Control of Pink Bollworm Moth (Lepidoptera: Gelechiidae) with Insecticides and Pheromones (Attracticide): Lethal and Sublethal Effects

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ABSTRACT Effectiveness of combinations of insecticides with pheromones (attracticides) for control of *Pectinophora gossypiella* (Saunders) may depend on, among other factors, males freely contacting attracticide sources, insecticide-induced mortality, and sublethal interference with the mate-locating sequence in poisoned males. In flight-tunnel tests, males readily contacted pheromone sources containing permethrin, fenvalerate, or cypermethrin and suffered significant mortality. Moreover, after 24 h survivors were less likely to complete the normal behavioral sequence involved in sex pheromone-mediated mate location. The latter sublethal effects may contribute substantially to the effectiveness of the attracticide technique at the doses of insecticides used in the field. Cypermethrin induced lethal and sublethal effects at a lower concentration than other insecticides tested. Chlordimeform appears to be a poor candidate for attracticide formulations because males avoided contact with this insecticide. Recovery from sublethal effects of cypermethrin occurred after 48 h, and represents a potential limitation to sublethal modification of behavior for population control. However, repeated contact with a moderate concentration of permethrin attracticide enhanced sublethal and lethal effects.

KEY WORDS *Pectinophora gossypiella*, sex pheromone, insecticides, mating disruption

THE POTENTIAL FOR MATING disruption with sex pheromones to control populations of pink bollworm moth, *Pectinophora gossypiella* (Saunders), was first established by Gaston et al. (1977) after the identification of *P. gossypiella*'s pheromone components as a 1:1 blend of (*Z*,*Z*)- and (*Z*,*E*)-7,11-hexadecadienyl acetate (*Z*,*Z*- and *Z*,*E*-7,11-16:Ac, known as gossyplure) (Hummel et al. 1973, Bierl et al. 1974). Since then, gossyplure has been used as a mating disruptant in the desert Southwest of the United States (Doane & Brooks 1981, Henneberry et al. 1982), as well as in many other cotton-growing areas around the world (Doane & Brooks 1981, Critchley et al. 1983). In the United States, growers began adding small amounts of insecticides to the sticker used to adhere the pheromone sources to cotton plants because they believed that effective and efficient use of pheromone and a more selective use of insecticides resulted. Subsequent data often supported these contentions of efficiency and selectivity (Butler & Las 1983, Beasley & Henneberry 1984; R. Staten, personal communication).

The effectiveness of insecticides used in attracticide formulations depends on their 1) degree of interference with pheromonally mediated attraction caused by perception and avoidance of volatile insecticides, 2) sublethal effects on the behavioral response after contact with the insecticide-laced sticker, and 3) ability to induce mortality. We performed a flight tunnel behavioral test to define the potential impact of these factors clearly, and to identify the behavioral stages affected by attracticides.

Materials and Methods

Insect Culture. Methods for rearing larvae have been described previously (Haynes & Baker 1985). Pupae were separated by sex and males were kept in an environmental cabinet with a photoperiod of 14:10 (L:D) and temperature of ca. 26°C. Pupae were removed from a cage (25 by 25 by 25 cm) daily, leaving behind adult males that had emerged over the previous 24 h. Males were used in behavioral tests when they were 3–6 days old.

Chemicals. The following technical grade insecticides were used: chlordimeform (95% AI; CIBA-Geigy, Greensboro, N.C.), fenvalerate (95% AI; Shell, Modesto, Calif.), cypermethrin and permethrin (77 and 94% AI, respectively; ICI Americas, Goldsboro, N.C.). The insecticides were diluted in hexane and the resulting solutions were mixed thoroughly into the sticker (BioTac).

