

ATTRACTION OF WILD AND LABORATORY-STRAIN MEXICAN
FRUIT FLIES (DIPTERA: TEPHRITIDAE) TO TWO SYNTHETIC
LURES IN A WIND TUNNEL

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ABSTRACT

Attraction of laboratory-strain Mexican fruit flies, *Anastrepha ludens* (Loew), and wild-type flies to two synthetic lures was evaluated in a wind-tunnel. The lures were BioLure® (ammonium acetate and putrescine) and AMPu (ammonium carbonate, methylamine HCl, and putrescine). In one experiment, wild-type flies from the state

of Nuevo Leon, Mexico, were evaluated against laboratory-strain flies that originated in Nuevo Leon. Yellow panels containing AMPu attracted >2.5 times more females and >3.5 times more males of both fly strains than panels containing BioLure®. In another experiment, wild-type flies from the state of Chiapas, Mexico, were evaluated against the Nuevo Leon laboratory strain. Results of this experiment were similar to the first except the differences in attractiveness between AMPu and BioLure® to flies of both strains were less pronounced. The difference in relative attractiveness of AMPu and BioLure® in the two experiments was related to the time of year when the experiments were conducted rather than to inherent differences between the fly strains. In both experiments, BioLure® was about two times more attractive to females than to males whereas AMPu was only slightly more attractive to females. Both lures were more attractive to laboratory-strain flies than to wild-type flies from either region of Mexico.

Key Words: *Anastrepha ludens*, attractants, ammonia, ammonium acetate, putrescine, methylamine

RESUMEN

La atracción de las moscas mexicanas de la fruta (*Anastrepha ludens* [Loew]) silvestres y unas de laboratorio a dos cebos atrayentes sintéticos fue evaluada en un túnel de viento. Los cebos utilizados fueron BioLure® (acetato de amonía y putrecina) y AMPu (carbonato de amonía, hidrócloro de metilamina, y putrecina). En un experimento, moscas silvestres del estado de Nuevo León, México, fueron evaluadas en comparación con moscas del laboratorio criadas originalmente de moscas que se obtuvieron en Nuevo León. Paneles amarillos con AMPu atrajeron >2.5 de veces más de moscas hembra y > 3.5 de veces más de moscas macho de las dos líneas de moscas que paneles con BioLure®. En otro experimento, moscas silvestres del estado de Chiapas, México, fueron evaluadas en comparación con las del laboratorio de origen de Nuevo León. Los resultados de este experimento fueron similares al del primero, con la excepción de que las diferencias de atracción entre AMPu y BioLure® para las moscas de las dos cepas fue menos pronunciada. En los experimentos, la diferencia de la atracción relativa de AMPu y BioLure® estuvo más relacionada a la época del año en que los experimentos se llevaron a cabo más que a diferencias inherentes entre las dos líneas de moscas. En los dos experimentos, BioLure® fue aproximadamente dos veces más atractivo para las hembras que para los machos, mientras que AMPu fue ligeramente más atractivo para las hembras. Los dos cebos fueron más atractivos para las moscas de la línea del laboratorio que para las silvestres de cualquiera de las dos regiones de México.

Robacker & Warfield (1993) developed and Robacker (1995) modified an attractant for the Mexican fruit fly, *Anastrepha ludens* (Loew), called AMPu consisting of ammonium carbonate, methylamine HCl and putrescine. Later, Heath et al. (1995) developed an attractant for the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann), consisting of ammonium acetate and putrescine. The latter attractant, commercially available as BioLure®, was improved by addition of trimethylamine (Heath et al. 1997). Field tests showed that the original two-component BioLure® also attracted the Mexican fruit fly (Heath et al. 1995) and that addition of trimethylamine did not affect attractiveness to the Mexican fruit fly (Heath et al. 1997).

Wind-tunnel bioassays demonstrated that AMPu was about two times more attractive than BioLure® to laboratory-strain Mexican fruit flies in experiments designed to test effects of hunger and gamma irradiation of flies on their responses to

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the two lures (Robacker 1998). One-day experiments conducted in citrus orchards in south Texas with fresh AMPu lures each day and BioLure® lures ranging in age from 0-24 days confirmed the wind-tunnel bioassays. In these field tests in which the lures were tested on sticky traps, AMPu was about 2.5 times more attractive than BioLure® to sterile, laboratory-strain Mexican fruit flies.

