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## ATTRACTION OF MEXICAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) TO TWO SYNTHETIC LURES: EFFECTS OF WATER AND THERMAL STRESS

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### ABSTRACT

Experiments were conducted in a flight chamber in a controlled-environment greenhouse to determine if thermal and water stress differentially affects attraction of Mexican fruit flies, *Anastrepha ludens* (Loew), to BioLure MFF 2-component lures and AFF lures. For most combinations of air temperature, water vs. no water in traps, and non-thirsty vs. thirsty flies, responses to traps with BioLures or AFF lures were equal. Generally, higher temperatures, water deprivation of flies, and especially presence of water in traps, increased attraction to both lures. Results indicate that observed greater attractiveness of BioLures compared with AFF lures in Multi-Lure traps in the field is not due to water in traps or thermal stress. Results are consistent with observed greater attractiveness of Multi-Lure traps containing water compared with sticky traps under hot, dry field conditions.

**Key Words:** *Anastrepha ludens*, BioLure, AFF lure, environment, weather, temperature, thirst, water deprivation

### RESUMEN

Se realizaron experimentos en una camara de vuelo en un invernadero con ambiente controlado para determinar si el estrés termal y el estrés de agua pueden afectar la atracción de la mosca mexicana de fruto, *Anastrepha ludens* (Loew), hacia el señuelo de BioLure del componente MFF 2 y el señuelo de AFF. Para la mayoría de las combinaciones de la temperatura de aire, agua vs. no agua en las trampas, y moscas sin sed vs moscas con sed, las respuestas hacia las trampas con BioLures o el señuelo AFF fueron iguales. Generalmente, las temperaturas mas altas, la privación de agua en las moscas, y especialmente la presencia de agua en las trampas, aumentaron la atracción hacia ambos señuelos. Los resultados indican que la mayor atracción observada con BioLures comparada con la del señuelo AFF en el campo no es debida al agua en las trampas o el estrés termal. Los resultados son consistentes con la mayor atracción de las trampas Multi-Lure que tienen agua comparada con las trampas pegajosas bajo condiciones calidas y secas en el campo.

McPhail and McPhail-type traps with hydrolyzed protein have been the standard traps for monitoring populations of numerous species of fruit flies for most of the last century (Cunningham 1989; Aluja 1994). However, BioLure MFF 2-component (ammonium acetate, putrescine) lure (hereafter called BioLure), first marketed in the mid 1990s, has proven superior to protein baits for Mexican fruit flies (*Anastrepha ludens* Loew) in recent field tests (Thomas et al. 2001). In 2002, IPM Tech (now Advanced Pheromone Technologies, Inc., Marylhurst, OR) introduced another synthetic lure, the AFF lure, for *Anastrepha*. AFF lures are similar to BioLures because they emit ammonia and putrescine as active components. Both lures also emit 1-pyrroline as a byproduct of putrescine (Robacker & Czokajlo 2005). The two lures differ in that AFF lures emit methylamine whereas BioLures emit acetic acid, and AFF lures emit more ammonia and 1-pyrroline than BioLures (Heath et al. 1995; Robacker & Czokajlo 2005). All of these compounds have been demonstrated attractive to Mexican fruit flies (Robacker

& Warfield 1993; Robacker & Flath 1995; Robacker 2001).

Although little has been published comparing the attractiveness of these two synthetic lures, Robacker (1999) demonstrated that AFF lure components formulated in agar were more than 2× as attractive as BioLures to both wild and lab-strain Mexican fruit flies when lures were tested exposed on yellow panels in a wind tunnel. Robacker and Czokajlo (2005) confirmed these results in field tests for lures exposed on sticky traps but found that BioLures were superior to AFF lures in Multi-Lure traps containing 10% propylene glycol antifreeze in water. Hall et al. (2005) also showed that BioLures in Multi-Lure traps with antifreeze were much more attractive than AFF lures to Caribbean fruit flies (*Anastrepha suspensa* Loew). Although wet traps such as Multi-Lure traps are generally more effective than sticky traps, Robacker & Czokajlo (2005) observed that sticky traps with AFF lures were more effective than Multi-Lure traps with either AFF lures or BioLures when daytime tempera-

tures were low but Multi-Lure traps with either lure became dominant as weather became hotter.

