Journal of Chemical Ecology, Vol. 23, No. 5, 1997

MEXICAN FRUIT FLY ATTRACTANTS: EFFECTS OF 1-PYRROLINE AND OTHER AMINES ON ATTRACTIVENESS OF A MIXTURE OF AMMONIA, METHYLAMINE, AND PUTRESCINE

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(Received May 13, 1996; accepted December 17, 1996)

Abstract—Several amines were tested alone and in combination with AMPu, an attractant mixture containing ammonium bicarbonate or ammonium carbonate, methylamine hydrochloride, and putrescine, for attractiveness to Mexican fruit flies (*Anastrepha ludens* Loew). In laboratory bioassay, 1-pyrroline, 3-pyrroline, 2-(methylamino)ethanol, spermidine, spermine, and indole-3-acetic acid were significantly more attractive than solvent controls. In orchard tests, traps baited with combinations of AMPu with dimethylamine hydrochloride, ethylamine, 2,5-dimethylpyrazine, or pyrrolidine captured fewer flies than traps baited with AMPu alone. Traps containing AMPu plus additional ammonium bicarbonate were much less attractive than AMPu alone. Combinations of AMPu with 1-pyrroline were about 50% more attractive than AMPu alone to both males and females. Combinations of AMPu with 3-pyrroline were not significantly more attractive than AMPu alone.

Key Words—Attractants, Mexican fruit fly, Diptera, Tephritidae, *Anastrepha ludens*, ammonia, methylamine, putrescine, 1-pyrroline.

INTRODUCTION

Robacker and Warfield (1993) described a three-component attractant for *Anastrepha ludens* Loew consisting of ammonium bicarbonate, methylamine hydro-

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0098-0331/97/0500-1263$12.50/0

chloride, and putrescine (AMPu). Robacker (1995) showed that yellow sticky panel traps baited with AMPu formulated into agar were equal in attractiveness to McPhail traps baited with *Torula* yeast, a standard attractant for many Tephritidae. Robacker et al. (1993) and Robacker and Flath (1995) demonstrated that attractiveness of bacterial odor to the Mexican fruit fly is largely due to chemicals containing protonatable nitrogen, such as the components of AMPu. The purpose of the current research is to evaluate attractiveness to Mexican fruit flies of various nitrogen-containing chemicals alone and in combination with AMPu, as part of ongoing research to develop more powerful attractants for the Mexican fruit fly and other Tephritidae.

Test chemicals were selected based on several factors. Ammonium bicarbonate was selected, even though it is already a component of AMPu, primarily to determine if attractiveness of the mixture could be increased with a greater concentration of this important component. Ammonia, dimethylamine, ethylamine, isoamylamine, and 2,5-dimethylpyrazine were identified in volatiles above bacterial cultures attractive to the Mexican fruit fly (Robacker and Flath, 1995). Ethanolamine, 2-(methylamino)ethanol, isoamylamine, spermidine, and spermine were selected because they were reported as products of various other microorganisms (Miller, 1961). Most of the aforementioned chemicals as well as pyrrolidine, indole-3-acetic acid, and 1-pyrroline are also known amino acid metabolites (Lehninger, 1970; Amoore et al., 1975). Amino acid metabolites were considered important because hunger for protein has been hypothesized as the driving force in attraction of fruit flies to proteinaceous baits (Wakabayashi and Cunningham, 1991; Heath et al., 1995), to bacterial odor (Drew and Lloyd, 1989; Robacker and Moreno, 1995), and to AMPu (Robacker and Warfield, 1993). Furthermore, pyrrolidine is a known attractant for the melon fly, *Bactrocera cucurbitae* Coquillett (Wakabayashi and Cunningham, 1991), and both pyrrolidine and ethanolamine proved attractive to Mexican fruit flies in laboratory experiments (Robacker and Warfield, 1993). 1-Pyrroline has been identified as an attractive pheromone component produced by male Mediterranean fruit flies (*Ceratitis capitata* Wiedemann) (Baker et al., 1985; Flath et al., 1993; Jang et al., 1994). It has also been suggested that 1-pyrroline, a natural contaminant of putrescine formed by spontaneous oxidation (Amoore et al., 1975), may be responsible for the attractiveness of putrescine to fruit flies (Robacker and Warfield, 1993). 3-Pyrroline was selected because of its structural similarity to 1-pyrroline.