The purpose of this research was to test the hypothesis that wild-type Mexican fruit flies, or flies that originated from different populations, may not respond to these two lures in the same way as the laboratory-strain flies. Wild-type flies from both northeastern and southern Mexico were tested against the laboratory strain. The experiments were conducted using the wind-tunnel bioassay described in Robacker (1998).

MATERIALS AND METHODS

Insects and Laboratory Conditions

Laboratory-strain flies were from a culture that originated from fruit of chapote amarillo, *Sargentia greggii* S. Wats., a native host of the Mexican fruit fly, collected in Nuevo Leon, Mexico, in 1987. The culture has been maintained on laboratory diet since establishment. Wild-type flies were from the Montemorelos area of Nuevo Leon in northeastern Mexico and the Tapachula area of Chiapas in southern Mexico. Nuevo Leon flies were obtained from larvae that egressed from either grapefruit or chapote amarillo collected during the spring of 1997. Chiapas flies were obtained from larvae that egressed from either sweet or sour orange collected during the winters of 1997 and 1998. Adult flies were maintained on sugar and water (provided separately) because previous work showed that this feeding regime maximized responses to both types of lures used in this work (Robacker 1998). Laboratory conditions for holding flies were $22 \pm 2^\circ\text{C}$, $50 \pm 20\%$ relative humidity and photophase from 0630 to 1930 h provided by fluorescent lights.

Lures

BioLure® lures were obtained from Consep, Inc. (Bend, Oregon). They consisted of an ammonium acetate packet and a putrescine packet. The 2 packets were taped together with their membrane openings unobstructed and separated from each other for use in the wind tunnel bioassay. BioLure® lures were used each day for 5 days following removal from refrigeration. The BioLure® lures produce stable emissions of their components for at least 4 weeks in the laboratory (Heath et al. 1995). Laboratory tests to measure emissions of BioLure® components indicated that the lures emit (at laboratory temperature 23°C) about $300 \mu\text{g/h}$ of ammonia (Heath et al. 1997). Emission of acetic acid is probably about $3\text{-}12 \mu\text{g/h}$. These rates for acetic acid were reported by Heath et al. (1995) for a similar ammonium acetate packet (Consep, Inc.) that emitted $100\text{-}500 \mu\text{g/h}$ of ammonia. Putrescine emission has not been determined for BioLure®.

AMPu was used in an agar formulation in 1.9 ml polypropylene microcentrifuge tubes (A. Daigger & Company, Inc., Wheeling, Illinois) (Robacker 1995, 1998). AMPu/agar lures were prepared by mixing equal volumes of hot agar solution (Bacto Agar, Difco Laboratories, Detroit, Michigan) and aqueous AMPu containing 120, 200, and 20 mg/ml, respectively, of ammonium carbonate (ACS Reagent quality, Aldrich Chemical Co., Inc., Milwaukee, Wisconsin), methylamine hydrochloride (99%, Sigma Chemical Co., St. Louis, Missouri) and putrescine (98%, Aldrich). Final concentrations in

AMPu/agar tubes were 60, 100, and 10 mg/ml of the three chemicals and 1% agar in a final volume of 1.7 ml. The pH of the AMPu/agar formulation was 8.7-8.9. AMPu tubes were capped and stored in a refrigerator. They were used in tests for 1 day after removal from refrigeration. Lures were discarded after 1 day because this agar formulation was developed only for short-term delivery of the AMPu components. Previous laboratory tests to measure emissions of AMPu components indicated that these lures emit (at 35°C) about 300 µg/h of ammonia, 40 µg/h of methylamine, 17 ng/h of putrescine, and 20 ng/h of 1-pyrroline, a chemical that forms spontaneously in the lures (Robacker and Bartelt 1996).

WIND-TUNNEL BIOASSAY

Bioassays were conducted in a plexiglass wind tunnel with dimensions 0.3 × 0.3 × 1.2 m. Wind tunnels of similar dimensions have been used successfully for bioassays of fruit fly semiochemicals (Landolt et al. 1992, Epsky et al. 1997). The bioassay method used for this work was modeled after that used by Landolt et al. (1992) for the Mediterranean fruit fly and was used previously with the Mexican fruit fly (Robacker 1998).

