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# Elevation of pheromone response threshold in almond moth males pre-exposed to pheromone spray

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**Abstract.** High percentages of naive *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) males not pre-exposed to pheromone flew upwind to sources containing 50 ng (83%) and 500 ng (97%) of pheromone, but not to sources containing 5 μg (23%) and 50 μg (4%). Of the naive males that flew upwind in response to 50 ng sources, 67% located and landed on the source, whereas fewer than 19% of the naive males that flew upwind in response to higher doses located and landed on the sources. A 2-minute pre-exposure of *C. cautella* males to a spray cloud containing 50 ng, 500 ng, 5 μg or 50 μg of pheromone, induced shifts in response levels such that in wind-tunnel bioassays performed 1 h later, there was an increase in the doses that optimally elicited upwind flight and landing on the source that was proportional to the pre-exposure dose. Few of the pre-exposed males flew upwind to (10–43%) and landed on (0–33%) 50 ng sources, whereas they now perferentially flew upwind to (58–81% and 52–73%) and landed on (33–68% and 55–60%) pheromone sources of doses of 500 ng and 5 μg, respectively. Therefore pre-exposure to pheromone promoted a shift of threshold for response, and not an overall reduction in responsiveness to pheromone.

**Key words.** Almond moth, *Cadra cautella*, Lepidoptera, sex pheromone, pre-exposure, attraction, disruption, adaptation, habituation.

## Introduction

To find a mate, males of most moth species must follow a plume of sex pheromone upwind to a calling female (Baker, 1989). This chemically-mediated orientation of males can be disrupted by the introduction of synthetic pheromone into the environment, which prevents mating through a process commonly called 'mating disruption.' Several behavioural and physiological mechanisms have been proposed to explain how this is achieved: by elevation of pheromone response threshold, by camouflaging the boundaries of the pheromone plume, by disruption of the structure of the pheromone plume, or by attraction to the synthetic sources (Bartell, 1982; Cardé, 1990; Cardé & Minks, 1995).

Previous studies have shown that the pre-exposure to Lepidoptera males to pheromone sources results in diminution of their responsiveness to subsequent exposures. The reduction of response is directly related to the duration of the pre-exposure (Bartell & Lawrence, 1973; Traynier, 1970), dose (Bartell & Lawrence, 1976), and inversely related to the time interval since pre-exposure (Bartell & Lawrence, 1973; Shorey & Gaston, 1964). The temporal pattern of presentation of pheromone during the pre-exposure is known to affect the degree of reduction of responsiveness achieved, with pulsed presentations having the strongest effect (Bartell & Lawrence, 1977a, b; Farkas *et al.*, 1975; Kuenen & Baker, 1981).

The effects of pre-exposure on male response have been traditionally examined using 'activation bioassays' (e.g. Bartell & Lawrence, 1973, 1976, 1977a, b; Bartell & Roelofs, 1973; Farkas *et al.*, 1975; Shorey & Gaston, 1964; Traynier, 1970). Activation bioassays are restricted to evaluating male responses to pheromone only with regard to pre-flight behaviour such as walking or wing fanning. However, wind-tunnel bioassays permit the evaluation of the male's ability to perform the entire sequence of pheromone-mediated behaviour from activation, to upwind flight, and finally source location. As such, wind tunnels have the potential to be more discriminating in assessing male responses (Baker & Linn, 1984). Here we pre-exposed males of the almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae), to air permeated with their sex pheromone, and then examined the effect of the exposure on subsequent responses of the males in a wind tunnel.

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## Material and Methods

*Insects.* *Cadra cautella* larvae reared on a stored-products diet (Mafra-Neto, 1993) were separated by sex at their last larval instar, and males were reared in a separate room from females. Pupae were transferred to 25 × 25 × 25 cm netting cages placed inside pheromone-free environmental chambers (25°C, 75–80% r.h.). Daily transfer of the pupae to a new cage kept only 1-day-old adult males in the old cage. Males were tested during the initial 2 h of their first scotophase.

