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Behavioral Reaction Times of Male Moths to Pheromone Filaments and Visual Stimuli: Determinants of Flight Track Shape and Direction

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Introduction

Over the years, there have been several explanations for the existence of zigzagging upwind flight in male moths responding to sex pheromone [e.g., 1–7]. Far from being a frivolous argument about the superficial shape of the flight tracks, the root of the discussion involves the very mechanisms that the moths use to maneuver and reach the source of pheromone in wind. The behavioral mechanisms need to be precisely understood if we are to make sense of the underlying neuronal responses at the sensory, central nervous system (CNS), and motor levels and create a robust neuroethological knowledge about this powerful and agriculturally important biological process called attraction.

At the last meeting of the International Symposium on Olfaction and Taste (ISOT), Baker [7] proposed, using the results of previous studies performed predominantly with *Grapholita molesta* and *Heliothis virescens*, that each contact with a filament of pheromone would produce an upwind surge and high frequency of counterturning, and each pocket of clean air, if long enough, would produce a decrease in counterturning frequency and a subsiding of the surge. Sustained upwind flight would occur under conditions favoring the stringing together, or reiterations of, the upwind surging response to appropriately frequent contacts with filaments. The upwind surge was viewed as involving predominantly the anemotactic response system and was hypothesized to be tied to underlying blend-enhanced phasic neuronal pathways demonstrated in other species [8–10]. Casting flight was viewed as involving predominantly the counterturning program known to be switched on by pheromone and independent of the anemotactic system [11]. Counterturning was viewed as being driven by underlying, blend-dependent tonically firing neuronal elements demonstrated in other species [12–14]. It was envisioned that an increased frequency of exposure to filaments relative to the reaction time to the loss of pheromone would result in more straight-upwind flight with little zigzagging, because more frequent upwind surges would occur with less time for the counterturning program to subside in frequency and allow wide casting flight to be expressed fully [7]. Conversely, less frequent exposure to filaments would result in a greater degree of zigzagging in tracks due to the more visible expression of casting during lower-frequency counterturning occurring in the longer periods of clean air [7].

Recently, Willis and Arbas [15] found that their *Manduca sexta* males did not increase their rate of counterturning as the windspeed increased. They reasoned that the higher airspeeds generated by the moths in order to maintain constant groundspeed in elevated windspeeds should have produced more frequent contacts with filaments and resulted in a higher frequency of counterturning (and a narrower upwind path). Since their males did not exhibit increased counterturning, they argued that their results did not support the Baker model [7] and offered an alternative model in which an overall average level of stimulation by filaments, not reactions to individual filaments or the frequency thereof, would determine both the rate at which counterturning is performed and the intensity with which the anemotactic program is expressed.

Windspeed and Filament Frequency

Further examination of the supposition [15] that the moth's frequency of contact with filaments will increase with the moth's airspeed as windspeed increases reveals that it is likely to be flawed. Electroantennographic (EAG) measurements of the pheromone plume for *G. molesta* [16] showed that when windspeed was increased from 30 cm/s to 100 cm/s, the frequency of filaments contacting a stationary antenna 3 m from a point source increased only from 2.0 filaments/s to 2.6 filaments/s, a significant increase but far from the tripling of frequency one might assume would occur with this increase in airspeed over the antenna. Interestingly, when the

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EAG preparation was pushed up the tunnel in order to increase the antenna's airspeed from 30 to 50 to 80 cm/s (the windspeed held constant at 30 cm/s), a more proportionate, expected increase in filament frequency occurred with readings of 1.6, 2.2, and 3.8 filaments/s at those three airspeeds, respectively [16].

These results suggest that if an increase in windspeed does not result in a significant increase in the frequency with which filaments are *generated*, then *in order to increase the frequency of filament contact when wind increases, a flying moth must increase the proportion of its groundspeed contributing to the airspeed*, as in the way that pushing the EAG up the wind tunnel did increase the frequency of filament contacts [16]. However, in no case have moths been shown to do this as a response to increased wind velocity. Rather, they maintain a constant groundspeed, and this is the now classical anemotactic response shown to be used by moths when they are exposed to pheromone [1,15,17–22].

