

ATTRACTIVENESS OF A MIXTURE OF AMMONIA,
METHYLAMINE AND PUTRESCINE TO MEXICAN FRUIT
FLIES (DIPTERA: TEPHRITIDAE) IN A CITRUS ORCHARD

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ABSTRACT

A mixture of ammonium bicarbonate or ammonium carbonate, methylamine HCl and putrescine (AMPu) was evaluated for attractiveness to gamma-irradiated Mexican fruit flies, *Anastrepha ludens* (Loew), in a citrus orchard in 1-day tests. AMPu (10:10:1 mixture of ammonium bicarbonate:methylamine HCl:putrescine) was tested both in dilute aqueous solutions in the reservoir of McPhail traps and in more concentrated form in polypropylene tubes suspended in McPhail traps or fastened to yellow sticky ball traps. The most attractive concentration of AMPu used in aqueous solution captured only half as many flies as *Torula* yeast in McPhail traps. AMPu (6:10:1 mixture of ammonium carbonate:methylamine HCl:putrescine) formulated into agar and tested in tubes fastened to sticky ball traps captured as many male and female flies as *Torula* yeast in McPhail traps.

Key Words: *Anastrepha ludens*, trapping, lures, amines.

RESUMEN

Fue evaluada la atractividad de una mezcla de bicarbonato de amonio o carbonato de amonio, metilamina HCl y putrescina (AMPu) sobre moscas mexicanas de la fruta gamma-irradiadas, *Anastrepha ludens* (Loew), en un campo de cítricos en pruebas de un día de duración. La mezcla AMPu (mezcla de 10:10:1 de bicarbonato de amonio:metilamina HCl:putrescina) fue probada en soluciones acuosas diluidas en el reservorio de trampas de McPhail y en forma más concentrada en tubos de polipropileno suspendidos en trampas de McPhail o atados a trampas pegajosas de bolas amarillas. La concentración más atractiva de AMPu, usada en solución acuosa, capturó solamente la mitad de las moscas capturadas con levadura *Torula* en las trampas de McPhail. La mezcla AMPu (mezcla de 6:10:1, de carbonato de amonio:metilamina HCl:putrescina) formulada en agar y probada en tubos atados a trampas pegajosas de bola capturó tantos machos y hembras como la levadura *Torula* en las trampas de McPhail.

Recently, I reported on development of a three-component attractant (AMPu) for the Mexican fruit fly (*Anastrepha ludens* (Loew)) containing metabolites from biological degradation of amino acids (Robacker & Warfield 1993). AMPu was more attractive than *Torula* yeast, the most commonly used bait for Mexican fruit fly, in flight chamber tests in a greenhouse. This research was analogous to work by Wakabayashi & Cunningham (1991) with the melon fly (*Bactrocera cucurbitae* (Coquillett)). More recently, Heath et al. (1995) have reported on a similar attractant for the Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)) and the Mexican fruit fly.

The purpose of the current research was to evaluate the initial attractiveness of AMPu compared to *Torula* yeast in a citrus orchard. Various concentrations of AMPu

were tested in McPhail traps and yellow sticky ball traps. The ultimate goal of this research is to develop an attractant that can be used with a dry trap to replace the McPhail trap/Torula yeast trapping system.

MATERIALS AND METHODS

Insects

The test flies originated from a culture from Morelos, Mexico, that had been maintained on laboratory diet for about 400 generations with no wild-fly introductions. A recent study has shown that the mating behavior of this culture has changed only slightly from that of wild flies (Robacker et al. 1991). Likewise, I presume that the response of laboratory-reared flies to food-based attractants does not differ markedly from that of wild flies.

Flies were irradiated with 7000-9200 rads (Cobalt 60 source) 1-2 days before adult eclosion, to comply with quarantine laws for releasing *A. ludens*. Irradiated flies were shown to be 20% less responsive than unirradiated flies to bacterial odor (Robacker & Garcia 1993), the attractant on which development of AMPu was partially based.

Mixed-sex groups of 180-200 flies were kept in 473 ml cardboard cartons with screen tops until used in tests. Laboratory conditions for holding flies were $22 \pm 2^\circ\text{C}$, $50 \pm 20\%$ relative humidity and photophase from 0630 to 1930 hours provided by fluorescent lights. Flies were fed sucrose and water up until the time of release.

