

Flying Beetles Respond as Moths Predict: Optomotor Anemotaxis to Pheromone Plumes at Different Heights

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*The current level of understanding of orientation mechanisms used by flying insects responding to pheromone sources, based almost entirely on studies of moths and flies, allows clear predictions to be made of how other, hitherto little-studied insect taxa, such as beetles (Coleoptera), should behave if the same mechanisms are used. Results are presented of the first test of such set of predictions, the effect of flight height on ground speed, on a beetle, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). The beetle *P. truncatus* flew upwind toward the source of horizontal pheromone plumes and responded to the movement of visible patterns on the floor of a sustained flight tunnel. Beetles flying at a greater height from the floor were less responsive to moving floor patterns. The flight speeds of *P. truncatus* increased with flight altitude, as found with moths, suggesting that they use orientation mechanism similar to those of moths.*

KEY WORDS: *Prostephanus truncatus*; larger grain borer; pheromone; flight orientation; optomotor anemotaxis; wind tunnel.

INTRODUCTION

There is a growing consensus about the orientation mechanisms used by moths to locate a distant odor source in flight: upwind orientation combines two programs, optomotor anemotaxis and self-steered counterturning (Kennedy, 1977; Baker, 1990). Both behaviors are initiated by pheromone contact, with casting

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flight produced on subsequent loss of pheromone. There is also increasing understanding of the relationship between the fine structure of pheromone plumes (Murlis *et al.*, 1992) and the detail of the moths' behavioral responses to the fine filaments of pheromone-laden air (Mafro-Neto and Cardé, 1994; Vickers and Baker, 1994).

The majority of experiments have been carried out with moths and, to a lesser extent, with Diptera. The focus on a limited number of model animals has been very productive but the assumption is often made that all insects will use the same orientation mechanisms. However, as Cardé (1990) pointed out, whether the detailed in-flight orientation mechanisms used to locate distant odor sources by different insect taxa are the same may depend on when such mechanisms evolved compared with the divergence of insect groups. The current level of understanding of orientation mechanisms in moths and flies does allow clear predictions to be made of how other insect taxa should behave if similar mechanisms are used.

It is surprising that the flight optomotor reactions of the largest group of insects, the beetles, have not been investigated. In part this is because beetles have a reputation for being difficult wind-tunnel subjects, although Choudhury and Kennedy (1980) demonstrated upwind anemotaxis in the bark beetle *Scolytus multistriatus* and wind-tunnel bioassays of beetles to pheromones or kairomones have been made (e.g., Birch and White, 1988; Domeck *et al.*, 1990; Salom and McLean, 1991; Wyatt *et al.*, 1993, 1997; Facundo *et al.*, 1994).

A flying insect needs to determine the wind's direction by judging visually how wind has deflected it from its heading, in order to make upwind flight (e.g., Kennedy, 1951; David, 1979; Murlis *et al.*, 1992). This optomotor reaction (comparing the discrepancy between heading and track over ground as visually detected drift or side slip) has been demonstrated for moths in the laboratory (Kennedy and Marsh, 1974; Marsh *et al.*, 1978; Kuenen and Baker, 1982; Baker *et al.*, 1984). Optomotor responses to moving floors (or their equivalent) by insects flying upwind to odor sources have also been demonstrated in fruitflies (David, 1979) and tsetse flies (Colvin *et al.*, 1989). We predict that if the beetles are using the same optomotor response, then they will show responses, in ground speed and air speed, to the effects of flight height and floor speed similar to those of moth species studied by Kuenen and Baker (1982). In this paper we provide the first tests of this in a beetle, using *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae).

MATERIALS AND METHODS

Insects

A Tanzanian strain of *P. truncatus* reared on whole, clean maize at $30 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH under a L12:D12 photoperiod with no dusk or dawn

was used in this study. Beetles of known age (10–15 days old) and mixed sex were collected from cultures established on milled grain that passed through an Endecotts sieve of mesh size 3.35 mm to ease removal of beetles from culture and were preselected for flight activity, selecting only beetles that displayed flight potential for the experiments (Fadamiro, 1995).