*Wind tunnel.* The pulling-type low-turbulence wind tunnel used to create the laminar airflow of 50 cm $s^{-1}$ was 2.5 m long and 0.9 m in diameter with a semi-cylindrical shape similar to that described in Mafra-Neto & Cardé (1995). It was constructed from transparent Plexiglas® (floor) and Vivac® (walls). Each end was covered by a layer of fine polyester mesh screen. Air crossed an Aluminium Honeycomb® laminizer (cells 1.9 cm height × 15 cm width) and a fine polyester mesh screen before entering the working section of the tunnel.

A downwind screen separated the working section of the tunnel from a downwind manipulating chamber where pheromone sources used in a bioassay could be temporarily stored. This chamber was connected by a flexible 40-cm-diameter exhaust pipe to a fume hood that drew all the air from the tunnel and exhausted it to the outside of the building. The downwind end of the exhaust pipe had a gate valve used to regulate the airflow leaving the pipe, and thus to control the airflow in the working section of the wind tunnel. The wind in the working section of the tunnel was continuously monitored by a digital anemometer.

A light box containing five red and five white 25 W incandescent light bulbs and a filter/diffuser made of a sheet of white Plexiglas® (0.5 cm thick) was placed above the working section of the wind tunnel. Light conditions were adjusted with a voltage regulator to 5.5 lux at moth flight height; relative humidity ranged between 60% and 85%. Dots of 9.5-cm-diameter red transparent plastic randomly arranged on the floor provided 'non-directional' optomotor cues for the flying males (David, 1986).

*Chemicals.* The two acetates making up *C. cautella* female sex-pheromone, (*Z,E*)-9,12-tetradecadienyl acetate (96.7% pure, Bedoukian, U.S.A.) (Brady *et al.*, 1971; Kuwahara *et al.*, 1971) and (*Z*)-9-tetradecenyl acetate (97.3% pure, Bedoukian, U.S.A.) (Brady, 1973), were formulated gravimetrically to a ratio of 10:0.9, respectively. The ratio of the neat material blend was determined by GC analysis of dilutions in HPLC grade hexane. The neat material blend was subsequently serially diluted in petroleum distillate, and formulated in metered aerosol spray canisters (Waterbury Co., Waterbury, Ct.). The spray canisters were equipped with metered valves that emitted 50 µl of a mixture of propellant (butane), petroleum distillate and pheromone per spray. Canisters were formulated with 38 ml of one of the serial dilutions so that they delivered 5 ng, 50 ng, 500 ng, 5 µg or 50 µg of sex pheromone per spray. A canister formulated with the same amount of petroleum distillate, but with no pheromone, served as control.

*Pre-exposure.* Fifty 1-day-old males were transferred with an aspirator from the emergence cage into a 3-litre, narrow-mouth glass Mason jar that was sealed with Parafilm®. The males were acclimated in the jar for 10–30 min. The inside of the jar was covered with thick absorbent (ink blotter) paper, which resulted in an even distribution of the males, and enabled them to sit on the vertical surfaces of the jar. More importantly, by absorbing the excess petroleum distillate, the blotter paper prevented oil damage to the moths' wings.

Once the males were acclimatized in the jar, the seal of Parafilm® was perforated, the nozzle of a spray canister introduced through the hole, pointed to the bottom of the jar, and pressed to discharge 50 µl of the canister's mixture. This procedure generated a fine aerosol mist that created a visible fog throughout the jar. The mist contained 50 µl of a mixture of propellant, petroleum distillate and 0 ng, 5 ng, 50 ng, 500 ng, 5 µg or 50 µg pheromone.

The nozzle was then retracted, the Parafilm® resealed, and the males left in the pheromone-permeated atmosphere for 2 min. The males were then transferred by means of an aspirator to a pheromone-free, clear plastic, cylindrical holder (9 cm height × 5 cm diameter) sealed with Parafilm® and perforated with small holes to allow for air movement. Males stayed in the cylinder until used for testing, 1–3 h later. The containers with treated males were transferred to the experimental wind tunnel room 30 min before the end of the photophase.

