Timed, Metered Sprays of Pheromone Disrupt Mating of *Cadra cautella* (Lepidoptera: Pyralidae)[1]

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ABSTRACT A controlled release system for dispensing insect pheromones and other semiochemicals at a specified release rate over long periods of time was developed and tested. Large quantities of pheromone were stored in pressurized canisters of metered semiochemical timed release system (MSTRS) devices kept in rooms with little thermal insulation at ambient field temperatures for more than 90 d during the summer. The devices were tested in experimental storage rooms for their efficacy as mating disruptants by releasing pheromone only or pheromone-plus-pyrethroid. Pheromone required for mating disruption experiments was released upon demand from canisters for the desired duration, with no detectable decrease in effectiveness in promoting suppression of mating as the canisters aged. MSTRS devices placed in 3 m × 3 m × 3 m rooms disrupted up to 100% of the mating attempts of the almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae), in populations consisting of freely flying virgin males and females at a 2:1 ratio, respectively, for 24 h, and disrupted up to 92% of the mating attempts for 72 h. Disruption of mating was measured directly by recapture of moths and examination of the females' bursa copulatrices for the presence of spermatophores, which were used as indicators of successful mating encounters. MSTRS devices emitting different doses of pheromone were tested for mating disruption. Strong sources, dosed with 50 µg of pheromone per spray at 15 min intervals, were the most efficacious, promoting more than 90% mating disruption, and their effect was independent of either moth population density or number of MSTRS devices used per room. Sources receiving 5 µg of pheromone per spray at 15 min intervals disrupted 60%–70% of mating, which still represented a significant level of disruption. Mating disruption was enhanced (to >80%) by the addition of 1% natural pyrethrin extract to the 5 µg pheromone sprays; 96.1% of the male population was dead after 24 h.

KEY WORDS Lepidoptera, Pyralidae, almond moth, sex pheromone, mating disruption, control release, attraction, pyrethroids

Males of most moth species must follow a plume of sex pheromone upwind to a calling female to mate (Baker et al. 1989). The chemically mediated orientation of

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males can be disrupted, and mating prevented, by the introduction of synthetic pheromone into the environment. Two methods are used to prevent mating with synthetic pheromones: "mating disruption," in which pheromone sources (usually at high doses) are dispensed to impair male orientation, and "attract-and-kill," in which pheromone sources (usually at low doses) are laced with small amounts of insecticides that poison the males attracted by the pheromone.

The sex pheromone of the almond moth, *Cadra cautella* (Walker) (Lepidoptera: Pyralidae), is comprised of two components (*Z,E*)-9,12-tetradecadienyl acetate (Z9,E12-14:Ac) (Brady et al. 1971, Kuwahara et al. 1971a, b) and *(Z)*-9-tetradecenyl acetate (Z9-14:Ac) (Brady 1973) in a 10:1 ratio, respectively (Coffelt & Vick 1987). Mating disruption of stored products phycitine pests with the major component of their pheromone and associated minor components (Brady 1973) has been attempted since the early 1970s; however, thus far only partial control has been achieved. Early mating disruption involved laboratory experiments conducted in small boxes or cages (Wakamura et al. 1975, Brady et al. 1971, Brady & Daley 1975, Barrer et al. 1987), and the presence of pheromone successfully disrupted mating of several species of phycitine moths, including *C. cautella* (Wakamura et al. 1975, Brady et al. 1971, Brady & Daley 1975, Barrer et al. 1987). The transition from the laboratory to simulated commercial use conditions, however, proved to be more difficult than expected (Brady & Daley 1975). Although mating of phycitine moths could be reduced substantially with pheromone when moths were present at low population densities in the field, mating could not be suppressed when moths were present at high population densities (Hagstrum & Davis 1982). It was concluded that control of *C. cautella* populations by using pheromone for mating disruption was neither practical nor economical (Hagstrum & Davis 1982, Vick et al. 1981).

Both the formulation and the matrix used to deliver pheromone are of paramount importance in determining the mode of action and the success of mating disruption programs (Cardé & Minks 1995, Leonardt et al. 1990, Weatherston 1990). Pheromone molecules are highly volatile and usually chemically unstable. To maintain attractancy for the necessary field lifetime, pheromones have been formulated in polymeric matrices designed to slow the rate of pheromone release and to confer protection to pheromone molecules from ultraviolet light, extremes in pH, and free oxygen (Leonardt et al. 1990, Weatherston 1990). There are five major polymeric matrices commercially available: micro-capsules, trilaminates, capillaries, ropes, and liquid flowables (Weatherston 1990). Each releases pheromone passively and continuously. These polymeric matrices also expose the pheromone pool to the environment continuously, which leads to uncontrolled temporal changes in the rate of pheromone emission and in the blend of pheromone components emitted (Leonardt et al. 1990, Weatherston 1990). Temporal changes in rates of emission can be caused by depletion of the reservoir contents by accumulation of debris, which may impede or enhance evaporation, or simply by variation in temperature or wind speeds. Depletion of the more volatile components of the blend and enrichment of the semiochemical storage pool with the less volatile components occurs as the source ages and results in the emission becoming less

behaviorally active. Furthermore, desired 'controlled release' has not been fully achieved with the development of polymeric matrices; release of pheromone from such systems is continuous and passive and cannot be actively controlled. Once polymeric matrices are deployed in the field, pheromone emission cannot be altered to address changes in population levels.

Here we describe a metered semiochemical timed release system (MSTRS) developed to release pheromone or other chemicals upon demand. This actively controlled release device to dispense pheromone was used to suppress mating in populations of the stored products pest *C. cautella* in experimental rooms. In contrast to the inability of previous efforts to achieve *C. cautella* mating disruption at high population densities (Ganyard & Brady 1971, Hagstrum & Davis 1982, Haines 1976, Hodges et al. 1984, Minks & Cardé 1988, Vick et al. 1981), our experiment resulted in nearly total suppression of mating by these moths, even when they were present at high density similar to, or even higher than, that used in previous studies that failed.

Materials and Methods

Insects. *Cadra cautella* larvae were reared from eggs in a stored-products-moth diet (Mafra-Neto & Cardé 1995). They were separated by sex at their last larval stage and reared to pupae. Pupae were transferred to 25 cm × 25 cm × 25 cm screen cages, and the males were transferred to an environmental chamber (25°C, 75%–80% RH) located in a pheromone-free room isolated from the main colony and from the females. Female pupae were transferred to an environmental chamber (25°C, 75%–80% RH) located in the same room as the main colony. Adults were transferred daily to clean cages. The experimental adults were 1–3 d old.

