in the flight maneuvers, which generated indistinguishable flight tracks (for leg distance, leg angle, distance and rate of movement upwind and crosswind) among treatments. Although the pulse frequency used in these experiments were ca. 10 times slower than the frequency of small scale pulses characteristic of turbulent plumes (Murlis and Jones 1981, Willis et al. 1991, **Chapter III**), this slow frequency was similar to that reported by Baker and Haynes (1989), Murlis (1986), and Murlis and Jones (1981) for the encounter of stationary probes sampling plumes in shifting winds.

The concept of apparency (sensu Cardé *et al.* 1984) was supported by Kramer (1992) with two non-pheromone compounds, one as a "stimulant" and the other as an "inhibitor" of firing the pheromone receptors of *Bombyx mori* males. Although males did not walk toward either the stimulant or the inhibitor, they move toward the sources when the stimulant is presented and followed by pulses of the inhibitor. Some of these regimens of stimulant pulses followed by inhibitor pulses elicited faster and more direct source location than the female pheromone alone. The recovery of the receptor to the baseline of stimulation in pulsed plumes is conceivably achieved by the absence of pheromone when moths transect volumes of clean air. In *B. mori* case the recovery of the receptor from stimulation was "forced" by the inhibitor, which rendered the stimulant plume more apparent than plumes of pulsed pheromone (Kramer 1992).

Using a pulser similar to the one used in this study (Syntech, SFC-2), Vickers & Baker (1992) created plumes consisting of pulses of pheromone isolated by gaps of clean air. Although *Heliothis virescens*, their experimental insect, was able to sustain upwind flight toward the pulsed source, the overall levels of source location were relatively low (max. 44%). They suggested that the lack of interpulse strands of pheromone, usually present in plumes generated by point sources that continuously emit filaments, was the factor responsible or the resultant low levels of source location, a concept initially suggested by Dusenbery (1989). Since that experiment did not compare pulsed and continuous plumes, it was impossible to determine if source location was suppressed due to the lack of interpulse strands or to other undetermined variables.

Our data suggests that, unlike *L. dispar* (Cardé *et al.* 1984), *C. cautella* males perceive pulsed pheromone plumes as more apparent than continuous plumes. It is interesting to note that using plumes of equal volume, the increased apparency of pulsed plumes is evident only when comparing, for *C. cautella*, flight to continuous plumes with the flight to plumes with high pulse frequency (i010) (Fig. 29). Due to the mechanical characteristics of the pulser apparatus, the pulsed plumes tested by Cardé et al. (1984) were constrained to frequencies below 1.5 pulses per second. The high pheromone pulse frequencies to which *C. cautella* males respond better (ca. 10 Hz) were not tested for *L. dispar* (Cardé et al. 1984). The proposed inhibitory effect of the lack of interpulse strands in the plumes generated by the pulser (Dusenbery 1989; Vickers & Baker 1992) does not seem to occur for *C. cautella* since there was no evidence of suppression of the upwind flight and source location. In fact, *C. cautella* males fly slightly faster and more directly upwind to high frequency pulsed pheromone plumes, i.e., treatment i010, than to a continuous plume of same volume (c50). The percentage of males locating the source is slightly higher, although the differences are not statistically significant, for most of the pulsed treatments (an exception is i150=63%) than to the continuous plumes (i010=100%, i025=96%, i050=93%, contrasted with c50=93%, or c05=83%). The latency for source location was significantly shorter for the higher frequency pulsed pheromone plume than to the continuous plume of same volume (Fig. 29f).

The exclusion of fluctuations within the internal structure of a plume is technically difficult, and it was fully achieved in this experiment only by the low volume continuous plume c05, assuming that structure of smoke plumes accurately depicts the structure of pheromone plumes. The largest volume plume c50 was intermittent (Table 2), although with different characteristics than the other pulsed plumes tested, e.g., the pulses were longer and the interburst spaces of clean air were shorter than the pulsed plumes. This level of intermittency in the continuous plume might explain why several of the parameters of behavior and flight of moths to this plume were similar to those of high frequency intermittent plumes (i010 and i025).

Higher pulse frequency induces not only straighter upwind flights and faster location of the source, but also faster transitions of behavior leading to source location than with the non-pulsed plumes. With a decrease in pulse frequency, the net upwind flight velocity becomes slower, the time necessary for source location increases, the latency of late-in-the-sequence behaviors (sensu Chapter I) increases, the time spent on each behavior increases, and the success in source location diminishes. Our data indicate that there is a setting of pheromone pulse frequency (and volume) that will elicit optimal levels of pheromone-mediated behaviors and upwind flights on *C. cautella* males. For pulses of the same characteristics as the ones generated here, a hypothetical optimal setting for *C. cautella* would be located between 5 and 10 pulses per second (if all other variables are maintained constant).

Intermittency of the signal

If we assume that when flying upwind males contact every pulse (sic.), it is conceivable that the average *C. cautella* male was contacting up to 10.25 pulses of pheromone per second when exposed to plumes using treatment i010. The fastest net upwind velocity (YT=92.12 cm sec^{-1}) was recorded for a male flying to this treatment (i010), therefore it is possible that this male was transecting up to 16.52 bursts of pheromone per second. The characteristic tracks of males flying to this high pulse frequency shows low incidence of zigzagging flight, and sustained suppression of counterturning, which results in consistently straight and fast upwind flights leading directly the odor source.

It was expected from previous experiments (Chapter III) that *C. cautella* males would fly straight upwind in treatments i010, i025 and in the continuous plume with higher volume (c50), since they flew optimally to large pheromone plumes with mean pulse frequency of ca. 4.5 pulses per second. The maximum mean pulse frequency encountered by *C. cautella* males flying upwind to i010 plumes (10 to 16 pulses per second) is comparable to the highest level of pulse determination measured for a moth so far, i.e., 10 pulses per second (Christensen & Hildebrand 1988). It was expected that when a male achieved the i010 mean net upwind velocity and transected more than 10 pulses of pheromone per second, he would perceive that pheromone plume as a fused continuous string of pheromone, which in turn would trigger the switch from straight flight to crosswind flight, the behavior presumed to be associated with constant, homogeneous stimulation. The observed high success of source location and efficient upwind flight observed are, therefore, unexpected in light of the higher physiological limit pulse resolution measured for a moth so far.

There are several conceivable alternative explanations for the fact that *C. cautella* males sustained upwind flight under these circumstances. *C. cautella* males may have a higher limit for pulse resolution than that determined for other moth species. It is also conceivable that the flying males were not contacting with every pulse, there was, therefore, no "fusion" of the signal. Alternatively these males could be flying upwind while contacting all the pulses of the plume, they would experience fusion of the signal and the turn crosswind, exiting the plume. Outside the plume the fusion of the signal vanishes, allowing the male to reenter the plume and fly straight upwind again. A variation of this motif is that the male could fly fast and straight upwind up to the moment of the signal fusion, when the male would reduce its velocity, and as soon as the fusion of the signal vanishes, the male would accelerate to the previous speed.

Males flying to i025 plumes also showed flight characteristics similar to those of treatment i010, fast and very straight upwind, but the "rapidity of source location" (or "efficiency") of flight is consistently faster, although not always statistically significant, for the latter. When the frequency of pulses is reduced, the efficiency of the upwind flight also decreases, this is due primarily to a higher incidence of casting (casting was embedded under the general classification of "oriented flight") in the flight of tested males. The proportion of males locating the pheromone source is similar for all treatments (except for i150), although the latency for this behavioral transition increased substantially with the decrease of pulse frequency.