Each end of the wind tunnel was screened to allow airflow. The downwind end contained a baffle system to create a uniform airflow through the chamber. Air was pulled through the chamber at 0.4 m/sec by an exhaust fan connected to the downwind end. Air leaving the chamber was vented from the room by a ceiling exhaust fan. The top of the chamber had 2 circular service openings (12.8 cm diam) with plexiglass covers, one located near each end of the chamber to allow easy access to the chamber interior. A 100 W "soft white" light bulb (General Electric Co., Cleveland, Ohio) in a reflecting lamp was positioned 17 cm above the downwind end of the chamber. The purpose of this light was to hold flies non-responsive to lure odors in the downwind end by positive phototaxis and thus minimize random flying into the upwind end of the chamber. Overhead lighting was provided by 2 banks of 4 fluorescent "cool white" lights each (F40CW, General Electric).

For each bioassay one AMPu tube or BioLure® lure was attached to the side of a yellow plastic panel (10 by 13 cm). The panel was suspended in a fixed position from the service opening at the upwind end of the chamber so as to provide a broad visual stimulus to responding flies downwind and with the lure on the upwind side of the panel away from responding flies. In this configuration the panel was 21 cm away from the upwind end and nearly in the center of the air stream (midway between top and bottom and the sides).

Flies were introduced one at a time into the downwind end of the chamber in clear plastic vials (7 cm by 3 cm diam) placed on top of a beaker on the bottom of the chamber directly below the downwind service opening. In this configuration, the top of the vial (where the fly would emerge) was located in the center of the air stream. Each fly was allowed 5 min to leave the vial (fly or walk off of the vial). Flies that did not leave in 5 min were not included in the data. Once a fly left the vial, the fly was allowed 5 min to fly or walk upwind or contact the panel. Upwind movement was scored if flies passed a point 2/3 of the distance from the release vial to the panel.

As a control, each test of a wild fly was followed by a test of attraction of a laboratory-strain fly to the same lure. Also, tests were conducted in two identical chambers. One chamber was used for AMPu and the other for BioLure® for a series of 40-50 tests. Chambers then were washed with soapy water (all test chemicals are water soluble) and the lures were tested in the other chambers for a series. Comparison of data obtained in the two chambers indicated that fly responses were not affected by chamber.

Statistical Analyses

Effects of fly type, sex, lure type, time of year when bioassays were conducted, and various factor interactions on responses of flies in the wind-tunnel bioassay were tested by Chi-square using the Loglinear Model procedure of SYSTAT 7.0 (SYSTAT 1997). The effect of fly age on response was tested using option Cochran (Cochran's test of linear trend) of the XTAB procedure of SYSTAT 7.0.

RESULTS AND DISCUSSION

Wild-Type vs Laboratory-Strain Flies from Nuevo Leon

Loglinear models containing the factors fly type, sex, and lure type and all interactions of these factors with response (response or not) were constructed. Pearson χ^2 was not significant for either upwind movement ($\chi^2 = 6.4$; $df = 5$; $P = 0.27$) or contact with panels ($\chi^2 = 3.9$; $df = 5$; $P = 0.57$) indicating that the complete models fit the observed response frequencies.

Fly type had significant effects in models for both upwind movement and contact with panels. Wild-type flies from Nuevo Leon moved upwind ($\chi^2 = 163.6$ for both lures combined; $df = 1$; $P < 0.001$) and contacted ($\chi^2 = 175.3$ for both lures combined; $df = 1$; $P < 0.001$) panels at much lower rates than laboratory-strain flies that originated from Nuevo Leon (Table 1). Wild-type flies whose larvae had egressed from chapote amarillo did not respond differently to the lures than flies from grapefruit.

Sex of flies had little effect on upwind movement of either strain toward either lure or on contact with panels with AMPu. Females of both strains contacted panels with BioLure® at higher rates than did males ($\chi^2 = 4.9$ from a reduced model containing data for BioLure® only; $df = 1$; $P < 0.05$). Although sex had less effect on responses to AMPu than to BioLure®, the interaction of sex and lure type was not significant.

