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Short Communication

Harmonic Radar as a Means of Tracking the Pheromone-Finding and Pheromone-Following Flight of Male Moths

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INTRODUCTION

Over the past 30 years radar has emerged as an essential tool for investigating the migratory flight of insects at high altitude, providing, as it does, a unique means of measuring their altitudinal distribution, speed, and direction, and even their orientation (Riley, 1989; Reynolds, 1988). Until recently, however, it was not practicable to apply this powerful technique to low-altitude "foraging" flights (defined by Kennedy, 1985), i.e., flights to find food, mates, egg-laying sites, and other resource items (Bell, 1991). This is because the strong radar echoes from ground features and vegetation ("clutter") usually mask the very tiny returns from insects. In principle, this constraint can be overcome by using the *harmonic transponder* technique first suggested in 1967 (Vogler *et al.*, 1967) and subsequently investigated for automobile collision avoidance systems (Shefer *et al.*, 1974). In this technique, the target to be tracked is "tagged" with an harmonic transponder, in the form of an electrically nonlinear conductor (usually a diode) attached to some form of antenna. The transponder picks up energy from the illuminating radar and reradiates some of it at an harmonic of the original frequency: the radar receiver is selectively tuned to this new frequency and so is able to detect the transponder even in the presence of very strong clutter.

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The obvious attraction for entomological studies is that the harmonic tag needs no battery and, thus, can be made *extremely* lightweight. An early attempt was made by a group at the University of North Dakota (Klempel, 1977; Barbie, 1978; Shiao, 1978; Boyd, 1979) to exploit this potential by designing a system to track flies of the screw-worm, *Cochliomyia hominivorax* (Coquerel), but unfortunately the work did not lead to a usable radar, and the first successful entomological application of the harmonic tag principle was that of Mascanzoni and Wallin (1986). They showed that a hand-held transmitting/receiving device, built (commercially) for finding buried skiers, could also be used to locate burrowing Carabid beetles to which small custom-made harmonic tags had been glued (see also Wallin and Ekbom, 1988; Wallin, 1991; van der Ent, 1989; Hockmann *et al.*, 1989; Kennedy, 1994). More recently, Roland *et al.* (1996) described tags that weigh, remarkably, less than half a milligram, and showed that when these were fitted to flies and small moths, the insects could be located from ranges of up to 50 m. Although the devices used by Mascanzoni and Wallin, and by Roland *et al.*, were described as radars, they were actually portable *direction finders*—very useful for finding stationary or slowly moving insects from short range but not suited to measuring real-time flight trajectories.

Our scanning harmonic radar system (Riley *et al.*, 1996) was built with the specific aim of measuring low-altitude flight paths of insects. The first application of the radar was to study foraging flights by bumblebees and honey bees (Riley *et al.*, 1996; Carreck, 1996; Osborne *et al.*, 1997), but we briefly report here a recent extension of the method to observe male turnip moths, *Agrotis segetum* (Denis & Schiffermüller) (Noctuidae), flying in the presence of pheromone plumes in the field. The flight of male moths to female sex pheromones has hitherto been recorded with film or video techniques (Riley, 1993; Baker and Haynes, 1996)—methods which yield quantitative and fine-scale measurements of flight trajectories, but over ranges of only a few meters. The ability of harmonic radar to extend greatly the range of trajectory measurements should give new insights into basic pheromone-mediated behavior and, in particular, should improve our understanding of the mechanisms underlying the use of pheromones to control pest insects, e.g., by mating disruption.

METHODS

A. segetum moths, from a laboratory culture maintained at the Department of Ecology, Lund University, Sweden, were first tested in a wind tunnel to establish their ability to fly satisfactorily with our transponders attached. Males to be tagged were pressed down against soft foam rubber with a net to immobilize them. All scales on top of the scutum and the surrounding area were removed with a pair of tweezers. A plastic, concave disk 2.5 mm in diameter (normally used to number-tag bees) was further cut down in size to fit on top

of the scutum and glued to the insects with Evo-stik impact adhesive glue (Evode Ltd., Common Road, Stafford, England). The tag was then glued onto this disk using Super Attak glue (Loctite Sweden AB, Gothenburg, Sweden). During this handling, care was taken not to harm the insects: males that looked damaged in any way were not used. Males were released into a 40 × 60-cm screened cage until 1–3 h before the start of experiments, when they were individually transferred to glass tubes, closed with gauze cloth at both ends.

