Host Odor Mediated Response of Female Navel Orangeworm Moths (Lepidoptera: Pyralidae) to Black and White Sticky Traps

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ABSTRACT In laboratory and field experiments, we used fatty acids found in crude almond oil and a crude almond oil/fatty acid blend (1:1) as attractants and found highly significant differences in numbers of navel orangeworm, *Amyelois transitella* (Walker), female moths that were attracted to and caught in black versus white sticky traps. In a mark-release-recapture experiment, 98.2% of the recaptured females were recovered in black traps after 7 d. Black traps also caught more than 85% of the wild *A. transitella* females compared with white traps. Wind tunnel bioassays on the upwind flight behavior of *A. transitella* females showed that females were more likely to approach, land, and become caught in black traps than in identically baited white traps.

KEY WORDS Insecta, *Amyelois transitella,* female attraction, almonds

PROMISING ALTERNATIVES to conventional insecticide applications for controlling navel orangeworm, *Amyelois transitella* (Walker), populations in almonds, *Prunus amygdalus* Batsch, were shown by Phelan & Baker (1987) and Van Steenwyk & Barnett (1987). These control strategies involve the use of almond by-products in an attracticide bait (Phelan & Baker 1987) and oviposition disruption (Van Steenwyk & Barnett 1987). The objective of the first strategy is to reduce *A. transitella* population densities by attracting gravid females to bait laced with insecticide. The objective of the second is to reduce almond infestation by promoting egg laying on or near attractants that were applied before hull-split of new-crop nuts or away from new-crop nuts after hull-split.

Additional research has shown that fatty acids in crude almond oil are responsible for eliciting the complete behavioral sequence of upwind flight and host source location in mated *A. transitella* female moths (P. L. Phelan & T.C.B., unpublished data). Thus, another major use for these highly attractive fatty acids may lie in the direct monitoring of the oviposition flights of adult females.

Rice (1976) was the first to selectively monitor *A. transitella* in almonds by counting eggs laid on a specialized trap baited with standard *A. transitella* rearing media. The Pherocon IV egg trap (Trece, Salinas, Calif.) is the commercial version of this trap that consists of a 92.4-ml clear plastic vial with two large openings covered with organdy. Although Pherocon IV traps are useful for indicating general population trends, data based on egg counts are inherently more variable than data that are based on actual female moth counts because the eggs deposited on one trap may be the result of many females depositing a few eggs each, or one female depositing many eggs.

Our objective was to develop a sticky trap for *A. transitella* female moths that could be used either in conjunction with, or as an alternative to, counting eggs on Pherocon IV egg traps. Presented herein are results from laboratory and field experiments that compare the behavior of female moths and moth catches, respectively, between a black version of the Pherocon 1C trap (Trece, Salinas, Calif.) and the white Pherocon 1C trap that is commercially available.

Materials and Methods

Mark-Release-Recapture Experiment. In 1987, a mark-release-recapture experiment was conducted to compare catches of *A. transitella* female moths in black and white traps with identical bait. The rationale for comparing only black and white traps in our bioassays was based on a study that showed significantly higher egg deposition on Pherocon IV egg traps that were painted black compared with all other colors that were tested (Van Steenwyk & Barnett 1985). We used a white Pherocon 1C trap and constructed our own black traps from 15-mil black polyvinyl, because commercial black traps were not available. Tangletrap (Tanglefoot Company, Grand Rapids, Mich.), was used with Pherocon 1C traps, and Stickem Special (Seabright Enterprises, Emeryville, Calif.) was used

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in the black traps. Wind tunnel bioassays ($n = 18$ replicates of 20 moths each) did not reveal significant differences (t test, $t = 1.37$, $df = 34$, $P = 0.18$) in the number of female moth catches between identically baited (10 mg almond oil fatty acids) black polyvinyl cards coated with either Tangle-trap (3.3 ± 0.4 [$\bar{x}$ ± SD]) or Stickem Special (2.6 ± 0.4).

Larvae were reared on a modified diet (Finney & Brinkman 1967) of bran, honey, and glycerol and maintained in a temperature cabinet at 25 ± 2°C and a 16:8 (L:D) photoperiod. Male and female larvae were marked internally with red dye following a modification of the procedure described by Andrews et al. (1980). One gram of Solvent Red 26 dye (Passaic Color & Chemical Company, Patterson, N.J.) was dissolved in 30 ml of heated vegetable oil (Wesson) and incorporated into 9 liters of regular rearing media. Larvae reared on diet containing this dye appear bright red whereas larvae reared without dye are cream colored. Pupation did not diminish the dye's intensity because the distinctive red coloration was also readily apparent in the moths.

