Till Tom med bästa hälsningar
från Glenn

Entomologia Experimentalis et Applicata **101:** 111–121, 2001.

Mating disruption in *Agrotis segetum* monitored by harmonic radar

Glenn P. Svensson[1], Peter G. Valeur[1], Donald R. Reynolds[2], Alan D. Smith[2], Joseph R. Riley[2], Thomas C. Baker[3], Guy M. Poppy[4] & Christer Löfstedt[1]

[1]*Department of Ecology, Lund University, SE-223 62 Lund, Sweden;* [2]*Radar Entomology Unit, Natural Resources Institute, University of Greenwich, North Site, Malvern, Worcestershire, WR14 1LL, UK;* [3]*Department of Entomology, Iowa State University, Ames, IA 50011, USA;* [4]*IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, UK; Present address: Division of Biodiversity and Ecology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK*

Accepted: September 11, 2001

Key words: *Agrotis segetum*, Lepidoptera, Noctuidae, sex pheromone, mating disruption, harmonic radar, mating table, field trapping

Abstract

The long-range, pheromone-mediated, flight behaviour of male moths under natural and mating disruption conditions was monitored by means of harmonic radar. Individual male turnip moths, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae), tagged with radar transponders, were released and tracked in plots with or without disruptive doses of sex pheromone. In addition, male attraction to pheromone-baited traps and mating of calling females in treated and untreated plots was investigated. High doses of a four-component pheromone blend reduced trap catch by 79% and mating of females by 62% when compared with control plots in pre-radar experiments. Surprisingly, this effect was not associated with any pronounced differences in flight behaviour of males between a treatment and a control plot as revealed by harmonic radar recordings. In total, 20 flight tracks from a control plot and 22 flight tracks from a treatment plot were analysed. Moths could be followed for up to 77 min, corresponding to a track length of 7350 m. Mean ground speed ranged from 0.7 m s^{-1} to 5.4 m s^{-1}. There was a strong trend ($P = 0.06$) for a greater number of male orientations to traps from downwind in the control field compared to the treatment field. Many flight tracks were fragmented due to radar shadow. Advantages and constraints using harmonic radar to study the pheromone-mediated flight behaviour of nocturnal moths are discussed.

Introduction

The ultimate practical goal of research on moth sex pheromones is their use in pest management. However, until now, commercially acceptable, effective population suppression by pheromone-based mating disruption has only occurred for a few species and such successes have been mixed with ineffective suppression for species in other Integrated Pest Management (IPM) systems (Cardé & Minks, 1995). A more complete understanding of the mechanisms behind the disruption of pheromone-mediated mate finding in moths should help in facilitating more successful uses of the mating disruption technique.

Whereas a thorough understanding of how male moths orient towards and locate pheromone sources has now been gained through wind tunnel experimentation (see reviews by Baker & Vickers, 1997; Cardé & Mafra-Neto, 1997), monitoring the flight of males under mating disruption conditions in the field has been difficult because of the lack of suitable techniques. Recently, however, a novel scanning harmonic radar system for measuring low-altitude insect flight over long distances (≈700 m) was developed (Riley et al., 1996). In their pioneering radar work the foraging behaviour of bumblebees and honey bees was studied (Riley et al., 1996, 1999), and the technique has also been used to analyse the flight of male moths towards

pheromone sources under field conditions (Riley et al., 1998). In the current study, we attempted to use this technique for the first time to analyse the behaviour of freely flying male moths in the field exposed to conditions of mating disruption.

Several mechanisms have been suggested as contributing to mating disruption in moths. At the physiological level, adaptation of receptor neurones and/or habituation at the CNS may cause sensory fatigue (Sanders, 1997). Arrestment behaviour of male turnip moths, *Agrotis segetum* (Denis & Schiffermüller) (Lepidoptera: Noctuidae), when exposed to high concentrations of pheromones in wind tunnel experiments has been associated with either total adaptation or maximal spike activity of pheromone-sensitive receptor neurones (Baker et al., 1988; Valeur et al., 2000). Another proposed mechanism behind mating disruption is 'false trail following', i.e., males orientating towards pheromone dispensers instead of calling females. The disruption effect here will depend on the quality and quantity of pheromone dispensers as well as the density of calling females in the treated area (Sanders, 1997).

The present study was performed to investigate the pheromone-mediated flight behaviour of male *A. segetum* under natural and mating disruption conditions and link the flight performance to the attraction to synthetic and natural pheromone sources. For disruption a system of widely spaced, high-emission-rate dispensers was used. One of our main goals was to see what useful behavioural parameters were possible to gain from harmonic radar tracking in evaluating the effects of pheromone communication disruption on free-flying males over long distances. In addition, we wanted to use levels of behavioural disruption that were moderate compared with the usual 95-100% suppression of trap catch so that we could determine how males behave under such conditions.

Materials and methods

Insects. Turnip moths were obtained from a laboratory culture maintained at the Department of Ecology in Lund, Sweden, for more than 15 years and genetically refreshed on a yearly basis by mating of ca. 100 wild males and females from the culture with ca. 100 field-collected moths. Larvae were reared on a semi-synthetic bean diet described by Zhu et al. (1996) and kept in a L17:D7 photoperiod at 25 °C. Pupae were separated according to sex and kept in different rearing rooms at 21 °C. Males used in radar experiments were selected as fresh pupae in Lund and sent by mail to England five days before the start of radar experiments and kept outdoors to acclimatise them to the weather and natural photoperiod.

