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Pheromone puffs suppress mating by *Plodia interpunctella* and *Sitotroga cerealella* in an infested corn store

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Abstract

The efficacy of pheromone mating disruption was investigated in a 7 × 6 × 3 m corn storage room harboring a high population density of Indian meal moth, *Plodia interpunctella* (Hübner) and Angoumois grain moth, *Sitotroga cerealella* (Olivier). Pheromones were released from a controlled release dispenser, the metered semiochemical timed release system (MSTRS^{TM}) at emission rates of $\sim$0.6 μg min^{-1} (Z9,E12:14:Ac for Indian meal moth) and $\sim$0.2 μg min^{-1} (Z7,E11-16:Ac for Angouimois grain moth). Mating disruption efficacy was evaluated using three parameters: male capture in pheromone traps, visual examination of mating behavior, and the incidence and frequency of mating as measured by spermatophores. In three trials, comparisons were made between data collected before pheromone treatment and during treatment. Disruption of pheromone source location by males averaged 70% and 40% for *P. interpunctella* and *S. cerealella*, respectively, in the three trials. In addition, reduced levels of copulation by both species were recorded during pheromone treatment. More importantly, significant reductions were recorded in the incidence and frequency of mating by females of both species collected during the treatment period. While $\sim$85% of *P. interpunctella* females collected before pheromone treatment in three trials had mated at least once, only 50% of the females collected during treatment had mated. The mean number of matings, as measured by spermatophores, ranged between 0.8–1.1 and 0.5–0.7 before and during pheromone treatment, respectively. Similarly, a $\sim$20–30% reduction in the proportion of mated *S. cerealella* females was recorded during pheromone treatment. In the three trials, mean number of spermatophores per *S. cerealella* female averaged 1.0 and 0.7 during the pretreatment and treatment periods, respectively. Additional tests conducted in small boxes also recorded significant mating disruption of both species.

Introduction

The potential control of insect pests by pheromone-mediated mating disruption has received considerable research attention, particularly in the last decade. The blend formulation and release matrix of pheromone are known to be critical to the success of mating disruption programs (Cardé & Minks, 1995). Available formulations include capillary tube evaporators, liquid flowables, microcapsules, and sealed polyethylene tubes (ropes) (Weatherston, 1990; Shorey et al., 1994; Mitchell et al., 1997). Metered systems, either MSTRS^{TM} (Mafra-Neto & Baker, 1996; Fadamiro et al., 1998; 1999) or puffers (Shorey & Gerber, 1996a, b) that actively release desired amounts of pheromone over long periods of time in the field have recently been described. Another controlled release dispenser, the microsprayer was also recently developed for mating disruption of orchard pests (Isaacs et al., 1999). These systems allow control over the amount of pheromone released into the air on a daily or seasonal basis, in addition to their ease of deployment and retrieval in the field.

Much of the work on mating disruption has focused on field and orchard pests (Shaver & Brown, 1993; Deland et al., 1994; Shorey et al., 1994; Fitzpatrick et al., 1995; Shorey & Gerber, 1996a, b). The few studies on mating disruption of stored-product insects have been conducted either in the laboratory (Brady & Daley, 1975; Hagstrum et al., 1978), or at best, in simulated environments (Sower & Whitmer, 1977; Hagstrum & Davis 1982; Prevett et al., 1989; Mafra-Neto & Baker, 1996; Isaacs et al., 1999). The potential use of pheromone-mediated mating disruption to control insect pests in warehouse conditions remains largely unexplored. Mating disruption of insect pests in the storage environment presents a different challenge due to the uniqueness of the storage habitat (Vick et al., 1981) and the high intrinsic rates of increase of insects in such situations (e.g., Brower, 1975). Furthermore, stored grains enjoy stringent plant quarantine regulations and there is an ever-growing need for alternative pest control. Easily deployable dispensers that actively release high concentrations of pheromone may be suitable for the storage environment, and their potential use in stores merits research attention (Vick et al., 1978).

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), and the Angouimois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) are ubiquitous pests of stored grains, particularly in the tropics (Dobie et al., 1991). In grain stores where mixed-infestation of both species occurs, Angouimois grain moth larvae are usually the primary colonizers (Barney & Weston, 1996), while Indian meal moth larvae feed on the flour produced by the former's feeding activities.

Female-produced sex pheromones have been identified for both species, the major components of which are (*Z,E*)-9,12-tetradecadien-1-ol acetate (or Z9,E12-14:Ac) (Brady et al., 1971; Kuwahara et al., 1971; Teal et al., 1995; Zhu et al., 1999) and (*Z,E*)-7,11-hexadecadien-1-ol acetate (or Z7,E11-16:Ac) (Vick et al., 1974), for *P. interpunctella* and *S. cerealella*, respectively. Additional sex pheromone components are also known for the two species, although their behavioral roles remain largely unclear: (*Z,E*)-9,12-tetradecadien-1-ol (Z9,E12-14:OH; Sower et al., 1974; Teal et al., 1995; Zhu et al., 1999) and (*Z,E*)-7,11-hexadecanal (Z7,E11-16:Ald; Ando et al., 1985) for *P. interpunctella* and *S. cerealella*, respectively.