Slow-release hollow fibers (200 μm i.d.) containing a 1:1 blend of *Z*,*Z*- and *Z*,*E*-7,11-16:Ac (No-mate PBW) were obtained from the Controlled Release Division of Albany International (Needham, Mass.). A fiber coated with a thin layer (10

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Table 1. Evaluation of avoidance of insecticide-laced sticker plus sex pheromone at key behavioral stages in the response of male *P. gossypiella*

Treatment	Conditional probability of transition					
	Stationary to wing fanning[a]	Stationary to flight	Flight to upwind flight	Upwind flight to 3 cm	3 cm to source contact	(Overall) Stationary to source contact
Control	0.816a	0.976a	0.697a	0.788a	0.851a	0.456a
10% cypermethrin	0.760a	0.968a	0.727a	0.739a	0.846a	0.440a
10% fenvalerate	0.776a	0.960a	0.708a	0.788a	0.821a	0.440a
10% permethrin	0.792a	0.992a	0.694a	0.733a	0.714a	0.360ab
10% chlordimeform	0.768a	0.952a	0.727a	0.779a	0.478b	0.256b

Proportions in the same column are not significantly different if followed by the same letter ($P < 0.05$; Ryan's [1960] multiple-comparison test for proportions). The initial sample size for each treatment was 125 males. Stationary, stationary in release cage; wing fanning, wing fanning in release cage; flight, taking flight out of release cage; upwind flight, upwind progress in pheromone plume; 3 cm, landing and walking to within 3 cm of source; source contact, contacting the attracticide source.

[a] Wing fanning is not a necessary precondition for flight.

mg) of sticker except for the open pheromone-emitting end was placed horizontally on a no. 8 cork (2.7 cm tall by 1.7 cm diam by 2.2 cm diam). When coated with insecticide-laced sticker, these pheromone sources served as attracticides.

Flight Tunnel Tests. An attracticide source was placed on a metal sheet (15 by 15 cm) located equidistant from the sides of the plexiglas wind tunnel (Miller & Roelofs 1978, Vetter & Baker 1983) and 15 cm above its floor. A wind speed of 0.5 m/s was generated by a 0.5 horsepower (8.9×10^{11} ergs/min) electric fan, and the pheromone plume was exhausted from the tunnel with a hose (30 cm diam) connected to a fumehood. The temperature in the room housing the tunnel was kept constant at ca. 26°C. The tunnel was dimly illuminated with incandescent white light (0.3 lx at the center of the tunnel) as well as supplemental red light. These conditions allowed observations of critical behavioral stages without apparent interference with the response of males to the sex pheromone.

Male *P. gossypiella* are optimally responsive to pheromone between 7 and 9.5 h after the initiation of the scotophase under the specified conditions; therefore, all flight-tunnel tests were conducted at that time. During the photophase preceding the tests, males were placed in screen cages (3.3 cm diam by 3.5 cm) with plastic lids either individually for tests in which behavioral categories were noted, or in groups of three to five for the initial exposure to the attracticide. Males were transferred to the darkened assay room ca. ½ h before observations began. A sheet-metal platform 1.4 m directly downwind of the pheromone source served as the release point for the males.

Potential avoidance of attracticides was documented by noting the behavioral response of each male in the following categories: wing fanning in the release cage, flight initiation, upwind flight in the pheromone plume, contact with the cork holding the attracticide source, and contact with the insecticide-laced sticker.

In studies designed to detect sublethal and lethal effects of contact with the attracticide, males were removed from the tunnel after they contacted the sticker with a gentle vacuum flow that resulted in deposition of the male in a screen cage (5 cm diam by 8 cm high). These males were held in the dark until their normal photophase began, and then were

Table 2. Evaluation after 24 h of the effect of contact with an attracticide source on mortality and attraction to sex pheromone by survivors

Previous contact with attracticide containing	Initial sample size	Subsequent response to sex pheromone source conditional probability of transition					Mortality
		Stationary to wing fanning	Stationary to flight	Flight to upwind flight	Upwind flight to source contact	(Overall) Stationary to source contact	
Control	100	0.843a	0.989a	0.614a	0.889a	0.539a	0c
1% permethrin	83	0.380b	0.810b	0.438ab	0.821a	0.291b	0.048c
10% permethrin	88	0.158bc	0.421cd	0.250ab	0.750a	0.079bc	0.568b
1% fenvalerate	86	0.383b	0.630c	0.412ab	0.810a	0.210bc	0.058c
10% fenvalerate	88	0.167bc	0.458cd	0.227b	0.800a	0.083bc	0.455b
1% cypermethrin	92	0.310b	0.517cd	0.233b	0.857a	0.103bc	0.370b
10% cypermethrin	100	0c	0.261d	0c	—[a]	0c	0.770a

Proportions in the same column are not significantly different if followed by the same letter ($P < 0.05$; Ryan's [1960] multiple-comparison test for proportions). Sex pheromone source was a 58:42 blend of *ZZ* and *ZE* components free of insecticide.