Chemicals and dispensers. Chemicals for mating disruption were purchased from Bedoukian Research Inc, Danbury, USA. The four-component blend consisted of (*Z*)-5-decenyl acetate Z5-10:OAc, (*Z*)-7-dodecenyl acetate (Z7-12:OAc), (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), and (*Z*)-5-dodecenyl acetate (Z5-12:OAc) mixed in solution in the proportions 1/5/2.5/0.25, mimicking the Nordic pheromonal strain of the species (Löfstedt et al., 1982; Wu et al., 1995). Because no commercial disruptant dispensers were available that emitted the complex *A. segetum* four-component pheromone blend at the desired emission rates, we used Metered Semiochemical Timed Release System dispensers (MSTRS^{TM}) (Baker et al., 1997) with their bottle reservoirs loaded with dilute solutions of pheromone in 95% ethanol. The bottles contained 100 ml of a solution of the four-component blend. Concentrations of the components in ethanol solution were as follows: 0.063 μg μl^{-1} of Z5-10:OAc, 0.016 μg μl^{-1} of Z5-12:OAc, 0.313 μg μl^{-1} of Z7-12:OAc, and 0.156 μg μl^{-1} of Z9-14:OAc. The machines were programmed to emit a 70 μl spray of the solution onto an acrylic pad (12 cm diam.) hanging 5 cm in front of the spray nozzle.

Chemicals used for pheromone baits were purchased from DLO Plant Research International (PRI), Wageningen, the Netherlands. Rubber septa (red sleeve, 16 × 9 mm, catalogue no. 1780J07, Thomas Scientific, Swedesboro, USA) were used as lures and solutions were prepared in distilled hexane. Each septum was loaded with the four-component blend at an amount corresponding to 5 μg of Z5-10:OAc and then put in a fume hood for 3 h to allow the hexane to evaporate. Chemicals used for MSTRS^{TM} and pheromone baits were all >99% pure with respect to other active components as confirmed by gas chromatography.

Pheromone disruptant emission rates. Release rates from the MSTRS^{TM} pads were measured for the three most abundant components in the blend (Z5-10:OAc, Z7-12:OAc, and Z9-14:OAc–Z5-12:OAc not included due to its low percentage in the blend) by taking the pads from the machines and attaching them to the mouth (10 cm diam.) of a large glass funnel, the narrow end of which was packed with 3 cm of

densely packed glass wool. A vacuum was drawn from the narrow end of the funnel at 100 ml min^{-1} such that air passed through the entire surface of the pheromone-impregnated pad and adsorbed onto the glass wool. Previous trials with this system had shown negligible breakthrough of these components using this collection method. After 15 min of airborne collection from each pad, 5 μg of an internal standard, (Z)-8-tridecenyl acetate in 5 μl of hexane, was placed onto the glass wool via a micro-syringe and then the inner surface of the funnel and the glass wool were rinsed with 5 ml of distilled hexane. Two microliters of the eluent was injected onto a Hewlett-Packard 5890 mass selective detector operated in the total ion current recording mode. The areas of the peaks corresponding to compounds of interest were compared with the areas of the internal standard peak and the amounts calculated according to 5 μg of the standard divided by 15 min collection time for the release rate per minute. Collections were made at 23 °C from two pads each, 4 h into the emission period on Days 1, 7 and 14 after initiation of the spraying of the pads.

Field trapping experiments. In an earlier study of *A. segetum* (Svensson et al., 1995) moderate disruption was achieved using a three-component disruption blend (Z5-12:OAc not included). To achieve that level of disruption in our pre-radar experiments, we used similar emission rates per area unit as in Svensson et al. (1995), calibrating for differences in dispenser type, number of dispensers per ha etc. Field trapping was conducted from 19 to 30 June 1998, in sugarbeet fields and in potato fields at Barsebäck, 20 km northwest of Lund, Sweden. The plants were 20–50 cm high. A four-component blend was used as disruptant (N = 3). MSTRS^{TM} devices were placed on stakes at 1 m height. In each treatment plot, 16 dispensers were placed in a circle with a diameter of 230 m, giving a nearest-neighbour distance between disruptant dispensers of ca. 45 m. Inside this area eight Lund II sticky traps (Anderbrant et al., 1989) were placed in a circle with a diameter of 150 m. Plots were separated by at least 400 m to avoid chemical interference. Pheromone was sprayed from the bottles every fifth minute from 8 pm to 5 am at a rate of ca. 5 μg $spray^{-1}$ with respect to Z5-10:OAc. Sticky traps were checked and sticky bottom inserts were replaced every 1–2 days. Rubber septa were replaced once a week. Trap catch data within plots were pooled and treatments were compared using an unpaired *t*-test.

The disruption effect between treatment and control plots was calculated by the formula:

$$\% \text{ disruption} = \frac{\text{trap catch in control} - \text{trap catch in treatment}}{\text{trap catch in control}} \times 100.$$

Mating tables. Mating of virgin females was estimated using mating tables in plots treated with a four-component pheromone blend and in control plots at Barsebäck, Sweden. Mating tables (four tables per treatment) baited with one or two females (2–4 days old) were used. The wings were cut off and individuals were placed in a circular arena (25 cm diam.) with 5 cm high walls covered with silicone, which made it impossible for the moths to escape. Calling platforms were created by pieces of metal net (5 × 10 cm) attached onto the bottom of the arena. Mating tables were placed on stakes at 1 m height. Females were replaced twice a week and put in 250 ml plastic jars for oviposition. Mating success was estimated as presence or absence of larvae. In total, 21 females were used in treatment plots and 20 females were used in control plots. Differences in mating frequency between treatment and control plots were analysed using a χ^2-test.