Chemicals. The two acetates that make up the *C. cautella* female sex pheromone, Z9,E12–14:Ac (Brady et al. 1971, Kuwahara et al. 1971a, b) and Z9–14:Ac (Brady 1973), were obtained from Bedoukian Research, Inc. (Danbury, Connecticut), and were 93% and 95% pure, respectively. The acetates were formulated gravimetrically to a ratio of 10:0.9 (Z9,E12–14:Ac: Z9-14:Ac) as neat material. The ratio of the neat material blend was determined by gas chromatographic analysis of serial dilutions in HPLC grade hexane. The pheromone was serially diluted in tenfold steps by using two solvents to formulate the canisters—LPA-210 petroleum distillate (petroleum distillate) or HPLC grade ethanol (ethanol).

Pheromone release devices. The Metered Semiochemical Timed Release System (MSTRS) device (Waterbury Co., Inc., Connecticut) consisted of a spray canister, a spray dispenser unit, and a spray pad (Fig. 1). The pressurized spray canister contained a solution with the desired concentration of pheromone in inert materials (isobutane, ethanol, petroleum distillate, and paraffin). A valve on top of the canister delivered 52 mg of the complete mixture per spray. The canister was housed in the spray dispenser unit with the valve positioned under a lever controlled by a battery-powered timer mechanism. The timer mechanism was set to press the valve to deliver a new spray every 15 min. The spray was intercepted by a pad formed by a 1-cm-thick sheet of acrylic padding (No. 300 Mountain Mist® Fiberloft®, The Stearns Technical Textiles Co., Ohio)

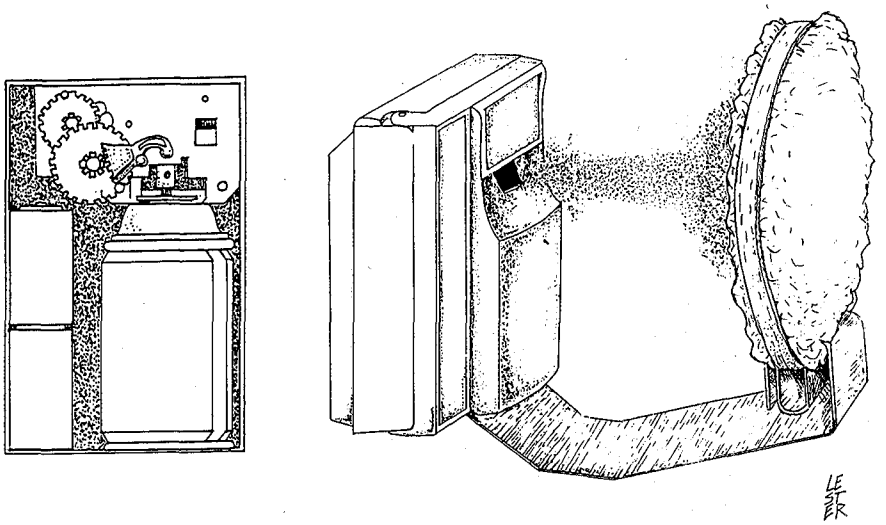

Fig. 1. The Metered Semiochemical Timed Release System (MSTRS) device consisted of a spray canister, a spray dispenser unit, and a spray pad. The spray canister contained the desired concentration of pheromone in a solution with inert materials under pressure. A valve on top of the canister delivered 52 mg of material per spray. The canister was housed in the spray dispenser unit with the valve positioned under a lever controlled by a battery-powered timer mechanism. The mechanism lowered the lever, delivering a new spray every 15 min. The spray was intercepted by a pad held 20 cm from the canister's nozzle, by an adjustable aluminum platform, where pheromone was deposited. A MSTRS device produced more than 3,000 recharging sprays of similar strength, sufficient to recharge a source pad every 15 min for 30 d without replacement of canister or batteries.

stretched on a needle-point frame (15 cm inner diam) and held in place 20 cm from the canister's nozzle by an adjustable aluminum platform. The acrylic padding intercepted and retained most of the aerosol particles of the spray jet. It also allowed the jet's airstream to pass through it, minimizing backdraft of pheromone onto the spray canister unit. The MSTRS device was set up to produce more than 3,000 recharging sprays of similar strength, sufficient to recharge a source pad every 15 min for up to 30 d without replacement of canister or batteries.

Canister formulations. For disruption experiments, the pheromone used to fill the spray canisters was diluted in either petroleum distillate or ethanol. Petroleum distillate dilutions were prepared so that aerosol canisters containing 31.8 g of pheromone solution in petroleum distillate and 127 g of propellant isobutane emitted 50 µg, 5 µg, 500 ng, 50 ng, or 5 ng of pheromone per spray. Ethanol dilutions were prepared so that aerosol canisters containing

31.8 g of pheromone solution in ethanol and 127 g of propellant emitted 50 µg, 5 µg, 500 ng, 50 ng, or 5 ng of pheromone per spray.

For pheromone-plus-pyrethroid experiments, dilutions were prepared so that aerosol canisters containing 22.4 g of pheromone solution in ethanol, 9.3 g of 20% natural pyrethrin extract (1% pyrethroid), and 127 g of propellant emitted 50 ng, 5 ng, or 0.5 ng of pheromone per spray. A second dilution series for canisters with pheromone and pyrethroid was prepared to emit the same doses of pheromone but now with only one-tenth the dosage of the pyrethroid. Thus, only 0.93 g of the natural pyrethrin extract was added to the canisters.

Experimental rooms. Five rooms that each measured 3 m × 3 m × 3 m were used for the experiment. The rooms were adjacent rental storage units located in an aluminum siding building with little thermal insulation. The walls of the rooms were lined with white housewrap paper (3 m wide, Tyvek®, DuPont) that was secured with staples and duct tape to the walls and floor of the room. A 2.2-m-high faux ceiling of housewrap paper was built to create an air buffer space of 0.8 m between the faux ceiling and a room's ceiling. The result of the lining was an internal, white tent of 3 m × 3 m × 2.2 m, which provided increased room isolation, reduced room contamination, and ease of observing moths. Released moths were present at 1.27 moths per m^2 (0.42 females and 0.87 males per m^2) when at low density, and at 4.27 moths per m^2 (1.42 females and 2.82 males per m^2) when at high density. Population densities of phycitines are reported in terms of insect per m^2 of wall and ceiling because calling and mating behavior occurs on those surfaces (Sower & Whitmer 1977, Hodges et al. 1984). The experiment was conducted during the summer of 1995 in Nevada, Iowa.

