Sublethal Effects of Permethrin on the Chemical Communication System of the Pink Bollworm Moth, *Pectinophora gossypiella*

K.F. Haynes and T.C. Baker

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside

Topically applied sublethal doses of permethrin can interrupt chemical communication between the sexes of *Pectinophora gossypiella* by affecting both the signaler and the responder. The probability of calling by females is reduced when they are treated with doses of permethrin which are much less than the LD_{50}. Similarly, key stages in the behavioral response of males to sex pheromone are effectively blocked at these low doses. Males recover from these effects 4 days after treatment, but calling by females is still significantly reduced at this time. Chemical control of *P. gossypiella* populations with permethrin may not be limited to mortality, and potentially includes effective control of behavioral aspects of chemical communication.

Key words: *Pectinophora gossypiella*, sublethal, permethrin, behavior, communication, pheromones

INTRODUCTION

To determine the full potential for chemical control of insect populations, it is critical to examine more than just the direct effects of compound on mortality, since sublethal doses may influence behaviors important in survival and reproduction. For example, sublethal doses of insecticides have been shown to adversely affect feeding of some blood-sucking Diptera [1], foraging by honeybees [2,3], pupation behavior of larval *Danaus gilippus berenice* (Cramer) [4], acoustical communication in *Acheta domesticus* L. [5], mating in *Glossina morsitans morsitans* Westw. [6], the wing fanning response of male *Pectinophora gossypiella* (Saunders) to pheromones [7], and the pher-

omone-mediated flight behavior of *Grapholita molesta* (Busck) [8]. The potential for control of behavior with neurotoxicants is just beginning to be realized, but these early studies have already revealed that the current paradigm for testing insecticides will have to be reexamined, since the objective of pest control is population management, which is not limited to mortality factors.

Our objective was to document the effects of sublethal doses of permethrin on the chemical communication system of the pink bollworm moth, *P. gossypiella*. We report on the effects of permethrin at low doses on key stages in the behavioral responses of males, including upwind flight to the pheromone source in a flight tunnel. In addition, our study includes an analysis of the effects of permethrin on the periodicity and probability of calling behavior by females. This behavior involves eversion of the ovipositor and the associated sex pheromone gland. The net effect of this sublethal behavioral modification is to interrupt chemical communication between the sexes.

MATERIALS AND METHODS

Insects

Larval *P. gossypiella* were reared on a shredded wheat germ diet modified from that described by Adkisson et al [9]. Pupae were separated according to sex, and males and females were held in 25 × 25 × 30 cm screened cages housed within separate environmental chambers (L:D 14:10; temperature ca. 30°C:25°C). Pupae were removed from the cages daily, leaving adults that had emerged over the previous 24-h period. This procedure ensured that the ages of the insects were known. Adults had access to 8% sugar water.

Insecticide and Dosing

Technical grade permethrin was provided courtesy of ICI Americas Inc., Pikeville, NC (91% active material), and was diluted in glass-distilled acetone. New solutions were prepared weekly. Moths were cold-anesthetized 0–3 h after the start of a photophase, and were dosed topically on the venter of the abdomen with 0.5 μl of insecticide solutions.

Behavioral Observations

The behavioral responses of males were observed in a flight tunnel described by Vetter and Baker [10]. Males dosed with permethrin were loaded into individual screen cages (3.3 cm dia. by 3.5 cm) with plastic lids. Caged males were transferred from their environmental chamber to the darkened room that housed the flight tunnel ca. 0.5 h before assays were to be started. Assays were run between 7 and 9.5 h after the initiation of scotophase under specific environmental conditions (0.3 lux from incandescent white lights; supplemental incandescent red lighting; temperature ca. 26°C; wind speed 0.5 m/s). Individual males were released from a sheet metal platform 15 cm above the floor of the flight tunnel and 1.4 m directly downwind from the pheromone source. The pheromone source was a rubber septum impregnated with 1 mg of a 56:44 blend of (Z,Z)-: (Z,E)-7,11-hexadecadienyl acetate (> 95% free of other pheromonelike volatile impurities as determined by

GLC* analysis on a 3 m × 4 mm (OD) glass column packed with 4 g of 10% Silar 10C on acid-washed 100 to 120-mesh Chromosorb at 175°C and N_2 flow rate of 30 ml/min). The septum was fixed to a sheet-metal platform 15 cm above the floor of the tunnel and equidistant from the sides of the tunnel.