The few attempts to investigate the efficacy of pheromone-mediated mating disruption of both *P. interpunctella* and *S. cerealella*, have yielded inconsistent results. Sower et al. (1975) and Sower & Whitmer (1977) recorded reductions in the mating frequency and population growth rate of *P. interpunctella* with high concentrations of Z9,E12-14:Ac. Brady et al. (1975), however, reported the inefficacy of pheromone for the disruption of *P. interpunctella* in the field. For *S. cerealella*, Sower et al. (1973) first demonstrated the habituating-effect of high concentrations of Z7,E11-16:Ac on the male. Later, Vick et al. (1978) recorded significant mating disruption with the sex pheromone. The above studies by Sower & Whitmer (1977), and by Vick et al. (1978) demonstrated the important interplay among pest population density, pheromone dose, and disruption efficacy for both species. Two emerging trends from these studies were the reduced efficacy of mating disruption at high pest densities, as well as improved disruption with increasing pheromone concentrations (Sower & Whitmer, 1977; Vick et al., 1978). The development of dispensers capable of releasing high pheromone concentrations while optimizing efficiency is therefore central to the achievement of effective mating disruption, in the storage environment (Vick et al., 1978).

In this paper we report investigations of the effects of high emission rates of pheromone components on the mating disruption of *P. interpunctella* and *S. cerealella* in a corn-store harboring high populations of both species. Pheromone components were dispensed from a controlled release dispenser, the Metered Semiochemical Timed Release System, or MSTRS™ (Mafra-Neto & Baker, 1996; Fadamiro et al., 1998, 1999). Additional tests were also conducted in boxes to evaluate, on a smaller scale, the disruption efficacy of our pheromone blends. Our goal was to utilize available, although imperfect, blends for each species and emit higher rates of the major components than had been used before to see if a significant reduction in mating could be achieved. The potential for even higher efficacy of disruption using optimal blends in conjunction with these high-release dispensers would be indicated if significant effects could be observed with these imperfect blends.

Materials and methods

Pheromone dispenser and formulations. Pheromone components used for mating disruption of both species were 93–96% pure, and some formulations contained known amounts of inter-specific compounds (Bedoukian Research, Inc. Danbury, Connecticut).

The pheromone blend used for *P. interpunctella* disruption during the first trial was a mixture of Z9,E12-14:Ac and Z9-14: Ac in the ratio 10:0.9, which was originally formulated for the disruption of the almond moth, *Cadra cautella* (Mafra-Neto & Baker, 1996). During the second and third trials, however, a two-component *P. interpunctella* pheromone blend (Z9,E12-14:Ac + Z9,E12-14:OH in the ratio 5:1) was used for disruption. For *S. cerealella*, the major sex pheromone component, Z7,E11-16:Ac was not available from suppliers in its pure formulation. The only available formulation of Z7,E11-16:Ac, gossyplure (a 50:50 mixture of Z7,E11-16:Ac and Z7,Z11-16:Ac) was used for disruption trials.

During the first trial for *P. interpunctella*, pheromone was released from a pressurized canister version of MSTRS^{TM} (Mafra-Neto & Baker, 1996; Fadamiro et al., 1998). Dilutions were made so that the canisters containing 31.8 g of pheromone solution in 40 ml of LPA-210 petroleum distillate plus 127 g of propellant isobutane emitted ~0.5 mg of Z9,E12-14:Ac per spray. During both second and third trials, the two-component *P. interpunctella* blend was released from a non-pressurized version of the MSTRS^{TM} device, which was described in Fadamiro et al. (1998). The non-pressurized MSTRS^{TM} is similar to the pressurized version, except that its operation is based on the hydraulic principle, compared with the pressurized MSTRS^{TM} that utilizes gas pressure. Both devices basically consisted of a spray canister (or container), a spray dispenser unit, and a spray pad that receives each spray burst and emits pheromone from its surface (Mafra-Neto & Baker, 1996; Fadamiro et al., 1998, 1999). Dilutions were made such that non-aerosol cans containing 4 g of pheromone (Z9,E12-14:Ac + Z9,E12-14:OH in the ratio 5:1) in 150 ml ethanol emitted ~1.3 mg of pheromone per spray. Pheromone used for *S. cerealella* disruption was also released from non-pressurized MSTRS^{TM}. Dilutions were made such that canisters containing 5 g of pheromone (gossyplure) in 150 ml ethanol emitted ~0.8 mg of Z7,E11-16:Ac per spray.

Measurement of pheromone release rates from MSTRS pads. Release rates of the major pheromone components of the two species, Z9, E12-14:Ac or Z7, E11-16:Ac from MSTRS^{TM} pads after different days of emission in the experiment room were measured as described in Fadamiro et al. (1998, 1999). Briefly, a circular cutout of the pad, contained within a frame (9.2 cm ID), was placed on top of a glass funnel (10 cm ID). The stem of the funnel was connected with a Teflon connecting tube to a glass Pasteur pipette containing a 7-cm-long plug of packed glass wool. Air was drawn across the pad (2000 ml min^{-1}) and through the glass wool trap from the tip of the Pasteur pipette by means of a vacuum. After a 20-min collection time the vacuum was stopped, the pad removed from the funnel, and 20 μg (1 μg μl^{-1}) of the appropriate internal standard (Z11-16: Ac and Z11-14:Ac for Z9, E12-14:Ac and Z7, E11-16:Ac, respectively) was added to the glass wool. The funnel wall and glass wool plugs were washed with 3 ml of HPLC-grade hexane. One microliter of this solution was analyzed for the amount of pheromone relative to the internal standard by capillary gas chromatography.

Care was taken so that the pheromone pads did not come in contact with the glass funnel surface. Trap breakthrough was checked and confirmed negative by analyzing collected material in a second, in-series-connected, Pasteur pipette. Pad collections were performed in duplicate and collected amounts of pheromone from pads were calculated for the original pad diameter. All GC analyses were performed by using a Hewlett-Packard 5890 GC with a FID detector (30-m DB-225 capillary column).

