

# Sterile Pink Bollworm Moth (Lepidoptera: Gelechiidae) Pheromone Emission and Courtship Success

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**ABSTRACT** Sterile female pink bollworm moths, *Pectinophora gossypiella* (Saunders), originating from a mass-rearing facility and intercepted en route to be air-dropped over the San Joaquin Valley of California, emitted amounts and ratios of pheromone components that were not significantly different from field-collected females. Sterile males from the same shipments were as successful as field-collected males in mating with either sterile or field-collected females. However, sterile males spent significantly more time during courtship to secure copulations. Field-collected females mated at significantly lower frequencies than sterile females, most likely because of laboratory conditions that were not optimal to induce calling behavior. These results indicate that, for the most part, even after the rigors of mass rearing and shipment, sterile males and females are sexually competent and that much of the benefit of sterile pink bollworm releases may come from the sterile females. Sterile males might, under some conditions, be outcompeted by field males were they to court a calling female at approximately the same time.

**KEY WORDS** Insecta, *Pectinophora gossypiella*, mating competitiveness, courtship behavior

STERILIZED PINK BOLLWORM MOTHS, *Pectinophora gossypiella* (Saunders), have been used in sterile release programs since 1968 (Miller et al. 1984) to keep this species from becoming established in the San Joaquin Valley of California, where approximately 1 million acres of cotton are grown. Wild adult *P. gossypiella* are transported into the valley on storm systems originating from cotton-growing regions in Mexico, southern California, and Arizona (Stern 1979). The California Department of Food and Agriculture has financed the sterile release program, backed up by pheromone disruption when necessary, which thus far has been successful in keeping the species from becoming endemic. This effort has saved growers potentially millions of dollars and prevented environmental contamination from the many insecticide sprays that would be needed to control this species.

Currently, the release program strives for at least a 60:1 ratio of sterile/native males as monitored by pheromone trap catch. Steriles are identified by a pink dye ingested by larvae. The more competitive the steriles are, the lower the sterile/fertile ratio that would need to be released to obtain control, saving the state potentially millions of dollars in rearing costs. Both males and females are released, meaning that some benefit would accrue from sterile females which could attract and occupy native males (Van Steenwyk et al. 1979). Previous attempts to estimate competitiveness of sterile *P. gossypiella* have involved mate-pairing experiments that assessed successful mating in the laboratory (Henneberry & Clayton 1983) and in

the field (Van Steenwyk et al. 1979). Oviposition tables also have been used (Henneberry & Keaveny 1984, 1985). Our objective was to extend the tests of sterile moth mating competence to test, for the first time, moths that had undergone the stress of being transported to the air-drop location while being immobilized in chilled vessels each containing hundreds of thousands of moths. We also wanted to determine for the first time the rates and ratios of pheromone components emitted by these transported, sterile females to learn about the possible role of sterile females in luring wild males, thus contributing to population control.

## Materials and Methods

**Moths.** Sterile moths originated from the USDA-APHIS facility, Western Regional Cotton Research Laboratory, Phoenix, Ariz., and were obtained en route to Bakersfield, Calif. Under standard shipping procedures, moths are immediately chilled after eclosion and wing-hardening and are packaged in chilled containers (41 by 41 by 81 cm), with >400,000 moths per container. Each night they are flown via commercial airline to Los Angeles International Airport, where they are picked up at approximately 1900 hours (PST) and transported by truck to Bakersfield for aerial release just before and after sunrise. We removed approximately 1,000 moths from each of five shipments at the Los Angeles airport, brought them back to our laboratory, and placed them at approximately 23°C in a cage under full room lighting to deter

mating. They were segregated by sex the next morning. Moths were acquired weekly during the length of the study.

Field moths were obtained from cotton bolls collected in Westmorland, Calif., during September 1986. Procedures for the collecting of cutout larvae were similar to those used by Haynes et al. (1984). Pupae were segregated by sex and placed in 237-ml paper cans with screened lids. Emerged moths were removed daily and segregated by age (after emergence).

All moths (sterile and field) were kept in a 14:10 (L:D) photoperiod at approximately 25°C in either metal cages (30 by 25 by 26 cm) or in 237-ml paper cans with screened lids until the day of videotaping. Field moths were 2–4 d old after they emerged. Sterile moths were used 2–6 d after being brought into the laboratory, but the majority had been in the laboratory 3 d or less.

Although most sterile moths survived transportation, some were damaged and lay moribund on the cage floor. For this study, we chose the more robust moths found on the upper half of the screen cage. Although this selection process was not random, this study was a first attempt to determine sterile moth competitiveness (moths damaged in shipping would probably not be capable of mating in the field).