*Pheromone sources.* The pheromone source used in the subsequent wind tunnel bioassay was a disk of 5 cm diam. filter paper (Whatman No. 1) sprayed once with (control 0 ng), 5 ng, 50 ng, 500 ng, 5 µg or 50 µg of pheromone. The spray was applied to the centre of the paper which was held vertically, facing the canister, 10 cm from the nozzle opening. The paper was allowed to sit for 5 min in a fume hood. The pheromone source was then transferred to the wind tunnel and held in a horizontal position by a disposable glass tube attached to the wind-tunnel floor, 45 cm from either side of the tunnel and 10 cm downwind from the upwind screen. The sprayed side of the filter paper faced downwind. The pheromone source was replaced with a fresh one every 15 min.

*Wind-tunnel assay.* Males were tested during the initial 30–120 min of their first scotophase. The order of treatments was established using a randomized complete-block design. Blocks of treatments were repeated three times, with ten males tested per treatment per block. A randomly selected moth was removed with an aspirator from the plastic holding cylinder and transferred to an aluminium gauze release cage located in the wind tunnel. The release cage was held in place by a Teflon® tube that traversed the wind tunnel floor, so that its position and orientation could be regulated from outside the tunnel. The release cage was located in a position previously established using $TiCl_4$ 'smoke' plumes; it was positioned 45 cm from either side of the tunnel, 150 cm downwind from the pheromone source, and 5 cm below the pheromone plume. Each male was tested individually and only once. A male was held in the screen release cage with the open end of the cage facing downwind until he was quiescent for 10 s, whereupon the release cage was turned 180° with the open end facing upwind and raised to the level of the pheromone plume. To obtain consistent rates of upwind flight after the male initiated flight, the release cage was quickly lowered from the position where it intercepted the pheromone plume (Mafra-Neto & Cardé, 1995). This procedure enabled males that had locked onto the plume downwind from the point of release to fly upwind without re-encountering the release cage.

*Pheromone-mediated behaviour.* A record of the male's behaviour was started as soon as he was introduced into the pheromone

plume and ended after 2 min, or as soon as he landed on the source or on the upwind screen of the wind tunnel. The following sequence of mutually exclusive behaviours at the release platform and during upwind progression were recorded for each male: quiescent – no perceptible movement; wing fanning/walking – wing fanning, walking on the release platform or walking while wing fanning; flight initiation – first flight from the release cage; oriented flight – upwind flight (zigzag or straight) along the pheromone plume (it included brief casting); in-flight arrestment – flight in the plume without making upwind progress, but with narrow crosswind zigzags resulting in the male abandoning the plume; locating the source – landing or touching the pheromone source.

Males that did not take off were tested for their ability to fly. They were removed from the release cage with an aspirator and released in the air, about 30 cm above the floor. Those that did not fly were discarded; those that flew were scored as non-responders.

Treatments, both pre-exposure and subsequent assay doses, were presented in a randomized, complete-block design. Differences in the percentage of males responding were tested according to a $\chi^2$ $2 \times 2$ test of independence with Yates correction at $\alpha = 0.05$ (SAS, 1989).

## Results

Nearly all quiescent naive *C. cautella* males, i.e. those not pre-exposed to pheromone, walked while wing fanning and initiated flight in response to exposure to the pheromone plume, independent of dose (Fig. 1A). The transition from flight initiation to oriented upwind flight of naive males, however, depended on the dose of pheromone on the source. The majority of the males that initiated flight to the low doses (50 ng and 500 ng) engaged in upwind flight (89% for 50 ng, 93% for 500 ng). Significantly fewer naive males that initiated flight made the transition to oriented upwind flight in response to the high dose sources. Only 39% of the naive males that initiated flight to 5 μg plumes, and 4% of the males that initiated flight to 50 μg plumes made the transition to oriented upwind flight. The majority of the males engaged in oriented upwind flight landed on the 50 ng source (80%). Most of the males flying upwind in response to high-dose plumes became arrested and abandoned the pheromone plume before landing on the source; thus, only 17% of the naive males landed on 500 ng sources, 3% on 5 μg sources, and none on 50 mg sources. The majority of the naive males flying upwind in response to the high pheromone doses became arrested in flight (78% for 500 ng, 86% for 5 μg, 100% for 50 μg) eventually abandoning the plume, whereas only 4% of the males engaged in oriented upwind flight to 50 ng became arrested. Thus the pheromone source at the lowest dose tested was the optimal dose for attraction of naive *C. cautella* males under these experimental conditions.