The behavioral response of males was noted in the following categories: wing fanning in the release cage; flight; upwind flight (flying upwind within the pheromone plume); and contacting the pheromone source. Ryan's [11] multiple-comparison test for proportions was used to evaluate differences in the conditional probabilities of transitions between these behavioral categories. In addition, the latency of flight was recorded. Each male was given 30 s to complete each behavioral transition, with the exception of the moths that were flying upwind; these moths were allowed to locate the source or terminate their flight.

Treated females were individually transferred to screen cages (4.0 cm diam. × 3 cm) 0–1 h after the start of a photophase, and hourly observations of calling behavior were conducted throughout the following scotophase. The calling posture of the pink bollworm consists of a ventral flexion of the abdomen, extrusion of the ovipositor and associated sex pheromone gland, and elevation of the distal ends of the wings [12]. These behaviors were observed with dim red backlighting in an otherwise dark room. At an hourly observation period each female was noted as either calling or not calling. The probit analysis of calling behavior used data from the observations at 8 h into the scotophase.

The duration of the effects of permethrin was observed in both males and females. One-day-old adults were treated with the insecticide (6 ng/male and 10 ng/female) ca. 1 h after the end of the scotophase. On successive days, previously untreated individuals were dosed with permethrin. This procedure was repeated until there were four groups of individuals of the same age, but treated on 4 consecutive days. On the fourth day a control group was also treated with 0.5 μl of acetone. Observations of pheromone-stimulated behavior and calling were made during the following scotophase.

RESULTS

The probit lines (Fig. 1) for lethal effects 96 h after treatment revealed differences between males and females, reflecting only in part the difference in average body weight (males averaged 11.6 ± 1.14 (SD) mg; females averaged 15.8 ± 2.42 mg). The 96-h values were selected because they were reproducible and because they were utilized in a previous study by other researchers [7]. Very little mortality (<10%) occurred by 24 h after treatment even at the highest dose tested (50 ng/moth). The 96-h LD_{50} for males (n = 50) was 14.4 ng/moth, while that for females was 23.7 ng/moth.

Sublethal doses of permethrin (3–10 ng/moth) adversely affected several stages in the response of male *P. gossypiella* to sex pheromone (Table 1).

*Abbreviations: CNS = central nervous system; GLC = gas-liquid chromatography; LD_{50} = lethal dose resulting in 50% mortality.
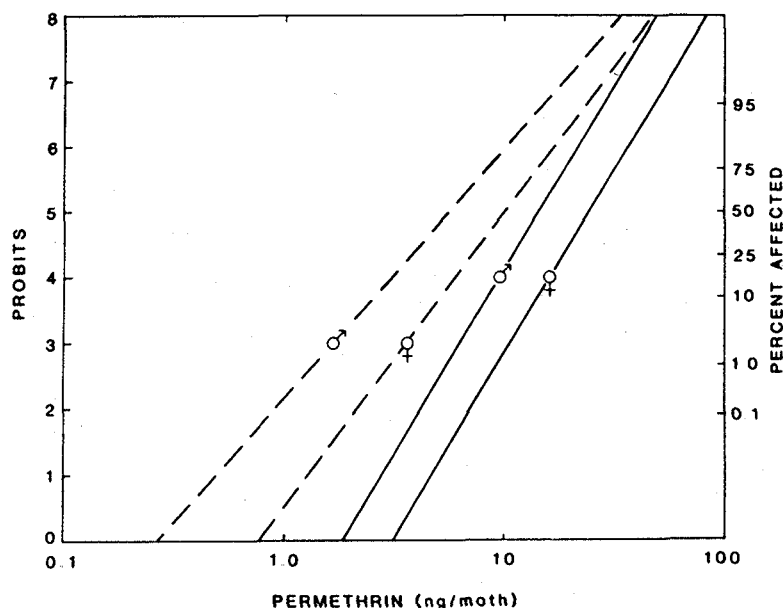
Fig. 1. Probit lines for lethal (solid lines) and sublethal effects (dashed lines) of permethrin on male and female *Pectinophora gossypiella*. Sublethal effects are probabilities of not calling for females, and probabilities of not locating the pheromone source for males. Both behavioral responses were corrected for control response by Abbott's formula.