**Collection and Quantification of Emitted Pheromone.** Pheromone was collected from individual female *P. gossypiella* during their peak calling period during the last half of the scotophase following the procedures described by Haynes et al. (1984). The female's wings were folded back over her head, and she was inserted abdomen first into a glass tube (2.0 mm inside diameter) with a hole (0.5 mm diameter) at the distal end. This hole was large enough to allow only the ovipositor and associated pheromone gland to emerge when light pressure was applied to the female's head with a pipe cleaner. The glass tube was then inserted through a teflon-coated gas-liquid chromatography (GLC) septum in the collector. Volatiles emitted from the gland's surface were collected for 10 min (at approximately 25°C). An internal standard (3.0 ng of (Z)-7-hexadecenyl acetate in 5  $\mu$ l of CS<sub>2</sub>) was added to the glass wool before the inside of the collector was rinsed with approximately 200  $\mu$ l of CS<sub>2</sub>. This volume of CS<sub>2</sub> was reduced under a nitrogen stream to approximately 6  $\mu$ l before it was pulled up into a 10- $\mu$ l syringe for injection onto a GLC. Analyses were performed on a Varian 3700 gas chromatograph (Varian Instruments, Sunnyvale, Calif.) equipped with a hydrogen flame detector, a Hewlett-Packard 3380A integrator (Fullerton, Calif.), and a Silar 10C (University of California, Riverside) packed column (approximately 4 g of 10% Silar 10C on acid-washed 100- to 120-mesh Chromosorb W; glass column 3 m; oven temperature 175°C; N<sub>2</sub> flow rate of 30 ml/min). The amount of each isomer was calculated from a standard curve that related peak to mass, equivalent to the peak

height times the retention time of each component. These values were corrected for recovery efficiency by standardizing the measurements relative to the internal standard. The lower analytical limit of our technique was approximately 0.1 ng (0.01 ng/min).

**Mating Success.** Moths were transferred into a darkened bioassay room (light intensity 0.3 lux) during the third hour of scotophase, placed in pairs in metal screen cages (85 mm long, 50 mm diameter), and covered with plastic Petri dishes. Moths were paired in the four possible combinations of field and sterile males and females. The pairs were observed for mating success every 30 min from the fourth through the 10th hour of scotophase. Moths were observed during this 7-h period and then discarded. Data were recorded on the success of mating, the hour of scotophase in which mating was first observed, and the minimum duration that moths remained in copula. Minimum duration was defined as the time that expired between first and last observation periods in which moths were seen in copula; this is a conservative measurement because moths were already mating before the first and after the last recorded coupling. It was assumed that no successful matings went unobserved because *P. gossypiella* remain in copula approximately 1 h (Henneberry & Clayton 1983).

**Behavioral Analysis.** During the third hour of scotophase, females were transferred to the bioassay room and set up in individual metal screen cages placed on their sides and covered with plastic Petri dishes. These were placed on a set of Plexiglas shelves behind which was a dimly lit light box. This backlighting procedure allowed females in the calling posture to be identified more easily. The array of cages was placed next to a hood to exhaust female pheromone from the bioassay room. Males were transferred to the bioassay room before videotaping (during the fifth hour of scotophase). This minimized possible pre-exposure and habituation to pheromone. Males were allowed to acclimate at least 30 min before taping.

Cages in which females were observed to be calling were carefully removed from the shelves and placed on the Plexiglas partitioning in front of the camera. If the female was observed to be calling after being transported, the Petri dish lid was removed and a male was added to the cage. The transparent lid was carefully replaced and videotaping commenced. No animal was recorded more than once.

The videorecording and lighting systems used in the mating analysis is described by Phelan (1984) with the following modification. The array of cages was set up in a partitioned Plexiglas tray taped to a pair of parallel-arranged model railroad cars resting on train tracks. Using a pulley system, the cages were moved smoothly back and forth in front of the stationary camera to facilitate filming of the moths without disturbing them.

