

Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths

Agenor Mafra-Neto* & Ring T. Cardé

Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003, USA

* Present address: Department of Entomology, Iowa State University, Ames, Iowa 50011, USA

IN studies of moths flying upwind to a pheromone source, attention has focused on the influence on flight orientation of the composition^{1,2} and concentration^{3,4} of the chemical message, and of changes in the visual environment^{5,6} and in wind speeds⁷⁻⁹. The chemical signal must be intermittent for moths to fly upwind¹⁰⁻¹², when they usually follow a zigzag track, the evident expression of a self-steered counterturning programme^{13,14}. The integration of counterturning and optomotor anemotaxis allows insects to polarize the zigzags upwind in odour plumes^{10,15}. Not all moths, however, zigzag along a plume^{16,17}. It has been suggested that the propensity to zigzag or to fly straight upwind is related to the frequency at which males encounter pheromone filaments that comprise the plume, as well as the male's latency of response, characteristic for each moth species, to both the onset and loss of contact with filaments¹⁸. Here we present evidence that flight manoeuvres are dictated by the interactions of the male with individual odour pulses. We use *Cadra cautella*, the almond moth, to show how the structure of an odour plume^{19,20} can greatly modify the flight track. Males following either turbulent or mechanically pulsed plumes fly faster and straighter upwind, and locate sources more frequently than males following continuous narrow plumes. Males also fly straighter upwind to fast-pulsed plumes than to slow-pulsed plumes. The temporally modulated interplay between counterturning and optomotor anemotaxis that is induced by the plume's structure therefore seems to explain the manoeuvres and resultant flight track shapes made by *C. cautella* males when flying upwind towards a pheromone source.

Cadra cautella males were tested in a wind tunnel²¹ using either ribbon or turbulent pheromone plumes at two concentrations.

A ribbon plume consisted of a single continuous filament of odour. A turbulent plume was characterized as a stream of odour pulses separated by gaps of clean air (Table 1a). More males that had been exposed to turbulent plumes took off, locked onto the plume, and landed on the pheromone source, than males exposed to ribbon plumes (Table 1a). Males flying along ribbon plumes zigzagged slowly upwind, whereas males flying along turbulent plumes flew rapidly and often took nearly straight upwind courses irrespective of concentration²¹ (Fig. 1).

The tempo of a self-steered, counterturning programme in some moths is set by the instantaneous concentration of pheromone²². Although an increase in pheromone dose slightly decreased the tempo of self-steered turns in *C. cautella* males, the manipulation of the plume structure had strong effects on form of turns and interturn duration (Table 1a). In ribbon plumes, the frequency of turns across the wind line and lateral displacement were maximized: track angles were steered almost perpendicular to the wind line, which resulted in substantial lateral excursions (Fig. 1a, b). Males flying in turbulent plumes appeared to suppress counterturning, so that interturn duration and lateral displacement were minimized (Fig. 1b, c and Table 1a).

To isolate the effect of intermittency of turbulent plumes on male flight, plumes of defined pulse frequencies (Table 1b) were generated with a mechanical pulser^{21,23}. Continuous ribbon plumes resulted in slow zigzag flights (Fig. 2a). High-pulse frequency resulted in fast flights and straight tracks (Fig. 2c, d). The flights in low-pulse-frequency plumes alternated between a stationary zigzag during clean air intervals (casting) and short upwind surges, suggesting that males were responding to single pulse contacts²¹ (Fig. 2b). To determine whether the flight changes in response to an encounter with a single puff of pheromone, casting *C. cautella* males were exposed to a single, brief 0.25-s pulse (Fig. 3). The typical response of casting *C. cautella* males after a short delay was to turn upwind and engage briefly in a faster and straighter flight along the wind line (Fig. 3), a result found for another moth species²⁴.