Preparation of the rooms. The experimental rooms were thoroughly cleaned before each experiment. The Tyvek lining was inspected for leaks and mended with duct tape if needed. Because some of the moths tended to get caught on the sticky surface of exposed tape, the old duct tape was reinforced with a new layer of tape. The two MSTRS devices per room were placed diagonally from one another. The first was positioned 1.3 m above the floor, 20 cm from the far right corner of the room, and the second was placed at the same height on the near left corner. Treatments requiring one MSTRS per room had the device placed on the near left corner of the room, 1.3 m in height and 20 cm from the corner. Spray pads were replaced with clean ones.

Determining mating disruption. During mating, male Lepidoptera deposit a durable spermatophore in the bursa of the female. In many species, including *C. cautella*, a chitinous, articulated portion of spermatophore remains intact in the bursa for the entire life of the female. By counting the number of spermatophores or their remains in the bursa of recaptured females, one can deduce how often the females mated (Eberhard 1985). In the following experimental-room study, the *C. cautella* moths released were recaptured, separated in the room into groups of alive or dead, transferred to vials with 70% alcohol, and segregated by sex. A moth was considered "alive" if it moved its appendages when touched. Both dead and live moths were segregated by sex. Females were dissected under the microscope at 20x, and their bursa copulatrices isolated and examined to determine presence and number of spermatophores.

Sequence of experiments. The effect of MSTRS devices on the mating success of *C. cautella* was determined by a sequence of seven experiments, with the first five experiments being conducted in experimental storage rooms, and the last two experiments being performed in the laboratory (one in a wind tunnel, the other in small screened cages). The experiments conducted in the experimental rooms first tested the efficiency of the MSTRS devices in promoting mating disruption, then tested the trade-offs involved in cost reduction (reduction in the number of devices per room and in the dose of pheromone used) against the effectiveness of disruption.

The first experiment, disruption vs. pheromone-plus-pyrethroid in the experimental rooms, tested the mating reduction effects over 24 h that two MSTRS per room have when they emit either a high dose of pheromone alone or a low dose of pheromone plus 1% pyrethroid. The second experiment, disruption with two different pheromone concentrations and formulations, tested the mating disruption effect of two MSTRS per room emitting pheromone at two doses (either high or low), formulated by using two solvents (either petroleum distillate or ethanol), over 72 h at high population densities. The third experiment, number of MSTRS devices per room, was designed to reduce the number of MSTRS needed per room. The effect on mating disruption with a low density of moths and a single MSTRS device per room emitting a high dose of pheromone formulated with petroleum distillate was compared with that of two MSTRS devices per room emitting a high dose of pheromone formulated with ethanol over 24 h with the assumption that the actual pheromone volatilization rate from the MSTRS formulated with petroleum distillate was lower than that of the MSTRS formulated with ethanol. Thus, if mating disruption could be achieved in a room by using a single MSTRS formulated with petroleum distillate as compared to disruption levels achieved with two ethanol MSTRS per room, then it could certainly be achieved with a single strong MSTRS per room formulated with ethanol, which would be tested in a later experiment. The fourth experiment tested the mating disruption effect of one MSTRS device formulated with ethanol per room at two doses, on moths at low population density over 24 h. The fifth experiment tested the mating disruption effect of a single MSTRS device per room formulated with ethanol and emitting low pheromone dosages on a high-density population of moths over 24 h.

Experiments six and seven were performed in the laboratory and were designed to determine the mode of action of the pheromone-plus-insecticide MSTRS system. The sixth experiment determined the effect of the addition of 1% pyrethroid on the orientation behavior of individual virgin males to spray pads with pheromone, and their subsequent mortality, in a wind tunnel. The seventh tested the effect of adding 1% pyrethroid to pheromone on the response, and on the subsequent mortality, of groups of virgin male moths in small screen cages.

Disruption vs. pheromone-plus-pyrethroid. The effect of two MSTRS devices per experimental room on mating disruption was tested by using a population density of 30 males and 15 females per room. The spray pad was renewed every 15 min either with 50 µg of pheromone or 5 µg of pheromone plus 1% pyrethroid. New spray pads were 'primed' with two sprays from the appropriate canister and the MSTRS devices set to discharge on a timed basis.

Jars containing males or females were transferred to an experimental room, and the door was closed. Males were released immediately in the center of the room's floor; 1 h later, females were released at the same location. The room was closed for 24 h. At the end of 24 h the moths were recaptured by using an aspirator.

Disruption with two different pheromone concentrations and formulations. Two MSTRS devices per experimental room were tested by using population densities of 100 males and 50 females per room. Success of mating was assessed under four pheromone treatments and a control: a room with two MSTRS devices spraying 50 µg pheromone in petroleum distillate; a room with two MSTRS devices spraying 50 µg pheromone in ethanol; a room with two MSTRS devices spraying 5 µg pheromone in petroleum distillate; a room with two MSTRS devices spraying 5 µg pheromone in ethanol; and a room without pheromone serving as untreated control. Two minutes before the males were released, the new spray pads were primed with 10 sprays, and the MSTRS devices activated for timed release. Jars containing either virgin males or females were transferred to the room and the door closed. Males were immediately released onto the floor in the rear left corner of the room, and females were released 4 h later on the floor at the near right corner. The door of each room was closed and sealed with duct tape for 72 h. At the end of 72 h following female release, moths were recaptured by using an aspirator.

Number of MSTRS devices per room. The mating disruption effect of a single MSTRS device per room spraying 50 µg pheromone was compared to the effect of two MSTRS devices per room, each spraying 50 µg pheromone, and to a control room with no synthetic pheromone. The pheromone treatment with one device per room was set with the MSTRS spraying 50 µg pheromone in petroleum distillate, which would release less pheromone at a slower rate than ethanol, whereas the room with two devices was set with the MSTRS spraying 50 µg pheromone in ethanol. As usual, the MSTRS devices produced a new spray onto their pads every 15 min. Two minutes prior to the release of the males, the new spray pads were primed with two sprays. Each room received 100 virgin males and 50 virgin females with the males being released onto the floor at the far left corner, and the females being released 1 h later at the near right corner. After 24 h the moths were recaptured with an aspirator.

One MSTRS device per room at two doses. The mating disruption effect of a single MSTRS device per room spraying 50 µg pheromone formulated in ethanol was compared to the effect of a single MSTRS device per room spraying 5 µg pheromone, and to a control room with no synthetic pheromone. New spray pads were primed with two sprays 2 min prior to the release of the males, and they were activated for 15-min-interval timed release of sprays. Each room received 30 virgin males and 15 virgin females with males being released at the far left corner on the floor, and the females being released 1 h later in the near right corner. After 24 h the moths were recaptured with an aspirator.