The 2 min pre-exposure of males to the single spray of pheromone resulted in a shift in the optimal pheromone dose for upwind flight, the magnitude of which was dependent upon the dose of pheromone sprayed on the males during the pre-exposure (Figs 1B–1E). A higher percentage of the males pre-exposed to 50 ng (50 ng males) flew upwind and landed on 500 ng sources (70%), than on 50 ng (10%), 5 μg (5%), or 50 μg (10%) sources. All the 50-ng-exposed males that engaged in oriented upwind flight to 500 ng sources located and landed on the source, whereas only one-third or less of these males flying upwind landed on sources at other doses.

The behavioural sequence leading to source location of the 50 ng pre-exposed males had a bottleneck of flow at the transition from walking and wing fanning to upwind flight for sources of 50 ng, where *c.* 70% of the males walking and wing fanning failed to engaged in upwind flight. At higher doses, however, the sequence shifted to having a reduced transition at later stages. For instance, the response to 5 μg sources shifted to having a reduced transition from oriented upwind flight to landing on the source. Although 80% of the 50 ng pre-exposed males flew upwind in response to 5 μg sources, only 5% landed on the source; 94% of the males flying upwind became arrested in flight and eventually abandoned the plume, landing elsewhere. At the highest source dosage tested (50 μg) a low percentage (45%) of the 50 ng pre-exposed males exhibited upwind flight but none landed on the source.

A pheromone pre-exposure dose of 500 ng (Fig. 1C) resulted in a shift of the threshold dose of response similar to pre-exposure to 50 ng: a higher percentage of the 500 ng males landed on the 500 ng source (68%) than on the other sources (33% on 50 ng and on 5 μg sources, and 3% on 50 μg sources). Slightly higher success in behavioural transitions resulted in the higher percentage of 500-ng-exposed males locating and landing on 500 ng sources. Fewer 500 ng males initiated the pheromone related behaviour sequence by walking and wing fanning to 50 ng sources (70%) and 50 μg sources (77%) than to 500 ng (100%) and 5 μg (100%). Only *c.* 50% of the 500-ng-exposed males initiated flight in response to the 50 ng and 50 μg sources, whereas nearly 80% and more than 90% did so to 5 μg and 500 ng sources.

Most of the 500 ng pre-exposed males that initiated flight engaged in oriented upwind flight (>70%), except in response to 50 μg sources to which only 50% of the males that initiated flight engaged in oriented upwind flight. The majority of the 500-ng-exposed males flying upwind located the 50 ng source (77%), and the 500 ng source (84%). Interestingly, pre-exposure to this dose now elevated the percentage of success of males flying upwind to locate the 5 μg source to 57%, resulting overall in 33% of the 500-ng-exposed males landing on the source. Again, as with pre-exposure to 50 ng, 500-ng-exposed males were able to locate the 50 μg source (34%).

Less than one-third of the males pre-exposed to 5 μg dose sprays (Fig. 1D) initiated the behavioural sequence by walking and wing fanning in response to 50 ng dose pheromone sources, whereas nearly all 5 μg males walked and wing fanned in response to the higher-dose sources. Nearly all males pre-exposed to 5 μg initiated flight in response to 5 μg sources (97%), whereas 77% and 74% initiated flight to 500 ng and 50 μg sources, respectively. Only 22% of the males initiated flight to 50 ng sources. Pre-exposure to 5 μg now elevated the percentage of males locating the 50 μg source to 33% and again a high percentage located the 500 ng (55%) and the 5 μg sources (60%). Less than one-third of the 5-mg-exposed males flying upwind to 500 ng (35%), and to 5 μg (27%) sources became arrested, in contrast to the high levels of arrestment at lower pre-exposure dosages.