Males thus change their flight in response to changes in the plume's overall shape (Fig. 1), and also to fine-scale variations in its internal structure (Figs 2 and 3). The characteristics of upwind flight in a plume may be predicted by a model based on the interactions of flying males and the individual pheromone pulses, assuming that straight flight results from the suppression

TABLE 1 Factors influencing male moth flight in odour plumes

Plume (a)	Pulse duration (s)	Air-gap duration (s)	Males landing (%)	Interturn duration (s)	Ground speed (cm s ⁻¹)	Track angle (deg)
Ribbon, low dose	Continuous		20 C	0.20 ± 0.02 C	36.17 ± 7.26 B	62.02 ± 7.96 A
Ribbon, high dose	Continuous		54 B	0.21 ± 0.04 C	35.19 ± 6.41 B	58.53 ± 9.37 A
Turbulent, low dose	0.17 ± 0.4	0.25 ± 0.04	69 A	0.30 ± 0.06 B	49.92 ± 9.62 A	41.46 ± 12.05 B
Turbulent, high dose	0.17 ± 0.04	0.25 ± 0.04	63 AB	0.36 ± 0.01 A	43.65 ± 8.34 B	34.30 ± 11.60 B
(b)						
Ribbon	9.67 ± 0.03	0.017 ± 0.03	83 B	0.25 ± 0.03 B	38.8 ± 7.24 B	63.5 ± 6.59 B
Slow pulse	0.17 ± 0.03	1.450 ± 0.04	63 C	0.29 ± 0.07 B	42.5 ± 4.25 B	73.9 ± 7.19 A
Fast pulse	0.13 ± 0.03	0.083 ± 0.01	100 A	0.40 ± 0.09 A	58.0 ± 11.05 A	40.4 ± 14.04 C

Values are means ± s.d. a, Males flying in turbulent plumes turn less frequently, fly faster, and give smaller track angles than males flying to ribbon plumes. A turn was scored when the track angle value changed signal relative to the wind direction from positive to negative or vice versa. Differences in pulse duration ($n=100$ pulses), air gap between pulses ($n=100$ gaps), percentage of males landing on the source, interturn duration ($n=20$ tracks), mean ground speed ($n=20$ tracks) and track angle ($n=20$ tracks) of flight are shown. Treatments were tested on a complete block randomized design. Values in the same column having none of the letters A-C in common are significantly different ($\alpha=0.05$, LSD, ANOVA, SAS). Methods as for Fig. 1. b, Males flying to quickly pulsed plumes turn less frequently, fly faster, and exhibit smaller track angles than males flying to ribbon plumes or to slowly pulsed plumes. These results illustrate the extremes of the plume structures tested in ref. 21. Flight tracks in plumes of intermediary pulse frequency showed intermediate values for the flight parameters measured. Methods as for Fig. 2.

FIG. 1 Example of *Cadra cautella* male flight tracks in ribbon plumes and turbulent plumes at two concentrations. Data points represent the position of the moth at 0.03-s intervals. Males flying to ribbon plumes produce zig-zagging flight tracks (a and b), and males flying to turbulent plumes produce straight tracks (c and d), independent of concentration. Frequency distribution histograms of pooled track angles (insets) are bimodal, with angles clustered around -90° and 90° , or unimodal with a mode of 0° (0° is upwind, 90° crosswind and 180° downwind). Bimodal distribution reflects crosswind tracks typical of flights in ribbon plumes (a and b). A mode of 0° reflects straight, upwind flight or a track with reversal legs nearly straight upwind as observed in turbulent plumes (c and d). METHODS. Pheromone sources (0.7-cm-diameter filter paper) containing 0.45 ng (low dose) or 45 ng (high dose) of pheromone were placed in 45 cm s^{-1} winds to generate continuous ribbon plumes of $0.8 \pm 0.2 \text{ cm}$ width and $0.1 \pm 0.1 \text{ cm}$ height. Addition of a $3 \times 3 \text{ cm}$ wind deflector standing 4 cm upwind created turbulent plumes. Flight tracks of 20 males for each combination of pheromone dose and plume structure were videotaped and the video images analysed frame-by-frame as described in ref. 25. Statistical analysis as in ref. 21.