Before attempting the field experiments, we studied the behavior of tagged male *A. segetum* when they were flying upwind toward a pheromone bait in a wind tunnel similar to the one used by Valeur and Löfstedt (1996) (Fig. 1). The pheromone dispensers were composed of rubber septa (red sleeve, 16 × 9 mm; Thomas Scientific, Swedesboro, USA) with an applied synthetic four-component pheromone blend, containing (Z)-5-decenyl acetate, (Z)-7-dodecenyl acetate, (Z)-9-tetradecenyl acetate, and (Z)-5-dodecenyl acetate in the proportions 1/5/2.5/0.25 (Wu *et al.*, 1995). For the wind tunnel trials we used a load of 0.5 μg with respect to (Z)-5-decenyl acetate, and we found that several tagged males responded and flew to the pheromone bait in the way that untagged *A. segetum*
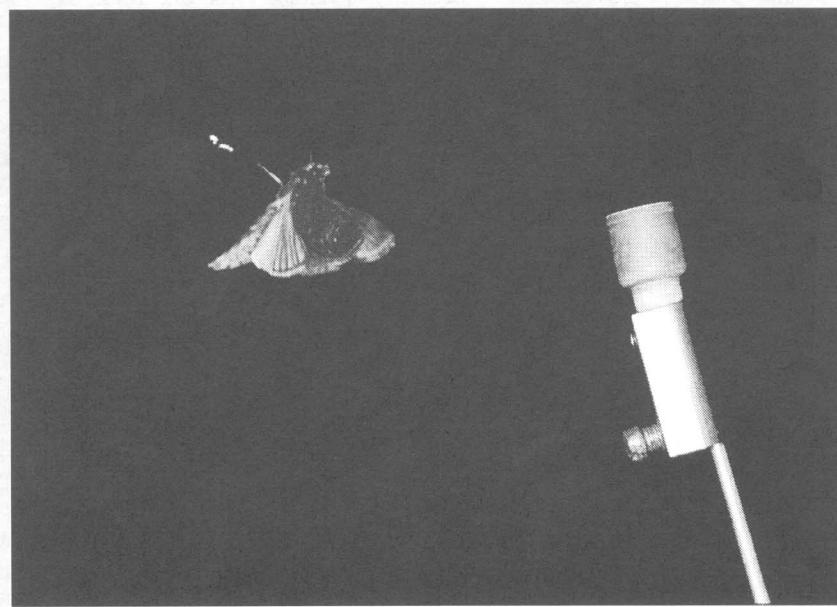

Fig. 1. A male *Agrotis segetum* approaching a pheromone dispenser in the wind tunnel. It is fitted with a tag consisting of a Schottky barrier diode mounted at the center of a 16-mm dipole antenna. The moths weighed 150–250 mg and the tags used during these flights weighed approximately 6 mg; equivalent to 3–4% of the body mass. More recent versions of the tag are made of lighter materials and weigh considerably less.

males normally do in a wind tunnel assay (see, e.g., Löfstedt *et al.*, 1985; Wu *et al.*, 1995).

Our field experiment was carried out at IACR—Rothamsted, Hertfordshire, UK, during August 1996. The radar was set up in a relatively flat and open area (Little Hoos) approximately 700 × 500 m and occupied principally by plots of agricultural crops or bare soil. The 3.2-cm-wavelength radar transmitted 25-kW pulses of 0.1 μs duration, at a repetition frequency of 2 kHz, and its receiver was tuned to receive 1.6-cm harmonic returns. The radar antenna assembly rotated in azimuth at 20 rpm, so the positions of tagged insects within range could be determined once every 3 s. Sequential positions were displayed on a conventional radar plane position indicator (PPI) display, and when filmed by time-lapse ciné photography, they provided a dynamic record of the insect flight tracks. Flying moths could be recorded over a circular area of about 1.4 km in diameter, but because vegetation strongly absorbs microwave transmissions, moths dipping below crop height or flying behind trees or hedges produced interrupted tracks. The nature of our general-purpose radar display limited the precision with which we could determine moth positions to ± 7 m in range and ± 3 m in azimuth (at a range of 700 m), but in future experiments we expect to be able to use improved signal processing to reduce these uncertainties by at least a factor of two.