One MSTRS device per room at a low dose, high moth density. The mating disruption effect of a single MSTRS device per room spraying 5 µg pheromone every 15 min on a spray pad was compared to a control room with no synthetic pheromone. New spray pads were primed with two sprays 2 min prior to the release of the males, and thereafter received emitted sprays at 15 min intervals. Each room received 100 virgin males, released on the floor of the

far left corner, and 50 virgin females, released 1 h later on the near right corner. Twenty-four hours later, the moths were recaptured with an aspirator.

Pheromone-plus-pyrethroid experiments in the laboratory. The effect on male behavior of the addition of 1% pyrethrin to the pheromone sprayed onto the MSTRS pads was investigated in two laboratory experiments, either in a wind tunnel or in cages. The effect of the addition of pyrethroid to the pheromone source on the males' long distance orientation and subsequent mortality was determined in wind tunnel bioassays. The effect of addition of pyrethroid to the pheromone source on the males' behavior and subsequent mortality was examined in small cages.

The upwind flight orientation of males responding to 9-cm-diam MSTRS spray pads that received either a spray of 500 ng of pheromone or a spray of 500 ng of pheromone and 1% natural pyrethrin was examined in a wind tunnel. The low-turbulence pulling wind tunnel used was a 2.5 m long × 0.9 m diam semicylinder of transparent Plexiglas® floor and Vivac® walls, similar to Mafra-Neto & Cardé's (1995). Wind speed was maintained at 50 cm/s, light level was 5.5 lux, temperature was 25 ± 2°C, and relative humidity ranged between 65%–85%. The MSTRS spray pad was located in the center of the wind tunnel, 20 cm downwind from the upwind screen. One-day-old males were tested during the initial 30 to 120 min of their first scotophase. A randomly selected moth was removed with an aspirator from a holding cage and transferred to an aluminum screen release cage located 150 cm downwind from the pheromone source. Each male was tested individually and only once. A record of the male's behavior was started as soon as the male was introduced into the pheromone plume and terminated at 2 min, or as soon as the male landed on the source or on the upwind screen of the wind tunnel. The following mutually exclusive behaviors at the release platform and during upwind progression were recorded for each male: quiescent—no perceptible movement; wing fanning/walking—either 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, including casting; in-flight arrestment—flight in the plume in a 'contained' crosswind zigzag without making upwind progress, which usually resulted in the male abandoning the plume; and 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. Males that did not fly were discarded; those that flew were scored as non-responders.

After the test, males were recaptured with an aspirator and transferred to a labeled 20 ml vial. Vials containing one male each were closed with Parafilm® with air holes and held in an environmental chamber for 24 h. After 24 h the males were scored alive or dead.

The effects of addition of pyrethroid to the pheromone pad on the behavior of males and their rate of mortality were studied in 25 cm × 25 cm × 25 cm screen cages with populations of 30 one-day-old males. The experiments were conducted in front of a fume hood, which provided unidirectional flow of clean air through two screen cages. A 9-cm-diam MSTRS pad treated with one spray of either 50 ng pheromone or 50 ng pheromone with 1% natural pyrethrin

extract was placed inside the cage, 3 cm from the upwind screen, with the sprayed side facing downwind. The number of males performing the following mutually exclusive behaviors was annotated every 2.5 min for 20 min, and then once at 60 min and again at 90 min: quiescent—no perceptible movement; wing fanning/walking—either wing fanning, walking, or walking while wing fanning on the cage; flying—flight in the cage; landing on the pad—landing or touching the pheromone source; knocked down—either quiescent on its back or side, i.e., not in a usual resting position, or performing uncoordinated wing fanning, walking, or walking while wing fanning.

Statistical analyses. The data were analyzed for differences by using ANOVA (GLM Procedures, SAS 1989) to test for the effect of treatment, block (each replicate was considered a block), and their interaction. The effect of block was not statistically significant. The data were tested for homoscedasticity; when the criterion was not met, the inverse of the variance was added as weight to the contrast model (SAS 1989). Probabilities of mean separation were obtained by using the least squares method (LSD, GLM Procedures, SAS 1989).

Results

Disruption vs. pheromone-plus-pyrethroid. At low density (30 males and 15 females per room), the pheromone-plus-pyrethroid treatment reduced mating by over 80% (n = 4 replicates), and the disruption treatment prevented mating entirely (Fig. 2). On average 12.0 ± 1.6 (SD) females and 16.3 ± 2.6 males were recaptured from the pheromone-plus-pyrethroid room, 12.0 ± 1.7 females and 18.3 ± 3.3 males were recaptured from the disruption room, and 10.3 ± 1.5 females and 21.3 ± 2.3 males were recaptured from the control room. All female *C. cautella* recaptured in the control room had mated at least once during the 24 h of the experiment, as indicated by successful spermatophore transfer. None of the females recaptured in the disruption room had mated, and more than 80% of the live females were still calling when recaptured in all three replicates of the disruption treatment, indicating their unmated status. All mated females in the pheromone-plus-pyrethroid room had only one spermatophore in their bursa copulatrix, whereas some of the mated females in the control room (7.4%) had two spermatophores in their bursa copulatrix.

Most of the moths recaptured alive in the pheromone-plus-pyrethroid room were females (86%, $\bar{x}$ = 5.25 ± 1.5). Fewer (3.9%, $\bar{x}$ = 0.9 ± 0.8, $P < 0.01$) males were recaptured alive in the pheromone-plus-pyrethroid room, as compared to 81.3% ($\bar{x}$ = 17.0 ± 3.5) recaptured alive in the disruption room and 80.9% ($\bar{x}$ = 18.5 ± 2.5) in the control room. Although the pyrethroid in the room also caused female mortality, 41% of the released females were recaptured alive in this room (compared with 97.5% in the disruption room and 83.8% in the control room), a 14-fold higher proportion than for males.