[a] Number of individuals completing the previous behavioral stage was too small to include this transition in the statistical analysis.

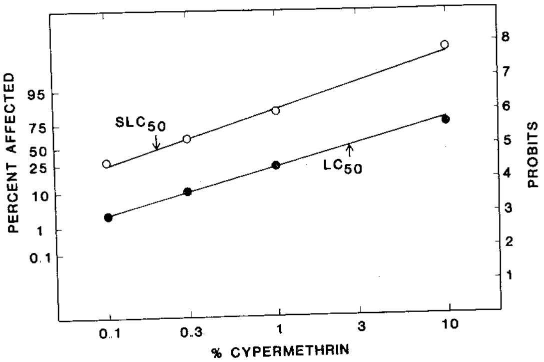

Fig. 1. Probit lines for sublethal (O) and lethal effects (●) of cypermethrin mixed with sticker. Arrows indicate the SLC_{50} for 24-h survivors and the LC_{50} at 24 h. The sublethal effect is the percentage of survivors that do not contact a pheromone source 24 h after pheromonally stimulated contact with attracticide source. Sublethal effects were corrected for the percentage not responding to the pheromone source in the control group (males that contacted insecticide-free sticker) using Abbott's (1925) formula.

transferred to individual screen cages for bioassay during the subsequent activity period. At that time, the behavioral response to 1 mg of a 58:42 blend of Z,Z-:Z,E-7,11-16:Ac loaded onto a rubber septum was noted in the following categories: wing fanning, flight initiation, upwind flight in the pheromone plume, and contact with the pheromone source. The source emitted pheromone at a rate and blend ratio that approximated that found from individual females (Haynes et al. 1984). Mortality was assessed at the time of the behavioral test; thus, both lethal and sublethal effects of insecticides were noted ca. 24 h after the initial contact with the attracticide. The experiment documenting the time course of sublethal effects was conducted 24, 48, 72, and 96 h after contact with the insecticide-laced sticker.

To determine if repeated contact with a permethrin-laced source intensified sublethal or lethal effects, males were allowed to contact the source one to three times before they were removed from the tunnel. Other males were allowed to contact insecticide-free sticker one to three times to ensure that the results reflected the presence of the insecticide and not deleterious effects of the sticker itself. Sublethal and lethal effects of the insecticides were assessed 24 h after contact with the attracticides.

A multiple comparison test for proportions (Ryan 1960) was used to determine significant differences between percent responses (behavioral and mortality) ($P < 0.05$). Probit analysis (Raymond 1985) was used to calculate probit regression lines for lethal and sublethal effects of cypermethrin attracticide. Sublethal effects were corrected for the percentage not responding in the control group using Abbott's (1925) formula.

Results and Discussion

Of the insecticides tested, only an attracticide formulation of chlordimeform resulted in a significant decrease in the proportion of males that completed the behavioral sequence ending in contact with the insecticide-laced pheromone source (Table 1). Chlordimeform fits the practical definition of repellent suggested by Barton Browne (1977): "a chemical acting in the vapor phase [that] prevents an insect from reaching a target to which it otherwise would be attracted." However, other definitions (e.g., Dethier et al. 1960) are more stringent and require knowledge that the insect was making directed responses away from a source.