Two types of experiments were conducted. Laboratory bioassays were first conducted to assess the attractiveness of individual test chemicals. Chemicals that were most attractive in these tests were then tested in orchard experiments in combinations with AMPu.

METHODS AND MATERIALS

Insects. Flies were from two cultures. Flies used in orchard tests involving aqueous AMPu in McPhail traps were from a culture that originated from mangoes (*Mangifera indica* L.) collected in Morelos, Mexico, in 1953. Flies used in all other experiments were from a culture that originated from yellow chapote fruit (*Sargentia greggii* S. Wats.), a native host of the fly, collected in Nuevo Leon, Mexico, in 1987. Both cultures had been maintained on laboratory diet since establishment. Flies used in orchard tests were irradiated with 70–84.7 Gy (^{137}Cs source) one to two days before adult eclosion for release into the orchard, to comply with quarantine laws for releasing *A. ludens*. Flies used in laboratory bioassays were not irradiated. Mixed-sex groups of 180–200 flies were kept in 0.5-liter cardboard cartons with screen tops until used in tests. Flies were fed sucrose and water but not protein until the time of testing. This feeding regime yields maximum attraction to volatiles from bacterial cultures (Robacker and Garcia, 1993) that contain chemicals similar to those tested in this work. Laboratory conditions for holding flies were 20–25°C, 50–70% relative humidity and photophase from 06:30 to 19:30 hr provided by fluorescent lights.

Test Chemicals. Test chemicals (purity) obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wisconsin) were ammonium carbonate (ACS Reagent quality), 3-pyrroline (97% pure), 2-(methylamino)ethanol (99%), spermidine (99%), spermine (99%), putrescine (98%), indole 3-acetic acid (98%), isoamylamine (99%), 2,5-dimethylpyrazine (99%), and ethanolamine (99%). Test chemicals obtained from Sigma Chemical Co. (St. Louis, Missouri) were ammonium bicarbonate (99%), methylamine hydrochloride (99%), dimethylamine hydrochloride (95%), 70% ethylamine in water (99%), and pyrrolidine (99%).

1-Pyrroline was synthesized by acid hydrolysis of 4-aminobutyraldehyde diethyl acetal by a method modified from that of Schopf and Oechler (1936), as follows: A 50-ml conical flask containing a magnetic stirbar was cooled in an ice-water bath and charged with 0.36 g of 90% 4-aminobutyraldehyde diethyl acetal (Aldrich) and 4 ml of 2 N HCl (Fisher Scientific, Fair Lawn, New Jersey). The reaction mixture was stirred for 25 min before 6 ml of 1.2 M potassium carbonate (J. T. Baker Chemical Co., Phillipsburg, New Jersey) was stirred into the flask (resulting pH > 12). The synthesis of 1-pyrroline was confirmed by extracting (3× 5 ml) the aqueous solution with diethyl ether (E. M. Science, Gibbstown, New Jersey) and analyzing the combined and dried (anhydrous magnesium sulfate, Fisher) extract by GC-MS using a Hewlett Packard (Avondale, Pennsylvania) model 5890A equipped with a 5971A mass selective detector (MSD) and HP5 bonded-phase fused-silica capillary column (25 m, 0.2 mm ID, 0.11 μm film). GC conditions were: split injection (100:1) at 250°C, col-

umn oven 50°C isothermal, and helium carrier gas flow rate 1 ml/min. MSD (electron impact ionization) conditions were: ionization voltage 70 eV, and ion source temperature 180°C. Under these conditions, 1-pyrroline eluted at 2.02 min. Ion masses and relative abundances were: 69 (M^+, 100), 68 (61.7), 67 (2.2), 54 (6.0).