Casting

It has been shown that males of numerous species perform three major patterns of flight when flying upwind to a pheromone source . The first pattern of **oriented flight** is seen when, in the presence of pheromone the male zigzags upwind, making forward progress over the ground, i.e., a positive net upwind velocity (+YT) (Kennedy & Marsh 1974; Mash *et al.* 1978, Kennedy 1983; David *et al.* 1983; Baker 1986). The second pattern, casting, occurs following loss of pheromone, after which the male switches to broad lateral flight excursions (**casting**), making little or no progress in reference to the ground (0 YT) (Kennedy & Marsh 1974;

Kennedy 1983; David *et al.* 1983; Baker 1986; Baker & Vogt 1988, Chapter I). The latency for this switch from upwind movement to casting movement following the loss of the plume is in the range of 0.5 to 1.0 sec for walking *Bombix mori* (Kramer 1975) and *Plodia interpunctella* (Marsh *et al.* 1981), in the range of 0.3 to 0.5 sec for *Antheraea polyphemus* (Baker & Vogt 1988), 1.0 sec for *Lymantria dispar* (Kuenen & Cardé, unpublished), 0.25 sec for *Heliothis virescens* (Vickers & Baker 1992), and as little as 0.15 sec for *Grapholita molesta* (Baker & Haynes 1987). The third flight pattern is **in-flight arrestment**, during which the male maintains contact with the pheromone plume in a contained (very small interleg distance), stationary (0 YT) zigzagging flight, that often results in the male abandoning the pheromone plume (Chapter I).

Casting was a predominant feature in the flight of *C. cautella* to the low volume continuous plume (i15) and the plume with 1.5 sec of interval between pulses (c05) (Fig. 24). Moths flying to the i150 casted, presumably, following the loss of pheromone and flew upwind, presumably, after intercepting the pheromone pulse. Moths flying to the c05, continuous, low volume plume, also casted, presumably, after loss of contact with the narrow pheromone plume and flew upwind, presumably, after intercepting the continuous pheromone plume. The tracks of males flying to these two treatments showed reduced YT, ground velocity, DZ and airspeed when compared to the other four treatments. Males submitted to these two treatments also showed bimodal distribution of their drift and track angles while flying upwind, in contrast to a clear unimodal distribution for males flying to the other treatments (Fig. 27a and 27c).

This contrast suggests that the upwind flight behavior of males is strongly modulated by the size of the plume and the pulse frequency. Small plume sizes (e.g., filamentous plumes) and low pulse frequencies make the plume less apparent and more difficult to locate. With a slowly pulsed plume the pheromone physically disappears for relatively long periods, when a flying male would start casting. In a small plume, the pheromone might be present all the time, but it is difficult to locate, and the males seem to lose contact frequently, switching from oriented upwind flight to casting flight (when it takes longer for the male to regain contact with the plume).

Because casting behavior was more common and evident in flight tracks for the i150 plumes, this was the only treatment yielding bimodal distribution of course angles (Fig. 27b). *C. cautella* males flying to the i150 plumes could take more than five minutes to move from the release cage to the pheromone source. These males alternated between straighter surges of upwind flights that lasted ca 0.33 sec, presumably just after encountering a pheromone pulse, and casting for long intervals until the interception of another pheromone pulse. During the casting part of the flight the male could either maintain his position in relation to the ground or slowly drift downwind. Males that flew for several minutes before locating the source were the ones that lost ground while casting between pulses. If the male maintained the position until encountering a new pulse (when he would fly upwind) he located the source in less than two minutes. This sequence of behaviors, casting, dashing upwind, and then casting again, switched concurrent to the arrival or loss of a pheromone pulse, indicating that casting males were responding to single pulses of pheromone when dashing upwind.

Wright (1958) suggested that the interval of a counterturning program was modulated by changes in chemosensory input, and this input was metered by the insect as the frequency of the odor pulses it detected while transecting an intermittent odor plume. He proposed that the insect would turn less often when the pulse frequency increased and more often when it decreased. Wright's proposed mechanism of olfactory guidance is generally vague in its definitions. In this study we show that males respond differently to various intermittent plumes. A deficiency in Wright's mechanism is that it does not address males responding to pulses, nor does it address the influence of the pulse and its form and dimensions on flight behavior.

An important aspect of the upwind flight of males toward turbulent plumes still remains untouched: do males respond behaviorally to **individual** pulses?

We believe that males do respond to individual pulses of pheromone and a clear evidence for this is seen in the sequence of flight behaviors (casting-dashing-casting) of males flying to the plume i150. Below we outline the male's expected in-flight behavior when encountering pheromone plumes containing pulses of different structures, with the perspective of the maintenance of a straight flight path. We showed that straight flights are the result of the suppression of the counterturning program, with drastic reduction of lateral velocity (XT) and maintenance of a positive net upwind flight velocity (YT). According to our hypothesis, males are able to suppress counterturning and fly straight upwind if: **(1)** the tempo of pheromone pulses is above a certain frequency, i.e., the pulse reaches the male antenna before the male starts counterturning. **(2)** a refractory period exists, during which contact with pheromone pulse or clean air has no affect on behavior. **(3)** the pheromone pulse is at a suitable concentration, i.e., the pheromone concentration is above the threshold for that behavior (sensu Chapter I), but below the levels that promote the switch to crosswind turning. **(4)** The pheromone pulse duration is short enough to hit the antenna and "disappear," being replaced by clean air (or pheromone concentrations below the threshold for that behavior), and finally **(5)** the lateral dimensions of the pulse are large enough, so that the male is very likely to contact a new pheromone pulse before the end of the period stated in (1).

The fact that straight flights were more frequent for the treatments of a large continuous plume (c50) and two high-frequency pulsed plumes (i010 and i025), and less frequent for both the smaller continuous plume (c05) and for the treatment with long intervals between pulses (i150), supports the model outlined above. In addition to the evidence already presented, in pilot studies we observed that *C. cautella* males, when flying (casting-dashing-casting) to the i150 plume, could be induced to fly more steadily upwind (higher net upwind velocity) if the frequency of pulses was elevated. The higher the frequency, the faster (and straighter) upwind that male would fly. If the male had not yet located the source, we could induce casting and downwind drift simply by reducing the frequency of pulses to levels lower than that delivered using treatment i150. The fact that one can so effectively modulate the pheromone-mediated upwind flight of a male by changing the pheromone pulse frequency is, as previously proposed by Vickers & Baker (1992) and Mafra-Neto and Cardé (Chapter III), evidence that, the pheromone pulse and the corresponding reaction are the *"basic building blocks*" (Vickers and Baker 1992) of the pheromone-mediated behavior, including upwind flight.

APPENDIX A

CHAPTER III FLIGHT TRACK ANALYSIS TABLES

Table 5. Track angle for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the track angle with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRACK ANGLE

TRT	N	Mean	SD
11A	20	62.02963	7.961151
12A	20	60.44709	8.310591
13A	20	58.53285	9.368357
11B	20	49.73311	13.50105
12B	20	43.38578	16.09288
13B	20	40.42903	10.12745
11C	20	41.46295	12.04776
12C	20	38.31903	10.75404
13C	20	34.29927	11.59957