Box experiment. In order to evaluate the efficacy of the pheromone blends deployed in the experimental room, a pilot study was conducted in 0.5 × 0.4 × 0.3 m wooden boxes with doors on one side. The top part of each box was constructed of transparent plexiglass and the wooden-joints were sealed to prevent escape. Air circulation in the box was minimal. Insects utilized in the experiments were reared and handled using standard procedures described in the literature (Mills, 1965; Silhacek & Miller, 1972; Sower et al., 1975). Larvae were reared on cracked wheat (*P. interpunctella*) or corn (*S. cerealella*) at 27 ± 2 °C and 60 ± 10% r.h. with a 12-h light-dark cycle. *Plodia interpunctella* pupae were held individually in 5/8 oz clear plastic cups (Fill-Rite Inc., Newark, NJ) until emergence. Similarly, corn kernels infested by *S. cerealella* were placed in 5/8 oz plastic cups until adult emergence.

Fifteen pairs of virgin 2–3-day-old adults of each species were placed in each box. This experimental density could be regarded as medium-to-high population density under the conditions of the tests (Sower & Whitmer, 1977; Vick et al., 1978). For *P. interpunctella*, the treated box contained a non-aerosol MSTRS^{TM} device which was programmed to deliver

onto its pad ~1.3 mg of a blend of Z9,E12:14:Ac and Z9,E12:14:OH in the ratio 5:1 every 25 min. The pad was primed with ~60 mg of the same blend. For *S. cerealella*, a device was programmed to deliver onto its pad ~0.8 mg of Z7,E11-16:Ac every 25 min, the pad having been primed with ~40 mg of pheromone. The control box for each treatment contained a similar MSTRS™ device programmed to deliver onto its pad 50 μl of ethanol every 25 min. The pad was 'primed' with 50 sprays of ethanol. After releasing moths into them, the boxes were placed in a shed where the temperature averaged 29 ± 2 °C during the day, and lighting was uncontrolled. Twenty-four hours later, moths in each box were knocked down by using ethanol sprays, collected and preserved in labeled glass vials containing 70% ethanol for dissection at a later date. The test was replicated three times for each species.

Room experiment layout. Experiments were conducted between November 1996 and October 1997 in a 7 × 6 × 3 m room located in the Molecular Biology building at Iowa State University, Ames. The test room is a seed preparation room and had also served as a store for experimental hybrids of corn for years. It was constructed of cement brick with concrete floors. The walls were painted pink, and fluorescent bulbs attached to the ceiling provided lighting in the room. Cobs of corn of several hybrids had been transported into the room at the end of the harvesting season in October 1996 and stored inside cupboards and drawers. The room had never before harbored any major insect infestations, except during the preceding storage season in 1995 when a minor infestation was recorded. This infestation was quickly brought under control by fumigation. Our opinion is that the major infestation that began in November 1996 started in the field, although a build-up following the 1995 minor infestation might have contributed to the high levels of infestation recorded in November 1996. Two treatments of insecticide fumigation in November 1996 failed to give a significant control of the infestation, after which we were consulted for advice. A mixed-infestation of two moth species, *Plodia interpunctella* and *Sitotroga cerealella* was identified in the room.

Insect monitoring. To give an indication of insect population dynamics, pheromone-baited Intercept W wing traps (IPM Technologies Inc., Portland, OR) were deployed in the room for two weeks. The lures used in traps were of two types: Z9,E12-14:Ac for *P. interpunctella*; and gossyplure for *S. cerealella*. Dilutions of each pheromone were made in hexane to give 0.5 μmg/μl^{-1} stock solutions. Aliquots (20 μl) of each stock solution were then measured using a micropipette onto a rubber septum, to give the desired pheromone concentration of 10 mg in a septum. Two traps of each lure type were randomly hung approximately 50 cm from the ceiling and spaced at least 1 m apart. Traps baited with commercial lures (Storgard cap lures supplied by Trécé Inc. Salinas, CA for *S. cerealella*; and *P. interpunctella* Bullet lures supplied by Insects Limited Inc., Indianapolis, IN) were also tested. Both commercial lures were not significantly more attractive than our laboratory-prepared lures, and their use was therefore discontinued.

Catches in traps containing conspecific pheromone lures in the room averaged 32.5 adults/trap/week and 97.25 adults/trap/week for *P. interpunctella* and *S. cerealella*, respectively. Inter-specific cross-attraction to traps was also recorded with *S. cerealella* being the least discriminatory. An average catch of 64 *S. cerealella* adults/trap/week was recorded in traps baited with the pheromone of *P. interpunctella*, compared with an average catch of 3.25 *P. interpunctella* adults/trap/week recorded in traps baited with *S. cerealella* pheromone.

Estimation of population density. Population density was estimated as the number of moths of each species per m^2 of wall and ceiling space, since calling and mating activity of both species occurred mainly on those surfaces (Sower & Whitmer, 1977; Mafra Neto & Baker, 1996; pers. observ.). Population density estimated by counting the number of adults in open surfaces (i.e., walls and ceiling) would only represent conservative estimates, since adults in cupboards, drawers and other hiding surfaces will not be counted. Estimates of population density were taken from different parts of the room, and an average calculated for the pretreatment and treatment periods of each trial. Using this procedure, average population density estimates of approximately 7 per m^2 and 25 per m^2 of ceiling were recorded for *P. interpunctella* and *S. cerealella*, respectively, during the pretreatment period of the first trial. Estimates taken during the other trials are shown in Tables 4 and 5.

Evaluation of mating disruption. In this study, both the control and treatment data were collected from the same experiment room. In all trials, disruption efficacy was compared between the pretreatment 'con-

trol' period when pheromone dispensers were not present in the room, and the treatment period during which pheromone dispensers were present in the room. The parameters used for assessing disruption efficacy were:

(1) disruption of pheromone source location as measured by male capture in traps;

(2) visual examination of mating behavior (number of coupling adult pairs per unit time);

(3) presence and number of spermatophores in captured females.