Lure type also had significant effects. More flies of both strains moved upwind ($\chi^2 = 45.9$ for both strains combined; $df = 1$; $P < 0.001$) and contacted ($\chi^2 = 57.9$ for both strains combined; $df = 1$; $P < 0.001$) panels with AMPu than panels with BioLure®. Lure type also had significant effects when only wild-type flies were included in the analysis. More wild-type flies moved upwind ($\chi^2 = 13.0$; $df = 1$; $P < 0.001$) and con-

TABLE 1. UPWIND MOVEMENT AND CONTACT WITH PANELS WITH SYNTHETIC LURES IN A WIND TUNNEL BY LABORATORY-STRAIN AND WILD-TYPE MEXICAN FRUIT FLIES, BOTH STRAINS ORIGINATING FROM NUEVO LEON, MEXICO.

		Upwind Movement		Contact with Source	
		BioLure®	AMPu	BioLure®	AMPu
laboratory strain	Males	22.8	47.1	9.8	36.6
	Females	27.7	51.1	17.5	44.9
wild-type	Males	5.2	15.7	0.5	5.2
	Females	5.6	10.2	1.7	4.5

Values are percentages with n's per cell: male flies with BioLure®, 193; females with BioLure®, 177; males with AMPu, 191; females with AMPu, 176.

tacted ($\chi^2 = 9.3$; $df = 1$; $P < 0.01$) panels with AMPu than panels with BioLure®. Relative responses of wild-type flies and laboratory-strain flies to the two lures differed as indicated by significant interactions of lure type by fly type for both upwind movement ($\chi^2 = 3.9$; $df = 1$; $P < 0.05$) and contact with panels ($\chi^2 = 7.2$; $df = 1$; $P < 0.01$). These interactions indicated that responses to BioLure® by wild-type flies were slightly less than expected based on main effects of lure type and fly type.

Wild-Type Flies from Chiapas vs Laboratory-Strain Flies

Loglinear models like those used to analyze the Nuevo Leon fly responses were constructed. Pearson χ^2 was not significant for either upwind movement ($\chi^2 = 3.0$; $df = 5$; $P = 0.70$) or contact with panels ($\chi^2 = 1.8$; $df = 5$; $P = 0.88$).

Wild-type flies from Chiapas moved upwind ($\chi^2 = 16.4$ for both lures combined; $df = 1$; $P < 0.001$) and contacted ($\chi^2 = 33.0$ for both lures combined; $df = 1$; $P < 0.001$) panels with lures at lower rates than the laboratory-strain flies that originated in Nuevo Leon (Table 2). Effects of larval host of the wild-type flies on responses of the flies to the lures could not be determined because the pupae from the two hosts were mixed together before eclosion.

Females of both strains moved upwind ($\chi^2 = 10.5$ for both strains combined; $df = 1$; $P < 0.01$) and contacted ($\chi^2 = 16.3$ for both strains combined; $df = 1$; $P < 0.001$) panels with lures at higher rates than did males. As with wild-type flies from Nuevo Leon, the effect was more pronounced for response to BioLure® than for response to AMPu although interactions of sex and lure were not significant for either upwind movement or contact with panels.

More flies of both strains moved upwind ($\chi^2 = 10.2$ for both strains combined; $df = 1$; $P < 0.01$) and contacted ($\chi^2 = 13.1$ for both strains combined; $df = 1$; $P < 0.001$) panels with AMPu than panels with BioLure® AMPu was not significantly more attractive than BioLure® when only wild-type flies were included in the analysis. Interactions of fly type with lure type were not significant for either upwind movement or contact with panels.

TABLE 2. UPWIND MOVEMENT AND CONTACT WITH PANELS WITH SYNTHETIC LURES IN A WIND TUNNEL BY WILD-TYPE MEXICAN FRUIT FLIES FROM CHIAPAS, MEXICO, AND BY LABORATORY-STRAIN FLIES ORIGINATING FROM NUEVO LEON, MEXICO.

		Upwind Movement		Contact with Source	
		BioLure®	AMPu	BioLure®	AMPu
laboratory strain	Males	15.6	23.8	4.8	17.0
	Females	18.3	29.8	12.5	22.3
wild-type flies	Males	10.2	12.2	2.0	4.1
	Females	9.2	18.2	4.2	5.8

Values are percentages with n's per cell: male flies, 147; females with BioLure®, 120; females with AMPu, 121.