Males were observed visiting the pheromone-plus-pyrethroid pad soon after they were released into the room. In wind tunnel experiments, MSTRS pads elicited walking and wing fanning, flight initiation, and oriented upwind flight in all males tested, independent of the presence of pyrethroid (Fig. 3A). The exposure of *C. cautella* to pheromone-plus-pyrethroid in the wind tunnel experiment resulted in high mortality of males. The majority (84.3 ± 11.7%) of

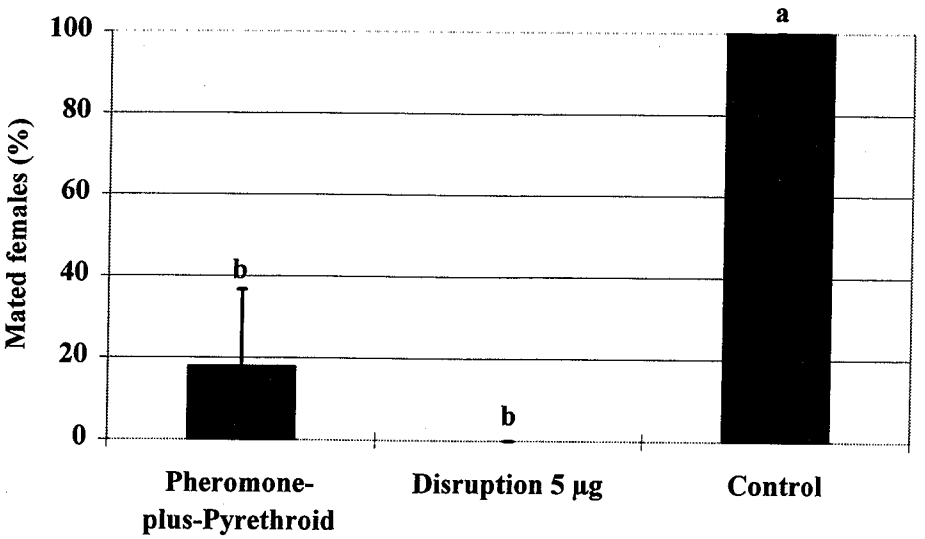

Fig. 2. Percentage of mated females under three treatments: pheromone-plus-pyrethroid room, where two MSTRS devices sprayed 5 µg pheromone in petroleum distillate and 1% natural pyrethroid extract on a spray pad every 15 min; disruption room, the two MSTRS devices sprayed 50 µg pheromone in petroleum distillate on a spray pad every 15 min; and a control room, where no synthetic pheromone was present. Virgin *C. cautella* males (30) and females (15) were released into the rooms and left for 24 h before they were recaptured and mating was assessed on females by dissection to determine the presence of spermatophores. Columns with no letters in common are significantly different at α = 0.05 level (n = 4 replicates) (ANOVA, LSD, SAS 1989).

the males that landed on the pheromone-plus-pyrethroid pad died within 24 h, whereas only a few (<5%) of the males that landed on the pheromone pads with no insecticide died during the same period (Fig. 3B). The pyrethroid even caused significant mortality (16.2 ± 5.2%) in the group of males that only oriented upwind, even though they did not contact the pheromone-plus-pyrethroid pad but did contact the plume (Fig. 3B). The pyrethroid was fast acting, killing most moths in less than 20 min (Fig. 3C). Addition of pyrethroid to the pheromone pad evoked an increased level of activity of males for sustained periods of time, resulting in more numerous and frequent visits to the pheromone-plus-pyrethroid pad than to the pheromone pad without the insecticide.

Disruption with two pheromone formulations at two concentrations. At high density (100 males and 50 females per room), mating was significantly reduced by two MSTRS devices per room emitting either 50 µg or 5 µg pheromone per spray, in solutions of either petroleum distillate or ethanol (n = 3 replicates) (Fig. 4). On average 32.3 ± 2.5 females and 63.7 ± 2.5 males were recaptured from the control room, 39.0 ± 1.9 females and 63.7 ± 2.7 males were

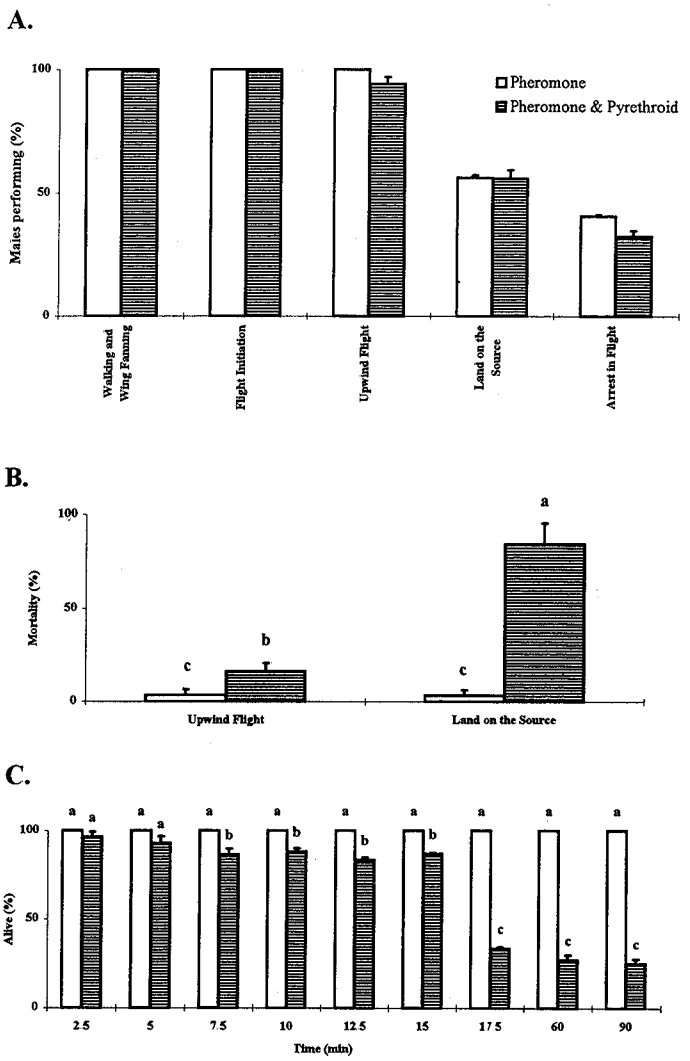

Fig. 3. Effects of the addition of 1% pyrethroid to pheromone sources on *C. cautella* males. (A) Percentage of males performing pheromone related-behaviors in response to a pad sprayed with 500 ng of pheromone and a pad sprayed with 500 ng pheromone and 1% pyrethroid (n = 30, 3 replicates). (B) Mortality of males that only flew upwind, and of males that landed on the sources of pheromone, or pheromone and 1% pyrethroid, after 24 h. (C) Survivorship of males exposed to pheromone pads, and to pheromone and 1% pyrethroid. The mortality effect of the pyrethroid is fully expressed at 17.5 min. Males were tested in groups of 30 (n = 5 replicates). Columns within each histogram with no letters in common are significantly different at $\alpha = 0.05$ level (ANOVA, LSD, SAS 1989).