Citrus Orchard and Test Procedures

A mixed citrus orchard located near the laboratory in Weslaco, Texas, was used for all experiments. The orchard contained several varieties of orange, lemon, grapefruit and tangerine trees of varying ages. One row of ruby red grapefruit (*Citrus paradisi*) and one row of Dancy tangerine (*C. reticulata*) were chosen for tests since they contained relatively large (2-3 m height) fruit-bearing trees. Two blocks of seven consecutive trees were used in each row, for a total of four blocks in the orchard.

Flies were released into the test orchard when 2-10 days old during the late afternoon of the day before a test. Robacker & Garcia (1993) showed that, within this age range, fly age had little effect on attraction to bacterial odor, an attractant similar to AMPu as discussed above. Approximately 2000 flies were distributed equally among the 28 test trees in the four blocks. Attractants were tested either in McPhail traps (Baker et al. 1944) or on sticky yellow ball traps (Robacker 1992) that were hung one to a tree, north of center, at 1-2 m height. Traps were placed in the orchard during the morning and removed for fly counts and cleaning on the following morning. Positions of treatments within each block of trees were randomized for the first 1-day test of most experiments. Positions of treatments in consecutive 1-day tests were not randomized but were moved sequentially within each block.

Experiments 1 and 2 were conducted to determine the most attractive concentration of AMPu in aqueous solution in McPhail traps. The AMPu tested was an aqueous solution of ammonium bicarbonate, methylamine HCl, and putrescine in the ratio 10:10:1 at pH 8.8 (adjusted with NaOH). The three chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and were >98% pure.

Experiment 1 evaluated three concentrations of AMPu. AMPu 2000 was prepared with the three chemicals at 2:2:0.2 mg/ml. AMPu 200 and AMPu 20 were 1:10 and 1:100 dilutions of AMPu 2000. Each AMPu trap contained 200 ml of one of these solutions in the trap's liquid reservoir. Traps baited with three Torula yeast/borax "bait

pellets" (Sit-Khem Corp., Michigan City, IN) and 200 ml of tap water were included in this and in Experiments 2 & 5 as attractiveness standards. Traps containing only 200 ml of water were used as blanks. All traps except Torula yeast contained amber coloring from a combination of red, yellow and green food colors (McCormick & Co., Inc., Baltimore, MD) to mimic the color of Torula yeast. These traps also contained 0.01% Triton® (Rohm and Haas Co., Philadelphia, PA) as a wetting agent. One each of the three AMPu traps, one Torula yeast trap and one water-blank trap were included in each of the four blocks of trees. Five 1-day tests were conducted for a total of 20 replications of each bait treatment. Baits were discarded after each 1-day test.

Experiment 2 compared AMPu 20 and AMPu 200 to three additional concentrations: AMPu 400; AMPu 100; and AMPu 50. The additional concentrations were 1:5, 1:20 and 1:40 dilutions, respectively, of AMPu 2000. Experiment 2 was identical to Experiment 1 except that each block contained five AMPu treatments instead of three. Seven 1-day tests were conducted for a total of 28 replications of each bait treatment.

Experiment 3 was conducted to develop a formulation of AMPu that could be used on a dry sticky trap. For this purpose, 1.9 ml polypropylene microcentrifuge tubes (A. Daigger & Company, Inc., Wheeling, IL) were used to hold various AMPu preparations. The objective was to determine how much AMPu was needed in the tubes to make them competitive with the best concentration used in McPhail traps.

AMPu was prepared in water at the concentration of 20:20:2 mg/ml at pH 8.8. Amounts ranging from 1.0-1.6 ml of this solution or various dilutions of the solution ranging down to 2:2:0.2 mg/ml were put into microcentrifuge tubes. Concentrations greater than 20:20:2 mg/ml were not used because all indications at the time this experiment was conducted were that greater concentrations would be less attractive. Tubes were suspended inside McPhail traps just above the inner rim and traps were filled with 200 ml of amber-colored water as described above. These traps were tested against McPhail traps containing 200 ml of AMPu 200 in a flight chamber in a greenhouse as described by Robacker & Warfield (1993). AMPu 200 was used as the standard because of its great attractiveness in previous flight chamber tests (Robacker & Warfield 1993). Briefly, the procedure was as follows. For each test, a trap containing AMPu 200 and a trap containing an AMPu tube were suspended side by side in the upwind end of the screened chamber (2.0 m long × 0.7 m wide × 1.3 m high) with an airflow of 0.1 to 1.0 m/sec. Traps were alternated between left and right sides of the chamber every 15 min for a 1-h test. Approximately 200 sugar-fed, protein-starved flies were released into the downwind end of the chamber at the beginning of each test. Four to 15 replications were conducted for each of seven concentrations tested in the microcentrifuge tubes.