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Effect of Fly Sex

Data from the current work and from Robacker (1998) indicated that BioLure® was much more attractive to females than to males (females/males = 1.2 to 6.1 in various experiments). Corresponding ratios for attraction to AMPu were smaller (females/males = 1.1 to 2.8). I conclude that AMPu and BioLure® differ in their propensities to attract males and females.

Effect of Fly Age

Bioassays were conducted with wild-type flies from both Nuevo Leon and Chiapas ranging in age from 3-60 days post eclosion. The relationship between age of flies and their responses to the two lures was investigated.

Table 3 shows the percentages of wild-type flies of 3 age groups that contacted panels with lures. The percentage of males responding to BioLure® appeared to increase with age of flies. Cochran's test of linear trend indicated the effect was not significant.

The percentages of older males (>18 days) that responded to both lures were higher than the percentages of 3-7 day old males that responded whereas the opposite was true for females (Table 3). A loglinear model containing the factors age and sex and the interaction of age, sex, and response (contact or not) was constructed to test whether age affected responses of males and females differently. Pearson χ^2 was not significant ($\chi^2 = 3.5$; $df = 4$; $P = 0.48$). The interaction of sex and age was significant ($\chi^2 = 4.3$; $df = 1$; $P < 0.05$) indicating that age affected responses of the sexes differently.

More flies contacted panels with AMPu than those with BioLure® at all fly ages (Table 3). Relative attractiveness of the lures at different fly ages was not significantly different.

Previous work showed that age of laboratory-strain flies between 6 and 17 days old had little effect on attraction of the flies to AMPu and BioLure® (Robacker 1998). The analysis conducted here indicates that age also did not have great effects on responses of wild-type flies to these two lures. I do not conclude that age would have little or no effect on responses to these lures or similar semiochemicals in nature where conditions differ.

TABLE 3. CONTACTS WITH PANELS WITH SYNTHETIC LURES IN A WIND TUNNEL BY WILD-TYPE FLIES (NUEVO LEON AND CHIAPAS FLIES COMBINED) OF DIFFERENT AGES.

		3-7 days	8-18 days	>18 days
males	BioLure®	0.0	0.6	2.0
	AMPu	3.0	5.2	4.8
	BioLure®	5.1	1.4	3.4
females	AMPu	5.4	5.5	4.2

Values are percentages with n's per cell: 0-7 days old, 33-39; 8-18 days old, 144-155; males >18 days old, 145-148; females >18 days old, 118-119.

Effect of Time of Year

AMPu was much more attractive than BioLure® to wild-type flies from Nuevo Leon but only slightly more attractive than BioLure® to wild-type flies from Chiapas. The following analysis indicates that this difference is not due to inherent differences between flies from Nuevo Leon and Chiapas, but more likely is due to the time of year when the bioassays were conducted.

Bioassays with wild-type flies from Nuevo Leon were conducted from June to September, 1997. Bioassays with wild-type flies from Chiapas were conducted during April and May, 1997, and during March and April, 1998. During each experiment, bioassays with laboratory-strain flies were conducted as controls. Laboratory-strain flies tested during June to September along with wild-type flies from Nuevo Leon responded like the wild-type flies in that their responses to AMPu were much higher than to BioLure® (Table 1). Likewise, laboratory-strain flies tested during March to May along with wild-type flies from Chiapas responded like the wild-type flies in that their responses to AMPu were only slightly higher than to BioLure® (Table 2). Thus, responses of wild-type flies from the two regions of Mexico were similar to responses of the laboratory-strain flies in each experiment.

Because the laboratory strain was the same in both experiments, the effect of time of year on responses of the laboratory strain was analyzed to determine if time of year may have affected the relative attractiveness of AMPu and BioLure® to the two wild-type strains. Table 4 shows responses in wind-tunnel bioassays of 4-17 day old, sugarfed, protein-deprived laboratory-strain flies to the two lures during spring (February-May) and summer (June-September). Table 4 includes data from the current work and from experiments conducted previously to test effects of food deprivation and gamma irradiation (Robacker 1998).

AMPu was about four times more attractive than BioLure® to males during both test periods (Table 4). However, the relative responses of females to AMPu and BioLure® changed. AMPu was only about 30% more attractive than BioLure® during spring bioassays but 2.5 times more attractive during summer bioassays.