Pheromone

The male-produced aggregation pheromone of *P. truncatus* is a two-component mixture. The major and minor components were identified as 1-methyl-ethyl(*E*)-2-methyl-2-pentenoate (Trunc-call 1; T1) and 1-methylethyl(*E,E*)-2,4-dimethyl-2,4-heptadienoate (Trunc-call 2; T2), respectively (Cork *et al.*, 1991; D. Hall, personal communication). The pheromones (T1:T2 at the ratio of 1:1) were supplied by the Natural Resources Institute, Chatham, UK (NRI), in polythene vial dispensers (20 × 9 × 1.5 mm thick) and stored at –50°C before use. For the tests, a 0.2-mg dose of the pheromone was used, this being the dose that gave the best attraction in the wind tunnel (Fadamiro, 1996).

Wind Tunnel

The wind tunnel was a clear, rectangular glass flight tunnel 160 cm long and 75 cm high and wide. The floor of the wind tunnel had a white background scattered with 13- and 24-cm-diameter black dots to provide cues for visual feedback (Miller and Roelofs, 1978). The treadmill could be rotated in either direction of the wind by means of a joy stick connected to a sliding-gear variable transmission and electric motor. Experiments were carried out at $28 \pm 1^\circ\text{C}$, $30 \pm 5\%$ RH, and 20 ± 1 cm/s wind speed, at 10–12 h of the photophase under a light intensity of 3700 lux (Fadamiro, 1995).

Evidence of Optomotor Anemotaxis

The optomotor anemotaxis hypothesis proposes that a flying insect controls its ground speed (speed of displacement of an insect over the ground) by reacting to and controlling the flow of ground images across its eyes (Kennedy and Marsh, 1974; Marsh *et al.*, 1978). To test this response adult *P. truncatus* were flown in a horizontal pheromone plume in a wind tunnel with a moving floor (above).

The pheromone source was placed in the middle of the upwind end 20 cm above the floor. Beetles were released onto a 20-cm-high platform facing upwind 100 cm downwind of the pheromone source. Using ventilation smoke plumes of HCl, it was confirmed that beetles released at this height had maximum exposure to pheromone plume.

Usually, a beetle released into the downwind part of the windborne pheromone plume took off and flew upwind toward its source. If the floor patterns

were now moved downwind, thus increasing the apparent ground speed of the flying beetle, it reduced its airspeed (speed of the flying insect through air, calculated as the sum of upwind velocity and wind speed) accordingly and was consequently carried downwind at the same height while still facing upwind. In this way beetles could be prevented from alighting on the pheromone source. Conversely, if the floor patterns were moved upwind, thus decreasing its apparent ground speed, a flying beetle increased its airspeed accordingly and was consequently carried upwind past the pheromone source.

Quantitative results were obtained by noting the displacement of individual beetles from the moment they crossed an imaginary transverse line midway between the release platform and the pheromone source (i.e., 50 cm upwind of the beetle's release point). Beetles' displacement was scored as either (a) downwind (i.e., when moved past the release point downwind reaching at least 10 cm downwind of the release platform), (b) reached the pheromone source (i.e., within a distance of 5 cm to the source), or (c) moved past the pheromone source upwind (i.e. at least 10 cm upwind of the pheromone source). These parameters were noted when (i) the floor patterns were stationary, (ii) they were moved in a downwind direction, and (iii) they were moved in an upwind direction. In all cases (except when stationary), the floor patterns were moved at one of two speeds, 7.5 and 30 cm/s, to give an indication of the effect of the speed of moving the floor patterns. In this study a beetle was flown up to a maximum of three times and beetles of similar age were used throughout. Only beetles that oriented in the pheromone plume were recorded. The order of test regarding the direction and speed of moving floor patterns was randomized.

Effect of Flight Altitude on Optomotor Response

It has been reported that moths flying at greater heights (40–60 cm) from the floor showed less response to the moving floor patterns (Kuenen and Baker, 1982). To test this hypothesis, *P. truncatus* were also flown to pheromone at a height of 50 cm from the floor. This was achieved by placing both the pheromone source and the release platform at this height (50 cm from the floor), as orienting beetles usually fly at the level of the pheromone plume. If the beetles' upwind velocity changes were determined or influenced by height, we would need to move the floor patterns downwind at a greater speed in order to halt the moths' upwind progress when they flew at a greater height. The same parameters as above were noted and the results were compared with those for the 20-cm height.