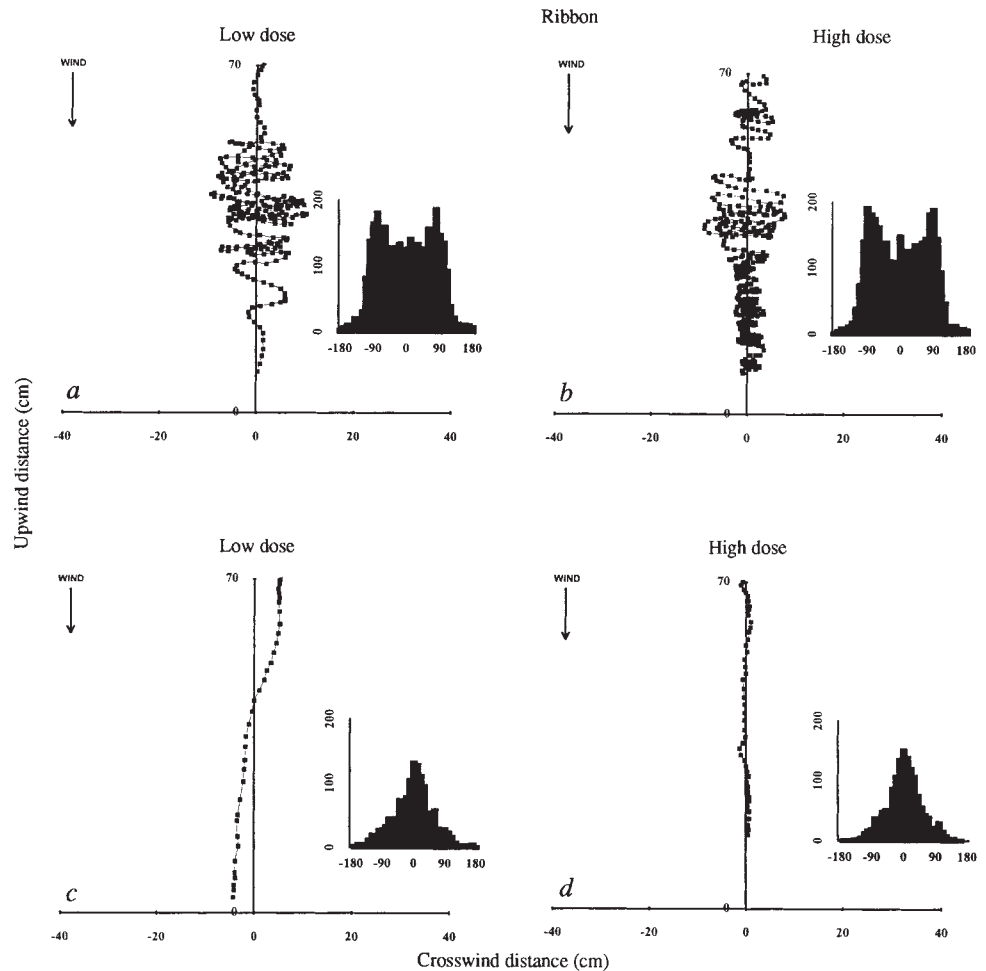


FIG. 2 a, The effect of manipulating the structure of pheromone plumes on the flight track of male moths by pulsing pheromone. Representative flight tracks and the corresponding distribution of flight-track angles of all males in each treatment are shown. Males flying to ribbon (a) and to slowly pulsed plumes (b) produced zigzagging flight tracks; and males flying to more quickly pulsed plumes (c and d) produced essentially straight tracks. Male lateral displacement in ribbon and slowly pulsed plumes is elaborated as frequent, conspicuous zigzags (a and b). Most flight tracks in quickly pulsed plumes lack the same temporal organization of lateral displacement (c), but some flight tracks have vestiges of counterturning (d). METHODS. Plumes were generated by forcing air regulated by a flow controller apparatus (SFC-2, Syntech)^{21,23} through a glass chamber containing a filter paper with 4.5 ng pheromone and injecting the exhaust in the centre of the working area of the wind tunnel with winds of 45 cm s^{-1} . The flight tracks of 20 males along structurally different plumes were videotaped and the resulting images analysed^{21,25}.