1-Pyrroline was used as an aqueous solution in behavioral bioassays within five days of its synthesis and was kept refrigerated until used. Gas chromatographic examination of the 1-pyrroline solution was conducted within a day of synthesis on three occasions to determine purity and concentration. GC analyses were conducted with a Shimadzu GC 17A (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) equipped with a flame ionization detector. The primary GC column was a DB-1 column (60 m, 0.32 mm ID, 5 μm film) (J & W Scientific, Folsom, California). 1-Pyrroline was sampled by solid-phase microextraction (SPME) with a 100-μm polydimethylsiloxane-coated fiber (Supelco, Inc., Bellefonte, Pennsylvania) that was inserted for 10 min into 0.2 ml of a 1 : 100 dilution of the 1-pyrroline reaction mixture. On-column injection was accomplished by thermal desorption of the fiber at 200°C in the tip of a 16-cm length of 0.53-mm-ID deactivated fused silica capillary tubing (Supelco) positioned in the on-column injection port. The 0.53-mm desorption tube was connected to the primary column by a GlasSeal connector (Supelco, Inc.). Column-oven conditions were 140–200°C at 5°C/min. Carrier gas was helium at a linear velocity of 30 cm/sec. Purity of the 1-pyrroline product was approximately 80% by total FID peak area comparisons. Quantitation of 1-pyrroline was done using a calibration curve constructed with known amounts of pyrrolidine, which is similar in structure and retention time to 1-pyrroline. Calculated concentration of 1-pyrroline (mean $\pm$ SE) was 14.5 $\pm$ 3.8 mg/ml ($N = 3$) in the reaction mixture. This concentration indicates that conversion of 4-aminobutyraldehyde diethyl acetal to 1-pyrroline (and ethanol by-product) was approximately 100%.

Laboratory Bioassays. Experiments were conducted to assess attractiveness of individual test chemicals that had not been evaluated previously using cage-top bioassays as in Robacker and Warfield (1993). Attractiveness of ammonia, dimethylamine, ethylamine, isoamylamine, 2,5-dimethylpyrazine, pyrrolidine, and ethanolamine to Mexican fruit flies was established in earlier work (Robacker and Warfield, 1993; Robacker and Flath, 1995). Four solutions of each chemical that had not been tested in earlier research were prepared. 1-Pyrroline, 3-pyrroline, 2-(methylamino)ethanol, and spermidine were prepared at concentrations of 0.001, 0.01, 0.1, and 1 mg/ml in water. Spermine was prepared at 0.01, 0.1, 1, and 10 mg/ml in water. Indole 3-acetic acid was prepared at 0.01, 0.1, 1, and 10 mg/ml in methanol. Test concentrations of the latter two chemicals were 10 times higher than those of the other chemicals because of their lower

volatilities. Chemicals were tested in 10 μl aliquots of the solutions, resulting in test quantities ranging from 10 ng to 100 μg of the individual chemicals.

Bioassays were conducted using aluminum-screened cages (30 cm/side) each containing one carton of 180–200 flies. Flies were tested when 5–14 days old between 10:00 and 14:00 hr under a combination of fluorescent and natural light. Tests were conducted by placing four filter paper triangles (3 cm/side), two containing test chemicals and two containing 10 μl of either water or methanol (controls for the respective solvents of the test chemicals), near the corners on the top of a cage. Papers containing test chemicals were located diagonally from each other on the cage top, as were the papers containing solvent. The filter papers were raised 5 mm above the cage top using plastic rings to ensure that olfaction and not contact chemoreception was solely responsible for the response of the flies. The numbers of flies beneath each paper were counted once each minute for 10 min. Totals of the 10 counts at each treatment and at the controls were calculated, and these totals were used in paired t tests to determine if each chemical was more attractive than a solvent control. Ten replications were conducted for each test chemical.

AMPu Preparations. AMPu was used either as an aqueous solution or an agar mixture. Each was adjusted to pH 8.8 with saturated NaOH (Fisher). For most experiments, the aqueous solution contained ammonium bicarbonate, methylamine hydrochloride, and putrescine at concentrations of 0.4, 0.4, and 0.04 mg/ml, respectively. In the experiment to evaluate additional ammonium bicarbonate, AMPu component concentrations were 0.2, 0.2, and 0.02 mg/ml. Both sets of AMPu concentrations were highly attractive in previous research (Robacker, 1995). The agar mixture of AMPu contained ammonium carbonate (instead of ammonium bicarbonate), methylamine hydrochloride, and putrescine at 60, 100, and 10 mg/ml. Molar ratios of ammonia to the other two components were approximately equal in the two preparations. Agar tubes were prepared by mixing equal volumes of hot agar solution (Bacto Agar, Difco Laboratories, Detroit, Michigan) and aqueous AMPu (120, 200, and 20 mg/ml of the three chemicals) in 1.9 ml polypropylene microcentrifuge tubes (A. Daigger & Company, Inc., Wheeling, Illinois) to a volume of 1.7 ml. Final agar concentration was 1% in the tubes.

