Efficacy of Two Synthetic Food-Odor Lures for Mexican Fruit Flies (Diptera: Tephritidae) Is Determined by Trap Type

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ABSTRACT Sterile mass-reared Mexican fruit flies, *Anastrepha ludens* (Loew), were trapped in a citrus orchard by using multilure traps and cylindrical sticky traps baited with Advanced Pheromone Technologies *Anastrepha* fruit fly (AFF) lures or Suterra BioLure two-component (ammonium acetate and putrescine) MFF lures (BioLures). The cylinder trap/AFF lure combination was the best trap over the first 6 wk, the multilure trap/BioLure combination was best during weeks 6–12, and the multilure trap/AFF lure combination was best during the last 6 wk. The multilure trap/BioLure combination was best overall by 36% over the cylinder trap/AFF lure combination, and 57% over the multilure trap/AFF lure combination. Cylinder traps with BioLures were the least effective trap/lure combination throughout the experiment, capturing only half as many flies as cylinder traps with AFF lures. Captures with cylinder traps baited with either lure and multilure traps with BioLures were female biased. For the most part, both lures remained highly attractive and emitted detectable amounts of attractive components under hot field conditions for the duration of the 18-wk experiment. Total emission of ammonia was 4 times greater and 1-pyrroline at least 10 times greater from AFF lures compared with BioLures. Correlations of trap and lure performance with ammonia emission and weather were determined, but no conclusions were possible. Results indicate that BioLures would be the lure of choice in multilure or other McPhail-type traps and AFF lures would be superior with most sticky traps or kill stations that attract flies to outer (not enclosed) surfaces.

KEY WORDS *Anastrepha ludens*, fruit fly, attractants, AFF lure, BioLure

MEXICAN FRUIT FLY, *Anastrepha ludens* (Loew) is an important pest of citrus and other fruits in Mexico and Central America (Enkerlin et al. 1989). The fly also poses a serious threat to the citrus industry in the United States where it is trapped annually in southern Texas and occasionally invades California and Florida (Nilakhe et al. 1991). Early and reliable detection of this invasive pest is critical to its eradication and control. McPhail traps baited with torula yeast or other proteinaceous baits have been the standard detection tools for most of the last century (Thomas et al. 2001). However, during the past 10 yr, synthetic food-odor lures such as BioLure MFF lures (BioLures) (Suterra LLC, Inc., Bend, OR) have been gaining favor for trapping both *Anastrepha* (two-component version of the lure) and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (three-component version) (Robacker and Landolt 2002). In many trials, these synthetic lures have been more attractive to fruit flies and less so to unwanted species of insects compared with torula yeast and other proteinaceous baits (Epsky et al. 1999, Katsoyannos et al. 1999, Thomas et al. 2001, Thomas 2003).

Robacker and Warfield (1993) invented a synthetic attractant (AMPu) for the Mexican fruit fly that is similar to BioLure. AMPu emits ammonia, methylamine, putrescine, and 1-pyrroline (Robacker and Bartelt 1996) compared with ammonia, acetic acid, putrescine (Heath et al. 1995), and 1-pyrroline (data presented herein) emitted by the two-component BioLures. An AMPu formulation in agar proved more attractive than BioLures in wind tunnel experiments using both laboratory-culture and wild-strain Mexican fruit flies (Robacker 1998, 1999). Despite invention and publication of AMPu preceding the ammonium acetate/putrescine lures (that are the basis of the BioLures) (Heath et al. 1995), a formulation of AMPu that was effective in the field was not developed for commercial sale until IPM Technologies, Inc. (now Advanced Pheromone Technologies, Inc., Marylhurst, OR), marketed the AFF lure in 2002. Preliminary field tests indicated that AFF lures were more effective than BioLures on sticky traps but that the reverse was true in multilure traps (unpublished data).

The main purpose of this work was to determine the validity of the interaction of trap type and lure type observed in preliminary testing. AFF lures were compared with BioLures in multilure (Better World Manufacturing, Inc., Miami, FL) traps and on recently developed cylindrical sticky traps (Robacker and Rodriguez 2004). Emissions of attractive chemicals from

each type of lure were monitored as they aged during the 18-wk experiment. Effects of ammonia emissions and weather on lure attractiveness were assessed to attempt to explain changing trends in attractiveness of trap/lure combinations.