recaptured from the 50 µg petroleum distillate room, 40.8 ± 1.4 females and 69.0 ± 0.8 males were recaptured from the 50 µg ethanol room, 31.8 ± 2.8 females and 66.0 ± 1.6 males were recaptured from the 50 µg petroleum distillate room, and 30.0 ± 3.2 females and 51.7 ± 3.3 males were recaptured from the 5 µg ethanol room. All females recaptured in the control room, dead or alive, had mated, with at least one, and as many as three spermatophores contained in their bursa copulatrices (8% had two, 1% had three). Thirty percent of the recaptured females had mated by the end of the 72 h in the rooms with the two MSTRS devices spraying only 5 µg pheromone per spray-burst. The solvent used to formulate the canisters had little effect on mating disruption. The pheromone in petroleum distillate solution prevented the mating of 66.1% of the females, and the pheromone in ethanol solution prevented the mating of 70.7% of the females. A 10-fold increase in the dose of pheromone to a 50 µg per spray-burst resulted in a higher degree of mating disruption. The majority of the females recaptured in the rooms with MSTRS devices emitting high-dose sprays of 50 µg pheromone per shot spray-burst were virgins (50 µg petroleum distillate 90.5%, 50 µg ethanol 92.0%) after the 72 h of close contact with males (Fig. 4).

Number of MSTRS devices per room. At high population densities, one MSTRS device emitting 50 µg pheromone per spray every 15 min was sufficient to reduce mating of *C. cautella* females by 95.5%, a mere 2.6% reduction in efficiency compared to 98.1% mating reduction for two MSTRS devices also emitting 50 µg pheromone every 15 min (Fig. 5). On average 39.5 ± 2.1 females and 70.0 ± 1.7 males were recaptured from the room with one MSTRS device, 46.5 ± 1.9 females and 75.5 ± 1.2 males were recaptured from the room with two MSTRS devices, and 44.5 ± 1.9 females and 69.8 ± 1.9 males were recaptured from the control room (n = 3 replicates).

At low population densities, one MSTRS device emitting 50 µg pheromone every 15 min reduced mating by 100%. A MSTRS device emitting only 5 µg pheromone per spray every 15 min reduced female mating by 97.5% (Fig. 6). On average 10.1 ± 1.0 females and 24.5 ± 2.1 males were recaptured from the room with the 5 µg MSTRS device, 9.5 ± 0.7 females and 31.0 ± 2.4 males were recaptured from the room with the 50 µg MSTRS device, and 14.0 ± 0.25 females and 22.5 ± 1.2 males were recaptured from the control room (n = 4 replicates).

At high population densities, one MSTRS device emitting 5 µg pheromone every 15 min reduced mating by 99.1% (Fig. 7). On average 49.5 ± 1.0 females and 73.0 ± 1.0 males were recaptured from the room with one 5 µg MSTRS device, and 37.0 ± 1.0 females and 69.5 ± 1.9 males were recaptured from the control room (n = 3 replicates). All mated females contained a single spermatophore in their bursa copulatrix.

Discussion

MSTRS for disruption and mating reduction with pheromone-plus-pyrethroid. These experiments demonstrated that it was possible to reduce mating of females by more than 90% in a high-density population of a stored-products moth species (>4 moths per m^2) in a closed room. It must be

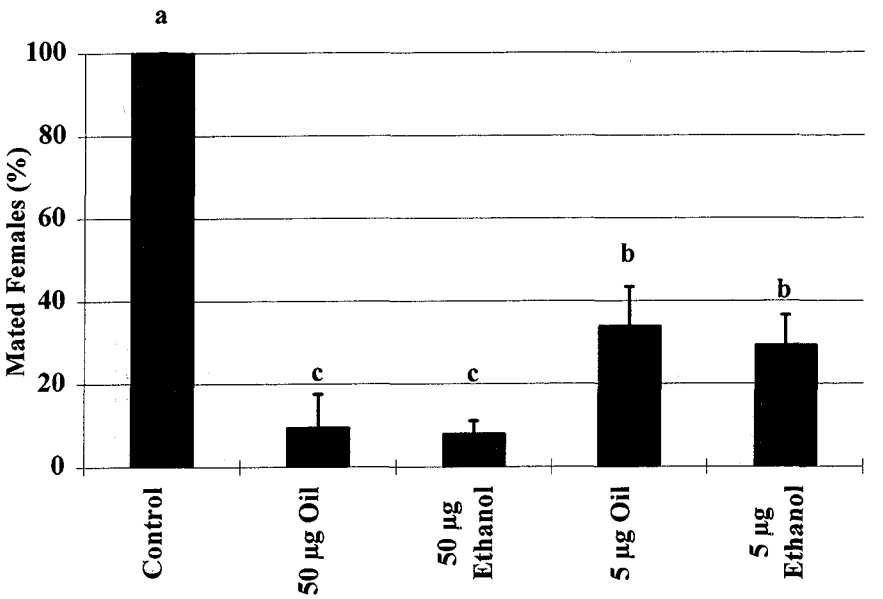

Fig. 4. Percentage of mated females under five treatments: disruption room; the two MSTRS sprayed 50 µg pheromone in petroleum distillate on a spray pad every 15 min; the two MSTRS devices sprayed 50 µg pheromone in ethanol on a spray pad every 15 min; the two MSTRS devices sprayed 5 µg pheromone in petroleum distillate on a spray pad every 15 min; the two MSTRS devices sprayed 5 µg pheromone in ethanol on a spray pad every 15 min; and a control room, where no synthetic pheromone was present. Virgin *C. cautella* males (100) and females (50) were released into the rooms and left for 72 h before recapture. Mating status of recaptured females was assessed by dissection of their bursa copulatrices to determine the presence of spermatophores. The effect of pheromone dose was more important than solvent (ethanol or petroleum distillate) in promoting mating disruption of moths. Columns with no letters in common are significantly different at $\alpha = 0.05$ level (ANOVA, LSD, SAS 1989) ($n = 4$ replicates).

emphasized that the mating activity was measured and assessed by the dissection of recaptured free-flying females that had been in the same room with males for periods of either 24 h or 72 h, rather than assessed according to the reduction of male trap capture in traps containing either synthetic pheromone sources (e.g., Doane & Brooks 1981) or caged live females as baits (e.g., Shorey et al. 1994), or from the dissection of tethered or restrained virgin females (e.g., Miller et al. 1990). All these techniques can be valid ways to assess pheromone disruption, but the use of freely flying and behaving males and females is the most direct measurement and the most relevant to