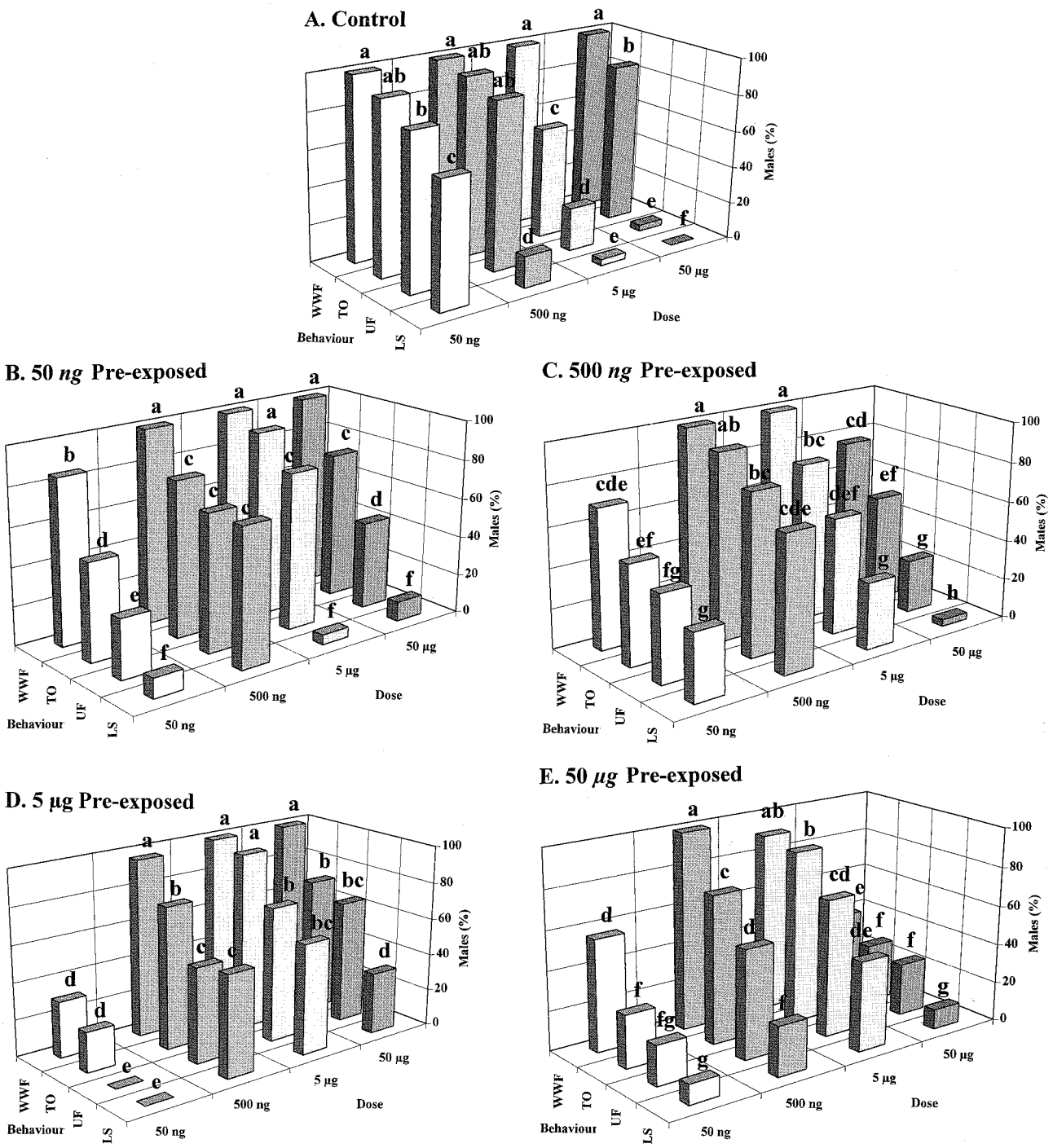


**Fig. 1.** Histograms of the percentage of *C. cautella* males performing a sequence of pheromone-related behaviours, from walking and wing fanning (WWF), to flight initiation (TO), to oriented upwind flight (UF), and ending with the percentage of males that landed on the source (LS) in response to pheromone sources of dose 50 ng, 500 ng, 5 µg or 50 µg. (A) Naive *C. cautella* males with no previous exposure to pheromone. (B) Males pre-exposed to a single pheromone spray of 50 ng. (C) Males pre-exposed to a single pheromone spray of 500 ng. (D) Males pre-exposed to a single pheromone spray of 5 µg. (E) Males pre-exposed to a single pheromone spray of 50 µg. Columns within each histogram with no letters in common are significantly different at $\alpha = 0.05$ level ($n = 30$ males for each treatment and control).