The courtship behavior of *P. gossypiella* was described in detail by Colwell et al. (1978). For

Table 1. Comparison of successful mating and successful courtship behavior of sterile versus field-collected *P. gossypiella* in the laboratory

Activity	Matings, $\bar{x} \pm SD^a$			
	S ♂ moth × S ♀	F ♂ moth × S ♀	S ♂ moth × F ♀	F ♂ moth × F ♀
<b>A. Mating</b>				
Time of mating (hours of scotophase)	6.3 ± 1.0a	5.9 ± 1.1a	7.3 ± 0.9b	7.2 ± 0.8b
Hours in copula	1.6 ± 1.2b	1.5 ± 1.0b	1.0 ± 0.5a	0.7 ± 0.6a
n	35	33	22	18
<b>B. Courtship behavior</b>				
Total no mating bouts	2.3 ± 1.8a	1.2 ± 0.4a	1.9 ± 1.5a	1.5 ± 0.5a
Duration of total courtship, s	25.3 ± 28.7a	5.8 ± 4.8b	25.6 ± 49.8a	6.8 ± 6.1b
Duration of successful mating bout, s	11.5 ± 19.1a	4.5 ± 3.7a	6.5 ± 7.1a	3.4 ± 2.7a
No. of copulatory attempts in successful mating bout	3.9 ± 4.4a	3.0 ± 3.3a	2.7 ± 2.2a	1.5 ± 1.2a
n	18	10	13	6

Means in same row having no letters in common are significantly different ( $P < 0.05$ ; ANOVA followed by Duncan's [1955] multiple range test).

<sup>a</sup> S, sterile; F, field-collected

<sup>b</sup> Data were arcsine $\sqrt{x}$ -transformed before statistical analysis was done

our study, tapes were analyzed with a Sony video cassette recorder (SLO 323) played back through a Sony SVM 1010 motion analyzer. Each recorded frame was  $\frac{1}{60}$  s. "Courtship" was defined as the total sequence of behaviors exhibited by one male after approaching the female. "Mating bout" was defined as the sequence of behaviors from initial physical contact of a female by a male until copulation (successful) or until one of the moths terminated the sequence by moving away (unsuccessful). A "copulatory attempt" was judged to have occurred when a male bent his abdomen more than 90° toward the female.

In addition to recording the number of mating bouts and copulatory attempts in each courtship, other parameters that were measured included the duration of each mating bout, the total time spent in courtship, the time from initial contact to copulation, and the duration of successful copulatory attempts (time from initiation of the attempt until end-to-end position was achieved).

**Data Analysis.** Data were analyzed with one of the following tests: Student's *t* test for unpaired data, 3-dimensional  $\chi^2$  test of independence (Everitt 1977), or analysis of variance (ANOVA) followed by Duncan's multiple range test or arcsine $\sqrt{x}$  transformation (SAS Institute 1982). Where data were transformed, the actual  $\bar{x} \pm SD$  are reported in Table 1. Differences were declared significant at  $P < 0.05$ .

## Results

**Pheromone Component Emission Rates and Ratios.** The female sex pheromone of pink bollworm consists of the components (Z,Z)-7,11-hexadecadienyl acetate and (Z,E)-7,11-hexadecadienyl acetate (Hummel et al. 1973). In airborne collections, sterile females released the Z,Z isomer at a mean rate ( $\pm SD$ ) of  $1.04 \pm 0.71$  ng/10 min and at a proportion relative to the combined

amounts of Z,E and Z,Z isomers of  $59.2 \pm 5.7\%$  ( $n = 10$ ). The field females released the Z,Z isomer at  $1.20 \pm 0.58$  ng/10 min in a proportion of  $58.5 \pm 6.7\%$  ( $n = 17$ ). There were no significant differences between the females for either the rate ( $t = 0.77$ ;  $df = 25$ ; NS) or ratio ( $t = -0.27$ ;  $df = 25$ ; NS). The emission rate from sterile females falls within the normal range of emission rates from pink bollworm females sampled from different parts of the world (Haynes & Baker 1988).

**Mating Success.** When paired in screen cages, field females mated with 28% of the field males ( $n = 64$ ) and 31% of the sterile males ( $n = 70$ ). In contrast, sterile females mated with 81% of the field males ( $n = 41$ ) and 78% of the sterile males ( $n = 45$ ). When analyzed with a three-dimensional  $\chi^2$  test, the success of mating was entirely dependent upon the female, regardless of origin ( $\chi^2 = 51.1$ ;  $df = 1$ ,  $P < 0.05$ ), and the origin of the male did not influence success of mating at all ( $\chi^2 = 0.30$ ;  $df = 1$ ; NS). The percentage of field-collected females mating in the lab was significantly lower than their sterile counterparts, which is likely because field females called less frequently than laboratory-reared females. In previous studies, despite adjusting the wind flow, cotton volatiles, and humidity, no more than 30% of field-collected females could be induced to call compared with 80–90% of laboratory females (K. F. Haynes, personal communication; Haynes & Baker 1988). Additionally, sterile females mated significantly earlier (approximately 1 h) than the field females (Table 1A,  $F = 36.04$ ;  $df = 1$ , 104;  $P < 0.05$ ). Sterile females also remained in copula significantly longer than their field counterparts ( $F = 13.60$ ;  $df = 1$ , 104;  $P < 0.05$ ). There were no differences between the males for either time of mating ( $F = 2.02$ ;  $df = 1$ , 104; NS) or copulation length ( $F = 0.61$ ;  $df = 1$ , 104; NS). Similar findings were made with irradiated laboratory (Phoenix) and field (St. Croix) *P. gossypiella* females (Henneberry & Keaveny 1984, 1985).