Generally, there was a decreasing probability that a male would complete the transitions from stationary to wing fanning, stationary to flight, and flight to upwind flight as the dose of permethrin was increased from 3 to 10 ng/moth. In addition, the behavioral response for the first two transitions was already significantly attenuated at the 3 ng/moth dose relative to the control treatment. Given that a male was progressing upwind in the pheromone plume, there was no longer an inverse relationship between the insecticide dose and the conditional probability of the transition to contacting the pheromone source. Thus the specific transition from orientation to source contact did not contribute to the overall decrease in source location by poisoned males. The latency of the flight responses was also affected by the dose of permethrin. Male *P. gossypiella* took significantly longer to take flight at the 10, 8, and 6 ng/moth doses than in the control or 3 ng/moth treatments (Table 1). These effects of permethrin cannot be attributed simply to a poisoning of the flight muscles or the motor output for flight since 95% of the males treated with 10 ng/moth, the highest dose tested, were capable of sustained flight when tossed into the air following behavioral assays.

The effects of permethrin on males at the 6 ng/moth level were temporary in that there was a significant trend toward recovery over the course of 4 days (Table 2). Four days after permethrin treatment none of the behavioral transitions in mate-locating was significantly different from that observed in the acetone-treated group. There was also a significant trend toward recovery in the latency of the flight response (Table 2).

TABLE 1. Influence of Sublethal Doses of Permethrin on Key Behavioral Responses of Male *Pectinophora gossypiella* to Sex Pheromone

Dose (ng/moth)	Conditional probability of transition					Mean latency* of taking flight (s ± SD)
	Stationary to wing fanning	Stationary to flight	Flight to upwind flight	Upwind flight to source contact	Stationary to source contact	
0**	0.744a	0.969a	0.761a	0.669a	0.494a	3.3 ± 2.35b
3.0	0.520b	0.831b	0.632ab	0.714a	0.375a	5.0 ± 4.36b
6.0	0.338c	0.625c	0.540ab	0.741a	0.250b	8.5 ± 6.90a
8.0	0.238c	0.369d	0.458b	0.815a	0.138c	9.8 ± 7.37a
10	0.075d	0.181e	0.517ab	0.733a	0.069c	9.2 ± 5.59a

Proportions in the same column are significantly different if they do not share a letter in common ($P < 0.05$; Ryan's [11] multiple-comparison test for proportions). Mean latencies in the same column are significantly different if they do not share a letter in common ($P < 0.05$; analysis of variance followed by Duncan's Multiple-Range Test).
The initial sample size at each dose was 160 males.
*Time from introduction of moth into wind tunnel to initiation of flight.
**Moths in this group were dosed with 0.5 μl acetone.

TABLE 2. Duration of the Effects of a Sublethal Dose (6.0 ng/moth) of Permethrin on Key Behavioral Responses of Male *Pectinophora gossypiella* to Sex Pheromone

Interval (days after treatment)	Conditional probability of transition					Mean latency* of taking flight (s ± SD)
	Stationary to wing fanning	Stationary to flight	Flight to upwind flight	Upwind flight to source contact	Stationary to source contact	
1	0.220c	0.440c	0.364a	0.750a	0.120b	7.2 ± 3.64a
2	0.300bc	0.540c	0.481a	0.846a	0.220ab	5.7 ± 3.66ab
3	0.380abc	0.860b	0.279a	1.000a	0.240ab	4.7 ± 4.15bc
4	0.540ab	0.940ab	0.468a	0.909a	0.400a	4.1 ± 2.57bc
Control**	0.620a	1.000a	0.480a	0.792a	0.380a	3.1 ± 2.22c

Proportions in the same column are significantly different if they do not share a letter in common ($P < 0.05$; Ryan's [11] multiple-comparison test for proportions). Mean latencies in the same column are significantly different if they do not share a letter in common ($P < 0.05$; analysis of variance followed by Duncan's Multiple-Range Test).
The initial sample size at each dose was 50 males.
*Time from introduction of moth into flight tunnel to initiation of flight.
**Moths in this group were dosed with 0.5 μl acetone 1 day before the behavioral assay.
Interval = time between treatment and observation.