The reasons for the different efficacies of these lures in different trap types are unknown. One possibility is that the lures function differently because of differences in emitted chemicals. Thus, the combination and amounts of chemicals emitted by AFF lures may work better on exposed traps whereas the combination and amounts emitted by BioLures may work better in enclosed traps. Further, interactions of various components with water or propylene glycol antifreeze in the traps may also play a role. Finally, the effects of water in traps may be magnified under conditions in which flies are thermally stressed, thus accounting for the increased attractiveness of Multi-Lure traps compared with sticky traps as field conditions became hotter (Robacker & Czokajlo 2005).

The primary objective of this work was to determine if the greater efficacy of BioLures compared with AFF lures in Multi-Lure traps is due to water in the traps, and whether the effects would be magnified under conditions of thermal stress. Secondly, I wanted to investigate whether thermal stress affects attraction of flies to McPhail-type traps as has been suggested in the literature. Experiments were conducted in a flight chamber in a controlled-environment to evaluate attraction of laboratory-strain Mexican fruit flies to BioLures and AFF lures in Multi-Lure traps with or without water. Flies were stressed by water deprivation and high air temperatures to determine the interplay of these factors with water in traps.

## MATERIALS AND METHODS

### Insects

Laboratory-strain Mexican fruit flies were obtained from a culture at our facility in Weslaco, TX. Laboratory stock originated from 2,000 pupae collected from yellow chapote (*Casimiroa greggii* S. Wats) fruit from the Montemorelos area of Nuevo Leon in northeastern Mexico in 2000 and was maintained on artificial diet for approximately 30 generations. Flies were irradiated, due to quarantine laws, with 70-92 Gray (Cobalt 60) 1-2 d before adult eclosion. Adult flies (160/carton) were held in 473-ml cardboard cartons where they were provided sugar and water. Flies were used in experiments at ages 2-20 days post eclosion. Laboratory conditions where test flies were housed were  $22 \pm 2^\circ\text{C}$  and  $50 \pm 20\%$  relative humidity with a photophase of 0630 to 1930 h provided by fluorescent lights.

### Traps and Lures

The Multi-Lure trap (Better World Manufacturing, Miami, FL) was used in all tests. This is a

plastic McPhail-type trap with a clear top that fits onto a yellow base containing liquid to drown trapped flies. Traps used in experiments either contained water with 0.01% Triton X-100 (Rohm and Haas, Philadelphia, PA) as the killing agent or Stickum Special (Seabright Laboratories, Emeryville, CA) coated onto the entire surface inside the trap bottom. The same trap top and two trap bottoms (one for water, the other for Stickum) were used for all replications of both experiments.

Two synthetic lures were used: BioLure 2-component MFF lure (Suterra, Inc., Bend, OR) and AFF lure (Advanced Pheromone Technologies, Marylhurst, OR). Each type of lure was adhered to the inside wall of the trap tops. Lures were used for no more than 104 d after removal from refrigeration. Robacker & Czokajlo (2005) showed that both of these lures function for at least 16 weeks under hot field conditions.

### Environmental Control

Tests were conducted in a greenhouse in which temperature and relative humidity were controlled by a remote multi-tasking computer. The computer received temperature and humidity data from sensors located in the center of the greenhouse and used the data to regulate conditions to set values. The computer controlled temperature by venting warm or cool air into the room on one end and venting it out at the other end, and humidity by switching on mist sprayers located at numerous points around the perimeter of the greenhouse. Air temperature set points were  $22^\circ\text{C}$  and  $32^\circ\text{C}$ . Overall, temperatures were maintained at  $22.3 \pm 1.3^\circ$  (SD) (range 20-26) for the low temperature setting and  $32.7 \pm 1.7^\circ$  (29-37) for the high setting. Control of temperature was more precise during cooler times of the year because it was easier to warm the greenhouse than to cool it. Relative humidity was set to 60-70% but was poorly maintained due to changes in outdoor temperature and humidity conditions and malfunctions of the misting system. Control of humidity was better during the hot, humid months because of less reliance on the misting system.