Orchard Tests. Experiments were conducted in a citrus orchard to assess the effects on attractiveness to Mexican fruit flies of combining test chemicals with AMPu. A mixed citrus orchard located near the laboratory in Weslaco, Texas, was used for all field experiments. The orchard contained several varieties of orange, lemon, grapefruit, and tangerine trees of varying ages. One row of Ruby Red grapefruit (*Citrus paradisi* MacFadyen) and one row of Dancy tangerine (*C. reticulata* Blanco) were chosen for tests. Two linear blocks of

eight consecutive trees each were used in each row, for a total of four blocks in the orchard.

In the first series of experiments, combinations of test chemicals with aqueous AMPu were tested in standard glass McPhail traps (Baker et al., 1944). Six experiments were conducted, each to test one chemical. In five experiments,

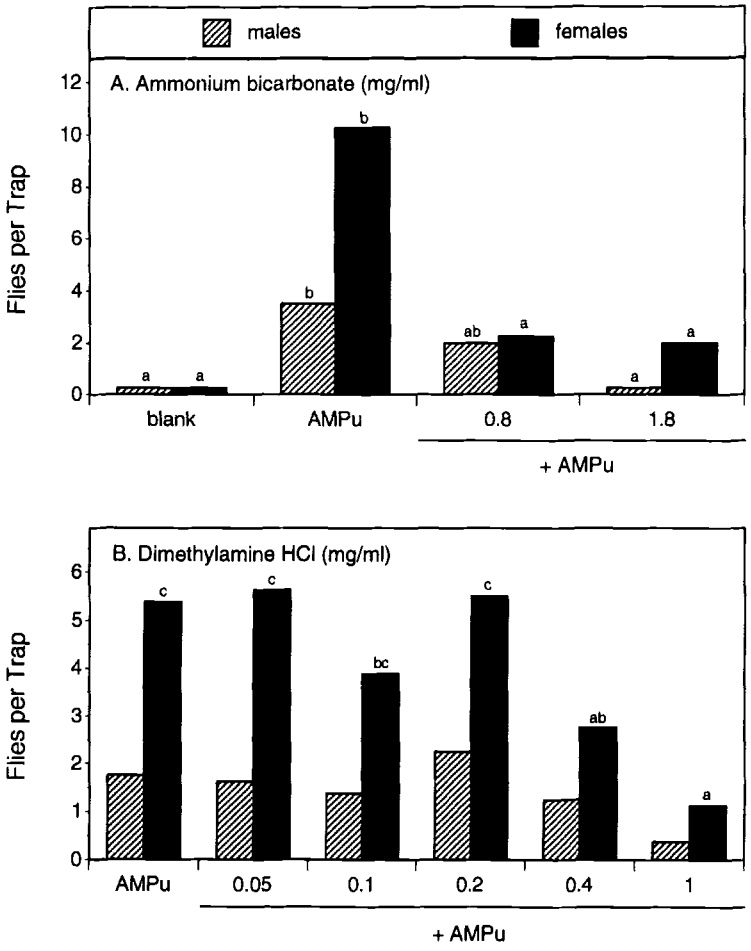

FIG. 1. Mean captures of *A. ludens* in McPhail traps containing AMPu or combinations of AMPu with different concentrations of test chemicals. (A) Ammonium bicarbonate ($N = 4$ replications of each treatment); (B) dimethylamine hydrochloride ($N = 8$). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$). Bars without letters indicate a nonsignificant F test.

five concentrations of the test chemical dissolved in 200 ml of AMPu solution were evaluated. Test chemicals were dimethylamine hydrochloride, ethylamine, isoamylamine, 2,5-dimethylpyrazine, and pyrrolidine. In the sixth experiment, only two concentrations of ammonium bicarbonate were evaluated. Concentrations of the six test chemicals are shown in Figure 1-3. Note that the concen-