Although the mating success of sterile males was as great as that of field-collected males, there was a tendency for the successful sterile males to take longer to achieve copulation than the field males (Table 1B). Sterile males spent significantly longer times courting females than did field-collected males ( $F = 4.45$ ;  $df = 1, 43$ ;  $P < 0.05$ ). This difference can be attributed to a trend for increased number of courtship bouts needed by sterile males ( $F = 2.19$ ;  $df = 1, 43$ ; NS) plus an increased duration of the bout that resulted in successful copulation ( $F = 2.43$ ;  $df = 1, 41$ ; NS), although these differences are not significant. Also, during the successful bout, the sterile males required a somewhat greater number of copulatory attempts (thrusts) to clasp the female's abdomen ( $F = 3.16$ ;  $df = 1, 40$ ;  $0.10 > P > 0.05$ ).

### Discussion

Despite mass rearing, irradiation, and mass aerial shipment of hundreds of thousands of moths packed together in chilled containers, there were surprisingly few differences between the mating competence of sterile and field-collected *P. gossypiella* moths. Sterile females that were intercepted following aerial shipment en route to their drop point emitted pheromone components at ratios insignificantly different from those of the field-collected females. Furthermore, the emission rate of pheromone produced by sterile females was comparable to those of fertile laboratory females that had not been chilled and shipped, measured over the course of 4 yr in a previous study (Haynes & Baker 1988).

It is unlikely that even a significant small reduction in pheromone production would contribute to a reduced mating success by sterile females in the field (Haynes & Baker 1988). Reduced mating success in sterile females would be especially unlikely if their propensity for increased levels of calling hence mating time 1 h earlier (observed in the laboratory) carried through under field conditions. This would allow them to outcompete field females, provided the field males were capable of responding to these sterile females' earlier pheromone emissions, which was true in the laboratory. In fact, several authors have suggested that the sterile females may be the greater contributors to the success of the sterile release program (Van Steenwyk et al 1979, Henneberry & Clayton 1980, Henneberry & Keaveny 1984). The longer copulation duration for sterile females implies that the females in some way determine copulation length. The implication of this increased duration for sterile females could be that males will be occupied with sterile females for longer periods than with field females, thereby enhancing the success of sterile releases.

Similarly, sterile males intercepted from the same shipments as the females mated as frequently as their field counterparts. However, the duration of

total courtship, number of courtship bouts, and number of copulatory attempts within a bout needed to accomplish copulation (Table 1B) could perhaps translate into a competitive disadvantage for a sterile male if a field male arrived to attempt courtship at the same time. If these trends carry over to the field situation, the ability of field males to finish mating in approximately 6 s compared with the 25 seconds for sterile males would make it more likely that sterile males arriving first at a female would find themselves in direct competition with field males but not the reverse. Mating by field males, more often than for sterile males, would be accomplished by the time competitors arrived because of the shorter time required to obtain copulation.

Although we tested the competency of sterile moths that had undergone nearly all the rigors of the rearing and shipping process, interpretation of the results is limited by factors unrelated to the artificial nature of the laboratory environment under which our measurements were made. Because we used moths that were 3 or more d old after emergence, it might be argued that air-dropped moths would only be 1 d old on their first night in the field and could suffer reduced competitiveness for several days until acclimated to ambient conditions similar to those for the moths in our study. Even if it were true that more than 1 d were needed for acclimation, given that moths are air-dropped virtually continuously in a region, this criticism would be valid only for the first few days in the field following the first air drop. After that, sufficiently acclimated moths would be available for competition and would be continuously replaced as younger air-dropped moths aged.

Our results suggest that shipped sterile moths are quite similar in pheromone emission and courtship competence to field moths that were chosen in the same way for study. Both sexes of sterile moths thus appear to be fully capable of contributing to population suppression following shipment under the procedures of the current program.

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