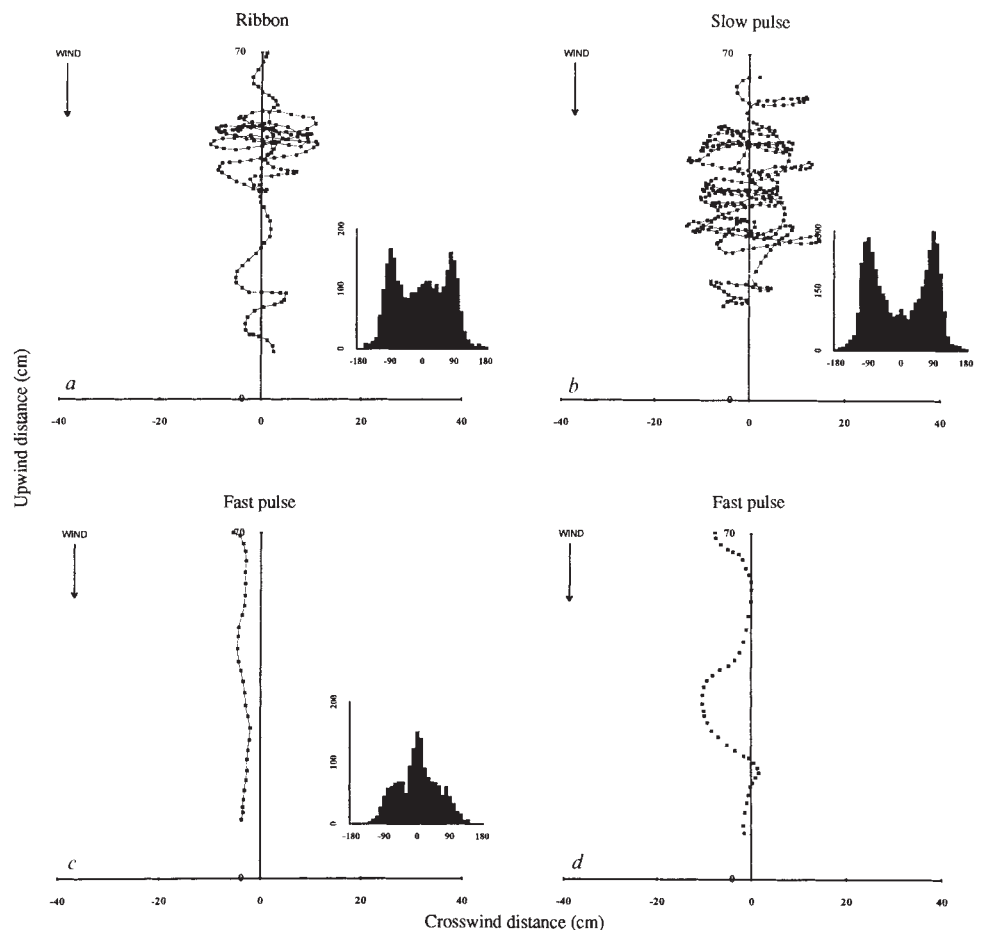
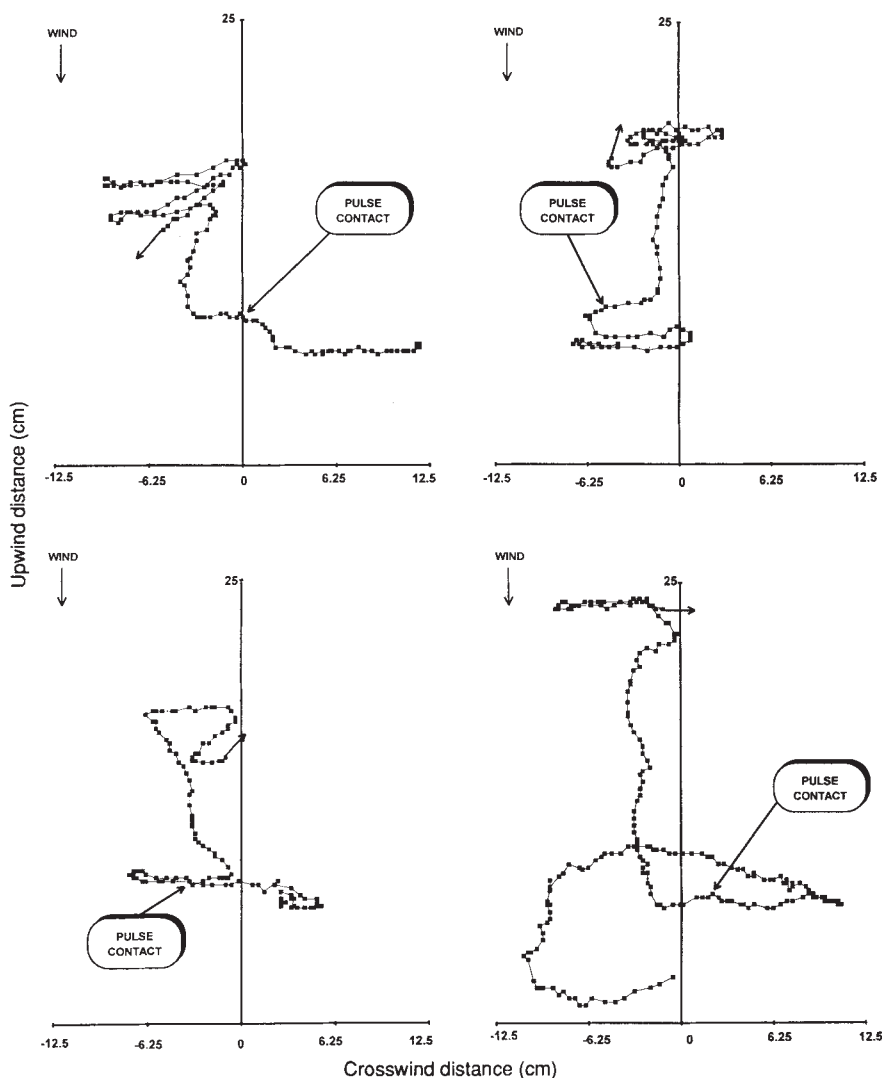