Effect of Flight Altitude on Flight Speeds

To test the hypothesis that insects increase their upwind velocity with increasing flight height (as recorded for moths by Kuenen and Baker, 1982), *P.*

truncatus were flown at 20 and 50 cm above a stationary floor and changes in upwind velocity and airspeed were examined. Upwind velocity (defined as the ground speed of an insect heading upwind) was measured by timing with a stopwatch, to the nearest 0.01 s, the duration of in-plume flight up the 100-cm distance between the beetle's release point downwind and the pheromone source upwind (100 cm/time) (Kuenen and Baker, 1982). Any large excursions from the plume (i.e., disoriented flights) were not measured. The wind speed in the tunnel was about 20 cm/s and the upwind velocity was measured and the airspeed calculated as described above. At least eight beetles were flown at each height and the order of observation was randomly determined.

RESULTS

Evidence of Optomotor Anemotaxis

The results of the tests are summarized in Table I. These show that *P. truncatus* flying upwind toward a pheromone source were responding to the visible floor patterns, as use of optomotor anemotaxis would require. Generally, a higher proportion of the flying beetles responded when the floor patterns were moved at the higher speed of 30 cm/s than when moved at 7.5 cm/s.

Effect of Flight Altitude on Optomotor Response

Flight altitude was an important factor influencing the response of flying *P. truncatus* to the moving visual floor patterns: beetles flying at 50 cm above

Table I. Sensitivity of *P. truncatus* Flying in a Pheromone Plume at Different Heights to the Direction and Speed of a Moving Floor Below Them^a

Direction of floor movement (speed; cm/s)	Displacement of beetles released (number of displaced beetles)					
	20 cm above the tunnel floor			50 cm above the tunnel floor		
	Flew downwind	Reached source	Flew past source	Flew downwind	Reached source	Flew past source
Stationary ^b	5 (11%)	41 (89%)	0 (0%)	3 (18%)	14 (82%)	0 (0%)
Downwind (7.5)	15 (75%)	4 (20%)	1 (5%)	1 (7%)	13 (93%)	0 (0%)
Downwind (30)	15 (88%)	2 (12%)	0 (0%)	7 (50%)	7 (50%)	0 (0%)
Upwind (7.5)	0 (0%)	1 (7%)	14 (93%)	1 (8%)	12 (92%)	0 (0%)
Upwind (30)	0 (0%)	0 (0%)	18 (100%)	0 (0%)	11 (79%)	3 (21%)

^aA beetle was tested up to three times and unequal numbers of tests were run for each direction of movement of the floor patterns.

^bThe floor was not moved when stationary.

the tunnel floor were less responsive to the moving floor patterns. Moving the floor patterns downwind prevented at least 75% of the beetles orienting to pheromone at a height of 20 cm above the tunnel floor from reaching the pheromone source. However, beetles orienting to pheromone at a height of 50 cm were less affected by the floor patterns even when moved at the faster speed of 30 cm/s (Table I). Similarly, by moving the floor patterns upwind, over 90% of the beetles orienting to pheromone at a height of 20 cm were moved past the pheromone source upwind, while only about 20% of the beetles flying at a 50-cm height were moved past the pheromone source, even at the faster moving floor speed of 30 cm/s.

Effect of Flight Altitude on Flight Speeds

Data from the experiment on flight speed versus flight height were square root transformed and analyzed using analysis of variance in GLM (SAS Institute, 1985). The results showed that flying *P. truncatus* significantly increased their upwind velocity ($F_{1,15} = 10.25$, $P = 0.006$) and airspeed ($F_{1,15} = 10.28$, $P = 0.006$), as their flight height increased (Fig. 1).