Disruption of pheromone source location was assessed by comparing the numbers of males of each species captured in traps containing conspecific pheromone during the pretreatment and treatment period for each trial. During each period, two traps containing 10 mg of either *P. interpunctella* pheromone, *S. cerealella* pheromone, or hexane (unbaited trap) loaded onto rubber septa were randomly hung approximately 50 cm from the ceiling and spaced at least 1 m apart in the room. Trap catch was counted every 2 or 3 days, and traps were randomly repositioned in the room after inspection. During treatment periods, new traps containing fresh lures were deployed in the room. Traps were hung at least 1 m on all sides away from the nearest pheromone dispenser. This method, together with the lower height at which the dispensers were deployed minimized possible contamination of traps. MSTRS^{TM} devices were hung approximately 15 cm away from the walls and 80 cm from the ceiling, and there was at least 1 m separation distance between two dispensers.

Visual examination of mating behavior was done by counting for 15 min the numbers of copulating pairs of each species on the walls and ceiling. Data were collected on the days when traps were inspected, during the pretreatment and treatment periods.

In order to determine incidence and frequency of mating, adults of each species were captured using an aspirator. Moths were collected during the pretreatment and treatment periods, and preserved in labeled vials containing 70% ethyl alcohol. Adults female moths were later dissected under the microscope at 30×, examining the bursae copulatrices for presence and number of spermatophores (Fadamiro & Baker, 1999).

Room experiments. Three trials were conducted in the infested room, each comprising of a pretreatment (control) and a treatment period. In the first trial, pretreatment data were collected for nine days (January 27, 1997 through February 4, 1997), prior to the deployment of MSTRS^{TM} devices in the room. MSTRS devices were deployed in the room after data collection on February 4, 1997, marking the beginning of the treatment period. Three aerosol MSTRS^{TM} devices each emitting ~0.5 mg of Z9,E12-14:Ac per spray (for *P. interpunctella*), and three non-aerosol MSTRS^{TM} devices each emitting ~0.8 mg of Z7,E11-16:Ac per spray (for *S. cerealella*) were hung in the room. The MSTRS^{TM} devices were programmed to deliver pheromone onto their pads every 15 min per 24 h period (=96 sprays per 24 h). Treatment data were collected in the room beginning February 6, 1997 for the next nine days (February 6, 1997 through February 14 1997). At the end of the first trial, all the MSTRS^{TM} devices were shut down, but were not removed from the room. Thus, the pads continued to emit pheromone in the room but at diminished rates.

During the second trial, pretreatment data were collected in the room from March 29, 1997 to April 6, 1997. Although the MSTRS^{TM} devices remained shut down during this period, the room was not absolutely free of pheromone since a small amount of pheromone was passively released from the MSTRS^{TM} pads. MSTRS^{TM} devices were deployed in the room on April 9, 1997. Three non-aerosol MSTRS^{TM} devices each with its pad primed with ~60 mg and emitting ~1.3 mg of pheromone (Z9,E12-14:Ac + Z9,E12-14:OH in the ratio 5:1) every 25 min (for *P. interpunctella*), and three non-aerosol MSTRS^{TM} devices each with its pad primed with ~40 mg and emitting ~0.8 mg of pheromone (Z7,E11-16:Ac) every 25 min (for *S. cerealella*) were deployed in the room. Treatment data were collected in the room beginning April 9, 1997 until April 19, 1997. At the end of the second trial, all MSTRS^{TM} devices were removed from the room.

The pretreatment period for the third trial was between July 25, 1997 and August 11, 1997. Pheromone treatment was applied from August 14, 1997 through August 30, 1997. The number of devices, deployment protocols, as well as pheromone formulation and concentration used during the third trial were as described for the second trial.

Statistical analyses. Percentage mated female *P. interpunctella* and *S. cerealella* were calculated for the control and treatment boxes, and significant differences established by using a χ^2 2 × 2 test of independence with Yates' correction of continuity (Parker, 1979).

Data collected from the room experiments were analyzed by considering each trial as a separate test. This was necessary since the three trials were not true replicates of one another. Average number of males captured per trap per sampling time was calculated for each species. These averages were used to calculate the mean trap catch for the pretreatment and treatment periods of each trial. Means were analyzed with a one-way ANOVA (SAS, 1985). Similarly, mean number of coupling pairs of each species before and during pheromone treatment was calculated for each trial and analyzed by using a one-way ANOVA (SAS, 1985). Data collected on the incidence of mating of females collected in the test room during the pretreatment and treatment periods of each trial were presented as percentages. Significant differences were established using a χ^2 2 × 2 test of independence with Yates' correction of continuity (Parker, 1979). Mean number of spermatophores per female collected before and during pheromone treatment was calculated for each trial and analyzed by using a one-way ANOVA (SAS, 1985). Significant differences were established at the 95% confidence level (Parker, 1979).

Results

Pheromone release rates. Mean emission rates of pheromone from MSTRS pads after 1 and 7 days in the test room were calculated for both pheromone systems. Release rates of Z9,E12-14:Ac from the pads containing *P. interpunctella* pheromone averaged 0.59 ± 0.09 μg min^{-1} and 0.60 ± 0.13 μg min^{-1} after 1 and 7 days in the room, respectively. Results of collection of Z7,E11-16:Ac from pads containing *S. cerealella* pheromone showed lower mean release rates of 0.18 ± 0.01 μg min^{-1} and 0.18 ± 0.07 μg min^{-1} after 1 and 7 days in the store, respectively. Therefore, *P. interpunctella* pheromone (14-carbon acetate) was released at a rate ~3 times higher than *S. cerealella* pheromone (16-carbon acetate).