None of the insecticides diminished the occurrence of wing fanning, flight initiation, or upwind flight. Avoidance of chlordimeform-laced pheromone sources was manifested only as a decrease in the probability of contacting the source after males had landed on the cork, and this occurred at very close range (<3 cm). The ability of male *P. gossypiella* to detect chlordimeform in the vapor state near the attracticide source and subsequently avoiding contact with that source makes this insecticide a poor candidate for attracticide formulations. This compound was chosen for study not because of its lethal effects (which are poor relative to the pyrethroids) but because of well-defined detrimental effects on the behavioral response of moths to sex pheromone. Males that receive a sublethal dose of chlordimeform subsequently behave as if they are too sensitive to pheromone; their flights are arrested downwind of a normally attractive pheromone source (Linn & Roelofs 1984; K.F.H., unpublished data). This sublethal effect did not occur even in the small group of individuals that contacted the 10% attracticide formulation of chlordimeform, suggesting that these males did not spend enough time in contact with the sticker to pick up such a dose.

Although certain pyrethroid insecticides are reported to be repellent to some insects (Gould 1984), we found no evidence that permethrin, fenvalerate, or cypermethrin resulted in any statistically significant reduction in source contact. Cypermethrin- and fenvalerate-laced sticker resulted in the least diminution in actual source contact, but even permethrin did not result in a significant decrease in performance of the overall sequence.

When tested in attracticide formulations, the pyrethroids significantly increased mortality as well as sublethal effects on the pheromone response in the survivors (Table 2). At the 1% concentration in sticker, permethrin and fenvalerate did not cause significantly more mortality than sticker alone, but at the 10% level mortality was significantly higher. Cypermethrin was more effective than permethrin and fenvalerate in inducing mortality at a given dose.

Some males that had contacted attracticide sources of permethrin, fenvalerate, or cypermethrin showed symptoms of sublethal poisoning that

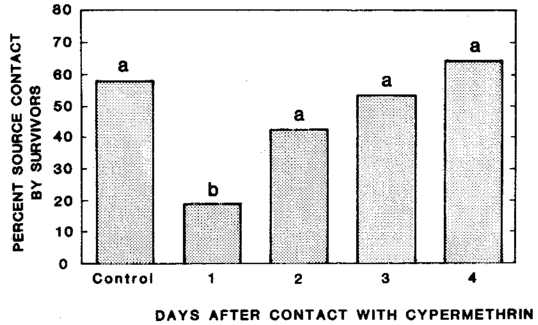

Fig. 2. Recovery from sublethal effects of contact with cypermethrin-laced attracticide sources. Control group contacted sticker without insecticide 24 h before the flight-tunnel test. Percentages are not significantly different if they do not share a letter in common ($P < 0.05$; Ryan's [1960] multiple-comparison test for proportions).

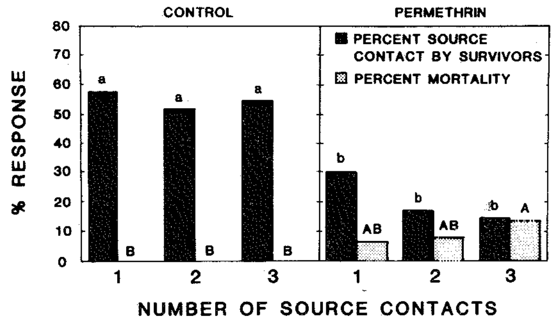

Fig. 3. Effect of repeated contact with sticker or permethrin-laced attracticide source on percent mortality and the percentage of surviving males contacting a pheromone source (both were evaluated 24 h after poisoning at an attracticide source). Percentages are significantly different if they do not share a letter in common ($P < 0.05$; Ryan's [1960] multiple-comparison test for proportions). Capital letters compare percent mortality and small letters compare percent source contact by survivors.

were only manifested in their ability to perform the behavioral sequence involved in locating a source of pheromone. Overall, after 24 h males surviving contact with pyrethroid attracticides at 1 or 10% concentration were significantly less likely to locate a pheromone source ($P < 0.05$). Cypermethrin was the most effective because it completely eliminated source location by males that had contacted the 10% concentration.