Radar experiments. The flight behaviour of male moths in a pheromone-treated and an untreated plot was investigated using a scanning harmonic radar system (Riley et al., 1996). For logistic reasons, flight recordings could not be carried out in Sweden, but had to be conducted at IACR-Rothamsted, Harpenden, England. Radar recordings of male flight behaviour were carried out from 4 to 13 August 1998. The radar transmitted 0.1 μs pulses at 25 kW with a wavelength of 3.2 cm and a repetition frequency of 1.5 kHz. The receiver was tuned to the 1.6 cm harmonic returns. The radar antenna rotated at 20 rpm so the position of the target could be estimated every third second. Radar returns from moths carrying miniature transponders (see below) registered as 'paints' on a Plan Position Indicator (PPI) type display generated on a computer screen, and the returns were simultaneously recorded on the computer's hard disk. Moth position co-ordinates were later digitised from the computer records. Moths could be tracked over distances of up to 700 m from the radar, provided that they remained above the radar's local horizon. The errors in fixing positions of the moths were approximately ±3 m in range and ±1.3 m

in azimuth (at a range of 300 m). Wind speed and direction were automatically recorded every 10 s at five locations within the area scanned by the radar, and temperature was recorded once every 15 min at one of the locations. The experimental area included hedges, different crops (corn, bean, wheat), and bare soil. The crop plants were up to 2.5 m high. A plot treated with the four-component blend and a control plot were used with similar design (size, number of dispensers, and traps) as for the field trapping experiments in Sweden. The distance between the respective release points in the two plots was 350 m.

Male turnip moths were kept in 40 × 60 cm screened cages. To immobilise them they were pressed down against soft foam rubber with a fine-masked net. Scales were removed and 16 mm radar transponders were glued on the top of the scutum using Evostick® Impact Adhesive (Evode Ltd., Common Road, Stafford, UK) or Bison-Tix® (Perfecta Chemie BV, the Netherlands). The transponders used to tag the males weighed ≈8 mg (including a small plastic base) which was less than ten percent of the body weight of a male (mean = 144.4 ± 2.9 mg; $n = 50$). Two to five days old males were used in radar experiments and they were all tagged and placed in small open glass cylinders within the experimental plots at least 2 h prior to the experiments. This was previously found to be the best method for obtaining moths suitable for recording flights with the radar (Riley et al., 1998). Recordings were carried out between 10 pm and 3 am (British Summer Time). Males were released from the centre of the plots (one individual at a time to avoid confusion of tracks). Two to 29 tagged males were released per night. Those that lost their tags were released as untagged individuals (control plot: $n = 10$, treatment plot: $n = 14$). In addition, during the last two nights of the experimental period males without tags ($n = 70$) were released in both areas.

By using the positions of successive paints, calculations of the ground speed of moths were carried out on a vector-by-vector basis. Mean ground speed between the fields was compared using an unpaired t-test. Number of paints per track, duration of track and number of upwind flights towards traps of males in the different plots were compared using Mann–Whitney U-tests. Also, ground speed of males estimated close to (inside a 20 × 20 m square surrounding a trap) or far away from pheromone traps were compared within plots using paired t-tests. When fragmented tracks were analysed, calculations of ground speed were not performed if two successive paints were more than 12 s apart (i.e., there were more than four missing paints in the track). In addition, landing frequencies of males in the different fields were estimated by calculating the ground speed within gaps of flight tracks. Males with ground speeds below 0.01 m s^{-1} within a gap were considered as 'non-flying'. Landing frequencies were compared using a Mann–Whitney U-test.

Performance of males in a wind tunnel. As the trap catch of tagged males differed from the trap catch of untagged males during the radar experiments, a wind tunnel assay was carried out to study the effect of the attached 16 mm radar tag on the flight behaviour of males towards a Lund II sticky trap. Tagged and untagged moths were tested in the 0.9 × 0.9 × 3 m plexiglas wind tunnel, described by Valeur & Löfstedt (1996). During flight tests the conditions in the wind tunnel were: temperature 21–22 °C; humidity 60–65%, wind speed 0.3 m s^{-1} and light intensity 3 lux. Two to four day old males were transferred individually to small glass cylinders and placed in the wind tunnel room before the initiation of the dark period. Flight experiments were conducted 2–5 h into the scotophase. Each male was first exposed to the pheromone plume for approximately 5 s and then allowed to leave the tube. It was then observed for 2 min and different behavioural steps were scored: take flight (TF), orientation in the plume (Or), orientation half way to the trap (HW), orientation 1 cm from the trap (1 cm), and entering the trap (T). Each male was used only once. Differences in response between tagged and untagged males were compared using Ryan's test for multiple comparisons of proportions (Ryan, 1960).

Results

Trap catch in control vs treatment areas. The ability of males to locate pheromone traps decreased significantly in plots treated with high doses of pheromones (treatment: 1.5 ± 0.2 males per trap per night, control: 7.4 ± 0.4 males per trap per night, $T = 6.95$; d.f. = 36; $P < 0.01$, Figure 1). In total, 228 males were trapped in pheromone plots and 1 101 males in control plots, corresponding to a disruption effect of 79%. No time effect was observed, i.e., the same level of disruption was achieved during the whole experimental period (12 nights).

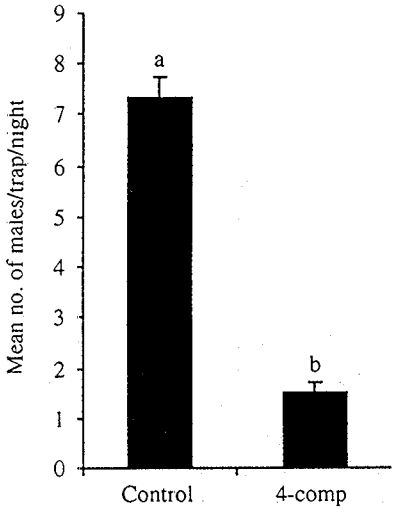

Figure 1 Mean (±se) number of male *A. segetum* trapped in control and treatment plots for mating disruption ($N = 3$). Trap catch differed significantly between treatments ($P < 0.01$).

Table 1 Matings of virgin female *A. segetum* in areas treated with the four-component pheromone blend and in control areas (four replicates per treatment)

	Treatment	Control
No. females in mating tables	21	20
No. of mated females	4[a]	10
Mating success (%)	19	50
Disruption effect relative to control (%)	62	

[a]Significant difference between treatment and control areas ($P < 0.05$).