To better visualize the situation, imagine a puffer device (e.g., as in [23]) generating filaments at 3/s, and the windspeed is doubled, then doubled again. Despite the quadrupling of windspeed, a stationary antenna downwind of the source will still only record 3 filaments per second, because this is the rate at which they were generated. A snapshot of the filaments will show them to be spaced more widely apart as the wind moves faster (Fig. 1), but nevertheless, increasing the stationary moth's "airspeed" by increasing the windspeed alone does absolutely nothing to increase the frequency of filaments arriving on the antenna. Next, assuming a known rate of filament generation from a typical pheromone point source, again, say 3/s [16], one can see that the calculated contact rate with filaments by a moth flying at 40 cm/s upwind at each windspeed would not increase, and will actually decrease substantially, under conditions in which its airspeed increase is due entirely to an increase in windspeed. In fact, it would take ca. a 50% increase in the filament generation rate with each doubling of the windspeed just to keep the rate of contact from diminishing (Fig. 1).

The data of Willis and Arbas [15] show that although their *M. sexta* males tripled their airspeeds with a quadrupling of the windspeed, they did this by maintaining groundspeeds of ca. 40 cm/s upwind (50 cm/s overall) and thus, as in every other moth species studied thus far, the increased airspeed was due entirely to the increased windspeed (as in Fig. 1) [17–20]. Given this information, the data of Willis and Arbas [15] actually support the Baker model [7]; the observed lack of increase in counterturning frequency is expected by the model, given the probability that filament contact frequency may not have increased significantly, let alone proportionally, with windspeed. The rate with which filaments contacted an antenna in the plume was never measured by the authors. Thus, it should now be clear that the filament generation rate, not the speed with which the filaments move through the air, is the key variable that must increase proportionately with windspeed if a moth flying upwind is to contact filaments more frequently.

Olfactory Reaction Times

Other recent results from orientation studies are supportive of the model [7] and not of other models

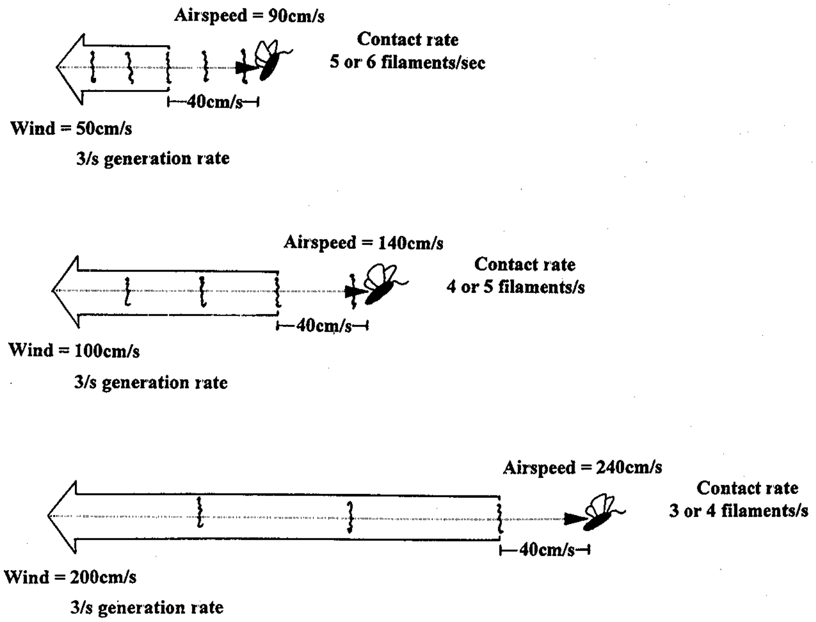

FIG. 1. Relationship between a moth's airspeed, groundspeed, and the frequency with which the moth contacts pheromone filaments as windspeed increases if the filament generation rate remains at 3/s and the moth maintains a preferred groundspeed of 40 cm/s

that have proposed that a counterturning program is not used by moths during pheromone-mediated flight [cf. 3,4]. Vickers [24] showed that male *H. virescens* do in fact respond to single filaments of pheromone by surging upwind. The reaction times of the males to both pheromone onset and offset are 0.23 s (−0.3 range) and 0.30 s (−0.4 range), respectively. This would translate into a frequency of contact of about 4 filaments per second if sustained upwind flight were to occur reliably, and in fact, frequencies of filaments puffed from the puffing device needed to exceed 4/s in order to evoke significant upwind flight and source contact [23]. The fact that casting across the windline occurred [24], always after flight into clean air, is evidence that a counterturning program is present and expressed in this species after emergence into clean air; any change in the direction of track legs to more crosswind must thus be viewed as the beginning of casting, and not as an error in the anemotactic system [3].