Box experiment. Significant reductions in mating by females of both species were achieved in pheromone-treated boxes. On average, 95% of *P. interpunctella* females had mated in the control box, compared with 47% mating recorded in the treatment box ($P < 0.05$, Figure 1). In the case of *S. cerealella* females, a comparatively lower proportion of mating (56%) was recorded in the control box, although this was significantly higher than the 12% mating recorded in the box treated with pheromone ($P < 0.05$, Figure 1).

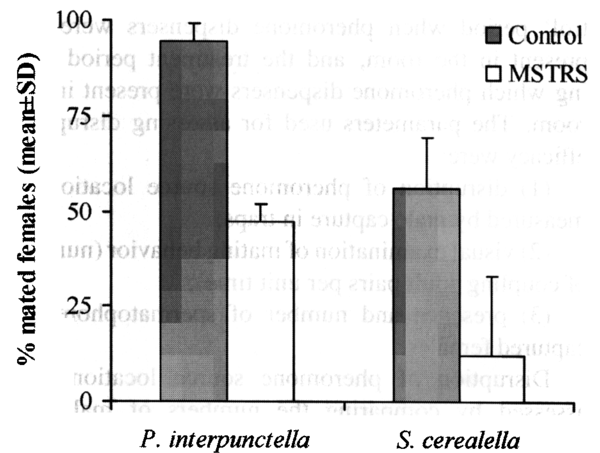

Figure 1. Incidence of mating by *Plodia interpunctella* and *Sitotroga cerealella* females in control and pheromone-treated test boxes. Means with different letters are significantly different ($P < 0.05$).

Male capture in pheromone-baited traps. Captures of *P. interpunctella* and *S. cerealella* males before and during pheromone treatment in the three trials are shown in Figures 2 and 3, respectively. For *P. interpunctella*, significantly higher numbers of males were captured in unbaited traps during the first-trial treatment period than during the first-trial pretreatment period, possibly indicating an increase in population ($P = 0.05$; Table 1). However, male capture in pheromone-baited traps were significantly lower ($P = 0.02$) during the treatment period of the first trial, compared with the first-trial pretreatment period, culminating in a 56% disruption of pheromone source location during pheromone treatment (Table 1). In the second trial, a significant, 61% reduction in trap catch was recorded when MSTRS™ devices were deployed in the room, compared with before treatment ($P = 0.03$, Table 2). Similarly, the deployment of MSTRS™ devices in the room resulted in a significant (93%) disruption of pheromone source location during the third trial ($P = 0001$), while catches in unbaited traps were approximately the same before and during pheromone treatment (Table 1).

For *S. cerealella*, in the first trial, a 40% disruption of pheromone source location by males was recorded during pheromone treatment, compared with before treatment, but this was not significant ($P = 0.10$, Table 2). Similarly, the 40% trap catch reduction recorded for *S. cerealella* males during pheromone