Materials and Methods

Insects and Handling Methods. Laboratory stock of *A. ludens* was started in 2000 from pupae collected from yellow chapote *Casimiroa greggii* S. Wats, a native host, from the Montemorelos area of Nuevo Leon in northeastern Mexico. Flies used in these experiments were reared on artificial medium and adults were held in 473-ml cardboard cartons with screen tops until released into the orchard. Flies were irradiated, due to quarantine laws, with 70–92 Grays (Cobalt 60) 1 to 2 d before adult eclosion. Flies were fed sugar and water until they were released in test plots 3–12 d after eclosion. Laboratory conditions where flies were housed were $22 \pm 2°C$, and $50 \pm 20\%$ RH with a photoperiod of 0630–1930 hours provided by fluorescent lights.

Lures and Traps. Two types of commercial synthetic food-odor lures were tested: the BioLure MFF lure (Suterra) used in the two-component version (ammonium acetate and putrescine) (hereafter called BioLure) recommended by Suterra for *Anastrepha* species (Anonymous 2003a); and the *Anastrepha* fruit fly (AFF) lure (Advanced Pheromone Technologies). Both lures were purchased just before testing and kept refrigerated until used.

Two types of traps were tested. The first was the multilure trap (Better World Manufacturing). This trap is a plastic McPhail-type trap with a clear plastic top that fits onto a yellow base containing liquid to drown trapped flies. The second trap was an experimental yellow, cylindrical, sticky trap that was 2.5 times more attractive than sticky panel traps in previous field tests (Robacker and Rodriguez 2004).

BioLures were deployed in multilure traps by adhering them on the inside wall of the plastic top. Individual plastic bags containing components of AFF lures were removed from their mesh bags, folded, and gently put into the lure basket of multilure traps so as not to damage the plastic bags. BioLures and AFF lures were deployed in the center of cylinder traps by suspending them from the trap hanger at the point where it attached to the trap.

Experimental Procedure. The experiment was conducted in a mixed citrus orchard located near the laboratory in Weslaco, TX. The orchard contained several varieties of oranges, lemons, and tangerines. One row of Valencia sweet oranges, *Citrus sinensis* (L.) Osbeck, and one row of Dancy tangerines (*Citrus reticulata* Blanco) were used for tests. Within each row, three linear blocks of six trees each were chosen with one buffer tree between blocks. All trees had ripe fruit initially, but most of the ripe fruits dropped from trees during spring as small green fruits grew.

Trap/lure combinations were multilure/BioLure, cylinder/BioLure, multilure/AFF lure, cylinder/AFF lure, multilure/unbaited, and cylinder/unbaited. All multilure traps contained 300 ml of 10% LowTox (Prestone Products Corp., Danbury, CT) antifreeze in water. LowTox is a propylene glycol-based antifreeze containing proprietary corrosion inhibitors. Lures and multilure traps were used for the duration of the experiment. Cylinder traps were replaced weekly. The antifreeze solution was replaced monthly. The six combinations were placed one to a tree, north of center, at 1- to 2-m height, in each linear block of six trees for a total of 36 traps in the orchard (six blocks by six trap/lure combinations). Positions of treatments within each block were randomized initially. A trial lasted 1 wk after which flies were counted and traps were serviced. Positions of treatments in consecutive weeks were not randomized but were moved sequentially within each block. Each week, ≈2,400 flies were distributed uniformly onto rows of trees adjacent to the test rows. Flies were released into the orchard one day after trap servicing. The duration of the experiment was 18 wk from 10 March to 14 July 2004.