### Flight Chamber used for Bioassays

Experiments were conducted in an aluminum-framed, aluminum-screened flight chamber (2.0 m long by 0.7 m wide by 1.3 m high) in a greenhouse with airflow of 1 m/sec. The cage contained an aluminum-sheet partition attached at the top and bottom of the cage, extending 0.6 m back into the cage from the upwind end. This partition created two zones in the upwind end of the chamber such that flies detecting odor in the air stream could choose the origination zone of the odor much as insects choose one arm in a Y-tube bioassay.

One trap was suspended in each of the two zones about 0.3 m apart, 0.3 m from the upwind end, and 0.4 m below the top of the cage.

#### Experimental Design and Procedure

Two experiments were conducted to test air temperature, effect of water in traps (trap state), thirst state of flies, and lure type. Each experiment tested the first three variables at two levels each in a factorial arrangement for a total of eight temperature/trap-state/thirst-state combinations. Temperature treatments were low (22°C) and high (32°C). Trap state was wet or dry. Flies were thirsty (deprived of water for 2 d) or not thirsty (sprayed with water daily and 1 h before testing). Each of the eight combinations was tested in a separate bioassay trial. Lure types were BioLure and AFF lure. Both lures were tested in each bioassay trial, one in each trap.

The first experiment was conducted with the low temperature in the morning and the high temperature in the afternoon. This experiment was conducted during May 2004, and Oct-Nov 2004, when outdoor temperatures were similar to the test temperatures. The second experiment was conducted with the temperatures reversed with respect to time of day. It was conducted from Jan-Mar 2005, when outdoor temperatures were cooler so that the lower temperature could be maintained in the greenhouse during the afternoon. One trial was conducted in the morning and one in the afternoon for four consecutive days to complete a replication. Morning trials were conducted between 0830 and 1030 h and afternoon trials between 1300 and 1530 h. Order of the factorial treatments was randomized within each replication of the experiments (with the exception that temperature treatments were not varied between morning and afternoon within each experiment).

To conduct a trial, 160 mixed-sex flies, either thirsty or not thirsty, were released into the

downwind end of the chamber containing two traps in the upwind end, both traps either wet or dry. After 0.5 h, the positions of the traps were exchanged. The traps were removed and the captured flies were counted after 1 h. Beginning positions of BioLure and AFF lure traps were alternated between the two sides of the chamber.

#### Statistical Analyses

All analyses were conducted with JMP (2002) programs. The responses to AFF lures and BioLures were analyzed as matched pairs (JMP: Analyze/Matched Pairs) with thermal stress factors (temperature, trap state, fly thirst, and their interactions) used as grouping variables in separate analyses. Because the overall effects of lure type were small, data were pooled over lures for further analysis. Effects and interactions of temperature, trap state, and fly thirst were determined by factorial analysis on the pooled data (JMP: Analyze/Fit Model/Macros/Full Factorial). Separate analyses were conducted for males and females.

#### RESULTS

BioLures and AFF lures were equally effective summed over both experiments and all treatments. Mean captures for the two lures were: BioLures,  $13.4 \pm 0.9$  (SE) males and  $11.7 \pm 0.8$  females; AFF lures,  $14.1 \pm 1.0$  males and  $12.0 \pm 0.8$  females. For individual thermal-stress treatments, attraction to BioLures vs. AFF lures differed only for the combination of low temperature, wet trap, and non-thirsty flies (Tables 1, 2). For both males and females, AFF lures were more attractive than BioLures under these conditions.

Only one interaction involving lure type with thermal stress factors was significant. The lure type  $\times$  trap state interaction was significant because for both males ( $F = 9.0$ ,  $df = 1, 142$ ,  $P < 0.01$ ) and females ( $F = 4.4$ ,  $df = 1, 142$ ,  $P < 0.05$ ), dry

TABLE 1. CAPTURES OF FEMALE MEXICAN FRUIT FLIES IN MULTI-LURE TRAPS BAITED WITH BIOLURES VS AFF LURES IN A FLIGHT CHAMBER AT DIFFERENT TEMPERATURES, TRAP STATES, AND THIRST STATES OF FLIES.