Experiment 4 evaluated AMPu tubes *vs* AMPu 400 in McPhail traps in the field. The purpose of this experiment was to determine if the optimum formulation of AMPu in microcentrifuge tubes that was developed in the greenhouse flight chamber would be equivalent to AMPu 400 in McPhail traps in the field. AMPu 400 was used as the standard because this was the most attractive concentration in previous field tests (Experiment 2). AMPu tubes contained 1.6 ml of AMPu at the concentration of 20:20:2 mg/ml at pH 8.8. Three traps of each type were placed alternately on six trees in each of the four blocks of trees used in previous tests. Two 1-day tests were conducted for a total of 24 replications of each bait treatment.

Experiment 5 evaluated AMPu tubes on ball traps *vs* Torula yeast in McPhail traps in the field. Initially, AMPu microcentrifuge tubes were prepared containing 1.6 ml of AMPu at the concentration of 20:20:2 mg/ml at pH 8.8, the formulation that proved equal in attractiveness to AMPu 200 in greenhouse tests (Experiment 3) and AMPu 400 in field tests (Experiment 4). Subsequently, AMPu solutions were mixed

with agar to increase durability of the AMPu tubes exposed to weather on tops of sticky traps. Concentrations of AMPu used in agar preparations were higher than 20:20:2 mg/ml because the initial tests with aqueous formulations indicated that 20:20:2 mg/ml was too low. Final AMPu concentrations in agar tubes ranged from 100:100:10 to 225:225:22.5 mg/ml of the three components at pH 8.5 to 8.8. Ammonium carbonate (A.C.S. Reagent quality; Aldrich Chemical Co. Inc., Milwaukee, WI) was substituted for ammonium bicarbonate in some preparations, keeping the molar concentration of ammonia equivalent to previous ammonium bicarbonate preparations. Agar (Bacto Agar, Difco Laboratories, Detroit, MI) concentrations ranged between 1-3% in final preparations. The AMPu/agar tubes were prepared by mixing hot agar solution with aqueous AMPu solution in microcentrifuge tubes.

AMPu tubes were fastened, with their caps open, to the tops of yellow ball traps (13 cm diam) that were coated with Tangle-Trap (Tanglefoot Company, Grand Rapids, MI). These traps were described previously in tests without olfactory lures (Robacker 1992). Ball traps with AMPu tubes, unbaited ball traps, and McPhail traps containing *Torula* yeast as described above were compared in 1-day tests. Two each of the three treatments were used in each of the four blocks in the citrus orchard for a total of eight traps of each treatment per 1-day test. Fourteen 1-day tests were conducted using various combinations of AMPu concentrations and agar percentages.

Statistical Analyses

Analysis of variance (ANOVA) was conducted for both males and females for Experiments 1, 2 and 5. Means separations were done by Fisher's protected least significant difference (LSD) method. *t*-Tests were used to determine if the ratio of females to males captured by AMPu traps was different from the ratio of flies captured in *Torula* yeast traps and to compare specific pairs of treatments in Experiments 3, 4 and 5.

RESULTS

Experiments 1 and 2: AMPu Dosage-Response

The results of Experiment 1 are shown in Fig. 1. AMPu treatments were generally more attractive than water but significantly less attractive than *Torula* yeast to both males ($F = 18.7$; $df = 4,91$; $P < 0.0001$) and females ($F = 15.8$; $df = 4,91$; $P < 0.001$). AMPu 200 was significantly more attractive than AMPu 2000 but was not significantly more attractive than AMPu 20. These results suggested that the most attractive concentration was probably between AMPu 20 and AMPu 2000.

The results of Experiment 2 are shown in Fig. 2. All AMPu treatments were significantly more attractive than water to both males ($F = 10.9$; $df = 6,189$; $P < 0.01$) and females ($F = 12.0$; $df = 6,189$; $P < 0.01$). *Torula* yeast was significantly more attractive to both males and females than all AMPu concentrations except AMPu 400 for males. Among AMPu treatments, AMPu 400 captured the most flies although it was not significantly more attractive than most of the other AMPu treatments. In both Experiments 1 and 2, the ratio of females to males captured by AMPu traps did not differ significantly from the ratio of flies captured by *Torula* yeast traps.