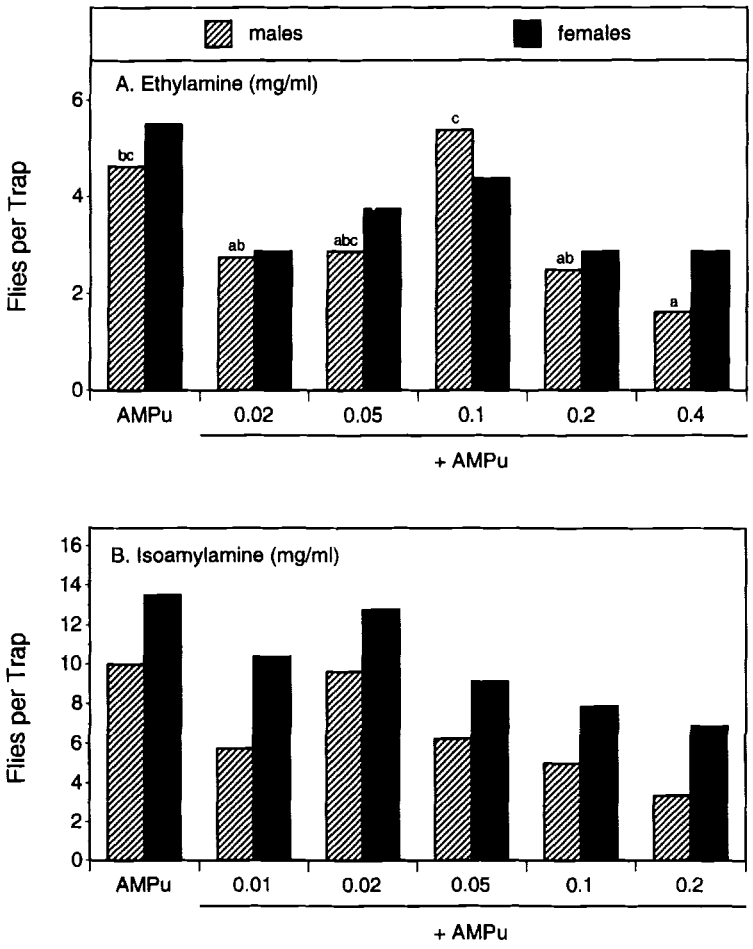

FIG. 2. Mean captures of *A. ludens* in McPhail traps containing AMPu or combinations of AMPu with five concentrations of test chemicals. (A) Ethylamine ($N = 8$ replications of each treatment); (B) isoamylamine ($N = 8$). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$). Bars without letters signify a nonsignificant F test.