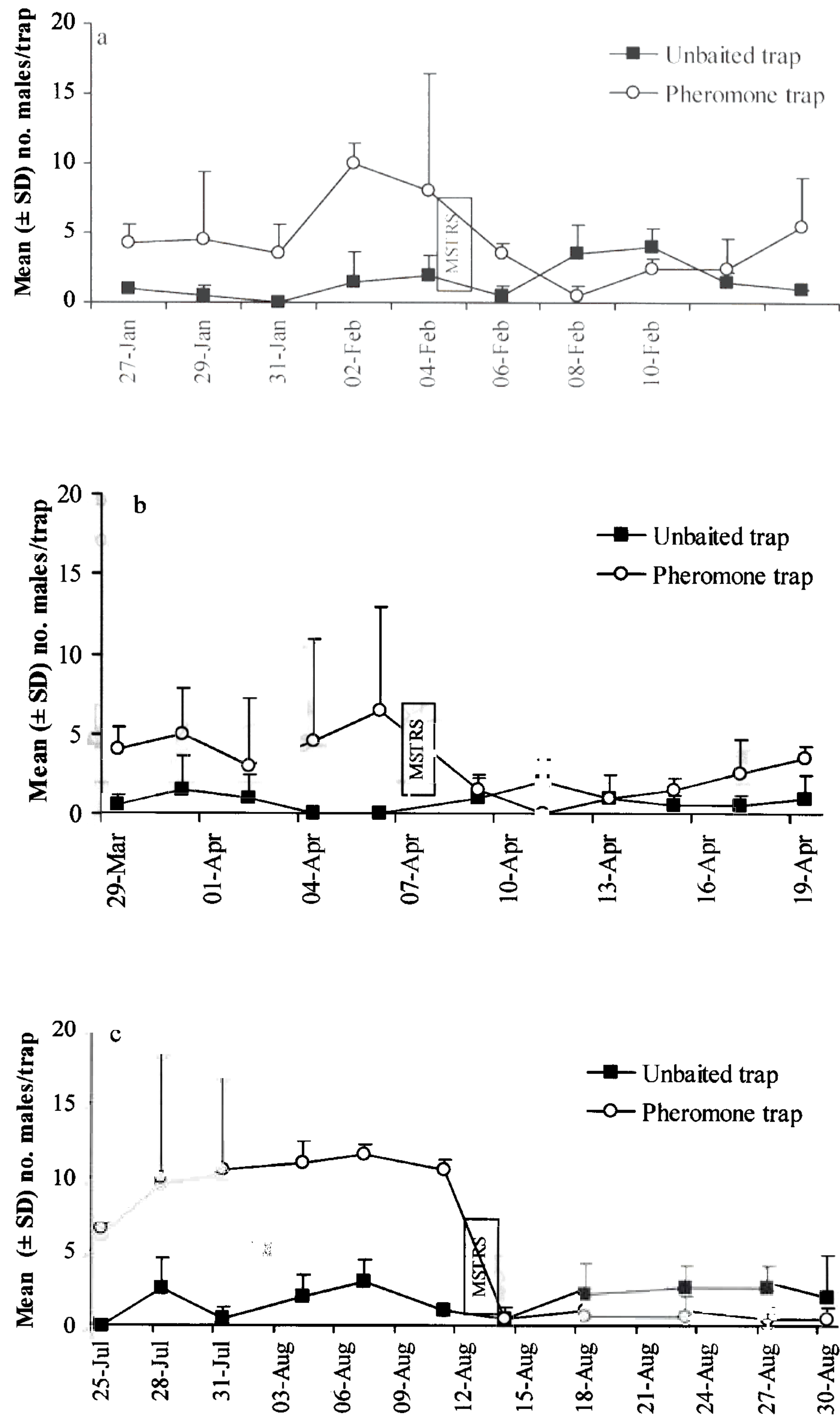

Figure 2. Number of *Plodia interpunctella* males captured in unbaited and pheromone-baited traps placed in the experimental room before and during pheromone treatment in three trials (a–c). Rectangle in the middle of chart indicates the date of deployment of MSTRS devices, marking the beginning of pheromone treatment.

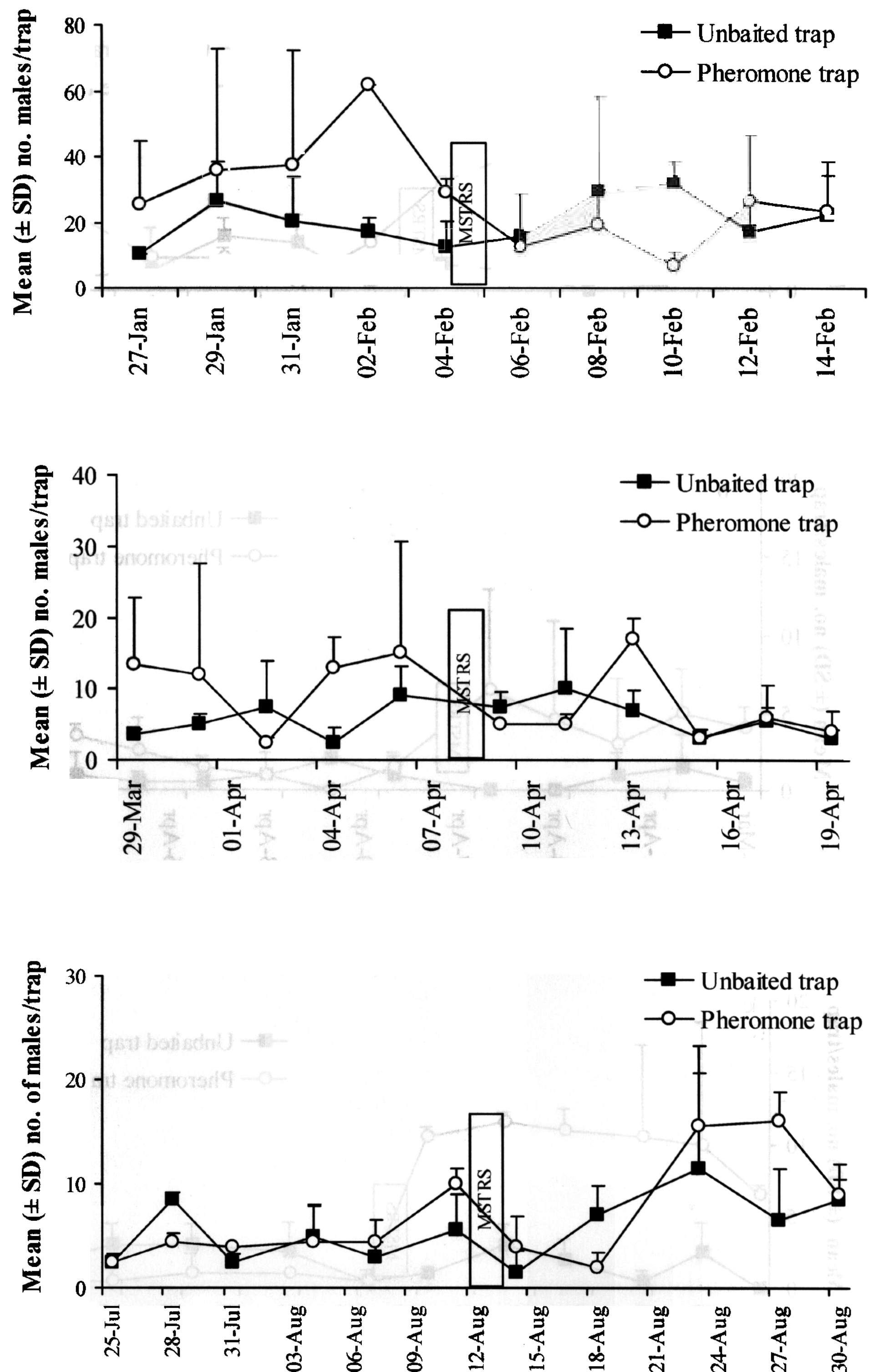

Figure 3. Number of *Sitotroga cerealella* males captured in unbaited and pheromone-baited traps placed in the experimental room before and during pheromone treatment in three trials (a–c). Rectangle in the middle of chart indicates the date of deployment of MSTRS devices, marking the beginning of pheromone treatment.