Experiment 3: Optimum Concentration of AMPu in Microcentrifuge Tubes

Only the microcentrifuge tube containing 1.6 ml of AMPu at the concentration of 20:20:2 mg/ml was comparable in attractiveness to the McPhail trap containing

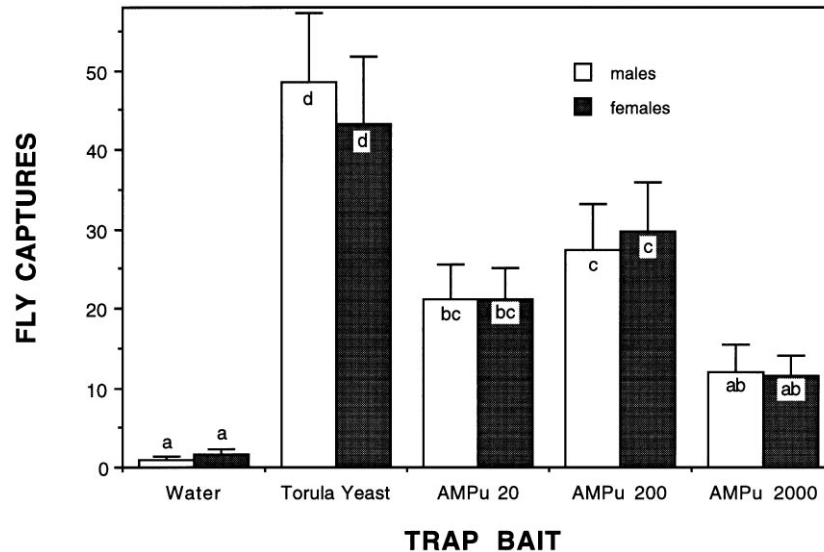


Figure 1. Mean captures (\pm SE; $n = 20$ each trap bait) of adult *A. ludens* in McPhail traps containing water, Torula yeast or three concentrations of AMPu (Experiment 1). Within each sex, bars with the same letter are not significantly different from each other by Fisher's protected LSD ($P < 0.05$).

AMPu 200. Mean captures by traps containing these AMPu tubes ($n = 12$) were 23.8 ± 2.2 (SE) flies. Mean captures by traps containing AMPu 200 ($n = 12$) were 21.6 ± 1.7 flies.

Experiment 4: Field Evaluation of AMPu Tubes vs AMPu 400 in McPhail Traps

Traps containing AMPu tubes at 20:20:2 mg/ml of the three components were equal in attractiveness to traps containing AMPu 400. Mean captures by traps containing AMPu tubes ($n = 24$) were 19.4 ± 3.1 (SE) flies. Mean captures by traps containing AMPu 400 ($n = 24$) were 17.2 ± 2.2 flies.

Experiment 5. Field Evaluation of AMPu Tubes on Ball Traps vs Torula Yeast in McPhail Traps

Ball traps with AMPu tubes containing aqueous AMPu at 20:20:2 mg/ml were much less attractive than McPhail traps containing Torula yeast. Mean captures on balls with AMPu tubes ($n = 16$) were 12.9 ± 1.7 (SE) flies compared with 28.9 ± 4.4 flies captured by Torula yeast traps ($n = 16$). The difference was highly significant ($t = 3.3$; $df = 30$; $P < 0.01$). Ball traps with AMPu tubes were much more attractive than unbaited balls ($n = 16$) which captured only 1.5 ± 0.4 flies per trap.

Ball traps with AMPu tubes containing AMPu in agar were competitive with McPhail traps containing Torula yeast. No consistent differences were observed for the various AMPu and agar concentrations, so they were combined for analysis. Table 1 shows captures of flies by the traps, summed over all AMPu and agar concentrations. Captures by unbaited ball traps were significantly lower than captures by the

TABLE 1. MEAN CAPTURES (\pm SE; N = 110 EACH TRAP/BAIT) OF MEXICAN FRUIT FLIES ON STICKY BALL TRAPS WITH OR WITHOUT AMPU TUBES AND IN MCPHAIL TRAPS CONTAINING TORULA YEAST (EXPERIMENT 5).

Trap/Bait	Males ¹	Females ¹	Males + Females ¹
Ball	0.21 \pm 0.05 a	0.30 \pm 0.06 a	0.51 \pm 0.08 a
Ball/AMPU Tube	1.83 \pm 0.33 b	3.15 \pm 0.34 b	4.97 \pm 0.61 b
McPhail/Torula Yeast	2.16 \pm 0.25 b	3.21 \pm 0.37 b	5.37 \pm 0.56 b

¹Means in a column followed by the same letter are not significantly different by Fisher's protected LSD.

other two traps for males ($F = 22.0$; $df = 2,314$; $P < 0.0001$), females ($F = 43.2$; $df = 2,314$; $P < 0.0001$), and males + females ($F = 39.6$; $df = 2,314$; $P < 0.0001$). Captures of males, females and total flies by AMPu traps and Torula yeast traps were not significantly different at the 5% level.