Calling behavior by females was significantly affected by sublethal doses of permethrin (Fig. 2). At the peak period of calling (8 or 8.5 h after the initiation of scotophase), there was a significant difference between the probabilities of calling of acetone-treated and both 15- and 10-ng-treated females (P < 0.05; Ryan's multiple-comparison test for proportions). In addition, the proportion of females calling decreased progressively from 3 to 15 ng/moth. These sublethal doses of insecticide apparently affected the probability of calling, but not its periodicity (Fig. 2).

Recovery of calling behavior of females dosed at 10 ng/moth was incomplete 4 days after treatment (Fig. 3). At the peak time of calling, 8 and 8.5 h after the initiation of the scotophase, there was a significant difference in the proportion of females calling between acetone- and permethrin-treated moths regardless of the interval between treatment and observation (1 to 4 days). There were no significant differences in the proportion of females calling among the females treated at the various intervals before observation at this time. However, there was a trend toward recovery (P < 0.05; Kendall rank correlation test).
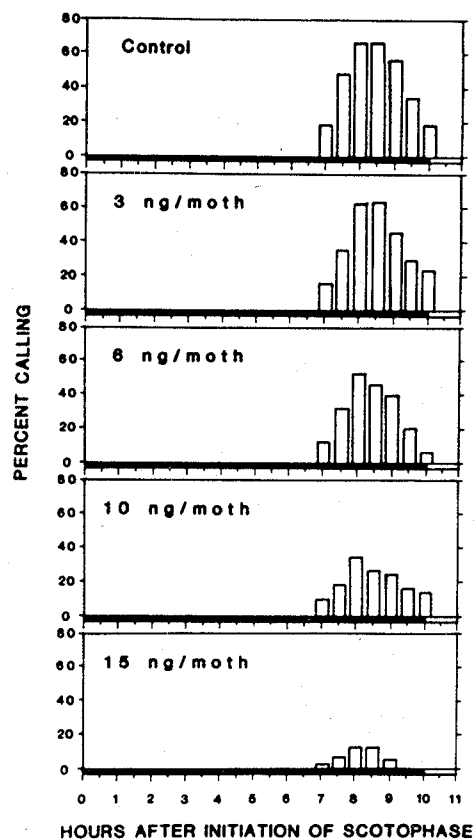

Fig. 2.   Sublethal effects of permethrin on calling behavior by female *Pectinophora gossypiella*. Horizontal black bars represent the scotophase. Control moths were dosed with 0.5 µl of acetone, and other moths were dosed with the designated quantity of permethrin in 0.5 µl of acetone immediately after the scotophase preceding observations.
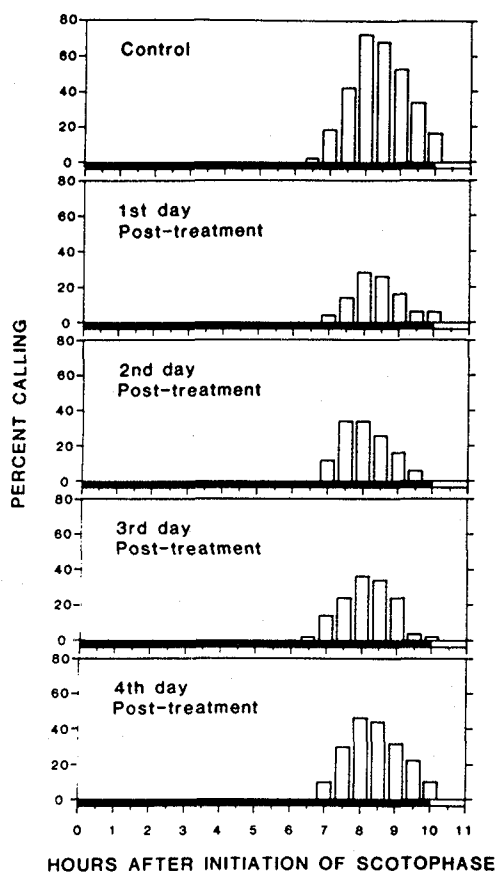
Fig. 3. Duration of sublethal effects of permethrin on calling behavior of female *Pectino-phora gossypiella*. Control moths and first-day posttreatment moths were dosed immediately after the scotophase preceding observations. Other groups were dosed at 24-h increments before this time. Horizontal black bars represent scotophase.