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	355.9811683			
TRT	8	184.9811085	23.1226386	23.12	0.0001
Error	171	171.0000598	1.0000003		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	12.31016454	12.31016454	12.31	0.0006
11a vs 11c	1	40.5687801	40.5687801	40.57	0.0001
11a vs 12a	1	0.37818308	0.37818308	0.38	0.5394
11a vs 12b	1	21.56545362	21.56545362	21.57	0.0001
11a vs 12c	1	62.80463793	62.80463793	62.8	0.0001
11a vs 13a	1	1.61796914	1.61796914	1.62	0.2051
11a vs 13b	1	56.23381712	56.23381712	56.23	0.0001
11a vs 13c	1	77.70150347	77.70150347	77.7	0.0001
11b vs 11c	1	4.17775449	4.17775449	4.18	0.0425
11b vs 12a	1	9.13404798	9.13404798	9.13	0.0029
11b vs 12b	1	1.82607086	1.82607086	1.83	0.1784
11b vs 12c	1	8.74584218	8.74584218	8.75	0.0035
11b vs 13a	1	5.73502744	5.73502744	5.74	0.0177
11b vs 13b	1	6.07814607	6.07814607	6.08	0.0147
11b vs 13c	1	15.03675855	15.03675855	15.04	0.0002
11c vs 12a	1	33.64820322	33.64820322	33.65	0.0001
11c vs 12b	1	0.18297512	0.18297512	0.18	0.6694
11c vs 12c	1	0.75799902	0.75799902	0.76	0.3852
11c vs 13a	1	25.02041292	25.02041292	25.02	0.0001
11c vs 13b	1	0.08630878	0.08630878	0.09	0.7693
11c vs 13c	1	3.66953881	3.66953881	3.67	0.0571
12a vs 12b	1	17.74673301	17.74673301	17.75	0.0001
12a vs 12c	1	53.01691106	53.01691106	53.02	0.0001
12a vs 13a	1	0.46729088	0.46729088	0.47	0.4952
12a vs 13b	1	46.69584432	46.69584432	46.7	0.0001
12a vs 13c	1	67.15664805	67.15664805	67.16	0.0001
12b vs 12c	1	1.37052469	1.37052469	1.37	0.2434
12b vs 13a	1	13.23348887	13.23348887	13.23	0.0004
12b vs 13b	1	0.48361204	0.48361204	0.48	0.4877
12b vs 13c	1	4.19609765	4.19609765	4.2	0.042
12c vs 13a	1	40.17388042	40.17388042	40.17	0.0001
12c vs 13b	1	0.40804811	0.40804811	0.41	0.5238
12c vs 13c	1	1.2916499	1.2916499	1.29	0.2573
13a vs 13b	1	34.43982933	34.43982933	34.44	0.0001
13a vs 13c	1	52.83168113	52.83168113	52.83	0.0001
13b vs 13c	1	3.16925863	3.16925863	3.17	0.0768

Table 6. Course angle for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the course angle with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

COURSE ANGLE

TRT	N	Mean	SD
11A			
12A	20	25.14869	6.829091
13A	20	20.26201	6.059041
11B	20	22.94107	4.805397
12B	20	20.55232	6.104638
13B	20	17.80879	6.597271
11C	20	17.16885	4.545608
12C	20	19.39988	5.221719
13C	20	17.13253	4.28901
	20	14.81089	4.690001

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	226.57586618			
Model	8	55.57585677	6.9469821	6.95	0.0001
Error	171	171.00000941	1.0000000 6		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	5.0359436	5.0359436	5.04	0.026
11a vs 11c	1	8.9438438	8.94384382	8.94	0.003
11a vs 12a	1	5.7300721	5.73007213	5.73	0.018
11a vs 12b	1	11.950712	11.9507115	11.95	7E-04
11a vs 12c	1	19.762145	19.76214463	19.76	1E-04
11a vs 13a	1	1.3978694	1.39786938	1.4	0.239
11a vs 13b	1	18.923816	18.92381592	18.92	1E-04
11a vs 13c	1	31.14264	31.14264032	31.14	1E-04
11b vs 11c	1	0.4116099	0.41160989	0.41	0.522
11b vs 12a	1	0.0227859	0.02278592	0.02	0.88
11b vs 12b	1	1.8633304	1.86333038	1.86	0.174
11b vs 12c	1	4.2021144	4.20211438	4.2	0.042
11b vs 13a	1	1.8907509	1.8907509	1.89	0.171
11b vs 13b	1	3.9523709	3.95237085	3.95	0.048
11b vs 13c	1	11.124716	11.12471585	11.12	0.001
11c vs 12a	1	0.232347	0.23234695	0.23	0.63
11c vs 12b	1	0.7152332	0.71523324	0.72	0.399
11c vs 12c	1	2.2517077	2.25170772	2.25	0.135
11c vs 13a	1	4.9803477	4.98034765	4.98	0.027
11c vs 13b	1	2.077036	2.07703603	2.08	0.151
11c vs 13c	1	8.5496599	8.54965987	8.55	0.004
12a vs 12b	1	1.500144	1.50014396	1.5	0.222
12a vs 12c	1	3.554352	3.55435199	3.55	0.061
12a vs 13a	1	2.4003175	2.40031748	2.4	0.123
12a vs 13b	1	3.3351392	3.33513915	3.34	0.07
12a vs 13c	1	10.122847	10.12284739	10.12	0.002
12b vs 12c	1	0.1477137	0.14771373	0.15	0.701
12b vs 13a	1	7.9081411	7.90814113	7.91	0.006
12b vs 13b	1	0.1276032	0.12760322	0.13	0.721
12b vs 13c	1	2.743399	2.743399	2.74	0.1
12c vs 13a	1	16.264759	16.26475908	16.26	1E-04
12c vs 13b	1	0.0006753	0.0006753	0	0.979
12c vs 13c	1	2.6688803	2.66888026	2.67	0.104
13a vs 13b	1	15.229814	15.22981427	15.23	1E-04
13a vs 13c	1	29.320451	29.32045123	29.32	1E-04
13b vs 13c	1	2.6067235	2.6067235	2.61	0.108

Table 7. Drift angle for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the drift angle with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

DRIFT ANGLE

TRT	N	Mean	SD
11A	20	36.88094	3.569737
12A	20	40.18508	9.457173
13A	20	35.59178	7.630183
11B	20	29.18078	8.883439
12B	20	25.57699	11.90869
13B	20	23.26018	6.286915
11C	20	22.06307	8.700876
12C	20	21.18649	7.976764
13C	20	19.48838	7.681837

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	382.3024			
TRT	8	211.3023	26.41279	26.41	0.0001
Error	171	171.0001	1		

C.

Contrast	*DF*	*Contrast SS*	*Mean Square*	*F Value*	*Pr > F*
11a vs 11b	1	12.937763	12.9377625	12.94	4E-04
11a vs 11c	1	49.649388	49.64938762	49.65	1E-04
11a vs 12a	1	2.1368602	2.13686016	2.14	0.146
11a vs 12b	1	16.534585	16.53458526	16.53	1E-04
11a vs 12c	1	64.504196	64.50419598	64.5	1E-04
11a vs 13a	1	0.4684007	0.46840067	0.47	0.495
11a vs 13b	1	70.990004	70.99000377	70.99	1E-04
11a vs 13c	1	84.316229	84.31622895	84.32	1E-04
11b vs 11c	1	6.5530818	6.55308178	6.55	0.011
11b vs 12a	1	14.385794	14.3857938	14.39	2E-04
11b vs 12b	1	1.1767479	1.17674785	1.18	0.28
11b vs 12c	1	8.9668657	8.9668657	8.97	0.003
11b vs 13a	1	5.9942125	5.99421248	5.99	0.015
11b vs 13b	1	5.919212	5.91921202	5.92	0.016
11b vs 13c	1	13.622191	13.62219083	13.62	3E-04
11c vs 12a	1	39.772486	39.77248578	39.77	1E-04
11c vs 12b	1	1.135304	1.13530401	1.14	0.288
11c vs 12c	1	0.1102932	0.11029321	0.11	0.74
11c vs 13a	1	27.3326	27.33260031	27.33	1E-04
11c vs 13b	1	0.2487324	0.2487324	0.25	0.619
11c vs 13c	1	0.9841482	0.98414822	0.98	0.323
12a vs 12b	1	18.455489	18.4554894	18.46	1E-04
12a vs 12c	1	47.161868	47.1618684	47.16	1E-04
12a vs 13a	1	2.8577473	2.8577473	2.86	0.093
12a vs 13b	1	44.423963	44.42396258	44.42	1E-04
12a vs 13c	1	57.71053	57.71052954	57.71	1E-04
12b vs 12c	1	1.8765499	1.87654992	1.88	0.173
12b vs 13a	1	10.027728	10.02772846	10.03	0.002
12b vs 13b	1	0.5919908	0.59199084	0.59	0.443
12b vs 13c	1	3.6918402	3.69184023	3.69	0.056
12c vs 13a	1	34.060533	34.06053323	34.06	1E-04
12c vs 13b	1	0.8337358	0.83373575	0.83	0.363
12c vs 13c	1	0.4702545	0.47025446	0.47	0.494
13a vs 13b	1	31.115311	31.11531057	31.12	1E-04
13a vs 13c	1	44.240754	44.2407537	44.24	1E-04
13b vs 13c	1	2.8875678	2.88756776	2.89	0.091