Because the speed and direction of the wind are both critical factors in understanding pheromone-modulated flight, we set up four anemometers in the experimental area so that they formed a quadrilateral centered on the area to the north of the radar where we expected the moths to fly. The lengths of the east–west sides of the quadrilateral were approximately 400 m, and those of the north–south sides 170 m. The anemometers recorded wind speed and direction once every 10 s, at 2.7 m above ground level, and thus provided a dynamic measure of the wind field over the flight area.

In the field experiments we used the same pheromone dispenser and blend as in the wind tunnel experiments, but this time with a pheromone load of 10 μg, with respect to (Z)-5-decenyl acetate. These baits were mounted in Delta pheromone traps (Oecos, Kimpton, UK) without sticky bottoms, fixed on poles at 1.3 m above ground. The traps were arranged in a line 145 m long, running 115–295° (i.e., approximately perpendicular to the direction of the prevailing wind), within the anemometry quadrilateral, and with the radar about 200 m to the southwest (see Fig. 2). Male pupae, from the same laboratory culture that we used for the wind tunnel trials, were kept at room temperature under natural light conditions. Moths were used at the age of 1–3 days and tags were applied 6–12 h before the start of experiments. Individual, tagged males in glass tubes were transported to the experimental field just before sunset. We exposed the males, still in the tubes, on a platform 0.5 m high, 150 m upwind from the
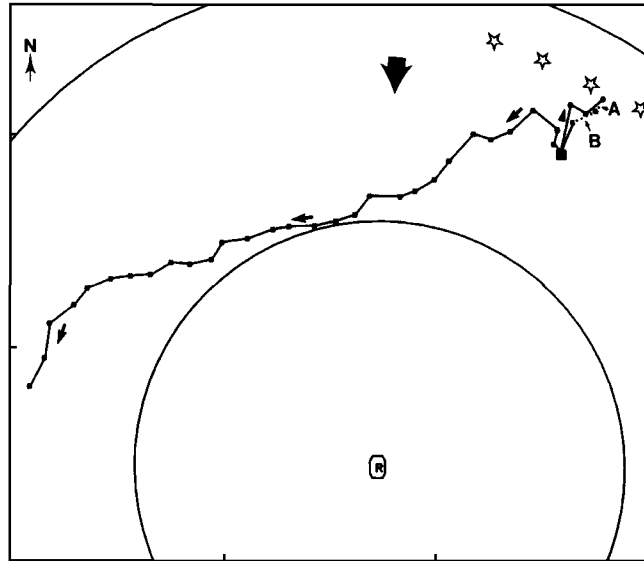
(a)

Fig. 2. Examples of moth flight trajectories: (*) a "paint" on the radar screen; (· · ·) gaps in the track where the moth had probably landed (letters A, B, etc., explained below); (■) the first appearance of the moth on the radar screen; (☆) the position of pheromone traps. Tick marks on the x and y axes = 100 m; R = position of the radar. Distance between the range rings = $\frac{1}{16}$ nautical mile (ca. 116 m). The broad arrows show the average direction in which the wind was blowing during the track. In the data presented below, the ± values attached to speed refer to the standard deviation; in the case of direction, they refer to Mardia's (1972) s parameter, which provides an analogous measure of angular dispersion. (a) Track recorded between 23:44:00 and 23:47:00 on 12 August 1996. A, 30-s break in track; B, 21-s break. Over the straighter portion of the track displacing toward the WSW, the moth's average displacement (ground) speed was 3.8 ± 1.1 ms^{-1} toward 241 ± 23°. The average wind speed and direction during the track, and at its midpoint, were calculated to be 1.9 ± 0.7 m^{-1} and 003 ± 22°. (b) Track recorded betweeen 21:50:06 and 21:58:06 on 13 August. C, break in track of ca. 7 min, during part of which the moth was stationary on a nearby pheromone trap. During the portion of track after the moth left the trap, its average displacement speed was 4.1 ± 1.8 ms^{-1} toward 111 ± 15°. The average wind speed and direction during the track, and at its midpoint, were calculated to be 2.0 ± 0.5 ms^{-1} and 009 ± 17°. (c) Track recorded between 22:34:33 and 22:35:18 on 14 August. The average wind speed and direction during the track, and at its midpoint, were calculated to be 2.0 ± 0.5 ms^{-1} and 022 ± 17°. (d) Track recorded between 23:25:18 and 23:27:51 on 14 August. D, 45-s break in track; E, 24-s break. The average wind speed and direction during the track, and at its midpoint, were calculated to be 2.3 ± 0.4 ms^{-1} and 008 ± 12°.
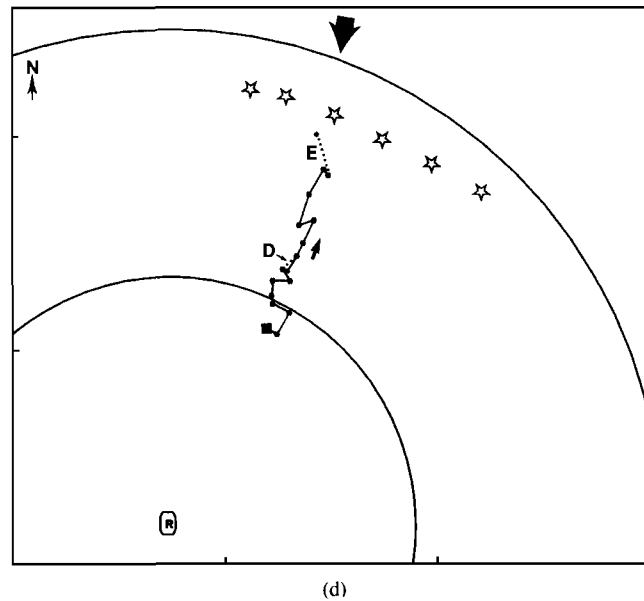
(b)