FIG. 3 Examples of the characteristic change of flight behaviour of *C. cautella* males in response to contact with a single pheromone pulse. Males flying upwind in a fast-pulsed pheromone plume were induced to cast as a result of removal of the plume. Once casting in clean air the male was exposed to a single pheromone pulse. Contact with pheromone (arrow) was followed by a sharp turn upwind, and a faster and straighter upwind flight. In the absence of new pheromone pulses, males returned to casting flight.

METHODS. Fast-pulsed plumes and single pulses were generated by forcing air regulated by the flow controller apparatus through a glass chamber containing a filter paper with 4.5 ng pheromone and injecting the exhaust in the centre of the working area of the wind tunnel with winds of 50 cm s^{-1} . Frame-by-frame video analysis (60 frames s^{-1}) of single TiCl_4 'smoke' pulses generated under the same experimental conditions as pheromone pulses allowed accurate prediction of the pheromone pulse position in the working area with time. An audio and a visual signal flagged the generation of single pulses. Flight tracks were analysed for males that intercepted the pheromone pulse. Moths intercepted pulses of $\sim 7.5 \pm 1.25 \text{ cm}$ cross-section along the wind line. Data points represent the position of the moth at 0.017-s intervals.



of a moth's internal counterturning programme¹⁸. For straight upwind flight to occur to turbulent plumes, the following elements are required: (1) a tempo of pheromone pulses above a certain frequency, so the male antennae encounter a pulse before the male starts to counterturn¹⁸; (2) a refractory period during which contact with pheromone or clean air has no effect on behaviour²¹; and (3) a level of pheromone concentration in the pulse above threshold for upwind flight behaviour, but below levels that promote turns more perpendicular to the wind line²¹. Pulses should be sufficiently brief along the wind line to promote brief encounters with the antennae and 'disappear' to then be replaced by clean air (or by low concentration) before the male starts counterturning. Crosswind diameter of pulses should be large enough that contact with a new pheromone pulse occurs before the end of the period in (1), even if the male's flight

track deviates slightly from the central axis of the plume. The integration of counterturning and optomotor anemotaxis modulated by the structure of an odour plume seems to explain many features of the moth's flight manoeuvres along plumes of differing form.

The fact that frequently straight flights were seen in moths flying along large turbulent plumes and high-frequency pulsed plumes, and rarely in moths flying along small, continuous plumes or low-frequency pulsed plumes²¹, supports this model. We conclude that the interaction of the moth with the structure of the plume is an integral part of the flight guidance system, modifying the output of the internal turn-generator and the optomotor anemotactic mechanism. Future studies of insect orientation to odour should account for the pronounced effects of plume structure. □

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