A conditional probability analysis allowed us to identify the specific behavioral transitions responsible for the diminished ability of males to perform the overall sequence involved in mate location. The effects of the insecticides were confined primarily to the early transitions in the behavioral response; initiation of wing fanning and flight were curtailed by all three pyrethroids. The transition from flight to upwind flight was diminished by 10% fenvalerate and 1% cypermethrin. No males that contacted the 10% cypermethrin attracticide completed this transition. The conditional probability of the transition from upwind flight to source contact was not diminished by contact with any attracticide formulation. Similarly, Haynes & Baker (1985) found that topical applications of permethrin diminished the conditional probabilities of the same early behavioral transitions, and not the specific transition from upwind flight to source contact.

Because cypermethrin showed the greatest potential in attracticide formulations, we examined the relationship between insecticide concentration and effects on mortality and sublethal effects on pheromone-source location in greater detail (Fig. 1). Probit analysis of the results indicated that the LC_{50} was 3.0% cypermethrin (95% CL = 2.2–4.3%; slope = 1.3 ± 0.12 [SEM]; n = 450), and the concentration that resulted in a 50% decrease in the ability of males to locate a pheromone source (SLC_{50}) was 0.2% (95% CL = 0.1–0.3%; slope = 1.5 ± 0.37 [SEM]; n = 347). Thus, there was a >10-fold difference between a dose sufficient to kill a male and a dose that was effective in removing the male from the mating population via sublethal interference with the behavioral response to pheromones.

Recovery from the sublethal effects of pheromone-stimulated contact with a 0.3% source of cypermethrin was complete after only 48 h (Fig. 2). One day after contact with the attracticide, only 18.8% of males (n = 64) reached the pheromone source. After 2, 3, and 4 days the response was significantly higher (42.2, n = 64; 53.1, n = 64; and 64.1%, n = 64, respectively) and not significantly different from the response of males that had contacted only sticker (57.8%, n = 64). Recovery from the sublethal effects of attracticide formulations might seem to be a limitation to this technique of population control. However, sublethal effects are likely to remove males from the potential mating population for a longer period of time than sensory adaptation and central habituation, two of the mechanisms thought to play a role in mating disruption when insecticides are not used (Bartell 1982). Males that recover from insecticidal effects are probably just as likely to contact another attracticide source as they were before the temporary poisoning. Thus, the number of chances a male has to mate with a female would be reduced when compared with mating disruption without insecticides.

An experiment documenting the effect of repeated contact with an attracticide source (permethrin) within a single activity period on the behavioral response 24 h later indicated a gradual and not significant diminution in the response as the number of contacts with attracticides went from one to three (Fig. 3). It was extremely difficult to collect enough males that had contacted the source three times, possibly because sensory adaptation, habituation, or sublethal effects of the insecticides (or all three factors) reduced the probability that these males would repeat the behaviors necessary for them to relocate the attracticide source. Mortality increased over this range until there was significant mortality at three contacts with the insecticide-laced sticker. Thus, the sublethal and lethal effects of attracticide formulations in the field may be more intense than our experiments allowing only one contact indicated.

Since attracticides are used in the field at a level comparable with the 1% concentration, our data indicate that one of the principal benefits from the addition of pyrethroids into the sticker is likely to be sublethal modification of behavior rather than direct lethal effects of the insecticide (Table 2; Fig. 1). Differences in environmental conditions between the laboratory and field may result in somewhat lesser or greater sublethal and lethal effects in the field. However, an evaluation of attracticide formulations that did not include an analysis of sublethal effects would consistently underestimate the potential impact of this technique for control of populations of the target pest.

Another benefit of the attracticide technique is that it allows a broad-spectrum insecticide to be used selectively. Butler & Las (1983) demonstrated that the use of attracticides for pink bollworm control preserves beneficial insect predators in cotton fields relative to conventional applications of insecticides. Beasley & Henneberry (1983) found some reduction of beneficial predators (26–29%) from the addition of pyrethroids into the mating disruptants when compared with disruptants without insecticide, but the population of predators was apparently preserved relative to conventional insecticide applications. Preservation of these natural enemies is likely to reduce problems with secondary pests (Van Steenwyk et al. 1975).

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