The almond orchard we used in this experiment was a 171-ha block of 'Nonpareil,' 'Mission,' 'NePlus,' and 'Merced' varieties planted in 1968 in Kern County, Calif. Trees were spaced 8.53 m between rows, with 4.27 m between trees within rows. The 0.26 ha-experimental plot was situated near the center of the orchard. A total of 24 trapping sites were located at eight directions and three distances around a central release point (Fig. 1). At each site, a pair of black and white traps was hung side-by-side (within 1 to 2 m of one another) on the north side of a tree at an aboveground height of approximately 3 m. Positioning of traps relative to one another within trees was randomized. All traps were baited with 60 mg of attractant, a 1:1 blend of crude almond oil (Liberty Vegetable Oil Company, Santa Fe Springs, Calif.) and fatty acids (from almond oil soapstock). The attractant dispenser consisted of a 3- by 5-cm piece of dark green construction paper with one end bent at a right angle so it could be raised approximately 1 cm above the sticky liner of the trap.

One of the by-products in the refinement of crude almond oil is a thick, viscous soapstock that contains the fatty acids. An acidification process was used to separate the fatty acids from the soapstock. The procedure involved slowly adding 2% sulfuric acid to a previously heated (approximately 35°C) mixture of distilled water (100 ml) and almond oil soapstock (150 ml) to attain a pH 3. Next, the oily layer containing the fatty acids was removed from the mixture and purified of sediment by centrifugation at 3,000 rpm for 5 min.

Two additional, unbaited Pherocon 1C control traps were hung in trees located 9.54 m NE and SW of the release point. Four black Pherocon IV egg traps baited with almond presscake (a by-product resulting from the compression of almonds with rice hulls in the production of crude almond oil [Phelan & Baker 1987]) plus 10% (by weight) crude almond oil, were spaced 10 trees apart from one another in a row located 205 m away from the main trapping area. This was done to check egg-laying activity of wild *A. transitella* females during the release-recapture interval. All traps were placed in the orchard on 29 September 1987.

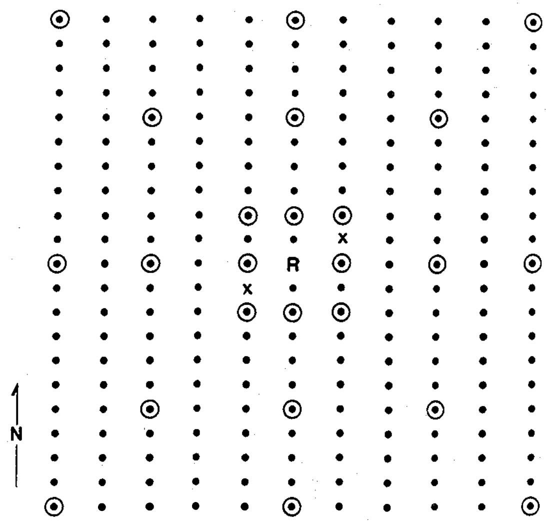

Fig. 1. Diagram of the trapping layout used in the mark-release-recapture experiment conducted in a commercial almond orchard in Kern County, Calif. Dots represent trees; encircled dots represent trees on which a pair of black and white traps were hung. Also represented are the release site (R) and the trees (X) on which an unbaited Pherocon 1C trap was hung. Trees were spaced 4.27 m apart within rows and 8.53 m apart between rows.

After harvest was completed for all varieties of almonds, the marked moths were released on 29 September 1987 at 1700 hours (PDST). Based on an approximate 1:1 sex ratio of the laboratory colony and moth counts made on 28 September 1987, we estimated that about 2,650 marked female and 2,650 marked male moths were released. The age of these moths varied from 1 to 7 d. On 6 October, all 50 sticky traps and the four Pherocon IV egg traps were removed from the orchard and taken to the laboratory at the University of California, Riverside to determine the number and sex of marked and unmarked (wild) moths in each trap. Data were analyzed by χ^2 using Yates correction factor (Little & Hills 1978) for single degree of freedom comparisons.

Wind Tunnel Experiment. We conducted a series of no-choice, wind tunnel bioassays to compare differences in the response of mated females between black and white traps with identical bait. However, only one adhesive, Stickem Special, was used in all traps. Thus, in the construction of the