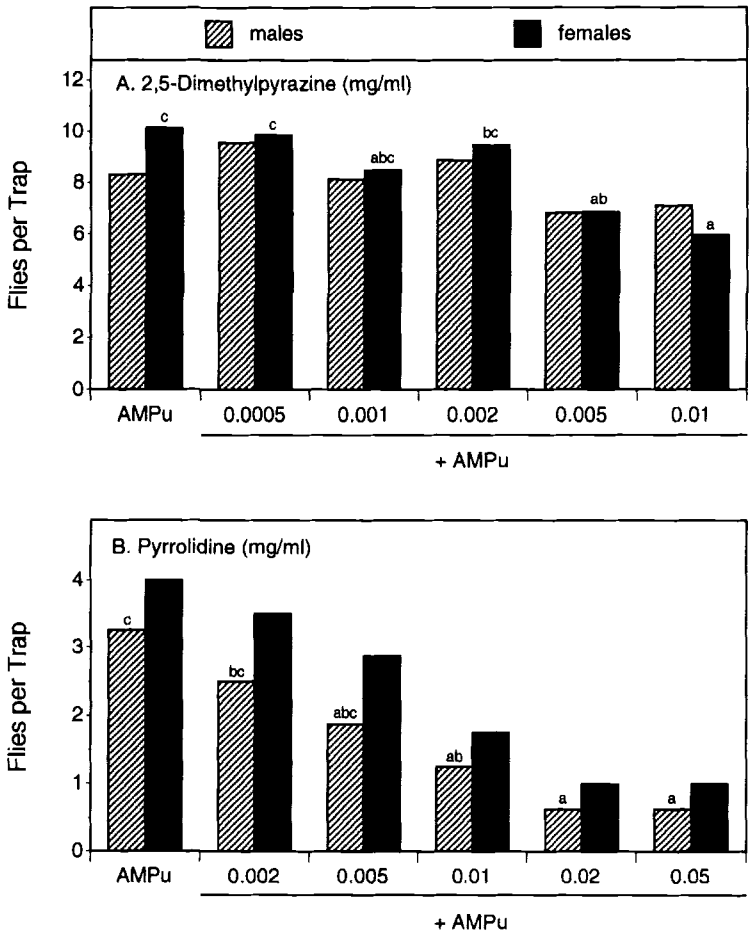

FIG. 3. Mean captures of *A. ludens* in McPhail traps containing AMPu or combinations of AMPu with five concentrations of test chemicals. (A) 2,5-Dimethylpyrazine ($N = 28$ replications of each treatment); (B) pyrrolidine ($N = 8$). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$). Bars without letters signify a nonsignificant F test.

trations of ammonium bicarbonate shown in Figure 1 (0.8 and 1.8 mg/ml) are the amounts that were added to that already in AMPu (0.2 mg/ml). A McPhail trap containing AMPu alone was included in each block in all experiments and a McPhail trap containing water alone was included in each block in the ammonium bicarbonate experiment. All traps contained 0.01% Triton X-100 (Rohm and Haas Co., Philadelphia, Pennsylvania) as a surfactant.

Concentrations chosen for testing were based primarily on previous research

that demonstrated the most attractive concentrations of the AMPu components in cage-top bioassays are about five times higher than the most attractive concentration in McPhail traps (Robacker and Warfield, 1993; Robacker, 1995). Therefore, test concentrations generally were centered at about 20% of the most attractive concentrations observed in cage-top bioassays. 2,5-Dimethylpyrazine was tested at lower concentrations than suggested by results of cage-top bioassays (Robacker and Flath, 1995) because preliminary bioassays in a flight chamber indicated these were the most attractive concentrations (D. C. Robacker, unpublished data).

Traps were hung one to a tree, 1–2 m above the ground, on the northwest sides of trees. 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 were randomized for the first replication of each experiment. Positions of treatments in consecutive replications were not randomized but were moved sequentially within each block. Flies were released into the test orchard when 4–12 days old during the late afternoon of the day before a test. Approximately 2000 flies were distributed equally among the 32 test trees in the four blocks. The test of ammonium bicarbonate was not replicated over time (N = 4 replicates of each treatment over space); two replications each were conducted for dimethylamine hydrochloride, ethylamine, isoamylamine, and pyrrolidine (N = 8 replicates of each treatment); and seven replications were conducted for 2,5-dimethylpyrazine (N = 28).

In the second series of experiments, combinations of test chemicals with AMPu–agar tubes were tested on Pherocon AM traps (Trece, Inc., Salinas, California). Pherocon AM traps are yellow cardboard panels (14 × 23 cm) with a sticky coating. Four chemicals were evaluated over the course of five experiments. Test chemicals were dimethylamine hydrochloride, ethanolamine, 1-pyrroline, and 3-pyrroline. In most experiments, five concentrations of the test chemical were evaluated in combination with AMPu–agar tubes. All chemicals except 1-pyrroline were tested in this way. In each experiment, the test chemical was formulated into agar in a 1.9-ml microcentrifuge tube by the same method as for AMPu. Tubes containing AMPu and those containing the various concentrations of test chemicals were fastened to opposite sides of the traps. Except for tubes containing aqueous 1-pyrroline solutions, all tubes were fastened to traps with their caps open. The pH of the dimethylamine hydrochloride tubes was adjusted to 8.8 with NaOH. The pH was not changed in the other test chemical tubes. A Pherocon trap containing only an AMPu tube, one containing only a tube with the middle concentration of test chemical, and one containing no bait were included in each block in each of the five experiments described above for a total of eight traps per block.