Table 1. Number of *Plodia interpunctella* males captured in unbaited and pheromone-baited traps before and during pheromone treatment in three trials

Data collection period	Mean (± SD) no. males captured per 2-day per trap					
	Trial 1		Trial 2		Trial 3	
	Unbaited trap	Pheromone trap	Unbaited trap	Pheromone trap	Unbaited trap	Pheromone trap
Pre-treatment	1.0 ± 1.2 b	6.1 ± 4.3 a	0.6 ± 1.1 a	4.6 ± 3.7 a	1.5 ± 1.4 a	10.0 ± 3.7 a
Treatment	2.4 ± 1.7 a	2.7 ± 2.1 b	1.0 ± 1.0 a	1.8 ± 1.5 b	2.2 ± 1.7 a	0.7 ± 0.7 b
% disruption		56		61		93

Means for the same trial having no letters in common are significantly different (P < 0.05).

Table 2. Mean number of *Sitotroga cerealella* males captured in unbaited and pheromone-baited traps before and during pheromone treatment in three trials

Data collection period	Mean (± SD) no. males captured per 2-day per trap					
	Trial 1		Trial 2		Trial 3	
	Unbaited trap	Pheromone trap	Unbaited trap	Pheromone trap	Unbaited trap	Pheromone trap
Pre-treatment	18.1 ± 9.3 a	38.1 ± 22.5 a	5.4 ± 3.8 a	11.2 ± 9.3 a	4.5 ± 2.7 a	5.0 ± 2.8 a
Treatment	21.4 ± 13.3 a	22.8 ± 19.8 a	6.0 ± 4.1 a	6.7 ± 5.1 a	7.0 ± 5.2 a	9.3 ± 6.8 a
% disruption		40		40		–

Means for the same trial having no letters in common are significantly different (P < 0.05).

treatment in the second trial was not significant (P = 0.16, Table 2). In the third trial, however, male capture in pheromone-baited traps during pheromone treatment was slightly, but not significantly higher than during the pretreatment period, indicating failure of pheromone treatment to cause disruption of pheromone source location (P = 0.06, Table 2). In all three trials, catches of *S. cerealella* males in unbaited traps were slightly higher during pheromone treatment, than before treatment, possibly indicating increased moth populations (Table 2).

Visual examination of mating behavior. There was a general trend for reduction in copulation by both species during pheromone treatment, compared with before treatment. For *P. interpunctella*, although reductions in the number of coupling pairs during pheromone treatment were recorded in two of the three trials, only during the third trial was this significant, averaging 1 and 0 coupling pairs per observation time for the pretreatment and treatment periods, respectively (P = 0.05, Table 3).

The decrease in the numbers of coupling *S. cerealella* adults recorded during pheromone treatment in the first two trials represents modest, but non-significant reductions (P > 0.05, Table 3). In the first trial, an average of six coupling pairs per observation time was recorded during the pretreatment period, compared with the two coupling pairs/time recorded during pheromone treatment (P = 0.13, Table 3). Similarly, whereas the number of coupling pairs averaged three per observation time during the pretreatment period of the second trial, it was less than one per observation during pheromone treatment (P = 0.13, Table 3). Approximately the same number of coupling pairs was recorded before and during pheromone treatment in the third trial (P = 0.74, Table 3).

Spermatophores. For both species, there was a general decline in the proportion of mated females collected during the treatment period, compared with the pretreatment period. Of the 37 *P. interpunctella* females collected during the pretreatment period of the first trial, 94% had mated at least once. This was significantly higher than the 63% ($n = 51$) mated females recorded during the treatment period (P < 0.05, Table 4). Similarly, a significant reduction was recorded in the mean number of spermatophores per *P. interpunctella* female collected during pheromone treatment (0.7 spermatophores/female), compared with before pheromone treatment (1.1 spermatophores/female; P = 0.0006, Table 4). The proportion of mated females

Table 3. Number of coupling *Plodia interpunctella* and *Sitotroga cerealella* adults observed per 15 min before and during pheromone treatment

Data collection period	Mean (± SD) no. of coupling pairs per observation time					
	P. interpunctella			*S. cerealella*		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Pre-treatment	0.8 ± 1.3 a	0.6 ± 0.9 a	1.0 ± 1.0 a	6.2 ± 5.0 a	2.8 ± 2.6 a	0.8 ± 0.8 a
Treatment	0.4 ± 0.6 a	1.4 ± 2.2 a	0.0 ± 0.0 b	2.0 ± 2.5 a	0.6 ± 1.3 a	1.0 ± 1.0 a

Means for the same trial having no letters in common are significantly different (P < 0.05).

Table 4. Percentage mated and number of spermatophores per *Plodia interpunctella* female collected before and during pheromone treatment

	Period	Estimated density (adults m^{-2})	No. females collected	% mated females	Mean (± SD) no. spermatophores
Trial 1	Pre-treatment	7	37	94 a	1.1 ± 0.5 a
	Treatment	8	51	63 b	0.7 ± 0.6 b
Trial 2	Pre-treatment	6	37	70 a	0.8 ± 0.6 a
	Treatment	9	26	50 a	0.6 ± 0.7 a
Trial 3	Pre-treatment	4	26	92 a	1.1 ± 0.6 a
	Treatment	5	18	44 b	0.5 ± 0.6 b

Means for the same trial having no letters in common are significantly different (P < 0.05).

and the frequency of mating were not significantly different during the second trial (P > 0.05, Table 4). In the third trial, 44% of females collected during pheromone treatment had mated with an average of 0.5 spermatophores/female, compared with the 92% matings and 1.1 spermatophores/female recorded during the pretreatment period (P < 0.05, Table 4). These results represented a ~50% reduction in mating incidence and frequency.