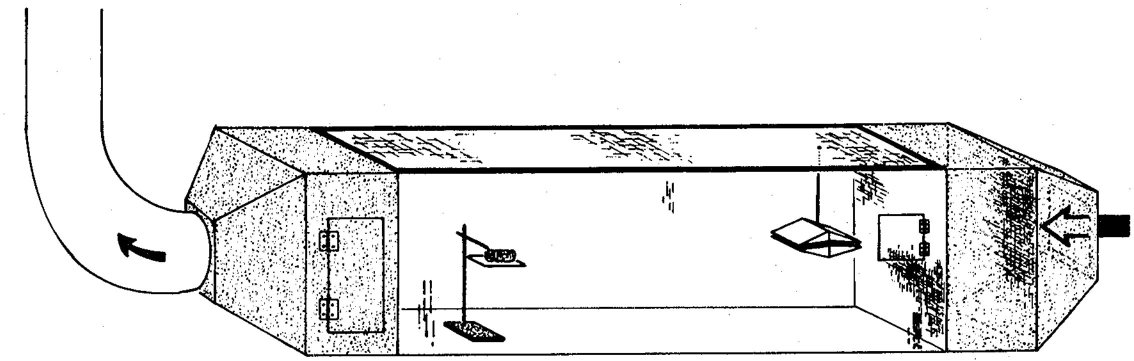

Fig. 2. Diagram of the wind tunnel used in laboratory bioassays Arrows indicate direction of air movement.

white trap, a Pherocon 1C top coated with Stickem Special was used in place of the commercial liner that normally is coated with Tangletrap.

The wind tunnel used for this experiment was constructed from 3-mm-thick Plexiglas supported on an aluminum frame (Fig. 2). The dimensions of the working section in which moths were flown were 1.8 by 0.6 by 0.6 m. For a more detailed description see Farkas et al. (1974) and Willis & Baker (1984). To simulate conditions approximating twilight in the wind tunnel, a 0.5-lux light level was used. Other variables included a constant windspeed of 0.5 m/s, 24–25°C temperature, and 39–45% RH.

Larvae were reared according to the procedure described previously. Because only mated female moths are attracted to crude almond oil (Phelan & Baker 1987), females were taken from mating cages with screen sides (25 cm^3) containing a minimum of 100 moths in about a 1:1 sex ratio. Dissections of differentially aged female moths revealed that all females contained at least one spermatophore by the fourth day after adult emergence. Thus, the age of females flown in the wind tunnel bioassays was standardized at 4 d.

We used the green construction paper dispensers to dispense the attractants in all tests. To make it easier to position the dispenser in the sticky liner of the trap, we bent 1 cm of the construction paper at a 90° angle. By placing the shorter end of the dispenser in the sticky liner, we could position the remaining 4 cm with the attractant parallel to the direction of air movement. Fresh dispensers, with 10 mg fatty acids, were prepared each day that the bioassays were conducted. To eliminate differences that might occur between two or more dispensers, the same dispenser was used in both traps.

Approximately 2 h before testing, 10 4-d-old female moths were transferred individually from their mating cage to a small, cylindrical screen cage (55 mm diameter by 80 mm long) with a plastic Petri dish lid covering the open end. Because no-choice bioassays were performed, the two treatments were randomly assigned to each pair of cages. The two treatments then were evaluated in succession for a 1-h interval.

After 10 moths in a cage were conditioned to low light (0.5 lx) for 30 min, they were placed on a 25-cm-high platform located 1.8 m downwind of either a black or a white trap in the wind tunnel (Fig. 2). Immediately after we put the cage in the wind tunnel, we removed the Petri dish lid. Observations of moth behavior lasted 30 min per treatment and included scoring the number of times females approached within 20 cm or less of the downwind side of the trap (approach), and the number of times females landed on the trap. A landing, regardless of whether or not it resulted in a capture, was defined as remaining on the trap for a minimum of 1 s. The landing category was included because females usually did not enter the trap and become stuck without first landing on either the downwind edge of the liner (without adhesive) or the top or bottom of the trap. We also recorded the number of females caught in the trap at the end of the 30-min bioassay. Any females not caught in the trap at the end of the period were removed from the wind tunnel before the beginning of the next bioassay. Three bioassays, comprising 60 moths, were typically performed each day. A t test was used to test for differences in the number of approaches, landings, and captures between black and white traps. Data pertaining to the number of approaches and landings were transformed using $(x + 0.5)^{1/2}$; capture data were transformed using $\arcsin(x)^{1/2}$.

Results

Mark-Release-Recapture Experiment. The difference in numbers of marked *A. transitella* females recaptured in black traps compared with white traps was highly significant ($P \leq 0.001$) with more than 98% recovered from the black traps (Table 1). Similar to marked females, wild *A. transitella* females also had a strong preference for the black traps in which nearly six times as many wild females (85.3%) were caught. As expected, num-

Table 1. Trap catches of laboratory-reared marked and wild *A. transitella* moths in commercial (white) Pherocon 1C traps and a black version of the trap

	Total no. caught					
	♀♀		χ^2	♂♂		χ^2
	Black	White		Black	White	
Marked	110	2***	102.2	29	0***	27.0
Wild	29	5***	15.5	50	1***	45.1
Total	139	7***	117.5	79	1***	74.1

***, Significant at $P \leq 0.001$ according to χ^2; Yates correction factor for single degree of freedom comparisons was used for all analyses (Little & Hills 1978).

bers of marked females in black traps were inversely related to distance; traps nearest (8.53 m) the release point averaged 15.5 ± 5.0 ($\bar{x}$ ± SEM) females per trap, whereas those farthest (60.35 m) from the release point averaged 1.0 ± 0.6 per trap. Also as expected, the captures of wild females were evenly distributed across the traps with an average of 1.4 ± 0.4 near the release point, 1.1 ± 0.6 in the middle, and 1.1 ± 0.4 at the edges.