Mating frequency in control vs treatment areas. Mating of females in mating tables occurred throughout the period in both treatment and control plots. Mating frequency was reduced by 62% in plots treated with pheromone compared to control plots ($\chi^2 = 4.36$; d.f. = 1; $P < 0.05$, Table 1).

Male flight behaviour in a control vs a treatment area. In total, more than 4300 paints were recorded from male moths during the experiments. Only flight tracks that could be linked with certainty to specific males were analysed and only tracks made up of five paints or more were included. Using these criteria, 20 tracks from the control plot including 1900 paints, and 22 tracks from the treatment plot including 700 paints, qualified for further analysis (Figure 2). Many of these tracks were fragmented, i.e., the moth was invisible on the radar screen for either short (10–30 s) or sometimes very long (>120 s) periods. A selection of tracks is shown in Figure 3.

The duration of the recorded flight routes ranged from a few seconds up to 77 min, corresponding to flight track lengths between 19 m and 7350 m. About three times as many paints were recorded from males in the control plot versus the treatment plot (control: 94.3 ± 175.0, treatment: 30.5 ± 32.7). However, this asymmetry was due to four very long tracks contributing to 82% of all paints recorded in the control plot and no statistically significant difference was observed in number of paints per male between the plots. The mean duration of the tracks recorded in the control plot was also nearly triple that in the pheromone-treated plot (control: 884.8 ± 1519.2 s, treatment: 302.5 ± 359.4 s).

Interestingly, in both plots significantly more males upon release flew in a downwind or crosswind direction instead of heading upwind (control: 17 out of 20, $\chi^2 = 9.80$, d.f. = 1, $P < 0.01$; treatment: 20 out of 22, $\chi^2 = 14.73$, d.f. = 1, $P < 0.01$), which indicates that the majority of males in both plots were not behaviourally stimulated to perform optomotor anemotaxis at the time of their release. However, at some point after release there were brief periods of males flying upwind towards the pheromone traps, sometimes from >30 m away. Only one male in the treatment plot was clearly attracted to disruption dispensers. There was a strong trend for more upwind-oriented movements close to the pheromone traps in the control plot (1.25 ± 0.70 orientations per male) versus the pheromone-treated plot (0.23 ± 0.11 orientations per male) ($P = 0.06$). Twenty-five clear instances of such orientation were seen in the tracks in the control plot, and only five instances of this movement were seen in the tracks examined in the treatment plot. Mean ground speed ranged from 0.7 m s^{-1} to 5.4 m s^{-1}. For all males in both plots, overall ground speed did not differ. Males flew at a mean ground speed of 2.82 ± 0.25 m s^{-1} in the control plot and at 2.90 ± 0.22 m s^{-1} in the treatment plot. Males in the disruptant-treated plot significantly reduced their ground speed when flying within 10 m of traps, regardless of wind direction compared with when they were flying further away than 10 m from traps (1.76±0.35 ms^{-1} when close, 2.64 ± 0.28 m s^{-1} when far away, $T = 2.82$, $P = 0.03$, Figure 4). Although no statistically significant difference was found in the control plot (2.70 ± 0.35 m s^{-1} when close, 3.12 ± 0.37 m s^{-1} when far away, $T = 1.65$, $P = 0.14$), the reduction in ground speed close to traps did not differ between