Table 8. Net crosswind speed (XY) for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the net crosswind speed with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

CROSSWIND SPEED

TRT	N	Mean	SD
11A	20	26.31308	8.234282
12A	20	20.63576	8.217599
13A	20	24.26136	5.392875
11B	20	24.08593	6.075711
12B	20	20.98686	6.944948
13B	20	21.00237	4.810494
11C	20	25.20115	6.281364
12C	20	22.54972	5.620387
13C	20	19.57404	4.87911

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	193.57247367			
TRT	8	22.57247038	2.82155880	2.82	0.0058
Error	171	171.00000329	1.00000002		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	0.94734	0.94733996	0.95	0.332
11a vs 11c	1	0.2305394	0.23053942	0.23	0.632
11a vs 12a	1	4.7633678	4.76336783	4.76	0.03
11a vs 12b	1	4.889626	4.889626	4.89	0.028
11a vs 12c	1	2.8498993	2.8498993	2.85	0.093
11a vs 13a	1	0.8689595	0.86895948	0.87	0.353
11a vs 13b	1	6.2023915	6.20239147	6.2	0.014
11a vs 13c	1	9.9148634	9.91486344	9.91	0.002
11b vs 11c	1	0.3257091	0.32570906	0.33	0.569
11b vs 12a	1	2.279454	2.27945397	2.28	0.133
11b vs 12b	1	2.255932	2.25593197	2.26	0.135
11b vs 12c	1	0.68901	0.68901003	0.69	0.408
11b vs 13a	1	0.0093264	0.00932637	0.01	0.923
11b vs 13b	1	3.1665435	3.16654348	3.17	0.077
11b vs 13c	1	6.7052604	6.70526038	6.71	0.01
11c vs 12a	1	3.8964169	3.89641685	3.9	0.05
11c vs 12b	1	4.050794	4.05079402	4.05	0.046
11c vs 12c	1	1.9790837	1.9790837	1.98	0.161
11c vs 13a	1	0.257725	0.25772499	0.26	0.612
11c vs 13b	1	5.6328465	5.6328465	5.63	0.019
11c vs 13c	1	10.010685	10.01068476	10.01	0.002
12a vs 12b	1	0.0212973	0.02129727	0.02	0.884
12a vs 12c	1	0.7391656	0.73916564	0.74	0.391
12a vs 13a	1	2.7211896	2.72118958	2.72	0.101
12a vs 13b	1	0.029646	0.02964595	0.03	0.864
12a vs 13c	1	0.2468405	0.24684047	0.25	0.62
12b vs 12c	1	0.6119982	0.61199822	0.61	0.435
12b vs 13a	1	2.7736687	2.77366872	2.77	0.098
12b vs 13b	1	6.739E-05	0.00006739	0	0.994
12b vs 13c	1	0.5541689	0.55416888	0.55	0.458
12c vs 13a	1	0.9657656	0.96576556	0.97	0.327
12c vs 13b	1	0.8749504	0.87495041	0.87	0.351
12c vs 13c	1	3.196945	3.19694495	3.2	0.076
13a vs 13b	1	4.0674968	4.06749679	4.07	0.045
13a vs 13c	1	8.3083723	8.30837233	8.31	0.005
13b vs 13c	1	0.8691235	0.86912354	0.87	0.353

Table 9. Net upwind speed for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the net upwind speed with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

NET UPWIND SPEED

TRT	N	Mean	SD
11A	20	18.10801	3.944561
12A	20	16.62499	4.712274
13A	20	19.38923	4.983181
11B	20	24.79281	8.150413
12B	20	29.60562	14.37408
13B	20	30.1906	8.465548
11C	20	37.13215	9.897557
12C	20	32.80496	8.254016
13C	20	34.73842	10.18689

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	360.8523933			
TRT	8	189.8532301	23.7316538	23.73	0.0001
Error	171	170.9991632	0.9999951		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	10.900612	10.90061151	10.9	0.001
11a vs 11c	1	63.761655	63.76165475	63.76	1E-04
11a vs 12a	1	1.1647388	1.16473877	1.16	0.282
11a vs 12b	1	11.900127	11.90012677	11.9	7E-04
11a vs 12c	1	51.619736	51.61973644	51.62	1E-04
11a vs 13a	1	0.8127957	0.81279568	0.81	0.369
11a vs 13b	1	33.474085	33.47408465	33.47	1E-04
11a vs 13c	1	46.352713	46.35271303	46.35	1E-04
11b vs 11c	1	18.524024	18.52402397	18.52	1E-04
11b vs 12a	1	15.053509	15.0535092	15.05	1E-04
11b vs 12b	1	1.6966652	1.69666518	1.7	0.195
11b vs 12c	1	9.5415393	9.54153926	9.54	0.002
11b vs 13a	1	6.398934	6.39893401	6.4	0.012
11b vs 13b	1	4.2197504	4.21975042	4.22	0.042
11b vs 13c	1	11.623265	11.62326457	11.62	8E-04
11c vs 12a	1	69.992609	69.99260931	69.99	1E-04
11c vs 12b	1	3.7198315	3.71983148	3.72	0.055
11c vs 12c	1	2.2547311	2.25473113	2.25	0.135
11c vs 13a	1	51.274638	51.27463819	51.27	1E-04
11c vs 13b	1	5.6812953	5.68129534	5.68	0.018
11c vs 13c	1	0.5680634	0.56806342	0.57	0.452
12a vs 12b	1	14.727462	14.72746171	14.73	2E-04
12a vs 12c	1	57.960168	57.96016761	57.96	1E-04
12a vs 13a	1	3.2488921	3.2488921	3.25	0.073
12a vs 13b	1	39.208251	39.20825061	39.21	1E-04
12a vs 13c	1	52.087499	52.08749947	52.09	1E-04
12b vs 12c	1	0.7451163	0.74511631	0.75	0.389
12b vs 13a	1	9.0193443	9.01934431	9.02	0.003
12b vs 13b	1	0.024594	0.02459404	0.02	0.876
12b vs 13c	1	1.6975996	1.69759957	1.7	0.194
12c vs 13a	1	38.721984	38.7219837	38.72	1E-04
12c vs 13b	1	0.9778506	0.97785063	0.98	0.324
12c vs 13c	1	0.4349305	0.43493048	0.43	0.511
13a vs 13b	1	24.180959	24.18095889	24.18	1E-04
13a vs 13c	1	36.638941	36.63894122	36.64	1E-04
13b vs 13c	1	2.3578338	2.35783379	2.36	0.127

Table 10. Ground speed for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the ground speed with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

GROUND SPEED

TRT	N	Mean	SD
11A	20	36.16606	7.267477
12A	20	29.82752	9.513238
13A	20	35.18736	6.414504
11B	20	38.92913	7.274697
12B	20	41.15997	12.72966
13B	20	41.20614	8.180126
11C	20	49.92372	9.621475
12C	20	43.82696	8.321089
13C	20	43.6563	8.340706