Black traps also attracted significantly ($P \leq 0.001$) more marked and wild males than white traps (Table 1). However, the occurrence of males in black or white traps was probably more a function of their attraction to pheromone emitted by females already caught in the trap, than to the almond odor attractant itself. In the process of attempting to free themselves from the sticker, we observed that females extended their abdomens into the air and extruded their pheromone glands. Additional support for this hypothesis is provided by the fact that males were found only in traps in which at least one female was present. No males were found in 23 traps that contained from one to nine females per trap after 7 d.

No moths were caught in the two unbaited Pherocon 1C traps that had been placed near (9.54 m) the release point at the same time as the other traps. The Pherocon IV egg traps averaged 32.8 ± 20.4 eggs per trap by the end of the trapping interval.

Wind Tunnel Experiment. The results of the no-choice, wind tunnel experiment agreed with those of the field experiment. Black traps resulted in significantly ($P \leq 0.05$) more female approaches, landings, and catches than white traps (Table 2). Observations during the initial phase (first meter) of odor-mediated upwind flight indicated that the percentage of females flying upwind to the black and white traps was similar. However, significantly more females were observed approaching to within 20 cm or less of the downwind entrance of a black trap compared with a white trap.

The greater trapping efficiency of the black trap compared with the white trap can be explained by inspecting the approach/landing and landing/capture ratios (Table 2). On average, every 1.5 approaches to a black trap resulted in a landing, whereas nearly twice as many approaches (2.8) were necessary for the white trap. This was not the case for the landing/capture ratios. The difference in landing/capture ratios between black (2.3:1) and white (2.0:1) traps were small by comparison indicating that both were equally acceptable to females once they had landed. These findings show that some form of visual repellency to white traps is expressed during flight. The findings of Van Steenwyk & Barnett (1985) of fewer eggs on white (clear) versus black Pherocon IV egg traps might be at least partially explained by the reluctance of gravid females to land on white surfaces.

Discussion

Attraction of *A. transitella* female moths to either fatty acids alone or blended with crude almond oil can be dramatically increased with black sticky traps compared with white sticky traps. Thus, strong consideration should be given to the visual aspect when monitoring tools and attract-and-kill baits are designed for this pest. Furthermore, the approximate 4% recapture rate of the marked females in the mark-release-recapture experiment demonstrates again the highly attractive nature of the almond-oil fatty acids to females, because even in experiments involving other species and highly attractive sex pheromone blends, recaptures of marked male moths do not generally exceed 5% (Elkinton & Cardé 1981).

Lieu et al. (1982) first reported the presence of free fatty acids (i.e., palmitic, oleic, and linoleic) in frass from infested almonds, but because they found no positive effect in egg-laying bioassays, they concluded that the fatty acids were not active constituents. However, using a wind tunnel bioas-

Table 2. No-choice wind tunnel bioassays

Treatment and *t* statistics	*n*	$\bar{x}$ ± SEM[a] no. of			Ratio	
		Approaches	Landings	Catches	Approaches/landings	Landings/catches
Black traps	14	12.4 ± 1.5*	8.2 ± 1.7**	3.5 ± 0.4***	1.5:1	2.3:1
White traps	14	7.4 ± 1.2	2.6 ± 1.1	1.3 ± 0.5	2.8:1	2.0:1

*, Significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; ***, significant at $P \leq 0.001$, according to a paired *t* test.

[a] Means (±SEM) are based on raw data; statistical analyses were performed on transformed data. Approaches, $t = 2.58$; landings, $t = 3.45$; catches, $t = 4.06$.

say recording female attraction, not egg-laying, P.L. Phelan & T.C.B. (unpublished data) showed that these same fatty acids, obtained from crude almond oil, would elicit upwind flight and host source location in mated female moths.

Current methods for monitoring populations in almonds rely predominantly upon the Pherocon IV egg trap. However, one drawback of the Pherocon IV egg trap is that after hull-split traps must compete with new-crop almonds, therefore egg deposition on traps is poorly related to moth flight patterns as determined by blacklight or live-female-baited traps (Rice 1976, Rice et al. 1976). After hull-split, catches of female moths in black sticky traps may be affected to a lesser extent than oviposition on Pherocon IV egg traps. If this competition is due to only greater dispersion of eggs on the greater number of available almonds after hull-split, rather than to attraction of female moths away from the trap, then a trap that captures females should be less vulnerable to a reduction in trap efficiency.

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