Pre-exposure of males to 50 µg sprays (Fig. 1E), the highest dose tested, resulted in less than 50% of the males locating the source at any given dose. Less than one-third of the males tested initiated flight to the sources with the lowest and the highest dose tested (27% for 50 ng, 30% for 50 µg), whereas more than two-thirds of the males initiated flight to the pheromone sources of intermediate doses (75% for 500 ng and 88% for 5 µg). Most of the males that initiated flight flew upwind (>70%) independent

of dose at the source. Only 20% and 27% of the 50 μg males flew upwind to 50 ng sources and 50 μg sources, respectively, compared to 55% and 70% to 500 ng and 5 μg sources, respectively. Because the success in the behavioural transitions was independent of the dose of the source, the differences observed in percentage of males initiating flight were maintained throughout the behavioural sequence. Thus a higher percentage of 50 μg pre-exposed males ended up landing on 5 μg sources (46%) than on any other source (10–25%).

## Discussion

Pre-exposure to pheromone resulted in a shift of the threshold and optimal concentrations for the expression of pheromone-related behaviours in male *C. cautella*, with the magnitude of the shift related to the dose during pre-exposure. This reduction in response to lower doses and increase in response to higher doses following pre-exposure could be explained by at least two physiological mechanisms, sensory adaptation (at the antennal neuronal level), and habituation at the central nervous system level. Preliminary data comparing EAG response of naive and pre-exposed antennae show long-term adaptation of the pre-exposed antennae (Mafra-Neto & Baker, unpublished). Therefore sensory adaptation probably contributed to the shift in optimal dosage eliciting behavioural responses in males pre-exposed to sprays of pheromone. However, such conclusions were complicated by the possibility that the pre-exposed *C. cautella* males contacted the aerosol particles in the jar, which could have promoted the observed 'long-term' adaptation. Nevertheless, regardless of the neuronal mechanism responsible for behavioral shift, under the experimental conditions we documented a significant elevation of thresholds at which males became activated and flew upwind to pheromone, as well as the thresholds at which they became arrested due to excessive concentration. This shift of threshold response to higher doses due to pre-exposure to pheromone is a phenomenon that must considered in mating disruption tactics.

The shift in threshold explains why *C. cautella* males in experimental rooms under mating disruption do not respond to nearby calling females but respond to spray shots of high pheromone dose (Mafra-Neto & Baker, 1996). The work of Doane & Brooks (1981) on mating disruption of the cotton pink bollworm, *Pectinophora gossypiella*, provides further evidence that optimal responsiveness to pheromone can be shifted to higher doses by pre-exposure to the disruptant. A 16-fold increase in pheromone emission rate of monitoring traps did not increase the number of males captured in untreated fields, but it promoted a 5-fold increase in the number of males captured in pheromone mating disruption fields. The lower emission rate traps caught insignificant numbers of males in such fields.

Nearly all pre-exposed quiescent *C. cautella* males responded to the plume from different source doses of pheromone by walking and wing fanning or taking flight (activation), which was the discriminating behaviour used in the majority of the previous work on the effects of pre-exposure to pheromone (e.g. Bartell & Lawrence, 1973, 1976, 1977a, b; Bartell & Roelofs, 1973; Farkas *et al.*, 1975; Shorey & Gaston, 1964; Traynier, 1970). Monitoring the entire sequence of pheromone-related behaviours in the wind tunnel, from quiescence to landing on the source, thus provided a sensitive and discriminating indicator of the effects of pre-exposure that an 'activation' assay might have missed.

