Direct Measurement of the Flight Behavior of Male Moths to Calling Females and Synthetic Sex Pheromones

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The approach of male *Lobesia botrana* (Lepidoptera: Tortricidae) in the wind tunnel to a source of sex pheromone was studied using two video cameras connected to image processing equipment. Three-dimensional records demonstrate that the flight to calling females is quite direct compared to the zigzag flight reported for other species. Convoluted paths displaying bimodal distribution of track and course angles were only obtained with a synthetic pheromone blend consisting of *E*-7,*Z*-9-dodecadienyl acetate, *E*-7,*Z*-9-dodecadien-1-ol and *Z*-9-dodecenyl acetate at the proportions of 1/0.2/0.05. This suggests that this blend is still suboptimal and that zigzag flight may be due to a lack of precision in upwind motion caused by inadequate composition of the stimulus.

Introduction

Male moths approach a source of female sex pheromone in a winding upwind path by what has been considered a combination of two mechanisms. One, called optomotor anemotaxis [1, 2], is upwind orientation based on visual cues. The other, called reversing anemomenotaxis [3, 4], is an internal program of counterturns yielding a zigzag path when viewed from above. Evidence for self-steered turns has been obtained from frequency distributions of course angles displaying distinct maxima on either side of the wind direction and from zigzag paths observed in the absence of wind [5–8].

We are studying the flight behavior of male moths with the aim to provide a complete chemical description of the female sex pheromones. In this paper we present a new technique of flight track recording which allows direct measurement of insect flight behavior to a pheromone source and report our first observations of male grapevine moths, *Lobesia botrana,* flying to calling females and a synthetic pheromone blend.

Materials and Methods

In our observation system, two CCD cameras (Henz Memo AG, Suhr) are placed *ca.* 100 cm above and on the side of a Plexiglas™ wind tunnel of 63 × 63 × 170 cm, lit diffusely from above at 3 lux. Each field of view covers an area of *ca.* 50 × 50 cm. A small light bulb is placed near the camera lens and a No. 7615 Scotchlite™ reflective foil on the opposite wall of the tunnel, rendering the insect visible as a silhouette on a bright background. A pattern of 28 dark circles per m^2 ranging in size from 2 to 12 cm covered the entire floor of the tunnel except for the 60 × 60 cm covered by the reflective foil.

The image processing system furnished by Eltec GmbH, Mainz (F.R.G.), records the insect's position at 25 frames/s. Reconstruction of actual object coordinates and analysis of flight paths are done off-line. Two angles were determined for each pair of successive frames: The track angle, reflecting the observed direction of the flight path, and the course angle or heading, reflecting the direction taken by the insect. The track angle was measured between flight direction and wind direction, and the course angle calculated from the velocities of the insect and the wind [2].

Synthetic pheromone composed of *E*-7,*Z*-9-dodecadienyl acetate, *E*-7,*Z*-9-dodecadien-1-ol and *Z*-9-dodecenyl acetate at the proportions of 1/0.2/0.05 [9], was deposited on a filter paper 1 cm square. GC analysis of extracted filter papers showed that the proportions of chemicals did not change over the course of an experiment. A 2 cm diameter glass tube with a capillary tip of 1 mm, containing either the filter paper or a calling female, was placed at the upwind end of the tunnel.

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It was supplied with air leaving the tip at the speed of the air in the tunnel (30 cm/s). Smoke generated by hydrolyzing $TiCl_4$ from an identical tube yielded a cylindrical plume ranging in diameter between 12 and 16 cm.

Results and Discussion

The flight tracks to calling females and to a blend containing 1 ng of *E*-7,*Z*-9-dodecadienyl acetate (Fig. 1) show deviations from the wind line in both projections, generally larger in the horizontal than in the vertical plane. A projection along the flight axis shows that what appears as zigzag in the other two projections is actually a path in space. Approaches to the synthetic blend deviate more from the plume axis than those to females (7.6 cm as compared to 5.5 cm; Mann-Whitney test, $N = 40$, $P = 0.05$). This is seen from the probabilities of male distributions in a plane across the flight axis (Fig. 2). In the approach to the pheromone blend, males fly at a lower average level than to females and frequently turn without net upwind movement.

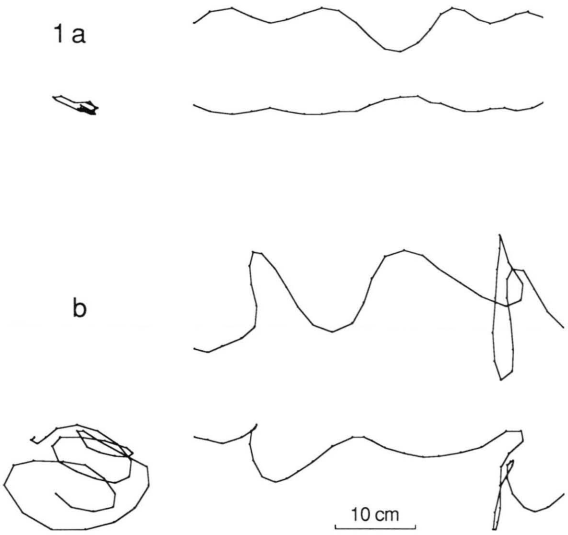

Fig. 1. Typical flight tracks of male *Lobesia botrana* to calling female (a) and synthetic blend (b). Source is located 50 cm to the left. Top: top view; bottom: side view; left: front view.