Lure Emissions Measurements. Emissions from two lures of each type were monitored by gas chromatography (GC) during the experiment. These lures were kept in dry multilure traps several rows away from the trapping experiment. Lures were brought into the laboratory during one day each week for emissions testing. To collect emissions, a lure was put into a 650-ml polypropylene container at 30°C. Volatiles were sampled using solid phase microextraction (SPME) with a polydimethylsiloxane (PDMS)-coated fiber (100-μm coating) (Supelco, Inc., Bellefonte, PA). PDMS has been reported highly efficient for trapping amines (Bartelt 1997). The fiber was inserted into the headspace through a small hole drilled in the lid of the container. Sampling time was 1 h. On-column injection of volatiles was by thermal desorption from the PDMS fiber at 210°C in a 10-cm retention gap (0.53-mm i.d. deactivated fused-silica) connected to the analytical column by a GlasSeal connector (Supelco). The analytical column was a DB-1 (60 m, 0.32 mm i.d., 5-μm film) (J & W Scientific, Folsom, CA). Column oven temperature was 40°C for 5 min then programmed at 10°C/min to 100°C. Carrier gas was helium at a linear velocity of 27 cm/s. Analyses were conducted using a Shimadzu GC-17A (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a flame ionization detector. SPME and GC have been used successfully to collect and quantify ammonia and other chemicals in static air containers like those used in this work (Robacker et al. 2004).

Quantifications were conducted for ammonia, methylamine, acetic acid, and 1-pyrroline. Ammonia, methylamine, and acetic acid were quantified because they are principal attractive components of the lures according to manufacturer's specifications. 1-Pyrroline was quantified because it is attractive to Mexican fruit flies (Robacker et al. 2000), it enhances attractiveness of ammonia and methylamine in laboratory experiments (Robacker 2001) and AMPu in field tests (Robacker et al. 1997), and its presence has been demonstrated in AMPu-based lures (Robacker and

Bartelt 1996). Ammonia, methylamine, acetic acid, and 1-pyrroline were identified by comparison of their retention times with those of standards. GC peak areas were measured using Millennium 2010 Chromatography Manager software (Waters Corporation, Milford, MA). Peak areas were used to compare relative amounts of chemicals emitted by the two lure types as they aged. Absolute emissions were not determined.

Statistical Analyses. Analyses of variance were done on capture rates of males, females, and total flies. To stabilize variance, the numbers of flies captured in traps were transformed by square-root. The transformed data were subjected to analysis of variance (ANOVA) by using SuperANOVA (Abacus Concepts 1989). Means separations were conducted by Fisher's protected least significant difference (LSD) in the transformed scale. χ^2 tests of significance of binomial proportions were conducted to compare the overall percentages of females captured over the entire experiment for each trap/lure combination (Snedecor and Cochran 1967).

Emissions from lures were analyzed by regression of peak areas on test week by using SuperANOVA. Linear and exponential decay models were evaluated. The exponential decay model was converted to a log linear model for analysis by SuperANOVA.

Effects of ammonia emission and weather on performance of trap and lure types were also assessed by regression analyses. For these analyses, numbers of flies in individual traps each week were converted into percentages of the total flies captured in each block each week. This conversion was done to keep overall capture rates constant at 100% from block to block and week to week so that performances of individual trap/lure combinations could be evaluated relative to other trap/lure combinations without variability due to changes in actual capture rates.

Results

Overall Trap Captures. The results of the field test, summed over the 18-wk duration, are shown in Table 1. The multilure trap/BioLure combination captured the most males and females, followed by both trap types containing AFF lures, the cylinder trap/BioLure combination, and the two unbaited traps.

The analysis of percentages of females in traps showed a trend in which cylinder traps captured more females than multilure traps and traps containing BioLures captured more females than those with AFF lures (Table 1). The cylinder trap/BioLure combination captured a significantly higher percentage of females than any other trap and the cylinder trap/AFF lure combination captured a higher percentage of females than the multilure traps with either lure and the unbaited cylinder trap.

Changes in Capture Rate over Time. Although the multilure trap/BioLure combination captured the most flies overall, it did not capture the most flies at all times during the experiment (Fig. 1). The cylinder trap/AFF lure combination captured the most flies during the first 6 wk. The effect was statistically sig-

Table 1. Overall captures of male and female Mexican fruit flies by two types of traps baited with two types of synthetic food-odor lures

Trap/lure combination[a]	Males[b]	Females[b]	Total[b]	% females[c]
Cylinder/BioLure	3.9b	6.2b	10.4b	61.6c
Cylinder/AFF lure	8.5c	11.3c	20.7c	57.1b
Cylinder/unbaited	0.8a	0.8a	1.6a	48.2a
Multilure/BioLure	12.9e	15.3d	28.2d	54.4a
Multilure/AFF lure	9.0d	8.9c	18.0c	49.7a
Multilure/unbaited	1.0a	1.1a	2.1a	53.9ab