In other studies, the first-ever EAG's performed on flying male moths have demonstrated that encounters with filaments of pheromone from a standard rubber septum point source need to exceed 4 per second in order to sustain upwind flight [25]. Reaction times measured from EAG's of flying *H. virescens* males were consistent with those from single filament studies, with contact evoking an upwind surge 0.23 s (±0.11 SD) later in males that had been casting. Loss of pheromone caused crosswind flight 0.30 (±0.17 SD) seconds later, as measured by in-flight EAG's. Increased groundspeed up the windline produced, as predicted, an increase in the rate with which filaments contacted the antennae of the flying males (see foregoing discussion).

Visual Reaction Time

In a shifting wind-field, as under natural conditions in the field, the shape of a male's flight track will be determined by the latency of the male's reaction to the change in wind-direction plus the concomitant loss of contact with pheromone filaments [cf. 2,6,26]. The optomotor anemotactic system is necessarily a visual one [17,21,22], and thus the latency of the male's visually-mediated reaction to a change in wind direction also must play a part in shaping the track. For instance, when the wind direction shifted in the field, *H. virescens* males seemed to favor the correct direction, i.e., toward the newly displaced plume along the new windline, in which to make their first long cast [24]. Likewise, the published flight tracks of male gypsy moths during shifting wind direction also reveal a tendency for the males to make their first long cast in the correct direction toward the shifted plume [6,26].

In order to measure the visual reaction latency and try to explain this tendency, we created a nearly instantaneous "shift" in wind direction with which we challenged male *H. virescens* flying upwind in a plume of their pheromone. A rubber septum loaded with the six-component blend at 100 μg was placed on a metal platform at the upwind end of the wind tunnel in a windspeed of 50 cm/s. As the male reached the field of view of a video camera positioned looking down on the male's flight path, a dotted pattern located on the floor was abruptly moved sideways, 90° across the windline from the moth's left to its right at 50 cm/s (Fig. 2). The combination of 50 cm/s wind from the fan at the front of the tunnel plus the visual equivalent of wind from the dots moving 90° sideways at 50 cm/s below the moth instantly created a new windline coming at 45° from the right—a wind-shift that the moth, now flying to the left of the windline, must respond to by turning to the right, using this new visual feedback. The reaction time until the moth made a rightward response revealed the latency of the visual reaction to the wind-shift. These data show that males took, on average, 0.41 s (±0.17 SD; $n = 14$ males) to react to the visual stimulation by exhibiting a significant rightward change in the track direction (Fig. 2).

One key way a moth loses contact with pheromone is to fly into a large pocket of clean air caused by a

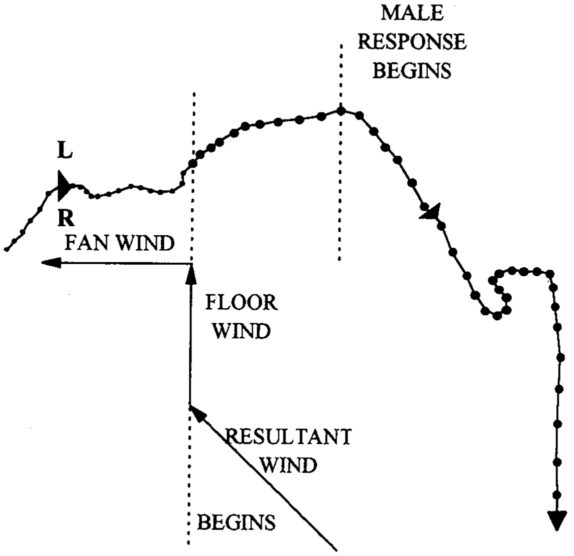

Fig. 2. The flight track of a male *H. virescens* that was flying upwind toward the pheromone source when the ground pattern was moved to create a new resultant wind direction at 45° from the moth's right while the plume remained unchanged in the center of the tunnel. *Larger dots* (every 1/30 s) on the flight track indicate the time period during which the ground pattern was moving and hence the period when the moth should have been responding to the new windline by steering to its right. The beginning of the rightward turn is indicated by male response begins

wind-shift [2,6,26]. Imagine a moth that in the field, is flying straight up the windline and encounters clean air at the shift-point of a plume that is being rapidly displaced to the left. During the 0.30 s that it takes the moth to begin responding to the loss of pheromone and begin casting [24], the inability of the moth to respond visually to the new windline for 0.41 s will allow it to be pushed off the windline *to the left, toward where the plume has gone*, and will facilitate recovery of contact with plume filaments. Thus, the similarities between olfactory [24] and visual reaction times, and indeed, the very existence of such time-lags, may explain why the flight tracks of moths seem to be biased toward the displaced plume in shifted wind-fields.

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