Two experiments were conducted to evaluate 1-pyrroline. The first experiment was conducted in the same manner as for the other test chemicals except that the 1-pyrroline tubes contained aqueous 1-pyrroline instead of agar for-

mulations. These tubes were fastened to traps with their caps closed but with a pinhole in the middle of the cap. The pH of the 1-pyrroline solutions was not changed from the high pH of the reaction mixture (> 12). The second experiment with 1-pyrroline differed from the first in two respects. First, only three concentrations of 1-pyrroline were evaluated and no tube containing 1-pyrroline alone was tested. Second, the 1-pyrroline was not put into separate tubes but

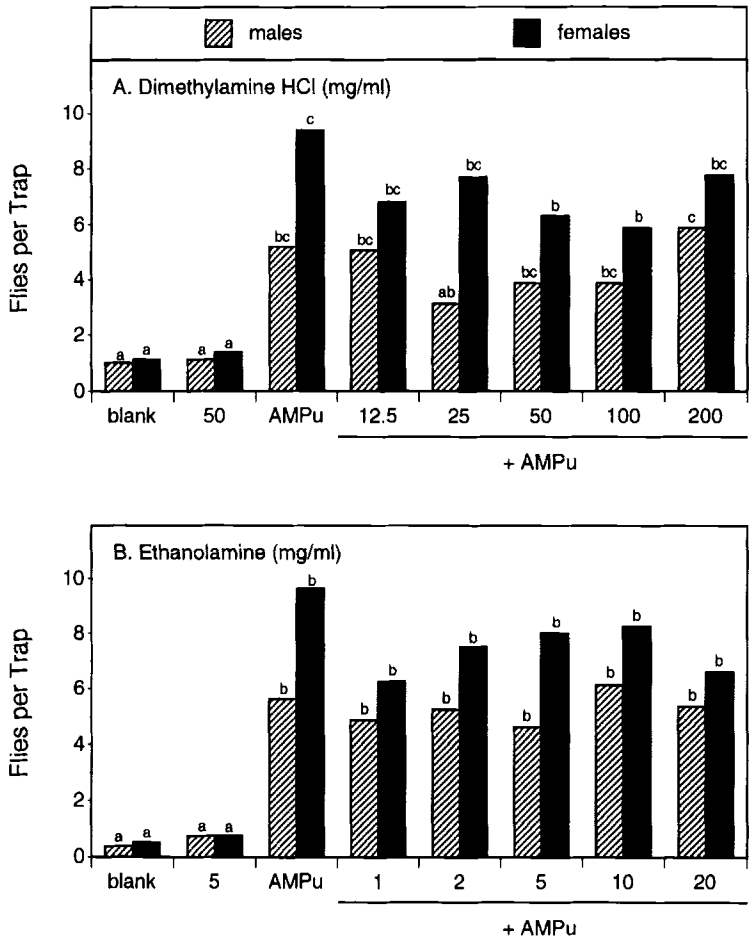

FIG. 4. Mean captures of *A. ludens* on Pherocon AM traps baited with a test chemical, AMPu, or combinations of AMPu with five concentrations of test chemicals. (A) Dimethylamine hydrochloride (N = 16 replications of each treatment); (B) ethanolamine (N = 8). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$).

was formulated directly into the AMPu–agar tubes. This was done by mixing 1-pyrroline solution with aqueous AMPu before the hot agar was added to the tubes. The pH of the AMPu–1-pyrroline–agar tubes was adjusted to 8.8 with NaOH. Thus, the pH of the AMPu–1-pyrroline–agar tubes and the AMPu–agar tubes was equal. Concentrations of the four chemicals tested in combination with AMPu/agar tubes are shown in Figures 4–6.