DISCUSSION

Wakabayashi & Cunningham (1991) developed a chemically defined attractant for the melon fly (*B. cucurbitae*) for use in McPhail traps. Their attractant was an aqueous preparation of ammonium bicarbonate, linolenic acid, putrescine and pyrrolidine in which concentrations of ammonium bicarbonate and putrescine were similar to those in AMPu 400. Wakabayashi & Cunningham's attractant was more attractive

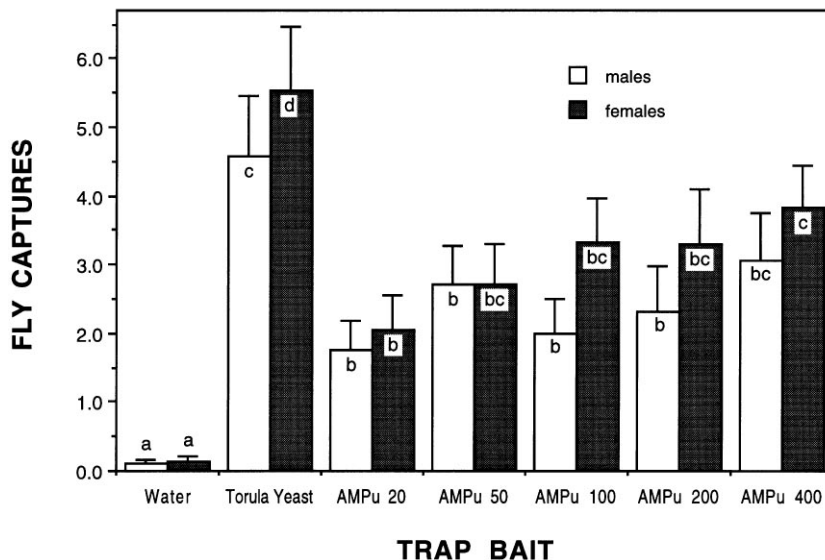


Figure 2. Mean captures (\pm SE; $n = 28$ each trap bait) of adult *A. ludens* in McPhail traps containing water, Torula yeast or five concentrations of AMPu (Experiment 2). Within each sex, bars with the same letter are not significantly different from each other by Fisher's protected LSD ($P < 0.05$).

than NuLure in 1-day orchard tests. They did not evaluate their attractant in tests lasting longer than 1 day, nor did they attempt to develop a formulation that could be used in a dry trap. However, their results were very important in showing that chemically defined "food bait" attractants are at least competitive with standard proteinaceous lures.

Heath et al. (1995) developed an attractant for Mediterranean and Mexican fruit flies for use with a newly designed dry trap. Their attractant contained ammonium acetate and putrescine in a formulation that released ammonia and acetic acid at stable rates for at least a month. Although putrescine emissions were not measured by Heath et al. (1995), their data indicate that the lures remained attractive to both species of flies for 6 weeks in the field in Guatemala. The lure was as attractive as *Torula* yeast to feral Mediterranean fruit flies, but despite its similarity to AMPu, it was less than 20% as attractive as *Torula* yeast to feral Mexican fruit flies. The explanation for the low attractiveness of their lures to Mexican fruit flies compared with the AMPu lures used here is unknown. It could be related to chemical differences between AMPu and their lure, laboratory flies *vs* feral flies, race differences between the Mexican and Guatemalan flies, or a combination of the three factors.

As in Wakabayashi & Cunningham (1991), all experiments conducted here with AMPu were 1-day tests. It would have been desirable to conduct tests for 1 week, the standard period that McPhail traps with *Torula* yeast are used. However, liquid formulations used in this work were not sufficiently attractive to warrant longer tests (Figs. 1 and 2) and the longevity of the agar preparations is unknown but likely less than 1 week. The initial attractiveness of the AMPu/agar preparations on sticky traps was sufficient to warrant longer tests with sticky traps if a longer-lasting AMPu formulation that can be used with the traps can be developed. The goal will be to develop a formulation that emits the three components at constant rates for 2 weeks or more. This should be possible in light of the success of Heath et al. (1995). It certainly will be necessary to develop such a formulation before AMPu lures will be practical for trapping programs by regulatory agencies.

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