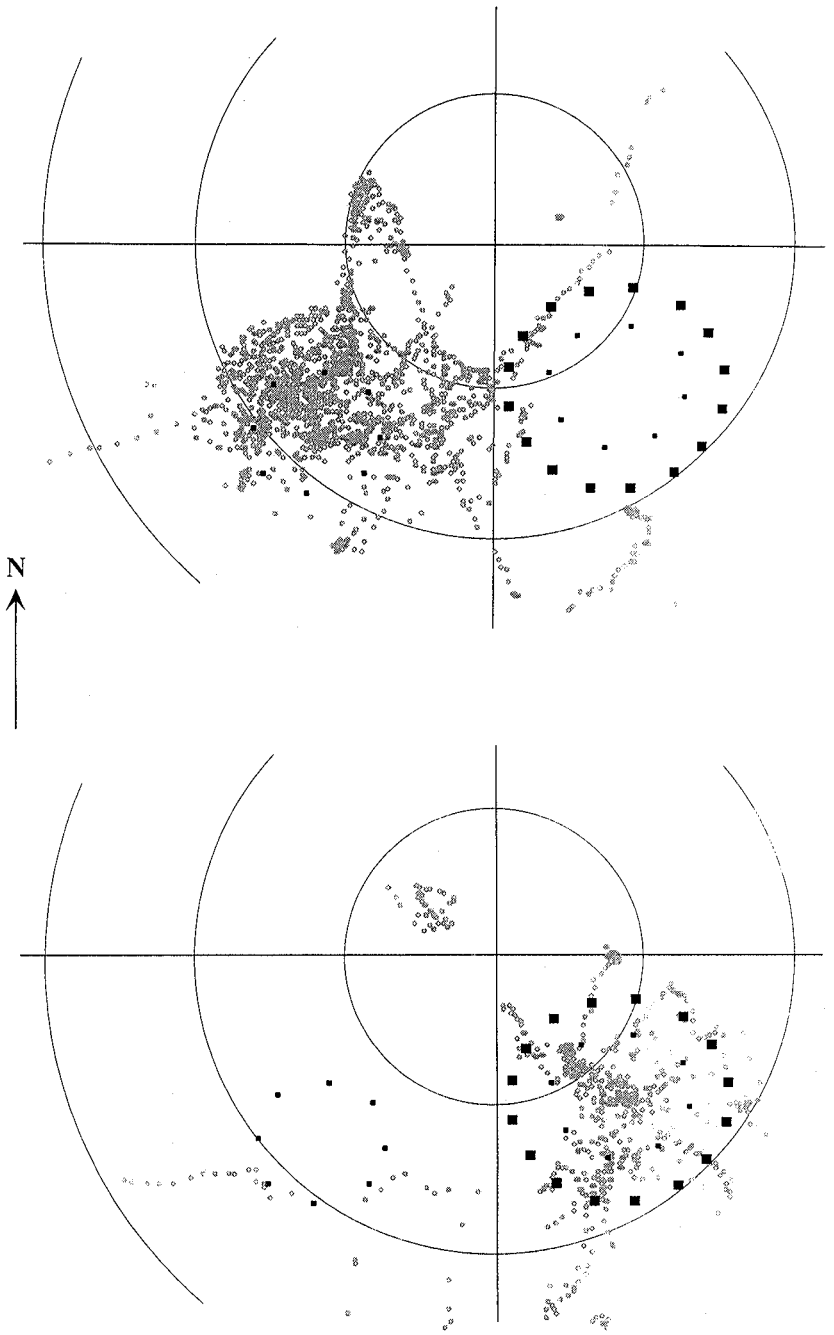

Figure 2. Recorded paints from male *A. segetum* tracked by harmonic radar. Males were released in a control plot (upper figure, $n = 20$) or in a plot treated for mating disruption (lower figure, $n = 22$). Small squares indicate positions of pheromone traps. Big squares indicate the positions of MSTRS^{TM} machines. Range rings are 100 m apart. The radar was positioned at the origin. The control plot was positioned ~~east~~ west of the treatment plot.

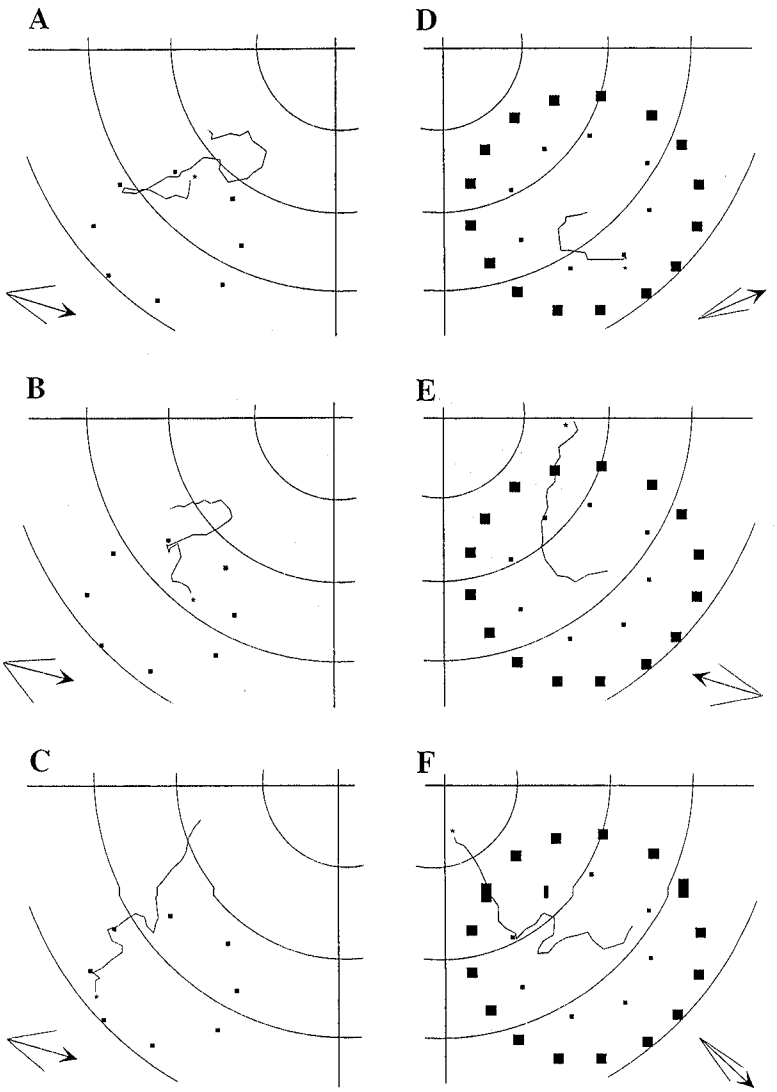

Figure 3. Examples of flight tracks of male *A. segetum* monitored by harmonic radar in plots with or without disruptive doses of pheromones. The radar is positioned at the origin. Range rings are 100 m apart. Small squares indicate positions of pheromone traps. Big squares show positions of MSTRS^{TM} machines emitting pheromones. Endpoints of recorded tracks are indicated by asterisks (*). Arrows show the wind direction during the recordings (mean direction: thick line; range of directions: thin lines). (A–C) Tracks recorded from the same male released in the control plot 11 August and followed for 90 min. Track A was recorded from 11.13 pm to 11.16 pm, track B from 11.22 pm to 11.24 pm, and track C from 11.46 pm to 11.48 pm. Temperature: 15 °C, mean wind speed: 1.2 m s^{-1}. (D) Treatment male recorded 8 August from 11.47 pm to 11.49 pm. Temperature: 20°C, mean wind speed: 3.3 m s^{-1} (this male was trapped). (E) Treatment male recorded 9 August from 10.43 pm to 10.45 pm. Temperature: 16 °C, mean wind speed: 3.8 m s^{-1}. (F) Treatment male recorded 10 August from 10.45 pm to 10.47 pm. Temperature: 18 °C, mean wind speed: 0.9 m s^{-1}.

the plots ($P = 0.94$). No difference was observed in landing frequency (periods of no movements) of males between plots.

Sixteen out of 80 untagged males were trapped in the control plot and one out of 84 untagged males was trapped in the treatment plot, i.e., a disruption effect of 93%. Surprisingly, however, during the radar tracking experiment, only two males carrying radar transponders were trapped (one in each plot).