(c)
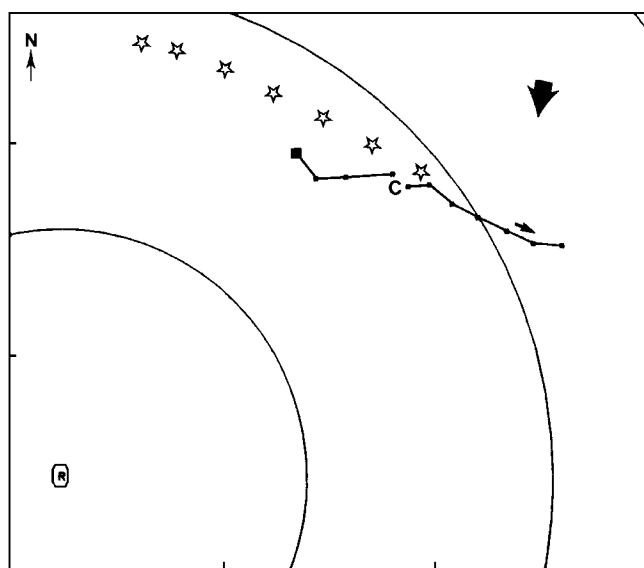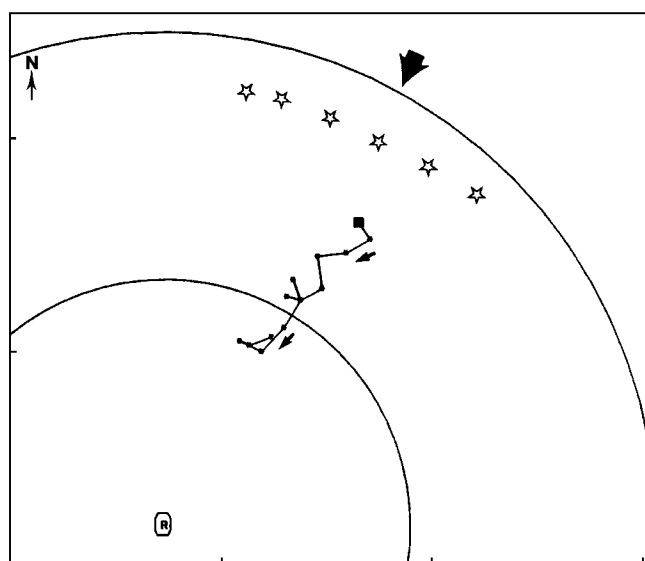
Fig. 2. Continued

(d)

Fig. 2. Continued

radar, and 30 m downwind from the line of pheromone traps. When a male started wing fanning, we opened his tube and allowed him to fly away. Flying moths appeared as moving targets on the radar PPI screen, which was recorded on 16-mm film (one frame for every revolution of the radar scanner). The tracks were later plotted from the film and entered into a computer as digitized coordinates.