A loglinear model containing the factors lure and time of year and all interactions of these factors with response (response or not) was constructed to test the hypothesis that laboratory-strain females responded differentially to the 2 lures at different times of the year. Pearson χ^2 was not significant ($\chi^2 = 0.9$; $df = 2$; $P = 0.64$). The interaction of lure and time of year was significant ($\chi^2 = 5.9$; $df = 1$; $P < 0.05$) indicating that

TABLE 4. CONTACTS WITH PANELS WITH SYNTHETIC LURES IN A WIND TUNNEL BY LABORATORY-STRAIN MEXICAN FRUIT FLIES AT 2 TIMES OF THE YEAR.

		BioLure®	AMPu
males	spring	7.0	27.8
	summer	8.9	39.0
females	spring	20.0	26.4
	summer	16.9	43.6

Values are percentages with n's per cell: male flies during spring, 227-229; males during summer, 210-213; females during spring, 200-201; females during summer, 195.

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the relative attractiveness to laboratory-strain females of AMPu compared with BioLure® changed from spring to summer. This analysis indicates that the difference in relative attractiveness of AMPu and BioLure® to wild-type females from Nuevo Leon compared with those from Chiapas probably is not due to inherent differences between flies from Nuevo Leon and Chiapas. The factors that caused the time-of-year effect are not known but they exerted their effects equally on laboratory-strain and wild-type flies.

Wind-Tunnel Responses in the Absence of Lures

Control bioassays were not conducted to determine how many wild-type flies would move upwind and contact panels when no lures were present. Previous research with laboratory-strain flies in this bioassay system showed that about 9% of males and 4% of females moved upwind and 0.6% of both males and females contacted panels that did not contain lures (Robacker 1998). Responses by wild-type females but not males to BioLure® were higher than responses by laboratory-strain flies when no lures were present (Tables 1 & 2), however, it was not possible to determine from this comparison if wild-type females actually responded to BioLure®. Positive responses to AMPu can be inferred because responses of wild-type flies (Nuevo Leon & Chiapas combined) to AMPu were significantly greater than responses to BioLure® ($\chi^2 = 8.3$ for upwind movements by males, $df = 1, P < 0.01$; $\chi^2 = 7.5$ for contacts by males, $df = 1, P < 0.01$; $\chi^2 = 6.6$ for upwind movements by females, $df = 1, P = 0.01$; $\chi^2 = 2.2$ for contacts by females, $df = 1, P = 0.14$).

Implications for Attractants Research

Laboratory-strain flies (not irradiated) responded to the lures at much higher rates (21.5%) (summed over both lures and both experiments) than wild-type flies (3.4%) in this work, and nonirradiated flies responded at higher rates (29.4%) than irradiated flies (14.4%) in previous work (Robacker 1998). However, attractiveness of the 2 lures relative to each other varied little with fly type: i.e. AMPu/BioLure® response (contacts) ratios were similar for wild-type flies (2.6) (summed over Nuevo Leon and Chiapas flies) vs laboratory-strain control flies from the experiments with wild flies (2.8) in this work, and for irradiated (2.8) vs nonirradiated laboratory-strain flies (2.4) in previous work. These data indicate that semiochemical research conducted with laboratory-strain Mexican fruit flies, irradiated or nonirradiated, should not differ qualitatively from that done with wild-type flies.

As discussed above, laboratory-strain flies responded quantitatively much differently than wild-type flies to both lures. It is not known if this result is due to morphological or physiological differences in neural apparatus between the fly types, or to differences in the way each fly type reacts to laboratory conditions.

Implications for Trapping Programs

Results in this work and in Robacker (1998) in which AMPu was consistently more attractive than BioLure® to both laboratory and wild-type Mexican fruit flies suggest that AMPu should be a better lure than BioLure® in trapping programs. However, other factors such as local climatic conditions and the type of trap used with the lures could have profound effects on the relative attractiveness of the lures. Thus, results of these laboratory bioassays and even the field experiments conducted with irradiated flies in south Texas (Robacker 1998) may be poor predictors of field-trapping experiments with wild flies in localities other than Texas.

Direct field comparisons of the two lures in populations of wild flies have not been conducted because a formulation of AMPu proven to function properly for more than 2 days in the field has not been developed. Obviously, a long-lasting formulation is needed before AMPu could even be considered for use in trapping programs.

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