Performance of males in a wind tunnel. No difference in overall ability to fly upwind in a pheromone plume in the wind tunnel was observed between tagged and untagged males, as evidenced by any of the behavioural steps up to trap capture (Figure 5).

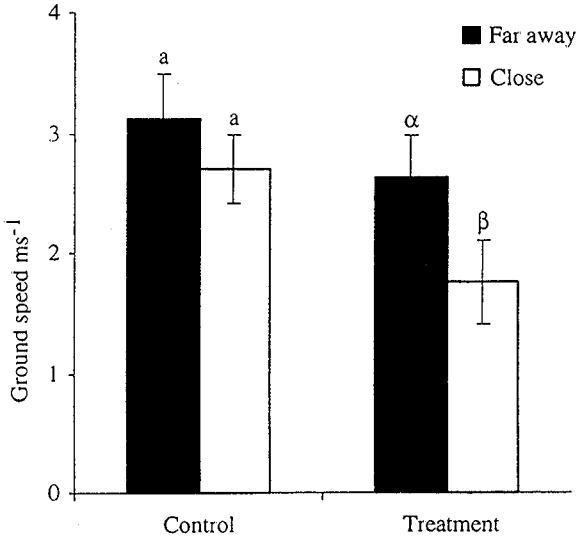

Figure 4. Ground speed (mean±se) of male *A. segetum* flying close to pheromone traps (<10 m) and far away from traps in the different plots (control: $n = 9$, treatment: $n = 8$). No difference in ground speed reduction was observed between plots.

However, direct observations showed that the ability of eight tagged males to enter the trap once close to it was hindered by the tag. The tags on these males that tried to fly directly into the trap bumped against the edge of the top of the trap, and the males bounced back without entering the trap. In fact, out of the eighteen males that flew within 1 cm of the trap, only two successfully entered the trap by flying into it. Ten of the males first landed on the roof of the trap and then walked underneath the lid inside to locate the bait.

Pheromone disruptant emission rates. Emission rates from the MSTRS^{TM} pads were as follows ($N = 2$): On Day 1, pads emitted 0.01 μg min^{-1} of Z5-10:OAc, 0.14 μg min^{-1} of Z7-12:OAc, and 0.08 μg min^{-1} of Z9-14:OAc. On Day 7, pads emitted 0.01μg min^{-1}, 0.16 μg min^{-1}, and 0.01μg min^{-1} of these same three components, respectively, and on Day 14 the values were 0.01, 0.06, and 0.02 μg min^{-1}, respectively. These values on Days 1 and 7 for Z7-12:OAc, the component with the highest concentration in solution, were similar to those emitted by commercially available polyethylene tube dispensers ('ropes') used for other moth species, which achieve a mean of usually 0.1–0.3 μg min^{-1} (Bäckman, 1997; Baker et al., 1997). These emission rates were ca. 10 times lower than those from MSTRS^{TM} pads receiving more highly concentrated pheromone sprays closer to those used in the standard MSTRS^{TM} system protocol (Fadamiro et al., 1999). Spraying of this more concentrated solution (0.4 g of Z5-10:OAc, 2.0 g of Z7-12:OAc and 0.8 g of Z9-14:OAc per 100 ml ethanol) four times per hour for 8 h resulted in the pads emitting 0.13 μg min^{-1}, 1.18 μg min^{-1}, and 0.77 μg min^{-1} of these three compounds, respectively.

Discussion

For the first time the long-range, pheromone-mediated, flight behaviour of individual male moths under natural and mating disruption conditions in the field has been studied. By using harmonic radar we have compared the performance of males with different exposure to sex pheromone. We also have attempted to link the behavioural observations to trap catch and mating of calling females. The use of a four-component blend as disruptant in the pre-radar experiments in Sweden resulted in moderate suppression of both trap catch and mating of females by 79% and 62%, respectively. Using this same disruptant blend in the radar experiments in England produced similar suppression of trap catch (93%) when untagged males were released. Trap-catch differences in the disruption versus the control plot did, however, not occur when transponders were attached to males, but these were due to low captures in the control plot and seem to be attributable to the tags interfering with the ability of males to enter the traps. Wind tunnel observations showed that the presence of the tag did not seem to impair the ability of males to fly upwind properly, but did impair direct flight into the opening of the trap.

Nevertheless, examination of the radar tracks produced some insights into behavioural differences that might have been occurring in the field, which were previously impossible to observe. Males 'investigated' pheromone traps, flying from one trap to another, a procedure that could continue for several minutes (see Figures 3A–C). Sustained flight towards pheromone sources for more than 30 min has been observed in wind tunnel studies on *Lymantria dispar* (L.) (Miller & Roelofs, 1978) and *Choristoneura fumiferana* (Clemens) (Sanders, 1998). Although the majority of flight tracks were shorter than 5 min, some moths could be followed for very long time periods. The longest track was 7350 m, showing that male moths have the potential to actively fly over very large distances when searching for mates. There was a strong trend ($P = 0.06$) for more instances of males closely approaching pheromone traps from downwind in the