Flight paths change with dose of chemicals; tracks recorded with the ternary blend at 1 ng of dienic acetate showed the greatest resemblance to those obtained with virgin females. At this dose, only 25% of the males reached the source as compared to 65% arriving at the females. Increasing the amount to 100 ng led to 57% arrivals; however, reduced flight speed and frequent path reversals were observed. For comparison, the female gland contains between 1 and 2 ng *E*-7,*Z*-9-dodecadienyl acetate [10]. The average track speed (40 flights) was 58 ± 18 (S.D.) cm/s to calling females, 61 ± 23 cm/s to the blend at 1 ng of diene and 40 ± 19 cm/s to 100 ng. Omitting the two secondary components resulted in a decrease of male response [9].

The frequencies of track angles and course angles to synthetic pheromone (Fig. 3 b) produced a bimodal distribution, as in *Adoxophyes orana* [4], *Grapholita molesta* [7] and *Lymantria dispar* [8]. A bimodal distribution is not obvious during the

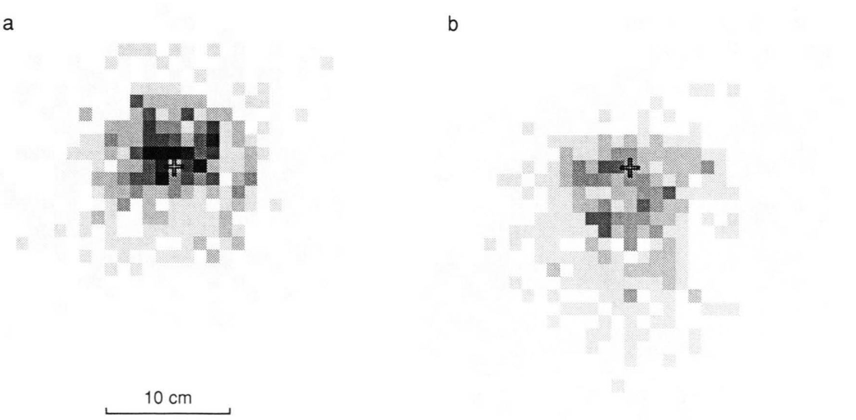

Fig. 2. Frequency distributions of *Lobesia botrana* males (40 tracks) over cells of 1 × 1 × 50 cm across the wind tunnel during the flight to calling females (a) and synthetic blend (b). Probabilities in 10 steps from white (0) to black (0.017). Cross: centre of the plume.

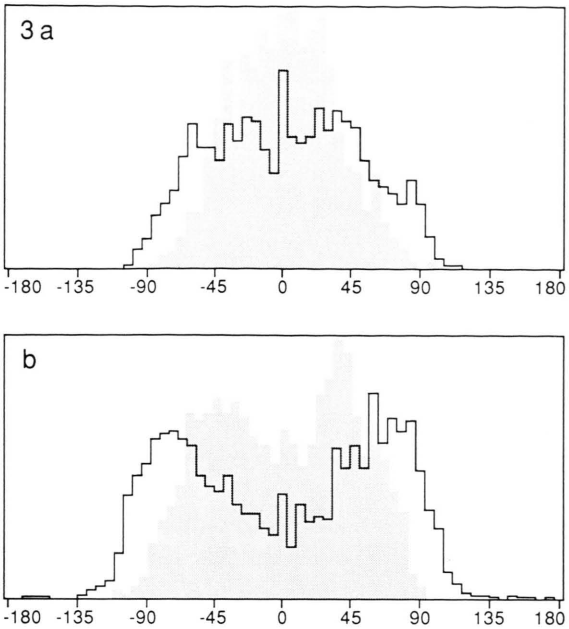

Fig. 3. Distribution of track angles (white) and course angles (shaded) of *L. botrana* flight to calling female (a, 1095 frames) and synthetic blend (b, 1939 frames).

flight to females (Fig. 3a). Therefore, our data show that *L. botrana* males steer more precisely into the wind towards calling females than they do to the synthetic blend. Similar observations have been made with *Choristoneura fumiferana* [11]. We assume that this difference in behaviors is due to the chemistry of the stimulus since physical factors such as visual cues produced by the females, pulsation of pheromone by the females, or acoustic signals have not been observed in tortricids and would have been largely eliminated by our capillary delivery system. Preliminary observations indicate that track angles increase further when only the main component is used (females, 39.4°; ternary blend, 52.3°; *E*-7,*Z*-9-dodecadienyl acetate, 67.4°). In *Euxoa ochrogaster* the flight to a complete blend is more direct than if one or two components are omitted [12].

The apparent unimodal distribution of course angles clearly shows that turns and counterturns in *L. botrana* are diminished in flights to females compared to the synthetic mixture tested. Preiss and Kramer [13] suggest that the apparent zigzag motion in *L. dispar* is merely a manifestation of the male's inability to fly precisely upwind. It is worth noting that most hypotheses on an internal counterturning program have been derived from experiments with synthetic compounds. Nearly direct flights to female gland extracts showing unimodal track and course angles have recently been observed in *Amyelois transitella* [14]. The results presented here indicate that the chemistry of the synthetic blend is different from the chemistry of the calling females. Further experiments to identify the stimuli responsible for direct upwind flight of *L. botrana* are underway.

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