Probit analysis can be applied to sublethal as well as lethal effects (Fig. 1). The probit lines for sublethal effects of permethrin occurred at lower doses than those for lethal effects in both males and females. Sublethal effects on males were analyzed at ca. 22 h after treatment because the effects were maximal at the time. Since 50.6% of control males did not contact the pheromone source and 34.0% of control females did not call at the peak time, Abbott's formula correcting for control response was utilized to determine the probit values. The lack of response in males and females was not due to a solvent effect, since similar levels of response were observed in untreated moths in other experiments. From this probit analysis it was calculated that a dose of 5.6 ng/male would result in a 50% decrease in location of the pheromone source 22 h after treatment. This dose corresponds to a calculated 96 h LD of only 1.1%. A dose 9.9 ng/female would result in a 50% decrease in calling behavior, whereas the same dose would lead to only 1.5% mortality at 96 h. No mortality or other symptoms of poisoning were observed at the

time of the behavioral assay even at the highest doses tested: 10 ng/male and 15 ng/female.

DISCUSSION

The response of male moths to sex pheromone is complex and often involves a sequence of behaviors that results in the males locating a source of pheromone [13]. The behavioral sequence often begins with the male fanning his wings when he first perceives the pheromone at a distance downwind from the source (called wing fanning or activation). The sequence then proceeds through a series of transitions from flight to upwind flight (which includes a series of narrow zigzags as the male progresses upwind in the pheromone plume) and upwind flight to contacting the source of pheromone. The flight behaviors have been found to result from a complex integration of visual feedback from wind-induced drift with a chemically modulated program of counterturning [14–16]. The complexity and sequential nature of the behavioral responses to sex pheromones suggest that their initiation and maintenance may be unusually vulnerable to neurotoxicants that affect the sensory system or the CNS pathways integrating olfaction and vision.

We have found that sublethal doses of permethrin affect the behavior that leads to a male locating a source of sex pheromone (Table 1). The activation response (the transition from stationary to wing fanning) was dramatically influenced by these low doses of permethrin. Floyd and Crowder [7] found this effect in male survivors of 96 h LD_{50} and LD_{80} doses of permethrin. We observed a significant effect on wing fanning 20–23 h after treatment at 3 ng/ moth; this dose corresponds to a calculated value of less than 1% mortality at 96 h. Floyd and Crowder did not observe a decrease in mating when the LD_{50} and LD_{80} survivors were confined in small cages with females; thus it would appear that close-range courtship behaviors were not affected by these insecticide treatments. We have documented that some of the behaviors that are involved in long-range mate-locating behaviors were affected by sublethal doses of permethrin, such as the transition from stationary to flight and the transition from flight to upwind flight, and thus the ability of males to locate females would be dramatically affected. The transition from flight to upwind flight includes the initiation of narrow counterturning modulated by pheromone (which has also been called "locking on" to the pheromone plume). The next transition from upwind flight to contacting the source of pheromone was not adversely affected by permethrin. This finding has two alternative explanations: 1) the behavior seen in orientation and source contact is not influenced by the sublethal doses of permethrin; or 2) the behaviors in upwind flight and source contact involve elements of the previous transitions, and thus selection against the affected individuals had already occurred. The latter explanation seems most likely. It is important to emphasize that the overall impact of the sublethal doses of permethrin was a substantial decrease in the ability of males to locate a source of pheromone.

Recently it has been reported that sublethal doses of permethrin adversely affected the number of males that initiated upwind flight in G. molesta [8].

Apparently, once upwind flight was initiated there was no further decrease in the behavioral response. In these aspects the effects of permethrin on G. molesta are strikingly similar to the effects on P. gossypiella. Significant effects on pheromone source location were observed at doses as low as 0.1 pg/moth of permethrin in G. molesta [8]. In contrast, in P. gossypiella similar effects were observed at 6 ng/moth. Apparently, G. molesta is much more sensitive to the sublethal effects of permethrin than P. gossypiella. However, G. molesta is also much more sensitive to lethal effects of permethrin [8] than P. gossypiella. In terms of ratio between sublethal and lethal effects the two species may be very similar, but differences in experimental design preclude more detailed comparisons.