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	240.07279556			
TRT	8	69.07268145	8.63408518	8.63	0.0001
Error	171	171.00011411	1.00000067		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	1.4440646	1.44406458	1.44	0.231
11a vs 11c	1	26.03678	26.03677983	26.04	1E-04
11a vs 12a	1	5.6067131	5.60671305	5.61	0.019
11a vs 12b	1	2.3214267	2.32142671	2.32	0.13
11a vs 12c	1	9.616709	9.61670904	9.62	0.002
11a vs 13a	1	0.2038825	0.20388246	0.2	0.652
11a vs 13b	1	4.2432795	4.24327949	4.24	0.041
11a vs 13c	1	9.1685486	9.16854861	9.17	0.003
11b vs 11c	1	16.616618	16.61661783	16.62	1E-04
11b vs 12a	1	11.551756	11.55175594	11.55	8E-04
11b vs 12b	1	0.4630174	0.46301744	0.46	0.497
11b vs 12c	1	3.9273601	3.9273601	3.93	0.049
11b vs 13a	1	2.9767854	2.97678537	2.98	0.086
11b vs 13b	1	0.8653183	0.86531828	0.87	0.354
11b vs 13c	1	3.64871	3.64871002	3.65	0.058
11c vs 12a	1	44.11932	44.11932019	44.12	1E-04
11c vs 12b	1	6.0328503	6.03285034	6.03	0.015
11c vs 12c	1	4.5942212	4.59422118	4.59	0.034
11c vs 13a	1	32.480089	32.48008949	32.48	1E-04
11c vs 13b	1	9.5300729	9.53007285	9.53	0.002
11c vs 13c	1	4.845253	4.84525295	4.85	0.029
12a vs 12b	1	10.170378	10.17037762	10.17	0.002
12a vs 12c	1	24.537456	24.5374559	24.54	1E-04
12a vs 13a	1	4.3643498	4.36434984	4.36	0.038
12a vs 13b	1	16.449797	16.44979698	16.45	1E-04
12a vs 13c	1	23.894112	23.89411208	23.89	1E-04
12b vs 12c	1	0.6150717	0.61507169	0.62	0.434
12b vs 13a	1	3.5112014	3.51120135	3.51	0.063
12b vs 13b	1	0.0001862	0.00018624	0	0.989
12b vs 13c	1	0.5381176	0.53811763	0.54	0.464
12c vs 13a	1	13.523818	13.52381752	13.52	3E-04
12c vs 13b	1	1.0089509	1.00895089	1.01	0.317
12c vs 13c	1	0.0041962	0.00419615	0	0.948
13a vs 13b	1	6.7047448	6.70474481	6.7	0.01
13a vs 13c	1	12.956569	12.95656945	12.96	4E-04
13b vs 13c	1	0.8797269	0.87972688	0.88	0.35

Table 11. Airspeed for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the airspeed with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

AIR SPEED

TRT	N	Mean	SD
11A	20	67.29538	4.422328
12A	20	63.49141	7.519972
13A	20	68.00789	6.373183
11B	20	74.27113	8.975521
12B	20	77.82386	14.67821
13B	20	78.88243	8.850872
11C	20	86.12779	10.75152
12C	20	81.97487	9.050851
13C	20	82.92343	10.28896

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	324.9768665			
TRT	8	153.9767492	19.2470936	19.25	0.0001
Error	171	171.0001173	1.0000007		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	9.7208512	9.72085118	9.72	0.002
11a vs 11c	1	52.483069	52.48306862	52.48	1E-04
11a vs 12a	1	3.8025809	3.80258094	3.8	0.053
11a vs 12b	1	9.433667	9.43366701	9.43	0.003
11a vs 12c	1	42.471038	42.4710384	42.47	1E-04
11a vs 13a	1	0.1687331	0.16873305	0.17	0.682
11a vs 13b	1	27.429365	27.42936482	27.43	1E-04
11a vs 13c	1	38.946885	38.94688539	38.95	1E-04
11b vs 11c	1	14.333591	14.33359085	14.33	2E-04
11b vs 12a	1	16.950232	16.95023178	16.95	1E-04
11b vs 12b	1	0.8528004	0.85280038	0.85	0.357
11b vs 12c	1	7.3053058	7.30530581	7.31	0.008
11b vs 13a	1	6.474535	6.47453497	6.47	0.012
11b vs 13b	1	2.6764499	2.67644988	2.68	0.104
11b vs 13c	1	8.0314378	8.03143779	8.03	0.005
11c vs 12a	1	59.531835	59.53183474	59.53	1E-04
11c vs 12b	1	4.1659121	4.16591212	4.17	0.043
11c vs 12c	1	1.7463889	1.74638887	1.75	0.188
11c vs 13a	1	42.036564	42.03656353	42.04	1E-04
11c vs 13b	1	5.413737	5.41373698	5.41	0.021
11c vs 13c	1	0.9273013	0.92730133	0.93	0.337
12a vs 12b	1	15.104337	15.104337	15.1	1E-04
12a vs 12c	1	49.345426	49.345426	49.35	1E-04
12a vs 13a	1	4.1986533	4.19865325	4.2	0.042
12a vs 13b	1	35.122999	35.12299927	35.12	1E-04
12a vs 13c	1	46.499132	46.49913245	46.5	1E-04
12b vs 12c	1	1.1588931	1.15889309	1.16	0.283
12b vs 13a	1	7.5256329	7.52563288	7.53	0.007
12b vs 13b	1	0.0762853	0.07628527	0.08	0.783
12b vs 13c	1	1.6187086	1.6187086	1.62	0.205
12c vs 13a	1	31.840144	31.84014375	31.84	1E-04
12c vs 13b	1	1.1934835	1.19348349	1.19	0.276
12c vs 13c	1	0.0958312	0.09583121	0.1	0.757
13a vs 13b	1	19.882446	19.88244595	19.88	1E-04
13a vs 13c	1	30.375939	30.37593896	30.38	1E-04
13b vs 13c	1	1.7730218	1.77302179	1.77	0.185

Table 12. Orientation angle for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the orientation angle with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

ORIENTATION ANGLE

TRT	N	Mean	SD
11A	20	0.150428	3.887611
12A	20	-1.350739	3.717614
13A	20	0.359417	3.677584
11B	20	0.631476	3.76987
12B	20	2.003244	5.043088
13B	20	-0.710703	3.586968
11C	20	-1.611404	5.865995
12C	20	2.176149	4.68427
13C	20	2.27399	5.516677

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	186.88132727			
TRT	8	15.88121992	1.98515249	1.99	0.0510
Error	171	171.00010735	1.00000063		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	0.1578201	0.15782006	0.16	0.692
11a vs 11c	1	1.2535695	1.25356947	1.25	0.264
11a vs 12a	1	1.5576745	1.55767449	1.56	0.214
11a vs 12b	1	1.6933402	1.69334022	1.69	0.195
11a vs 12c	1	2.2147859	2.21478588	2.21	0.139
11a vs 13a	1	0.0305023	0.03050226	0.03	0.862
11a vs 13b	1	0.5300581	0.53005812	0.53	0.468
11a vs 13c	1	1.980151	1.98015104	1.98	0.161
11b vs 11c	1	2.0692397	2.06923968	2.07	0.152
11b vs 12a	1	2.8032905	2.80329047	2.8	0.096
11b vs 12b	1	0.9493083	0.94930826	0.95	0.331
11b vs 12c	1	1.3199065	1.3199065	1.32	0.252
11b vs 13a	1	0.0533708	0.05337082	0.05	0.818
11b vs 13b	1	1.3305482	1.33054823	1.33	0.25
11b vs 13c	1	1.2085645	1.20856451	1.21	0.273
11c vs 12a	1	0.0281755	0.02817545	0.03	0.867
11c vs 12b	1	4.3666804	4.36668035	4.37	0.038
11c vs 12c	1	5.0913816	5.09138163	5.09	0.025
11c vs 13a	1	1.6206005	1.62060052	1.62	0.205
11c vs 13b	1	0.3432012	0.34320121	0.34	0.559
11c vs 13c	1	4.6562149	4.65621488	4.66	0.032
12a vs 12b	1	5.7315805	5.73158052	5.73	0.018
12a vs 12c	1	6.9562978	6.95629781	6.96	0.009
12a vs 13a	1	2.1390396	2.13903962	2.14	0.145
12a vs 13b	1	0.3070011	0.30700109	0.31	0.58
12a vs 13c	1	5.937789	5.93778902	5.94	0.016
12b vs 12c	1	0.0126211	0.01262105	0.01	0.911
12b vs 13a	1	1.3872452	1.38724519	1.39	0.241
12b vs 13b	1	3.8463167	3.84631671	3.85	0.052
12b vs 13c	1	0.0262425	0.02624253	0.03	0.872
12c vs 13a	1	1.8611749	1.86117491	1.86	0.174
12c vs 13b	1	4.7884067	4.78840667	4.79	0.03
12c vs 13c	1	0.0036555	0.00365548	0	0.952
13a vs 13b	1	0.8678414	0.86784137	0.87	0.353
13a vs 13c	1	1.6677585	1.66775852	1.67	0.198
13b vs 13c	1	4.1147307	4.11473069	4.11	0.044