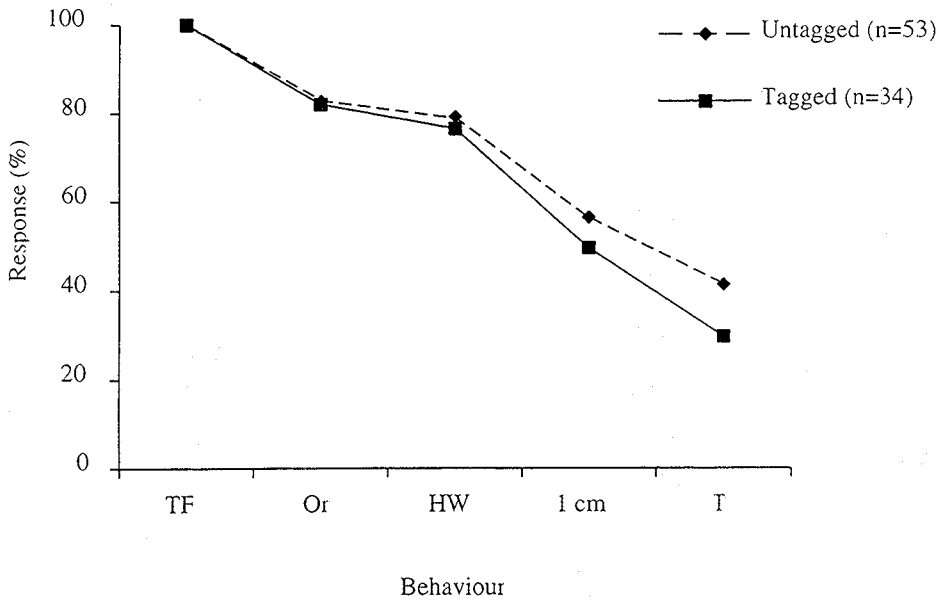

Figure 5 Response of *A. segetum* males with or without attached radar tags towards a trap baited with the sex pheromone in a wind tunnel. No differences between treatments were observed in any of the behavioural steps according to Ryan's test for multiple comparisons of proportions. TF = take flight, Or = orientation in the pheromone plume, HW = orientation halfway to the trap, 1 cm = orientation 1 cm from the trap, T = flight into the trap.

control plot than in the disruptant-treated plot. Although not statistically significant, this result may indicate a reduced ability for males to locate calling females under mating disruption conditions. However, as the trap catch of tagged males was impaired by the presence of the attached transponder interfering with the ability of males to enter the trap, no definite link between upwind flight towards pheromone traps and trap catch could be established, i.e., an upwind flight towards a trap could be due to other things than the male being stimulated by pheromone. Only one male was clearly attracted to disruption dispensers in the treatment plot, which may indicate a low level of false trail following. However, no conclusions concerning the exact mechanism behind the disruption effect observed in trap catch can be made based on the radar recordings.

The emission rates from the disruption dispensers in this study were at least 10 times lower than would be used in high-release-rate MSTRS^{TM}-type disruption in which 95–99% suppression of trap catch occurs (Fadamiro et al., 1998; Baker et al., 1997). Using emission rates causing complete shutdown of trap catch may be the only way to establish any behavioural differences in the delicate pheromone-mediated flight behaviour of male moths with the current resolution in time and space obtained by harmonic radar tracking.

Because of the limited detection range of the radar and the topography of the experimental area, the release points of the different plots had to be placed as close as 350 m, which might have caused chemical interference under certain wind regimes. Färbert et al. (1997) studied the transport of pheromones from an area treated for mating disruption of *Pectinophora gossypiella* (Saunders) and showed by electroantennogram recordings that significant amounts of pheromones could be measured 100 m downwind from the treatment area. In addition to wind effects, absorption and release from the vegetation may have an effect on the distribution of pheromones (Karg et al., 1994). However, for all nights except two, the mean wind direction was towards east which suggests that there would have been few instances of pheromone contaminated air being transported from the treatment plot into the control plot during the majority of flight recordings. Furthermore, the pronounced difference in trap catch of untagged males between the plots indicates that chemical interference had a minor impact on male flight behaviour in this study.

It was shown that a major constraint in the radar recordings was the fragmentation of flight tracks, probably due to radar shadow. Figure 2 illustrates how different parts of the plots were affected by radar shadow. Males flying in a southeastern direction upon

release in the control plot disappeared from the radar screen and were not detected until they had passed the southeastern traps, which was most probably due to high vegetation masking the signals from males flying behind it in relation to the radar. However, both plots seemed to be equally affected by this constraint. Experiments were carried out in the same agricultural field which has earlier been used for harmonic radar studies on bumble bees and honey bees (Osborne et al., 1999; Capaldi et al., 2000). As tracks were much less fragmented during those studies compared to the present study, it seems highly likely that the foraging bees had a higher mean flight altitude than the male moths searching for calling females. The problem with radar shadow could be reduced by choosing a more 'radar-friendly' site for flight recordings, e.g., a dry lake bed as in Loper et al. (1993), but recordings in such an area may not provide the information required for understanding how male moths actually behave in a typical agricultural environment. In this study we used the landscape of most relevance to the questions posed: although the patchwork of crops may have hindered radar observations, this type of vegetation may have been necessary to produce the pattern of pheromone dispersion characteristic of this environment.

In this study we have demonstrated both advantages and constraints in the use of harmonic radar to study the pheromone-mediated flight behaviour of nocturnal moths. Well-designed experiments using this technique have a great potential to give researchers new insights into mating disruption in moths and thus to facilitate pheromone-based methods of population control in Lepidoptera.

Acknowledgements

We thank Erling Jirle, Sara Ganestam, Mattias Larsson, Mikael Carlsson, and Andrew Martin for insect rearing and assistance in the field and Ann Edwards for analysis of data. Olle Anderbrant and two anonymous reviewers gave valuable comments on the manuscript. This study was supported by grants from the Swedish Council for Forestry and Agricultural research and the Knut and Alice Wallenberg foundation. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

Anderbrant, O., J. Löfqvist, J. Jönsson & E. Marling, 1989. Effects of pheromone trap type, position and colour on the catch of the pine sawfly *Neodiprion sertifer* (Geoffr.) (Hym., Diprionidae). Journal of Applied Entomology 18: 365–369.

Bäckman, A.-C. 1997, Pheromone release by codling moth females and mating disruption dispensers. Technology transfer in mating disruption. IOBC WPRS Bulletin 20(1): 175–180.

Baker, T. C. & N. J. Vickers, 1997. Pheromone-mediated flight in moths. In: R. T. Cardé & A. K. Minks (eds), Insect Pheromone Research: New Directions. Chapman & Hall, New York, pp. 248–264.