RESULTS AND DISCUSSION

The objective of this short paper is to demonstrate the potential of harmonic radar to investigate the flight of moths in the vicinity of pheromone plumes. We therefore present in Fig. 2 some examples of the flight trajectories which we recorded, and discuss them in the context of what is known about pheromone-mediated flight.

In Fig. 2a, the moth first flew to and fro over an area about 30 × 30 m downwind of the pheromone traps, landing twice for short periods. It then embarked on a relatively long and straight flight for about 270 m before landing again. The first, erratic part of the flight is difficult to interpret, but the second,

straight portion seems to be characteristic of the fast, linear displacements of male moths searching for a pheromone plume. This type of "ranging" flight (Bell, 1991) is said usually to be cross-wind or diagonal to the wind in noctuids such as *Heliothis/Helicoverpa* spp. (Lindgren *et al.*, 1978, 1986; Riley *et al.*, 1992). Elkinton and Cardé (1983) found no evidence for any preferred flight direction in plume-finding flights of male gypsy moths, *Lymantria dispar* L., but they were working in a forest habitat where the structure of plumes would be very different from their structure over flat open ground. Theoretical considerations suggest that the optimum flight direction tends toward the cross-wind for elongated plumes (Dusenbery, 1989), and we calculated by vector subtraction of the estimated wind velocity from the moth's average displacement velocity, that along the straight portion of the track in Fig. 2a, the insect's average orientation was toward 271°; i.e. it was heading almost exactly across the wind, which was toward 183°. Our calculations suggested that the moth's average air speed was 3.2 ms^{-1}. For the purposes of heading and air speed calculations, we assumed that the average wind vector experienced by the moth was the mean of the values recorded over the flight period from the two anemometers closest to the center of the flight path. In the relatively steady wind fields prevailing during the experiments, more elaborate interpolation routines using data from all four anemometers did not seem to be justified.

In the example shown in Fig. 2b, the moth flew for about 50 m from its release point almost directly to a pheromone trap, where it remained on the cardboard housing for about 7 min before it was disturbed by an observer and flew off eastward beyond the trap line. There was no evidence of zigzagging during the first half of the moth's flight, at least on scales resolvable by the radar, so it is not clear whether the insect arrived at the trap by searching or by chance. On leaving the trap the moth would have been outside the pheromone plumes, and this portion of the track could be another example of cross-wind "ranging" flight. We calculated that the moth's average orientation in this part of its flight was toward 083°, i.e., it was flying at 106° to the downwind direction, and that its air speed was 4.2 ms^{-1}.

In Fig. 2c, the moth followed a zigzag track over a straight-line distance of about 80 m, with lateral excursions of about 7–9 m, possibly "casting" (Kennedy, 1983), but its overall direction of displacement was *downwind* from the trap line. Short segments of downwind flight by pheromone-stimulated *Grapholita molesta* (Busck) have recently been reported by Baker and Haynes (1996), who speculated that this behavior might enable a male which has just lost a pheromone plume to relocate it, downwind, a few moments later. Our track covered a much longer downwind distance but may, perhaps, represent a similar type of plume recovery behavior. About 50 min later (Fig. 2d), the same moth appeared from a point very close to where it had last been detected by the radar. It was observed to zigzag about 90 m back toward the traps and was last detected

just a short distance (15 m) south of the trap line, where it presumably had landed. This track contained large (ca. 9-m) lateral movements (and a regressive movement at one point), and we conclude that it was probably an upwind anemotactic plume-following flight.

We do not suggest that the results of the exploratory study reported here constitute a significant contribution to knowledge about pheromone-mediated flight by male moths, but we do claim that they demonstrate that harmonic radar has the potential to make such a contribution. While flight maneuvers on the scale of centimeters seem certain to remain the province of video and ciné techniques (Riley, 1989, 1993), the unparalleled range of harmonic radar makes it possible for the first time to observe the nocturnal flight of individual moths over distances of hundreds of meters, and seems certain to lead to new insights into the effect of pheromones on moth flight behavior.

ACKNOWLEDGMENTS

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