Temperature	Trap State	Thirst State	BioLure	AFF Lure	Difference <sup>a</sup>
Low	Dry	Not thirsty	$3.9 \pm 0.7$	$4.2 \pm 0.9$	$-0.3 \pm 0.8$
		Thirsty	$7.7 \pm 1.0$	$6.6 \pm 1.0$	$1.1 \pm 1.2$
	Wet	Not thirsty	$9.8 \pm 1.7$	$13.1 \pm 1.7$	$-3.3 \pm 1.4$
		Thirsty	$20.6 \pm 2.3$	$20.1 \pm 1.9$	$0.5 \pm 1.8$
High	Dry	Not thirsty	$6.0 \pm 1.0$	$5.2 \pm 0.9$	$0.8 \pm 0.7$
		Thirsty	$9.6 \pm 1.3$	$8.2 \pm 1.0$	$1.3 \pm 1.0$
	Wet	Not thirsty	$16.1 \pm 2.1$	$16.4 \pm 2.5$	$-0.3 \pm 2.1$
		Thirsty	$19.7 \pm 2.5$	$22.2 \pm 2.1$	$-2.6 \pm 1.9$

<sup>a</sup>The mean difference ( $\pm$  SEM) for the combination "low, wet, not thirsty" was significant at the 5% level by paired *t*-tests across factorial treatment combinations ( $t = 2.5$ ,  $df = 17$ ,  $P < 0.05$ ).

TABLE 2. CAPTURES OF MALE MEXICAN FRUIT FLIES IN MULTI-LURE TRAPS BAITED WITH BIOLURES VS AFF LURES IN A FLIGHT CHAMBER AT DIFFERENT TEMPERATURES, TRAP STATES, AND THIRST STATES OF FLIES

Temperature	Trap State	Thirst State	BioLure	AFF Lure	Difference <sup>a</sup>
Low	Dry	Not thirsty	4.3 ± 0.9	3.8 ± 0.7	0.6 ± 0.7
		Thirsty	10.3 ± 1.2	8.9 ± 1.2	1.4 ± 0.9
	Wet	Not thirsty	9.4 ± 1.8	13.3 ± 2.5	-3.9 ± 1.6
		Thirsty	22.6 ± 2.8	25.5 ± 2.6	2.9 ± 1.5
High	Dry	Not thirsty	6.1 ± 1.0	4.6 ± 1.3	1.4 ± 0.8
		Thirsty	9.8 ± 1.9	9.6 ± 1.5	0.2 ± 1.8
	Wet	Not thirsty	20.3 ± 2.5	21.9 ± 2.5	-1.6 ± 2.1
		Thirsty	24.7 ± 2.9	25.7 ± 2.7	-1.0 ± 2.2

<sup>a</sup>The mean difference (± SEM) for the combination “low, wet, not thirsty” was significant at the 5% level by paired *t*-tests across factorial treatment combinations (*t* = 2.3, *df* = 17, *P* < 0.05).

traps with AFF lures captured about 10% fewer flies than dry traps with BioLures, but wet traps were the opposite by about the same percentage (Tables 1, 2).

Because lures performed equivalently for the most part, data were summed over lures for factorial analysis of effects of temperature, trap state, fly thirst state and the interactions of these variables for each experiment. No differences were found in the data for the two experiments so data were combined into one set for males and one for females for the final analysis shown in Table 3. Generally for both sexes, more flies were attracted to traps at the higher than at the lower temperature, more flies were attracted to wet than to dry traps, and more thirsty than non-thirsty flies were attracted to both dry and wet traps.

The interaction between temperature and thirst state was significant for males. Attraction of non-thirsty flies at the low temperature was lower than attraction of non-thirsty flies at the high temperature and thirsty flies at either temperature (Table 4). The same trend occurred for

females but the effect was not significant at the 5% level.

DISCUSSION

This work investigated whether water in traps and thermal stress on flies accounts for the higher efficacy of Biolures compared with AFF lures in McPhail-type traps. Results indicated that Biolures and AFF lures were about equally attractive to irradiated Mexican fruit flies regardless of temperature (within the limited range tested), whether or not traps contained water, and whether or not flies were thirsty. Only minor differences were found in efficacy of the two lures under the different thermal stress conditions in this study. Those effects were small, did not fit into observable trends, and may have been due to chance. Thus, the results indicate that the superiority of BioLures compared with AFF lures in Multi-Lure traps was not due to an interaction of active components of the lures with water in traps or because the active components elicited different physiological responses when flies were ther-