Table 13. Interleg angle for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the interleg angle with weighting inversely proportional to cell variance.

A.

INTERLEG ANGLE

TRT	N	Mean	SD
11A	20	150.8024	3.55077
12A	20	147.0825	6.191171
13A	20	149.2071	4.760964
11B	20	155.3945	4.817512
12B	20	156.2028	6.473446
13B	20	157.3315	3.813215
11C	20	159.8779	2.673268
12C	20	158.1143	3.880602
13C	20	158.4224	4.302321

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	353.0633885			
TRT	8	182.0635928	22.7579491	22.76	0.0001
Error	171	170.9997957	0.9999988		

Table 14. Transverse component of the visual flow for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the transverse component of the visual flow with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRANSVERSE COMPONENT OF IMAGE FLOW

TRT	N	Mean	SD
11A	20	18.04412	4.297516
12A	20	14.82409	3.961745
13A	20	16.67807	3.106511
11B	20	15.15534	4.093899
12B	20	12.94964	4.422807
13B	20	12.75828	3.125106
11C	20	13.95344	3.428406
12C	20	12.57555	3.037372
13C	20	11.04695	3.163035

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	232.14625860			
TRT	8	61.14617508	7.64327189	7.64	0.0001
Error	171	171.00008352	1.00000049		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	4.7376697	4.73766966	4.74	0.031
11a vs 11c	1	11.073601	11.07360072	11.07	0.001
11a vs 12a	1	6.0698921	6.06989214	6.07	0.015
11a vs 12b	1	13.649168	13.64916818	13.65	3E-04
11a vs 12c	1	21.596748	21.59674769	21.6	1E-04
11a vs 13a	1	1.3272851	1.32728506	1.33	0.251
11a vs 13b	1	19.791196	19.79119574	19.79	1E-04
11a vs 13c	1	34.390251	34.39025053	34.39	1E-04
11b vs 11c	1	1.0132216	1.01322164	1.01	0.316
11b vs 12a	1	0.0676149	0.06761494	0.07	0.795
11b vs 12b	1	2.6789355	2.67893551	2.68	0.104
11b vs 12c	1	5.1222857	5.12228574	5.12	0.025
11b vs 13a	1	1.7559104	1.75591041	1.76	0.187
11b vs 13b	1	4.3322173	4.33221734	4.33	0.039
11b vs 13c	1	12.612703	12.61270257	12.61	5E-04
11c vs 12a	1	0.5523106	0.55231056	0.55	0.458
11c vs 12b	1	0.6435364	0.64353637	0.64	0.424
11c vs 12c	1	1.8099284	1.80992842	1.81	0.18
11c vs 13a	1	6.9365108	6.9365108	6.94	0.009
11c vs 13b	1	1.3275047	1.32750471	1.33	0.251
11c vs 13c	1	7.7648444	7.76484435	7.76	0.006
12a vs 12b	1	1.9931451	1.9931451	1.99	0.16
12a vs 12c	1	4.0575612	4.05756119	4.06	0.046
12a vs 13a	1	2.7122716	2.71227158	2.71	0.101
12a vs 13b	1	3.3521563	3.35215627	3.35	0.069
12a vs 13c	1	11.102466	11.10246598	11.1	0.001
12b vs 12c	1	0.0972247	0.09722468	0.1	0.756
12b vs 13a	1	9.5175903	9.51759028	9.52	0.002
12b vs 13b	1	0.0249719	0.02497187	0.02	0.875
12b vs 13c	1	2.4489024	2.44890243	2.45	0.12
12c vs 13a	1	17.832846	17.83284575	17.83	1E-04
12c vs 13b	1	0.0351611	0.03516111	0.04	0.852
12c vs 13c	1	2.4301322	2.43013215	2.43	0.121
13a vs 13b	1	15.82633	15.82633027	15.83	1E-04
13a vs 13c	1	32.265742	32.26574191	32.27	1E-04
13b vs 13c	1	2.9625463	2.96254626	2.96	0.087

Table 15. Longitudinal component of the visual flow for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the longitudinal component of the visual flow with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

LONGITUDINAL COMPONENT OF IMAGE FLOW

TRT	N	Mean	SD
11A	20	29.0534	6.510906
12A	20	23.60829	9.036735
13A	20	28.72103	6.542553
11B	20	33.71329	8.094388
12B	20	36.9256	13.84327
13B	20	37.28371	8.642404
11C	20	45.98225	10.31005
12C	20	40.42986	8.862416
13C	20	40.6337	9.434097

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	272.9514995			
TRT	8	101.9514145	12.7439268	12.74	0.0001
Error	171	171.0000849	1.0000005		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	4.0245329	4.0245329	4.02	0.046
11a vs 11c	1	38.548377	38.54837677	38.55	1E-04
11a vs 12a	1	4.7800118	4.7800118	4.78	0.03
11a vs 12b	1	5.2960848	5.29608479	5.3	0.023
11a vs 12c	1	21.404065	21.4040653	21.4	1E-04
11a vs 13a	1	0.0259318	0.02593181	0.03	0.872
11a vs 13b	1	11.570954	11.57095418	11.57	8E-04
11a vs 13c	1	20.412404	20.41240385	20.41	1E-04
11b vs 11c	1	17.521931	17.52193145	17.52	1E-04
11b vs 12a	1	13.875474	13.87547423	13.88	3E-04
11b vs 12b	1	0.8025487	0.80254866	0.8	0.372
11b vs 12c	1	6.262957	6.26295698	6.26	0.013
11b vs 13a	1	4.60149	4.60148998	4.6	0.033
11b vs 13b	1	1.8184076	1.81840763	1.82	0.179
11b vs 13c	1	6.1987816	6.19878163	6.2	0.014
11c vs 12a	1	53.266024	53.26602357	53.27	1E-04
11c vs 12b	1	5.5061261	5.50612612	5.51	0.02
11c vs 12c	1	3.3357659	3.33576594	3.34	0.07
11c vs 13a	1	39.965864	39.96586437	39.97	1E-04
11c vs 13b	1	8.3612729	8.36127287	8.36	0.004
11c vs 13c	1	2.9295575	2.92955751	2.93	0.089
12a vs 12b	1	12.978522	12.97852233	12.98	4E-04
12a vs 12c	1	35.325412	35.32541173	35.33	1E-04
12a vs 13a	1	4.2003038	4.20030376	4.2	0.042
12a vs 13b	1	23.922287	23.92228716	23.92	1E-04
12a vs 13c	1	33.96884	33.96883982	33.97	1E-04
12b vs 12c	1	0.9090187	0.90901866	0.91	0.342
12b vs 13a	1	5.7425913	5.74259127	5.74	0.018
12b vs 13b	1	0.0096306	0.00963055	0.01	0.922
12b vs 13c	1	0.9799085	0.97990848	0.98	0.324
12c vs 13a	1	22.595808	22.5958079	22.6	1E-04
12c vs 13b	1	1.2919221	1.29192212	1.29	0.257
12c vs 13c	1	0.0049598	0.00495977	0	0.944
13a vs 13b	1	12.480337	12.48033692	12.48	5E-04
13a vs 13c	1	21.533238	21.53323847	21.53	1E-04
13b vs 13c	1	1.371152	1.37115198	1.37	0.243