[a] Cylinder sticky trap (Robacker and Rodriguez 2004), multilure trap (Better World Manufacturing, Inc.), BioLure MFF2-component fruit fly lure (Suterra, LLC, Inc.), and AFF lure (AdvancedPheromone Technologies).
[b] Mean flies per trap. Means followed by the same letter are not significantly different at the 5% level by Fisher's protected LSD conducted in the square root scale.
[c] Total females captured divided by total flies (excluding flies of undetermined sex) captured during the 18-wk experiment. Means followed by the same letter are not significantly different at the 5% level by χ^2 tests of binomial proportions conducted on all pairs of percentages.

nificant only during the first 2 wk for females ($F = 13.2$; df = 5, 65; $P < 0.0001$) and total flies ($F = 13.5$; df = 5, 65; $P < 0.0001$). The cylinder trap/AFF lure combination captured very few flies during the last 6 wk. The multilure trap/BioLure combination captured the
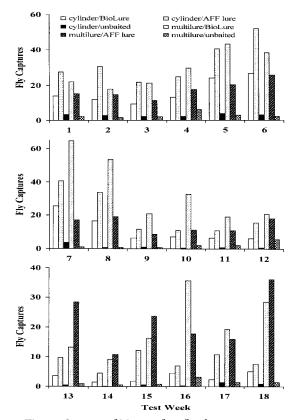

Fig. 1. Captures of Mexican fruit flies by two trap types baited with two lure types during an 18-wk experiment in a citrus orchard.
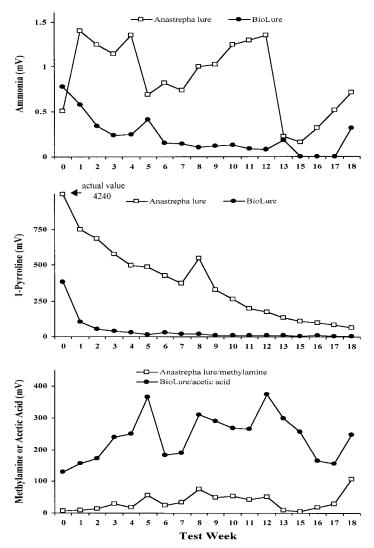
Fig. 2. Relative emissions of attractive chemicals from AFF lures and BioLures during 18 wk in a citrus orchard.

most flies during weeks 7–12 and was no worse than second best during the first and last 6 wk. The multilure trap/AFF lure combination captured the most flies during the last 6 wk, but the effect was significant only for males ($F = 41.5$; df $= 5, 205$; $P < 0.0001$). The multilure trap/AFF lure combination captured few flies during the first 11 wk. The cylinder trap/BioLure combination captured fewer flies than the other trap/lure combinations during most of the experiment.

Lure Emission Rates. Relative emission rates from lures held in dry multilure traps in the same orchard as the trapping experiment are shown in Fig. 2 (no data were recorded for week 14). Visual inspection suggested exponential decay functions for both 1-pyrroline curves and the BioLure ammonia curve. Therefore, data for these curves and the AFF lure ammonia curve were fitted to the model $Y = \beta_0 \beta_1^{-X}$, where Y is the area in millivolts, X is time in weeks, β_0 is the Y intercept, and β_1 is the regression coefficient. Re-

gressions were significant for all four curves (ammonia, AFF lure: $F = 6.2$, df $= 1, 34$, $R^2 = 0.15$, $P < 0.05$; ammonia, BioLure: $F = 34.3$, df $= 1, 34$, $R^2 = 0.50$, $P < 0.0001$; 1-pyrroline, AFF lure: $F = 214$, df $= 1, 34$, $R^2 = 0.86$, $P < 0.0001$; and 1-pyrroline, BioLure: $F = 130$, df $= 1, 34$, $R^2 = 0.79$, $P < 0.0001$).

Methylamine and acetic acid emissions did not decrease significantly during the experiment according to linear regression. Emissions of each increased significantly over the first 5 wk based on linear regression (methylamine: $F = 17.3$, df $= 1, 9$, $R^2 = 0.66$, $P < 0.01$; acetic acid: $F = 19.0$, df $= 1, 10$, $R^2 = 0.66$, $P < 0.01$).