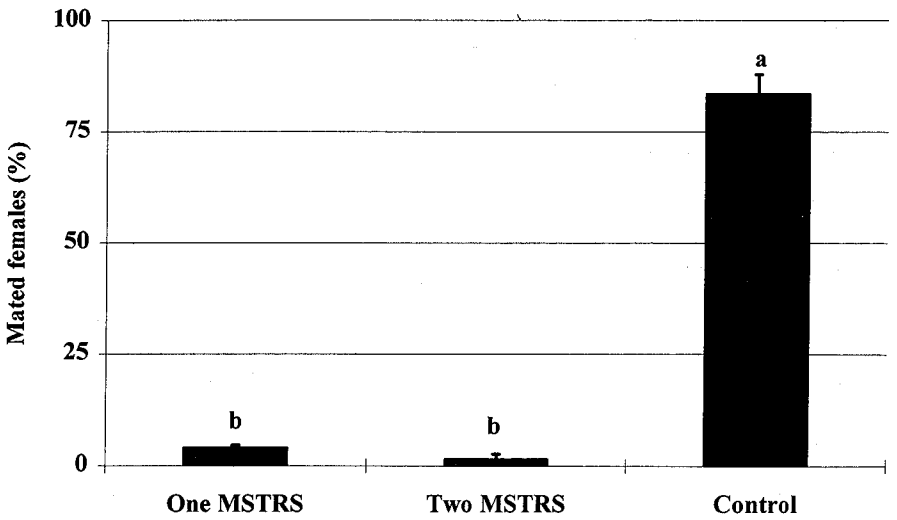

Fig. 5. Percentage of mated females under three treatments: disruption room with only one MSTRS device, which sprayed 50 µg pheromone in petroleum distillate on a spray pad every 15 min; a disruption room with two MSTRS devices that sprayed 50 µg pheromone in ethanol on a spray pad every 15 min; and a control room, where no synthetic pheromone was present. Virgin *C. cautella* males (100) and females (50) were released into the rooms and left for 24 h before recapture. Mating status of recaptured females was assessed by dissection of their bursa copulatrices to determine the presence of spermatophores. Columns with no letters in common are significantly different at α = 0.05 level (ANOVA, LSD, SAS 1989) (n = 4 replicates).

population control. It is also the most powerful way to evaluate the efficacy of a disruptant.

At high pest densities, mate finding may be facilitated simply due to the overall diminished distance between potential mates, which may require only close-range orientation and not long-distance pheromone-mediated orientation. At close range, males may switch to an orientation based on visual and tactile cues, which may override chemical communication in mate location (Charlton & Cardé 1990). Although long-distance orientation of *C. cautella* males to females is chemically mediated (e.g., Phelan & Baker 1990), close-range approach seems to be visually guided (Levinson & Hoppe 1983). There is evidence that *C. cautella* males under 'air permeation' rely predominantly on visual cues to locate their mates (Hagstrum & Davis 1982, Hagstrum et al. 1978). Thus, *C. cautella* mating in disruptant-treated rooms at high densities in previous studies, e.g., >0.1 moth per m^2 (Sower & Whitmer 1977) or >0.3 moth per m^2 (Hodges et al. 1984), probably was facilitated because the rooms were lighted,

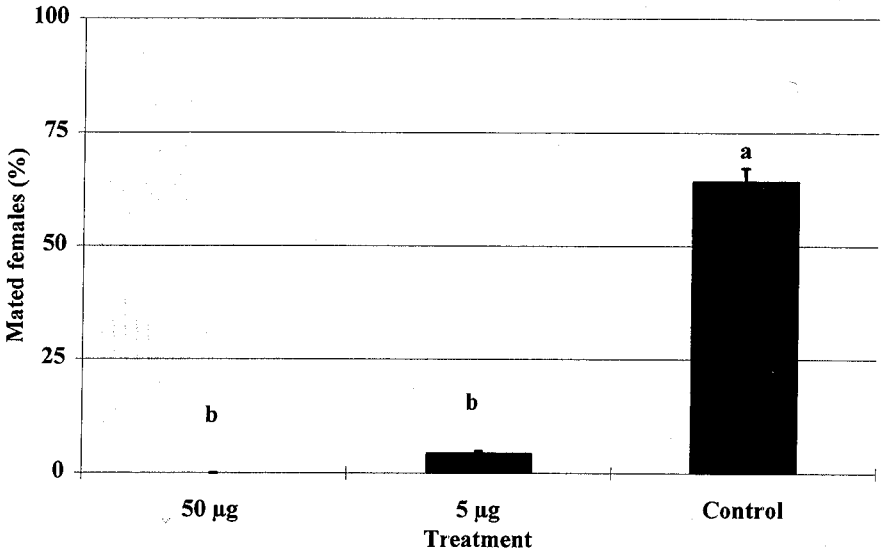

Fig. 6. Percentage of mated females under three treatments: disruption room with only one MSTRS device, which sprayed 50 µg pheromone on a spray pad every 15 min; only one MSTRS device, which sprayed 5 µg pheromone on a spray pad every 15 min; and a control room, where no synthetic pheromone was present. Virgin *C. cautella* males (30) and females (15) were released into the rooms and left for 24 h before recapture. Mating status of recaptured females was assessed by dissection of their bursa copulatrices to determine the presence of spermatophores. Columns with no letters in common are significantly different at $\alpha = 0.05$ level ($n = 4$ replicates) (ANOVA, LSD, SAS 1989).

mates were in close proximity, and there was a switch to visually mediated target orientation (Sower & Whitmer 1977, Hagstrum & Davis 1982). In addition, *C. cautella* moths tend to aggregate in crevices and corners of storage rooms, which facilitates mate finding. Our rooms, with either low or high population densities, were not lighted, although during daylight hours, when females were observed calling, significant amounts of light entered the room through the space under and around the door.

The addition of pyrethroid to the pheromone enhanced the pheromone's ability to reduce mating. The enhanced level of activity of the males exposed to pheromone-plus-pyrethroid probably increased the chances of males contacting lethal doses of the pyrethroid in comparison to the more quiescent females. It is possible that the higher level of activity of males observed in the laboratory cages also occurred in the pheromone-plus-pyrethroid field room, which may also have contributed to the somewhat surprising level of mating (17.9% of