An effect of sublethal doses of permethrin on calling by females has not been documented before this study. The release of pheromone in P. gossypiella and most other species of moths depends on an active behavioral process of everting a pheromone gland. In most species this activity is confined to a discrete period in the day/night cycle [17]. We found that sublethal doses of permethrin substantially decreased the probability that females would call, without affecting the periodicity of this activity (Fig. 2). Chadd and Brady [1] found a similar effect of permethrin on the unrelated behavior of probing in Glossina austeni Newst. The response was reduced substantially when treated with 0.6 ng/fly, but the periodicity of the response remained unchanged. The effect of permethrin on the behavior of calling in female P. gossypiella would be expected to curtail mating since the males are dependent on the volatile pheromone released by the female [18].

For the effects of permethrin to aid substantially in pest control, either they must be long-lived or there must a way to ensure continuous or frequent contact with the material. It appears the male P. gossypiella recovered from the sublethal effects of a 6-ng dose of permethrin over the course of 4 days. This contrasts to some extent with the finding in females, where a significant decrease in calling behavior was still observed 4 days after treatment (Fig. 3). A trend toward recovery in these females was apparent. Since permethrin has a relatively good residual activity on foliage [19], there is a good possibility for repeated contact with the toxicant. In addition, pyrethroid insecticides have been added to the polybutene adhesive (BioTac) that is used to stick the pheromone sources to the cotton plant when pheromone is used in mating disruption [20]. When male moths contact these pheromone sources, they may pick up sublethal doses of permethrin with the adhesive and thus carry the material with them after they leave the pheromone source.

The observed effects of the sublethal doses of permethrin could be due to action on the peripheral or central nervous system or the muscular system. Because 95% of males at the 10-ng dose were capable of sustained flight when thrown into the air, it seems likely that the effect is not on the muscular system. Miller and Adams [21], in reviewing the evidence for central vs peripheral action of pyrethroids on the housefly nervous system, conclude that there is evidence that some pyrethroids are acting at both levels. Kaissling [22] demonstrated that (+) trans-permethrin affected the response of olfactory sensory neurons of Antheraea polyphemus to pheromone. At first the effect was an enhancement of impulse-firing upon pheromone stimulation,

but over time (after 30 min) the receptor cell became blocked. Since a large number of poisoned male *P. gossypiella* were perceiving the pheromone (as evidenced by a behavioral change) but not going through the complete sequence of behavioral transitions, this suggests that the sensory system was not completely shut down. The signal from the sensory system could have been changed quantitatively or qualitatively. It seems most likely that the observed effect on behavior results from both sensory and central actions of permethrin.

Communication systems of insects seem to be logical targets for neurotoxicants, since both a signaler and a receiver are involved, and the behaviors involved are some of the most complex seen in arthropods. Acoustical communication in *Acheta domesticus* L. is interrrupted by sublethal doses of parathion, dieldrin, and carbaryl, resulting from an inability of males to produce the normal calling or courtship songs [5]. Schricker and Stephen [3] have documented that sublethal doses of parathion interfere with the communication of the direction of a source of food during the elaborate "communication dance" of scout honeybees (*Apis mellifera* L.). In both of these examples the effect was studied on the signaler. We have documented effects on both the signaler and receiver in the chemical communication system of the pink bollworm moth.

Perhaps the most important aspect of this research, and of all previous studies of sublethal effects of insecticides, is the emphasis on the need for a new dialogue on the methods that are currently used for selecting chemicals for control of insect populations. We documented distinct differences between the doses of permethrin necessary for lethal and sublethal effects, and the latter effects could be expected to interfere with reproduction in *P. gossypiella*. Thus, tests that utilize mortality for screening insecticides would overlook the materials that could actively interfere with behaviors important to survival or reproduction. In an extreme case a material could be rejected for economic or environmental reasons when it was assessed solely on the basis of mortality that could have been effective in population management at a fraction of the cost and with a minimal environmental impact.

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