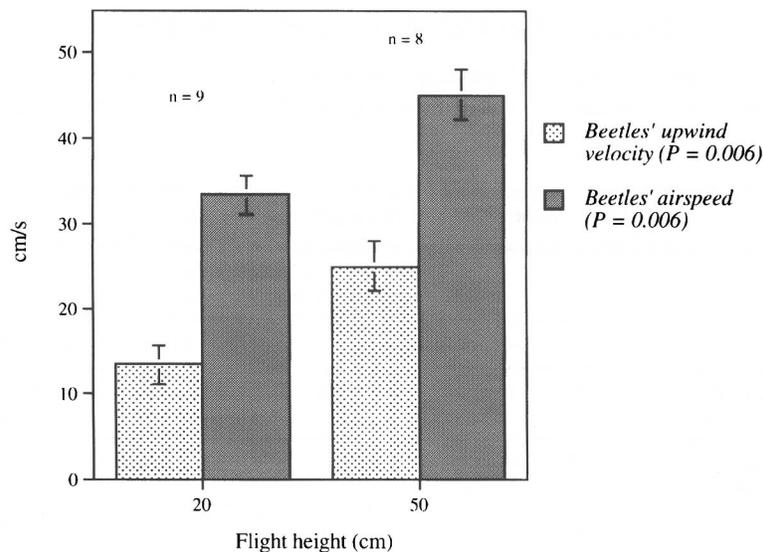


Fig. 1. Effect of flight altitude on the flight speed of *P. truncatus*. Mean upwind velocities and airspeeds of beetles flying at two heights. The differences between the two heights were significantly different (at $P < 0.05$).

DISCUSSION

To our knowledge this is the first detailed study on mechanisms of orientation in beetles. The beetles responded to changing flight height and floor speed in the ways that were predicted from David's (1979) study of the fruitfly and Kuenen and Baker's (1990) studies of moth species.

P. truncatus orienting upwind toward a pheromone source responded to the visible ground pattern, as use of optomotor anemotaxis would require (Table I). This has also been reported for other insects, notably moths (Kennedy and Marsh, 1974; Marsh *et al.*, 1978; Miller and Roelofs, 1978; Cardé and Haganman, 1979; Kuenen and Baker, 1982), but also flies (e.g., David, 1979; Colvin *et al.*, 1989).

In this study, the optomotor response to moving floor patterns was greatly reduced in beetles flying at the greater height of 50 cm from the tunnel floor (Table I). This may be due to one of the following conditions: (i) at a greater flight height, the ground pattern was less visible and no longer resolvable; (ii) at a 50-cm height, beetles were flying at a higher ground velocity than at 20 cm, and therefore the floor patterns needed to be moved at a higher speed (> 30 cm/s) for the beetles to respond.

The second experiment on the effect of flight height on flight speed lends support to the latter proposal (ii above)—the results show that while orienting to a pheromone source, *P. truncatus* increased their ground velocity as their height of flight increased (Fig. 1)—and supports the optomotor hypothesis of flight speed control in relation to height (Kennedy, 1951). Similar results have also been obtained for males of two moth species orienting to sex pheromone (Kuenen and Baker, 1982). They also observed a maximum height for each species where ground velocity no longer increased significantly—a “maximum compensatory height.” This was, however, not tested in the current study.

Upwind anemotaxis to odor sources seems well established in beetles, from the behavior observed in wind-tunnel experiments on odor responses (e.g., Choudhury and Kennedy, 1980; Birch and White, 1988; Salom and McLean, 1991; Wyatt *et al.*, 1993, 1997; Domeck *et al.*, 1990; Facundo *et al.*, 1994). Evidence of upwind anemotaxis also comes from field experiments. For example, the western pine beetle *Dendroctonus brevicomis* has been shown to orient upwind to pheromone anemotactically (Byers, 1988). In more indirect studies, Helland *et al.* (1984) and Schlyter *et al.* (1987) observed that the pattern of catch of the bark beetle *Ips typographus* in field traps was consistent with upwind anemotaxis.

The results presented here suggest the mechanism used by orienting *P. truncatus* is pheromone-modulated optomotor anemotaxis. These preliminary investigations set the stage for more critical research on maneuvers employed

by *P. truncatus* and other beetles, such as some of those above, for locating a distant odor source. A wider testing on beetles and other currently little-studied taxa of predictions from the rich moth and fly literature would be a worthwhile program.

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