Kuenen & Baker (1981), monitoring the behaviour sequence from quiescence to approach to less than 40 cm from the source, found that differences in the temporal pattern of pheromone pre-exposure were reflected primarily in the frequency at which a male performed behaviours late in the sequence, such as oriented upwind flight and approach to the source, rather than in behaviours early in the sequence, such as walking, walking and wing fanning or flight initiation. The behavioral data on pre-exposed *C. cautella* males show that a transition bottleneck between quiescence to walking and wing fanning occurred in only four treatments (500 ng males to 50 ng source, 5 μg males to 50 ng source, 50 μg males to 50 ng and to 50 μg sources) out of the twenty treatments tested. A bottleneck existed in transitions late in the sequence for ten treatments, from oriented upwind flight to source location (naive males to 500 ng and to 5 μg source, 50 ng males to 5 μg and to 50 μg source, 500 ng males to 5 μg and to 50 μg sources, 5 μg males to 50 μg source, 50 μg males to 50 ng, to 500 ng and to 50 μg sources) out of the twenty tested. The bottlenecks in the transitions late in the sequence thus appear to be diagnostic of the effects of pre-exposure to pheromone in the male response.

The elevation of response threshold by the pre-exposure to pheromone thus favours the response by males to the synthetic disruptant sources compared to females, even if females were nearby. The observed reduction in response to low doses of pheromone, but increased responses to higher doses, appears to be associated with the use of mating disruptants in the field (Cardé *et al.*, 1991; Doane & Brooks, 1981; Miller *et al.*, 1990; Mafra-Neto & Baker, 1996). Our results add to the evidence that although disruption is achieved at least in part by adaptation and habituation, such desensitization of the sensory pathways is not absolute and can be overcome by higher emission rate sources. Therefore other mechanisms such as camouflage of the natural plume, as well as competition between synthetic and natural (females) pheromone sources (Cardé *et al.*, 1991; Doane & Brooks, 1981; Miller *et al.*, 1990; Mafra-Neto & Baker, 1996) must still come into play if the strongly emitting disruptant point sources are to prevent a male from responding to a nearby calling female. Since habituation and adaptation can be incomplete, the use of attractive blends of components that promote upwind flight to the disruptant source should add to the effectiveness of mating disruption programmes.

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## References


Baker, T.C. (1989) Pheromones and flight behavior. *Insect Flight* (ed. by G. Goldsworthy and C. Wheeler), pp. 231–256. CRC Press, Boca Raton, Florida.

Baker, T.C., Francke, W., Millar, J.G., Löfstedt, C., Hansson, B., Du, J.-W., Phelan, P.L., Vetter, R.S., Youngman, R. & Todd, J.L. (1991) Identification and bioassay of sex pheromone components of carob moth, *Ectomyelois ceratoniae* (Zeller). *Journal of Chemical Ecology*, **17**, 1973–1988.

Baker, T.C., Hansson, B.S., Löfstedt, C. & Löfqvist, J. (1989) Adaptation of antennal neurons in moths is associated with cessation of pheromone-mediated upwind flight. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 9826–9830.

Baker, T.C. & Linn, C.E., Jr (1984) Wind tunnels in pheromone research. *Techniques in Pheromone Research* (ed. by H. E. Hummel and T. A. Miller), pp. 75–110. Springer, New York.

Bartell, R.J. (1982) Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. *Physiological Entomology*, **7**, 353–364.

Bartell, R.J. & Lawrence, L.A. (1973) Reduction in responsiveness of males of *Epiphyas postvittana* (Lepidoptera) to sex pheromone following previous brief pheromone exposure. *Journal of Insect Physiology*, **19**, 845–855.

Bartell, R.J. & Lawrence, L.A. (1976) Reduction in sexual responsiveness of male light brown apple moth following previous brief pheromone exposure is concentration dependent. *Journal of the Australian Entomological Society*, **15**, 236.

Bartell, R.J. & Lawrence, L.A. (1977a) Reduction in responsiveness of males of *Epiphyas postvittana* (Lepidoptera) to sex pheromone following pulsed pre-exposure to pheromone components. *Physiological Entomology*, **2**, 89–95.

Bartell, R.J. & Lawrence, L.A. (1977b) Reduction of responsiveness of males apple moths, *Epiphyas postvittana*, to sex pheromone following pulsing pheromonal exposure. *Physiological Entomology*, **2**, 1–6.

Bartell, R.J. & Roelofs, W.L. (1973) Inhibition of sexual response in males of the moth *Argyrotaenia velutiana* by brief exposure to synthetic pheromone or its geometrical isomer. *Journal of Insect Physiology*, **19**, 661–665.