TABLE 3. CAPTURES OF MEXICAN FRUIT FLIES IN MULTI-LURE TRAPS IN A FLIGHT CHAMBER AT DIFFERENT TEMPERATURES, TRAP STATES, AND THIRST STATES OF FLIES, SUMMED OVER LURE TYPE<sup>a</sup>

Temperature	Trap State	Thirst State	Males	Females
Low	Dry	Not thirsty	8.1 ± 1.5 a	8.2 ± 1.4 a
		Thirsty	19.2 ± 2.3 b	14.3 ± 1.6 ab
	Wet	Not thirsty	22.7 ± 4.0 b	22.9 ± 3.1 c
		Thirsty	48.1 ± 5.2 cd	40.6 ± 3.9 e
High	Dry	Not thirsty	10.7 ± 2.0 a	11.2 ± 1.7 ab
		Thirsty	19.3 ± 2.9 b	17.8 ± 2.1 bc
	Wet	Not thirsty	42.2 ± 4.6 c	32.5 ± 4.1 d
		Thirsty	50.3 ± 5.2 d	41.9 ± 4.2 e

<sup>a</sup>Means (± SEM) in the same column followed by the same letter are not significantly different at the 5% level by the LSD Method (Student's *t*) calculated from the error term from Factorial ANOVA (males: *F* = 36.8, *df* = 7,119, *P* < 0.0001; females: *F* = 29.1, *df* = 7,119, *P* < 0.0001).

TABLE 4. CAPTURES OF THIRSTY AND NON-THIRSTY MEXICAN FRUIT FLIES AT LOW (22°C) AND HIGH (32°C) TEMPERATURES IN MULTI-LURE TRAPS IN A FLIGHT CHAMBER, SUMMED OVER TRAP STATE AND LURE TYPE.

Temperature	Fly State	Males <sup>a</sup>	Females
Low	Not thirsty	15.4 ± 2.4	15.6 ± 2.1
	Thirsty	33.6 ± 3.7	27.4 ± 3.0
High	Not thirsty	26.4 ± 3.6	21.8 ± 2.8
	Thirsty	34.8 ± 3.9	29.8 ± 3.1

<sup>a</sup>The temperature X fly thirst state interaction was significant by Factorial ANOVA ( $F = 6.3$ ,  $df = 1,119$ ,  $P < 0.05$ ).

mally stressed. Results from a field experiment conducted concurrently with the present work indicate that antifreeze probably does play a role in the greater attractiveness of BioLures compared with AFF lures in Multi-Lure traps (Robacker & Czokajlo 2006).

Several published reports of field trapping experiments of fruit flies have noted a possible link between weather and trapping efficacy of McPhail-type traps containing liquid baits. Generally, field data indicate that various species of fruit flies are attracted more strongly to the traps when temperatures are high and/or water availability is low (McPhail 1937; Cunningham et al. 1978; Neuenschwander & Michelakis 1979; Houston 1981; Kapatos & Fletcher 1983; Heath et al. 1997; Robacker & Czokajlo 2005). Although each of these studies implicates water stress as an important factor in attraction to McPhail traps, most were conducted in the field where environmental variables could not be controlled. Also, comparisons of wet and dry traps were not always made (McPhail 1937; Neuenschwander & Michelakis 1979; Houston 1981; Kapatos & Fletcher 1983), or when they were made, they were not the same type of trap and utilized different baits (Cunningham et al. 1978; Heath et al. 1997).

The work presented here was a systematic, though only exploratory, approach to the question of whether thermal stress affects attraction of fruit flies to McPhail-type traps with liquid baits. Results clearly indicated that thermal stress was an important factor. Generally, attraction increased at the higher temperature, attraction to traps with water was greater than to traps without water, and attraction of thirsty flies was greater than non-thirsty ones. One surprising result was that thirst increased attraction to dry traps as well as wet traps. This effect may have occurred because thirsty flies sensed humidity (from the misting system) in the air entering the flight chamber and flew upwind even though none was coming from the traps. In this scenario, their higher response to dry traps compared with non-thirsty flies occurred because they were already near the traps, not because the dry traps were more attractive to thirsty than to non-thirsty flies.

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