Baker, T. C., B. S. Hansson, C. Löfstedt & J. Löfqvist, 1988. Adaptation of antennal neurons in moths is associated with cessation of pheromone-mediated upwind flight. Proceedings of the National Academy of Sciences U.S.A. 85: 9826–9830.

Baker, T. C., A. Mafra-Neto, T. Dittl & M. E. Rice. 1997. A novel controlled-release device for disrupting sex pheromone communication in moths. Technology transfer in mating disruption. IOBC WPRS Bulletin 20(1): 141–149.

Capaldi, E. A., A. D. Smith, J. L. Osborne, S. E. Fahrbach, S. M. Farris, D. R. Reynolds, A. S. Edwards, A. Martin, G. E. Robinson, G. M. Poppy & J. R. Riley, 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature 403: 537–540.

Cardé, R. T. & A. Mafra-Neto, 1997. Mechanisms of flight of male moths to pheromones. In: R. T. Cardé & A. K. Minks (eds), Insect Pheromone Research: New Directions. Chapman & Hall, New York, pp. 275–290.

Cardé, R. T. & A. K. Minks, 1995. Control of moth pests by mating disruption: successes and constraints. Annual Review of Entomology 40: 559–585.

Fadamiro, H. Y., A. A. Cossé, T. Dittl & T. C. Baker, 1998. Suppression of mating by blackheaded fireworm (Lepidoptera: Tortricidae) in Wisconsin cranberry marshes by using MSTRS^{TM} devices. Journal of Agricultural Entomology 15: 377–386.

Fadamiro, H. Y., A. A. Cossé & T. C. Baker, 1999. Mating disruption of European corn borer, *Ostrinia nubilalis*, by using two types of sex pheromone dispensers deployed in grassy aggregation sites in Iowa cornfields. Journal of Asia-Pacific Entomology 2: 121–132.

Färbert, P., U. T. Koch, A. Fäbert & R. T. Staten, 1997. Measuring pheromone concentrations in cotton fields with the EAG method. In: R. T. Cardé & A. K. Minks (eds), Insect Pheromone Research: New Directions. Chapman & Hall, New York, pp. 347–358.

Karg, G., D. M. Suckling & S. J. Bradley, 1994. Absorption and release of pheromone of *Epiphyas postvittana* (Lepidoptera: Tortricidae) by apple leaves. Journal of Chemical Ecology 20: 1825–1841.

Löfstedt, C., J. N. C. van der Pers, J. Löfqvist, B. S. Lanne, M. Appelgren, G. Bergström & B. Thelin, 1982. Sex pheromone components of the turnip moth, *Agrotis segetum*: chemical identification, electrophysiological evaluation and behavioural activity. Journal of Chemical Ecology 8: 1305–1321.

Loper, G. M., W. W. Wolf & O. R. Taylor Jr., 1993. Radar detection of drones responding to honeybee queen pheromone. Journal of Chemical Ecology 19: 1929–1938.

Miller, J. R. & W. L. Roelofs, 1978. Gypsy moth responses to pheromone enantiomers as evaluated in a sustained-flight tunnel. Environmental Entomology 7: 42–44.

Osborne, J. L., S. J. Clark, R. J. Morris, I. H. Williams, J. R. Riley, A. D. Smith, D. R. Reynolds & A. S. Edwards, 1999. A landscape-scale study of bumble bee foraging range and con-

stancy, using harmonic radar. Journal of Applied Ecology 36: 519–533.

Riley, J. R., A. D. Smith, D. R. Reynolds, A. S. Edwards, J. L. Osborne, I. H. Williams, N. L. Carreck & G. M. Poppy, 1996. Tracking bees with harmonic radar. Nature 379: 29–30.

Riley, J. R., P. G. Valeur, A. D. Smith, D. R. Reynolds, G. M. Poppy & C. Löfstedt, 1998. Harmonic radar as a means of tracking the pheromone- finding and pheromone-following behaviour of male moths. Journal of Insect Behaviour 11: 287–296.

Riley, J. R., D. R. Reynolds, A. D. Smith, A. S. Edwards, J. L. Osborne, I. H. Williams & H. A. McCartney, 1999. Compensation for wind drift by bumble-bees. Nature 400: 126.

Ryan, T. A., 1960. Significance tests for multiple comparisons of proportions, variances and other statistics. Psychological Bulletin 57: 318–328.

Sanders, C. J., 1997. Mechanisms of mating disruption in moths. In: R. T. Cardé & A. K. Minks (eds), Insect Pheromone Research: New Directions. Chapman & Hall, New York, pp. 333–346.

Sanders, C. J., 1998. Effect of pheromone permeation on sustained flight of male spruce budworm. Canadian Entomologist 130: 539–544.

Svensson, M., M. Bengtsson & J. Löfqvist, 1995. Communication disruption of male *Agrotis segetum* moths with one or several sex pheromone components. Entomologia Experimentalis et Applicata 75: 257–264.

Valeur, P. G. & C. Löfstedt, 1996. Behaviour of male oriental fruit moth, *Grapholita molesta*, in overlapping sex pheromone plumes in a wind tunnel. Entomologia Experimentalis et Applicata 79: 51–59.

Valeur, P. G., B. S. Hansson, K. Markebo & C. Löfstedt, 2000. Relationship between sex pheromone elicited behaviour and response of single olfactory receptor neurones in a wind tunnel. Physiological Entomology 25: 223–232.

Wu, W. Q., B. S. Hansson & C. Löfstedt, 1995. Electrophysiological and behavioural evidence for a fourth sex pheromone component in the turnip moth, *Agrotis segetum*. Physiological Entomology 20: 81–92.

Zhu, J., C. Löfstedt & B. O. Bengtsson, 1996. Genetic variation in the strongly canalized sex pheromone communication system of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Pyralidae). Genetics 144: 757–766.