Brady, U.E. (1973) Isolation, identification and stimulatory activity of a second component of the sex pheromone system (complex) of the female almond moth, *Cadra cautella* (Walker). *Life Sciences*, **13**, 227–235.

Brady, U.E., Tulminson, J.H., Brownlee, R.G. & Silverstein, R.M. (1971) Sex stimulant and attractant in the Indian meal moth and in the almond moth. *Science*, **171**, 802.

Cardé, R.T. (1990) Principles of mating disruption. *Behavior-modifying Chemicals for Insect Management. Applications of Pheromones and other Attractants* (ed. by R. L. Ridgway, R. M. Silverstein and M. N. Inscoe), pp. 47–92. Marcel Dekker, New York.

Cardé, R.T., Mafra-Neto, A., Staten, R.T., Koch, U. & Farbert, P. (1991) Evaluation of communication disruption in the pink bollworm in field wind tunnels. *International Organization for Biological Control. West Palearctic Regional Sector Bulletin*, **16**, 23–28.

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

David, C.T. (1986) Mechanisms of directional flight in wind. *Mechanisms in Insect Olfaction* (ed. by T. L. Payne, M. C. Birch and C. E. J. Kennedy), pp. 49–58. Clarendon Press, Oxford.

Doane, C.C. & Brooks, T.W. (1981) Research and development of pheromones for insect control with emphasis on the pink bollworm. *Management of Insect Pests with Semiochemicals: Concepts and Practice* (ed. by E. R. Mitchell), pp. 267–284. Plenum Press, New York.

Farkas, S.R., Shorey, H.H. & Gaston, L.K. (1975) The influence of prolonged exposure to pheromone on the behavior of males of *Trichoplusia ni*. *Environmental Entomology*, **4**, 737–741.

Kuenen, L.P.S. & Baker, T.C. (1981) Habituation versus sensory adaptation as the cause of reduced attraction following pulsed and constant sex pheromone pre-exposure in *Trichoplusia ni*. *Journal of Insect Physiology*, **27**, 721–726.

Kuwahara, Y., Kitamura, C., Takahashi, S., Hara, H., Ishii, S. & Fukami, H. (1971) The sex pheromone of the almond moth and the Indian meal moth: cis-9-trans-12-tetradecadienyl acetate. *Science*, **171**, 801.

Mafra-Neto, A. (1993) Effects of the structure and composition of pheromone plumes on the response of the male almond moth, *Cadra cautella*. Ph.D. dissertation, University of Massachusetts.

Mafra-Neto, A. & Baker, T.C. (1996) Timed metered sprays of pheromone disrupt mating of *Cadra cautella* (Lepidoptera: Pyralidae). *Journal of Agricultural Entomology*, **13**, 149–168.

Mafra-Neto, A. & Cardé, R.T. (1995) Effect of plume structure and pheromone concentration on the upwind flight of *Cadra cautella* males. *Physiological Entomology*, **20**, 117–133.

Miller, E., Staten, R.T., Nowell, C. & Gourd, J. (1990) Pink bollworm (Lepidoptera: Gelechiidae): point source density and its relationship to efficacy in attracticide formulations of gossyplure. *Journal of Economic Entomology*, **83**, 1321–1325.

SAS Institute, Inc. (1989) *SAS/STAT User's guide, Version 6, Fourth Edition*, Vol. 1. SAS Institute, Inc., Cary, N.C.

Shorey, H.H. & Gaston, L.K. (1964) Sex pheromones of noctuid moths. III. Inhibition of male responses to the sex pheromone in *Trichoplusia ni* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*, **57**, 775–779.

Todd, J.L., Millar, J.G., Vetter, R.S. & Baker, T.C. (1992) Behavioral and electrophysiological activity of (*Z,E*)-7,9,11-dodecatrienyl formate, a mimic of the major sex pheromone component of carob moth, *Ectomyelois ceratoniae*. *Journal of Chemical Ecology*, **18**, 2331–2352.

Traynier, R.M.M. (1970) Habituation of the response to sex pheromone in two species of Lepidoptera, with reference to a method of control. *Entomologia Experimentalis et Applicata*, **13**, 179–187.


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