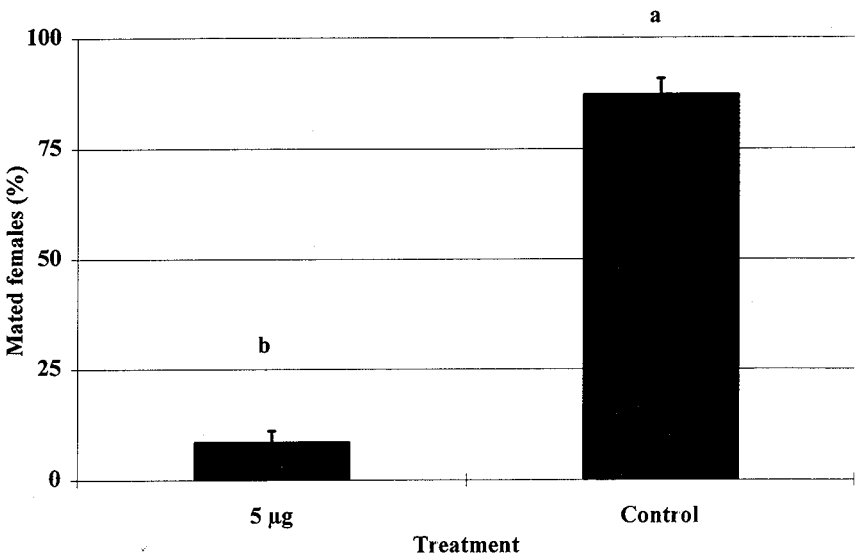

Fig. 7. Percentage of mated females under two treatments: disruption room with only one MSTRS device, which sprayed 5 µg pheromone on a spray pad every 15 min, and a control room, where no synthetic pheromone was present. Virgin *C. cautella* males (100) and females (50) were released into the rooms and left for 24 h before recapture. Mating status of recaptured females was assessed by dissection of their bursa copulatrices to determine the presence of spermatophores. Columns with no letters in common are significantly different at $\alpha = 0.05$ level (ANOVA, LSD, SAS 1989) ($n = 3$ replicates).

females mated), in light of the fact that nearly all (96.1%) males were dead at the time of recapture.

MSTRS as a strong source. One of the main thrusts of the development of commercial formulations for release of pheromone has been to make them sprayable through conventional farm equipment. This approach followed the early notion that pheromone had to be omnipresent in the area to be protected, thus the term pheromone air 'permeation,' which has been and still is widely erroneously used to describe an ideal pheromone emission scheme for a disruption program. However, stronger and more widely deployed dispensers can also be effective mating disruptants. Shorey and colleagues (Shorey 1977, Shorey et al. 1972) early on demonstrated that disruption could be just as effective with widely spaced but powerful emitters (up to 400 m apart) as with closely spaced, weak emitters. They concluded that the overall amount of pheromone emitted per field was critical for successful disruption and not the uniform permeation of the area. More recently, new formulations of very high pheromone per unit emitter-source, such as Shin-Etsu ropes, deployed widely

spaced in the field have proved to protect crops and to be a commercial success against several species of moths (Baker et al. 1990). The MSTRS devices issuing 50 μg per spray were shown here to promote disruption of mating of *C. cautella*: these devices fall into the category of strong, widely spaced pheromone dispensers. Here it has been demonstrated that even a single MSTRS device emitting high doses of pheromone could promote more than 95% mating disruption at high moth population densities. MSTRS devices might effectively protect spaces much larger and with much more complex topography than the simple interior of the rooms used for this experiment, which should have facilitated mate location by the habituated males.

During the writing of this paper, we became aware of the testing of a metered aerosol device by Thomas et al. (1975) for attracting southern pine beetles. They tested the advantages of such a controlled-release system for bark beetle pheromone but focused on attraction alone. Our efforts at employing such a system for moths with the aim of disrupting mating is to our knowledge the first of this type. It is interesting that moth pheromone researchers have not explored the use of metered aerosols for disruption of moth mating in the years since the Thomas et al. (1975) report, especially as these authors pointed out, they do offer so many advantages.

The inability of previous efforts to achieve *C. cautella* mating disruption at high population densities (Ganyard & Brady 1971, Hagstrum & Davis 1982, Haines 1976, Hodges et al. 1984, Minks & Cardé 1988, Vick et al. 1981) may have been caused by loss of activity of the pheromone sources used due to the hardening of the liquid phase of the Z9,E12-14:Ac reservoir, which occurs in ca. 6 d in the field (Shorey et al. 1994). Our experiment resulted in nearly total suppression of mating by *C. cautella* moths, independent of the age of the canister, even at population densities similar to those of previous studies that failed. The frequent renewal of the MSTRS pad with new pheromone sprays seemed to have circumvented the deterioration of the quality of the major pheromone component (Z9,E12-14:Ac) emitted, thus maintaining the high levels of mating disruption of the MSTRS devices.

Mechanism behind MSTRS disruption. As soon as the *C. cautella* males were released into the rooms containing MSTRS devices, they flew in the direction of the MSTRS devices, landed, and performed courtship. At the time we reentered the rooms to release the females, either 1 h or 4 h after male release, depending on the experiment, the majority of the previously released males were quiescent, sitting on the walls and floor of the pheromone-treated rooms. Also, at the end of the experimental period, 24 h or 72 h after the release, the majority (>90%) of the males in the pheromone-treated rooms were observed to be quiescent even though most of the females were still calling. However, we observed that after the MSTRS devices emitted a new spray, many of the quiescent males soon took off and flew. A few of these males landed on the pheromone pad, but most became arrested in-flight while still approaching the pad. Mating disruption of *C. cautella* by the MSTRS devices was, therefore, at least partially achieved by the mechanisms of false trail following and competition among synthetic (MSTRS) and natural (calling females) pheromone sources, in conjunction with (partial) habituation or sensory adaptation (Baker et al. 1989, Cardé 1990, Cardé & Minks 1995).

It is interesting that *C. cautella* males could orient at all to the high pheromone dose sources such as the ones receiving 50 µg of pheromone at 15 min intervals. Previous investigation on the pheromone dose-response in the wind tunnel has shown that *C. cautella* males fail to fly upwind to high doses of pheromone. The optimal pheromone dose to elicit oriented upwind flight and source location of *C. cautella* males in the wind tunnel was 4.5 ng of pheromone on filter paper (Mafra-Neto 1993). Increasing the pheromone dose one or two log steps significantly reduced orientation and landing (45 ng) or even eliminated flight orientation (450 ng). The few *C. cautella* males that engaged in oriented upwind flight to 45 ng and 450 ng sources in the wind tunnel became arrested in flight and abandoned the plume before they located the source (Mafra-Neto 1993). That *C. cautella* males in the field oriented to, and landed on, high-dose sources of at least 5 µg or 50 µg of pheromone may be due to partial habituation caused by the pre-exposure to high doses of airborne pheromone either due to false trail following or to the high ambient average pheromone concentration in the room. Mafra-Neto & Baker (unpubl. data) have found that once male *C. cautella* have been pre-exposed to pheromone, their response threshold shifts and they start responding optimally to higher-dose pheromone sources and fail to respond to the previously optimal low-dose pheromone sources. Thus, the pre-exposure effect may explain why *C. cautella* males in the field responded to high-dose pheromone sources but not to calling females.

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