Table 16. Transverse and longitudinal component of the visual flow for *C. cautella* males flying to nine treatments: three plumes of different structures at three pheromone concentrations. **A.** Mean (+SD; N=20) for nine treatments. **B.** Analysis of variance of the transverse plus longitudinal component of the visual flow with weighting inversely proportional to cell variance. **C.** Contrast table for the nine treatments. Column one, treatment contrasts, where 11a, 11b and 11c is the low concentration (11) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively; 12a, 12b and 12c is the intermediate concentration (12) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively, and 13a, 13b and 13c is the high concentration (13) filamentous plume (a), narrow turbulent plume (b), and wide turbulent (c) plume respectively. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRANSVERSE PLUS LONGITUDINAL COMPONENT OF IMAGE FLOW

TRT	N	Mean	SD
11A	20	47.09752	10.11267
12A	20	38.43238	12.65991
13A	20	45.3991	8.388269
11B	20	48.86862	8.646309
12B	20	49.87524	13.25827
13B	20	50.04199	8.886641
11C	20	59.93569	10.53935
12C	20	53.00542	9.255193
13C	20	51.68065	8.52126

B.

Source	DF	SS	MS	F Value	Pr > F
Total	179	216.13430887			
TRT	8	45.13440090	5.64180011	5.64	0.0001
Error	171	170.99990796	0.99999946		

C.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
11a vs 11b	1	0.3543922	0.35439218	0.35	0.552
11a vs 11c	1	15.450977	15.45097698	15.45	1E-04
11a vs 12a	1	5.7198842	5.71988424	5.72	0.018
11a vs 12b	1	0.5549932	0.55499323	0.55	0.457
11a vs 12c	1	3.714594	3.71459398	3.71	0.056
11a vs 13a	1	0.3341981	0.33419806	0.33	0.564
11a vs 13b	1	0.9567452	0.9567452	0.96	0.329
11a vs 13c	1	2.4022578	2.40225783	2.4	0.123
11b vs 11c	1	13.181441	13.1814405	13.18	4E-04
11b vs 12a	1	9.2681162	9.2681162	9.27	0.003
11b vs 12b	1	0.0808871	0.0808871	0.08	0.776
11b vs 12c	1	2.1335574	2.13355743	2.13	0.146
11b vs 13a	1	1.6589589	1.65895885	1.66	0.2
11b vs 13b	1	0.1791174	0.17911744	0.18	0.673
11b vs 13c	1	1.0731415	1.07314146	1.07	0.302
11c vs 12a	1	34.080758	34.08075795	34.08	1E-04
11c vs 12b	1	7.0565894	7.05658937	7.06	0.009
11c vs 12c	1	4.8825289	4.8825289	4.88	0.029
11c vs 13a	1	23.292681	23.29268089	23.29	1E-04
11c vs 13b	1	10.301	10.30100013	10.3	0.002
11c vs 13c	1	7.4196397	7.41963965	7.42	0.007
12a vs 12b	1	7.7927162	7.79271617	7.79	0.006
12a vs 12c	1	17.270904	17.2709038	17.27	1E-04
12a vs 13a	1	4.2088176	4.20881757	4.21	0.042
12a vs 13b	1	11.267372	11.26737223	11.27	0.001
12a vs 13c	1	15.07325	15.0732503	15.07	1E-04
12b vs 12c	1	0.7495382	0.74953821	0.75	0.388
12b vs 13a	1	1.6279673	1.6279673	1.63	0.204
12b vs 13b	1	0.002183	0.002183	0	0.963
12b vs 13c	1	0.2624466	0.26244657	0.26	0.609
12c vs 13a	1	7.416389	7.416389	7.42	0.007
12c vs 13b	1	1.0668558	1.06685582	1.07	0.303
12c vs 13c	1	0.2217719	0.22177188	0.22	0.638
13a vs 13b	1	2.8869887	2.88698866	2.89	0.091
13a vs 13c	1	5.5195437	5.51954374	5.52	0.02
13b vs 13c	1	0.3542854	0.35428541	0.35	0.553

APPENDIX B

CHAPTER IV FLIGHT TRACK ANALYSIS TABLES

Table 17. Track angle for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	63.50358	6.591365	70.09495
C50	25	44.82013	13.09386	57.91399
I01	25	40.43776	14.04361	54.48136
I02	25	44.77374	12.53843	57.31216
I15	25	73.931	7.185029	81.11603

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	36.523785	36.523785	36.52	0.0001
C05 vs I01	1	51.6406413	51.6406413	51.64	0.0001
C05 vs I02	1	41.4651726	41.4651726	41.47	0.0001
C05 vs I15	1	19.9490637	19.9490637	19.95	0.0001
C50 vs I01	1	1.2775639	1.2775639	1.28	0.2609
C50 vs I02	1	0.0001631	0.0001631	0	0.9898
C50 vs I15	1	83.24199	83.24199	83.24	0.0001
I01 vs I02	1	1.3491202	1.3491202	1.35	0.2481
I01 vs I15	1	102.6752164	102.6752164	102.68	0.0001
I02 vs I15	1	93.5935518	93.5935518	93.59	0.0001

Table 18. Course angle for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	30.03309	5.290876	35.32397
C50	25	24.08422	7.290758	31.37498
I01	25	22.67857	8.132908	30.81148
I02	25	23.1929	6.972766	30.16566
I15	25	36.10219	4.690336	40.79252

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	9.38703224	9.38703224	9.39	0.0028
C05 vs I01	1	12.87537742	12.87537742	12.88	0.0005
C05 vs I02	1	13.66013066	13.66013066	13.66	0.0004
C05 vs I15	1	12.92751984	12.92751984	12.93	0.0005
C50 vs I01	1	0.40650635	0.40650635	0.41	0.5251
C50 vs I02	1	0.1944944	0.1944944	0.19	0.6601
C50 vs I15	1	41.1617306	41.1617306	41.16	0.0001
I01 vs I02	1	0.05857861	0.05857861	0.06	0.8092
I01 vs I15	1	45.73616508	45.73616508	45.74	0.0001
I02 vs I15	1	52.66965611	52.66965611	52.67	0.0001

Table 19. Drift angle for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	33.47049	5.195139	38.66563
C50	25	20.7359	7.260205	27.99611
I01	25	18.25067	6.347539	24.59821
I02	25	21.58084	6.634236	28.21508
I15	25	37.82881	4.202072	42.03088

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	43.8807627	43.8807627	43.88	0.0001
C05 vs I01	1	74.4577189	74.4577189	74.46	0.0001
C05 vs I02	1	44.2837255	44.2837255	44.28	0.0001
C05 vs I15	1	7.4839612	7.4839612	7.48	0.0073
C50 vs I01	1	1.6219839	1.6219839	1.62	0.2056
C50 vs I02	1	0.1835712	0.1835712	0.18	0.6692
C50 vs I15	1	90.3160563	90.3160563	90.32	0.0001
I01 vs I02	1	3.3560884	3.3560884	3.36	0.0698
I01 vs I15	1	144.6253136	144.6253136	144.63	0.0001
I02 vs I15	1	96.649535	96.649535	96.65	0.0001