Emissions of ammonia were much higher from AFF lures than from BioLures over most of the experiment but became more comparable during the last 6 wk. Ammonia emission from BioLures was not detected during weeks 15–17. We have no explanation for the periodic declines and recoveries in ammonia emission for the two lures. Mean ± SEM ammonia emissions
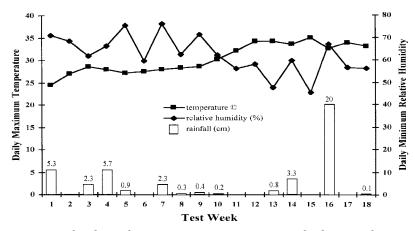
Fig. 3.   Weather during the trapping experiment in a citrus orchard near Weslaco, TX.

over the experiment were AFF lure, 0.88 ± 0.072 mV; and BioLure, 0.22 ± 0.036. These data show that AFF lures emitted 4 times more ammonia than BioLures. Note that the data are relative emissions (flame ionization detector response to the amount of chemical adsorbed onto the PDMS fiber in an hour), not absolute emissions such as micrograms per hour. Although emissions from AFF lures were more erratic from week to week, the relative standard error (SEM/ mean) was higher for the BioLures. This indicates that lure-to-lure variability was not responsible for the high weekly variability in AFF lure emissions of ammonia.

Emissions of 1-pyrroline were also much higher from AFF lures than from BioLures. Mean $\pm$ SEM 1-pyrroline emissions over the course of the experiment were AFF lure, 558 ± 164 mV; and BioLure, 42.6 ± 16.4. Emission of 1-pyrroline was detectable from both lures every week. These data show that AFF lures emitted >10 times as much 1-pyrroline as Bio-Lures.

Relative amounts of different chemicals emitted such as methylamine versus acetic acid, or ammonia versus 1-pyrroline, cannot be inferred from the data.

Weather. Effects of air temperature, relative humidity, and rain on performance of traps and lures were investigated by correlation of trap captures with daily maximum temperature, daily minimum relative humidity, and amount of rainfall. Figure 3 shows means of daily maximum temperatures and daily minimum relative humidities for the release day and the next 3 d of each test week, and rainfall for the period from 2 d before fly releases until 4 d after releases (including the release day for a total of 7 d). These periods were chosen because most fly captures occurred within three days after the day of a fly release. Rainfall before the release was included because its effects were usually evident in the orchard for several days (pooling, wet soil, and higher humidity).

Figure 3 shows that orchard temperatures generally increased from the beginning until the end of the experiment. Daily minimum relative humidity generally decreased during the experiment as daytime temperatures increased. Daily maximum relative hu-

midity was near 100% each morning (data not shown). Rain occurred frequently during the first 10 wk. The weather became hot and dry during most of the last 8 wk, but there were large rainfalls during weeks 14 and 16. Daily minimum relative humidity was higher during weeks with heavy rain.

Effects of Ammonia Emission and Weather on Trap and Lure Performance. Table 2 shows statistically significant correlations of trap/lure efficacies with changes in ammonia emissions, maximum daily temperatures, and daily minimum relative humidity. Efficacies of both cylinder trap/lure combinations were positively correlated with ammonia emission, whereas efficacies of both multilure trap/lure combinations were negatively correlated. Conversely, performance of both cylinder trap/lure combinations showed strong negative correlation with daily maximum temperature whereas the multilure/AFF lure trap showed a strong positive correlation. In the two instances in which efficacy was correlated with relative humidity, the correlations were opposite to those of temperature. No correlations with rainfall were significant. Maximum daily temperature was negatively correlated with daily minimum relative humidity ($F = 24.2$;

Table 2.   Correlations of captures of Mexican fruit flies with ammonia emission, daily maximum temperature, and daily minimum relative humidity

Trap/lure combination[a]	Factor	R	F	P
Cylinder/BioLure	Ammonia	0.62	10.3	<0.01
	Temp	−0.93	102	<0.0001
	RH	0.74	19.1	<0.001
Cylinder/AFF lure	Ammonia	0.50	5.3	<0.05
	Temp	−0.77	23.7	<0.001
Multilure/BioLure	Ammonia	−0.60	8.8	<0.01
Multilure/AFF lure	Ammonia	−0.55	7.1	<0.05
	Temp	0.81	29.9	<0.0001
	RH	−0.78	24.5	<0.0001

Captures defined as the percentage of flies captured in each trap/ lure combination each week.