For *S. cerealella*, significant reductions in the incidence and frequency of mating were achieved with pheromone in the first and second trials (Table 5). While 96% (n = 47) of females collected before pheromone treatment in the first trial had mated with an average of 1.3 spermatophores/female, only 71% (n = 45) of females collected during pheromone treatment in the same trial had mated, which resulted in a mean of 0.8 spermatophores per female (P < 0. 05; Table 5). In the second trial, a significant 20% decrease in incidence of mating (P < 0.05), as well as a significant reduction in the mean number of spermatophores per female (P = 0.02) was recorded during pheromone treatment (Table 5). However, the reductions in the incidence and frequency of mating recorded during pheromone treatment in the third trial were not significant (P > 0.05, Table 5).

Discussion

The results of the study described in this paper demonstrate the potential of mating disruption as a management strategy for stored-product moth pests. Significant disruption of pheromone source location was achieved for *P. interpunctella* averaging 70% in the three trials. A modest, but non-significant disruption of pheromone source location was also recorded for *S. cerealella* averaging 40% in the three trials. In addition, the results of the visual examination of mating behavior showed a general trend for moderate reduction in the number of copulating adults of both species during pheromone treatment.

The significant reduction in the proportions of mated females of both species, as measured by the number of spermatophores, observed during pheromone treatment is encouraging, particularly in view of the high population densities of the two moths. The interplay between population density and the efficacy of pheromone-mediated mating disruption has

Table 5. Percentage mated and number of spermatophores per *Sitotroga cerealella* female collected before and during pheromone treatment

	Period	Estimated density (adults m^{-2})	No. females collected	% mated females	Mean (± SD) no. spermatophores
Trial 1	Pre-treatment	25	47	96 a	1.3 ± 0.6 a
	Treatment	28	45	71 b	0.8 ± 0.6 b
Trial 2	Pre-treatment	20	63	76 a	0.8 ± 0.5 a
	Treatment	25	43	56 b	0.6 ± 0.6 b
Trial 3	Pre-treatment	12	26	81 a	1.0 ± 0.7 a
	Treatment	16	15	68 a	0.7 ± 0.6 a

Means for the same trial having no letters in common are significantly different ($P < 0.05$).

long been recognized (Cardé & Minks, 1995). High pest density is viewed as a constraint to the use of pheromone for mating disruption, since mate finding at close-range may involve the use of other cues, such as visual, tactile, or auditory (Cardé & Minks, 1995). It is worth noting that new batches of field-infested corn were periodically brought into the room for storage throughout the course of the study, and this may have contributed to the consistently high population densities observed in the room.

The few reports on mating disruption of stored-product insects have been conducted at comparatively low pest population densities (0.1 to 5 moths m^{-2}) in the laboratory or simulated environment (Brady & Daley, 1975; Sower & Whitmer, 1977; Hagstrum et al., 1978; Hagstrum & Davis, 1982; Prevett et al., 1989; Mafra Neto & Baker, 1996). Sower et al. (1975) investigated the pheromone-mediated mating disruption of *P. interpunctella* and showed that the efficacy of pheromone for mating disruption was density-dependent: they recorded higher disruption at low population densities (0.1 insect m^{-2}), compared with higher densities (1–3 insects m^{-2}). In a study similar to our box experiment, Vick et al. (1978) reported reduced mating by *S. cerealella* females released in a pheromone-laden room for 24 h. They also recorded a dose-density-dependent relationship with better disruption at high pheromone doses and low population densities. Thus, the relatively low disruption recorded for *S. cerealella*, compared with *P. interpunctella* in the room experiment may be explained by the much higher population density of *S. cerealella* relative to *P. interpunctella* in the test room. It may also be due to the lower emission rate of *S. cerealella* pheromone.

Widely spaced, high emission rate pheromone dispensers, such as MSTRS™, puffers or microsprayers are increasingly being evaluated for mating disruption of several insect pests (Mafra Neto & Baker, 1996; Shorey & Gerber, 1996a, b; Fadamiro et al., 1998, 1999; Isaacs et al., 1999). Shorey & Gerber (1996a, b) report on the use of aerosol canisters (puffers) for pheromone-mediated mating disruption of codling moths and beet armyworm in the field. Mafra Neto & Baker (1996) used the pressurized version of the MSTRS™ device described in this study to disrupt mating of *Cadra cautella* in a simulated store, while Fadamiro et al. (1998) used MSTRS™ to suppress mating activity of the blackheaded fireworm, *Rhopobota naevana* in cranberry marshes. MSTRS™ were also used to reduce mating frequency of European corn borer females in grassy aggregation areas surrounding corn fields (Fadamiro et al., 1999; Fadamiro & Baker, 1999). Another type of high emission rate dispenser, the microsprayer was recently evaluated for mating disruption of major tortricid pests of apple orchards (Isaacs et al., 1999).

The possible effect of the inter-specific compounds contained in the formulations used for disruption (particularly in the gossyplure formulation) cannot be overlooked. While we are not aware of any specific biological effects on male *S. cerealella* behavior of the Z,Z isomer in the gossyplure (*Z,E*-7,11-16:Ac/*Z,E*-7,11-16:Ac in the ratio 1:1) formulation, the potential of it acting as an antagonist cannot be ignored. Therefore, the presence of inter-specific compounds in the lure used to trap *S. cerealella* males may explain its relatively low attractancy, when compared with unbaited trap. Also, the secondary component, (*Z,E*)-7,11-hexadecanal was not available.

Despite the modest levels of disruption recorded for both species in the current study, the inability to achieve near 100% disruption with pheromone in the box test may indicate the possible involvement of cues other than pheromones in the mating behavior of both species when population densities are high. Mating at close-range, as presented in the box experiment, may not be solely pheromone-mediated and may involve visual cues, mechanoreception, or other forms of olfactory cues (Sanders, 1996). Further studies are necessary to determine if acceptable levels of mating disruption can be achieved with the use of complete, optimal pheromone blends for both species.

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