Table 20. Net crosswind speed (XY) for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	29.12399	6.093841	35.21783
C50	25	29.93376	8.798953	38.73271
I01	25	28.49674	7.61757	36.11431
I02	25	27.55723	7.94485	35.50208
I15	25	34.26961	4.381165	38.65078

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	0.12397973	0.12397973	0.12	0.7255
C05 vs I01	1	0.089741	0.089741	0.09	0.7651
C05 vs I02	1	0.54661888	0.54661888	0.55	0.4614
C05 vs I15	1	8.29455711	8.29455711	8.29	0.0048
C50 vs I01	1	0.37227475	0.37227475	0.37	0.5431
C50 vs I02	1	0.9989857	0.9989857	1	0.3199
C50 vs I15	1	4.31679923	4.31679923	4.32	0.0402
I01 vs I02	1	0.18587526	0.18587526	0.19	0.6673
I01 vs I15	1	9.65917822	9.65917822	9.66	0.0024
I02 vs I15	1	12.6675516	12.6675516	12.67	0.0006

Table 21. Net upwind speed for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	18.55068	3.573932	22.12461
C50	25	36.07567	9.671142	45.74681
I01	25	42.38321	14.72807	57.11127
I02	25	34.82612	10.9655	45.79162
I15	25	17.86447	3.10817	20.97264

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	66.66870434	66.66870434	66.67	0.0001
C05 vs I01	1	60.51290231	60.51290231	60.51	0.0001
C05 vs I02	1	49.65785414	49.65785414	49.66	0.0001
C05 vs I15	1	0.36848523	0.36848523	0.37	0.5451
C50 vs I01	1	3.1641329	3.1641329	3.16	0.0782
C50 vs I02	1	0.18321867	0.18321867	0.18	0.6695
C50 vs I15	1	74.27024833	74.27024833	74.27	0.0001
I01 vs I02	1	4.29351699	4.29351699	4.29	0.0407
I01 vs I15	1	65.02681236	65.02681236	65.03	0.0001
I02 vs I15	1	55.40122589	55.40122589	55.4	0.0001

Table 22. Ground speed for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	38.78694	7.242819	46.02976
C50	25	53.24132	8.671567	61.91288
I01	25	57.96386	11.05208	69.01594
I02	25	50.84471	9.377824	60.22253
I15	25	42.50546	4.252307	46.75777

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	34.54797739	34.54797739	34.55	0.0001
C05 vs I01	1	47.14644505	47.14644505	47.15	0.0001
C05 vs I02	1	23.08946008	23.08946008	23.09	0.0001
C05 vs I15	1	3.47597533	3.47597533	3.48	0.0651
C50 vs I01	1	2.78116969	2.78116969	2.78	0.0984
C50 vs I02	1	0.88153426	0.88153426	0.88	0.3499
C50 vs I15	1	27.46336331	27.46336331	27.46	0.0001
I01 vs I02	1	6.12966535	6.12966535	6.13	0.0149
I01 vs I15	1	40.16463943	40.16463943	40.16	0.0001
I02 vs I15	1	15.64131872	15.64131872	15.64	0.0001

Table 23. Airspeed for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	64.22121	5.416206	69.63741
C50	25	83.71165	10.1964	93.90805
I01	25	89.21296	13.12427	102.3372
I02	25	81.61972	10.62475	92.24447
I15	25	64.09002	4.418141	68.50816

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	63.71985396	63.71985396	63.72	0.0001
C05 vs I01	1	73.31215774	73.31215774	73.31	0.0001
C05 vs I02	1	50.69255186	50.69255186	50.69	0.0001
C05 vs I15	1	0.00619521	0.00619521	0.01	0.9374
C50 vs I01	1	2.696925	2.696925	2.7	0.1035
C50 vs I02	1	0.50453547	0.50453547	0.5	0.4791
C50 vs I15	1	70.25452659	70.25452659	70.25	0.0001
I01 vs I02	1	5.13349855	5.13349855	5.13	0.0255
I01 vs I15	1	78.52151913	78.52151913	78.52	0.0001
I02 vs I15	1	55.97308121	55.97308121	55.97	0.0001

Table 24. Interleg angle for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	153.7133	5.21828	158.9316
C50	25	159.1085	2.900901	162.0094
I01	25	160.3284	2.461904	162.7903
I02	25	156.9735	3.005349	159.9788
I15	25	152.8864	2.579859	155.4662

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	15.62059052	15.62059052	15.62	0.0001
C05 vs I01	1	24.93081189	24.93081189	24.93	0.0001
C05 vs I02	1	5.7138105	5.7138105	5.71	0.0186
C05 vs I15	1	0.35906571	0.35906571	0.36	0.5503
C50 vs I01	1	2.50932768	2.50932768	2.51	0.1162
C50 vs I02	1	6.53019147	6.53019147	6.53	0.012
C50 vs I15	1	52.16541449	52.16541449	52.17	0.0001
I01 vs I02	1	19.08267181	19.08267181	19.08	0.0001
I01 vs I15	1	87.36226275	87.36226275	87.36	0.0001
I02 vs I15	1	22.60691963	22.60691963	22.61	0.0001

Table 25. Transverse component of the visual flow for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	18.51888	2.774382	21.29326
C50	25	15.0763	4.046541	19.12284
I01	25	14.15639	4.497113	18.6535
I02	25	14.72461	4.008602	18.73321
I15	25	21.99589	2.327174	24.32307

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	10.67791976	10.67791976	10.68	0.0015
C05 vs I01	1	15.39026705	15.39026705	15.39	0.0002
C05 vs I02	1	13.76788802	13.76788802	13.77	0.0003
C05 vs I15	1	16.20173892	16.20173892	16.2	0.0001
C50 vs I01	1	0.56747688	0.56747688	0.57	0.4529
C50 vs I02	1	0.09512135	0.09512135	0.1	0.7584
C50 vs I15	1	47.84050041	47.84050041	47.84	0.0001
I01 vs I02	1	0.22626243	0.22626243	0.23	0.6353
I01 vs I15	1	54.50657627	54.50657627	54.51	0.0001
I02 vs I15	1	56.45021246	56.45021246	56.45	0.0001

Table 26. Longitudinal component of the visual flow for males flying to two continuous plumes and three pulsed plumes. **A.** Mean (+ 1 SD; N=20) for five treatments. **B.** Contrast table for the five treatments. Column one, treatment contrasts, where c05 as continuous low volume plume, c50 as continuous high volume plume, i01with a pulse per 0.1 seconds, i02 with a pulse per 0.25 seconds, i15 with pulse per 1.5 seconds. Column two, degrees of freedom, column three, contrast sum of squares, column four, mean square, column five, F value, and column six, p value.

A.

TRT	N	Mean	SD	CI
C05	25	32.12222	7.126273	39.24849
C50	25	49.36742	9.457362	58.82478
I01	25	54.51019	11.76754	66.27773
I02	25	46.8483	10.1	56.9483
I15	25	34.30979	4.485018	38.79481

B.

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
C05 vs C50	1	45.4173842	45.4173842	45.42	0.0001
C05 vs I01	1	59.95228621	59.95228621	59.95	0.0001
C05 vs I02	1	32.15181929	32.15181929	32.15	0.0001
C05 vs I15	1	1.19499871	1.19499871	1.19	0.2768
C50 vs I01	1	2.85439292	2.85439292	2.85	0.0941
C50 vs I02	1	0.82951315	0.82951315	0.83	0.3645
C50 vs I15	1	46.17777261	46.17777261	46.18	0.0001
I01 vs I02	1	6.20393814	6.20393814	6.2	0.0143
I01 vs I15	1	60.70244852	60.70244852	60.7	0.0001
I02 vs I15	1	30.78590323	30.78590323	30.79	0.0001

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