[a] Cylinder sticky trap (Robacker and Rodriguez 2004), multilure trap (Better World Manufacturing, Inc.), BioLure MFF2-component fruit fly lure (Suterra, LLC, Inc.), and AFF lure (AdvancedPheromone Technologies).

df $= 1, 16; R = -0.78; P < 0.001$), and daily minimum relative humidity was correlated with rainfall ($F = 3.9$; df $= 1, 16; R = 0.44; P = 0.06$).

Inspection of Figs. 1 and 2 gave a second indication that ammonia emission was correlated with performance of the trap/lure combinations. The multilure trap/AFF lure combination became much more attractive during week 13 at the same time that emissions from AFF lures (in dry multilure traps in the test orchard) decreased markedly.

Discussion

BioLures were superior to AFF lures in multilure traps, but AFF lures were even more dominant over BioLures on sticky cylinder traps. Although this is the first report of field comparisons of these two lures, numerous unpublished experiments by the authors strongly support the current results. Also, previous studies in wind tunnels comparing BioLures and an agar-based formulation of AMPu have shown that AMPu is more attractive than BioLures when presented to flies in an open-air (not enclosed) format (Robacker 1998, 1999). In these experiments, the AMPu formulations were >2 times more attractive to sterile and fertile laboratory-strain and fertile wild Mexican fruit flies.

Reasons for the differential attractiveness of the two lures in multilure versus on sticky traps are unknown. We presented correlations of attractiveness with ammonia emission, daily maximum temperatures, and relative humidity in an attempt to explain the differences. However, these results are inconclusive in part because temperature and relative humidity are themselves correlated. Correlation of ammonia emission with weather factors could not be determined because emissions were measured in the laboratory where weather effects would be latent at best (lures were brought in from the field 1–4 h before emissions were measured). Based on unpublished research, we suggest that ammonia emission is very important in determining attractiveness of these two lures on open traps versus inside enclosed traps. In preliminary tests with AFF lures that emitted greater or smaller amounts of ammonia than those used in this work, effectiveness in multilure and similar wet traps consistently diminished at higher emission rates (unpublished data). Also, high concentrations of AMPu in aqueous solutions in McPhail traps were less attractive than lower concentrations (Robacker 1995). More work is needed to verify this hypothesis.

Negative correlation of daily maximum temperature with effectiveness of the sticky cylinder traps was also strong, but again the results are not conclusive. Superiority of McPhail-type traps containing water relative to sticky traps during hot dry weather (Heath et al. 1997) has been reported, but no direct evidence to prove the effect has been published.

This work showed that both types of lures are effective in the field for 3 to 4 mo. Suterra recommends that BioLures be replaced every 4–6 wk (Anonymous 2003b), whereas Advanced Pheromone Technologies recommends 8 wk for AFF lures. Only two of the 12 AFF lures (the two on cylinder traps in blocks 5 and 6) became ineffective during the experiment and that did not happen until after 3 mo. Percentages captured by these two cylinder traps with AFF lures dropped to nearly the same level (4.5%) as unbaited cylinders (1.1%) during the last 6 wk. Examination of these two lures indicated the lure bags were empty. None of the BioLures failed during the experiment.

Sticky cylinder traps baited with AFF lures outperformed multilure traps with BioLures during the first 6 wk of the experiment. Reasons for the early success of the cylinder trap/AFF lure combination followed by a decline in its effectiveness could not be ascertained. However, AFF lures were consistently superior to BioLures on cylinder traps (Fig. 1), sticky yellow panel traps (D.C.R., unpublished data), and yellow panel targets in wind tunnels (Robacker 1998, 1999). These results indicate that AFF lures would be the lure of choice on sticky traps just as BioLures would be the better choice in wet traps, based on the overall superior performance of the multilure trap/BioLure combination. Also, the data indicate that AFF lures would be the lure of choice with kill stations that usually consist of a pesticide-coated surface that acts as a visual target to flies approaching a lure.

Acknowledgment

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