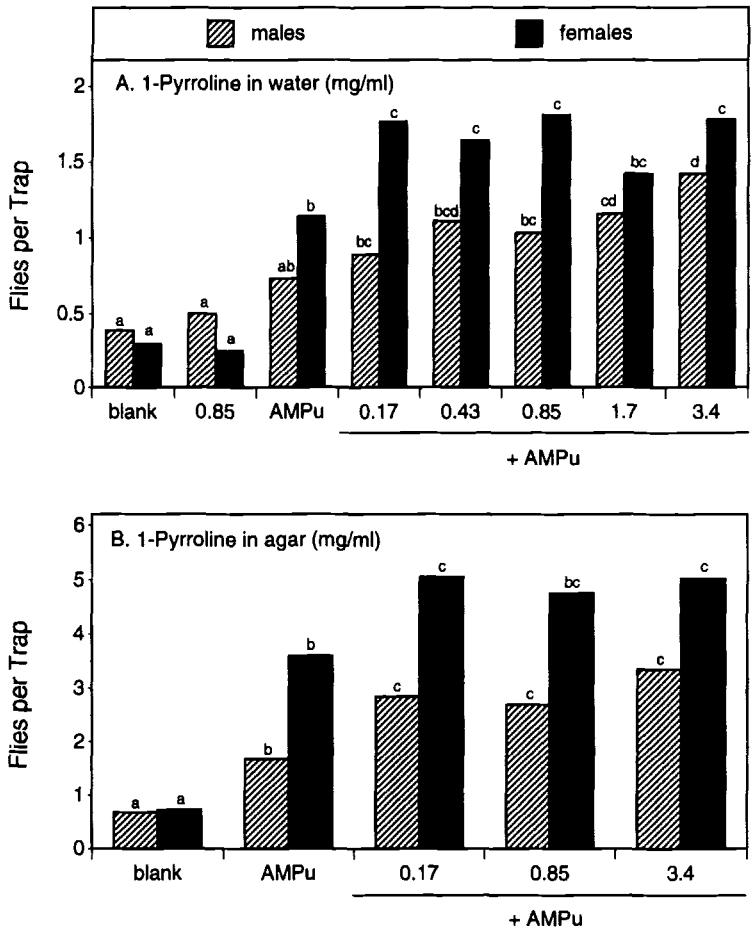

FIG. 5. Mean captures of *A. ludens* on Pherocon AM traps baited with 1-pyrroline, AMPu, or combinations of AMPu with three to five concentrations of 1-pyrroline. (A) 1-Pyrroline in water in one tube and AMPu in agar in a second tube ($N = 64$ replications of each treatment); (B) 1-Pyrroline and AMPu in agar in the same tube ($N = 40$). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$).

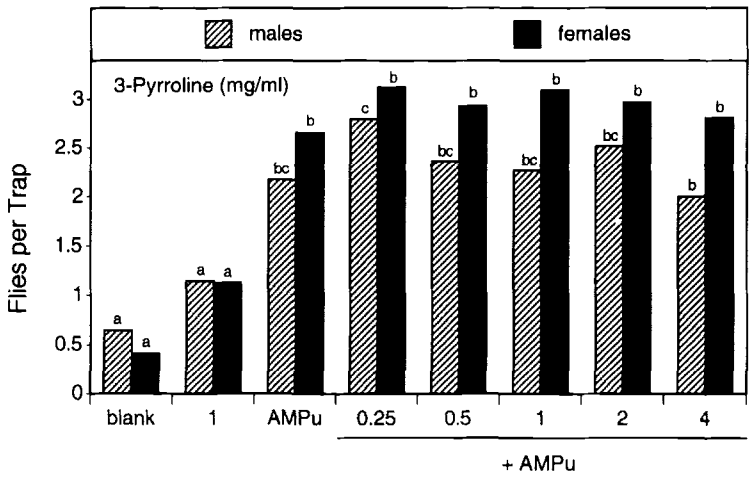

FIG. 6. Mean captures of *A. ludens* on Pherocon AM traps baited with 3-pyrroline, AMPu, or combinations of AMPu with five concentrations of 3-pyrroline ($N = 64$ replications of each treatment). Bars with the same letter, within males or females, are not significantly different by Fisher's protected LSD ($P < 0.05$).

The concentrations of dimethylamine hydrochloride and ethanolamine chosen for testing in agar formulations were based primarily on previous research that demonstrated that the most attractive concentrations of the AMPu components in agar are about 100 times higher than the most attractive concentrations in cage-top bioassays (Robacker and Warfield, 1993; Robacker, 1995). Therefore, test concentrations of these chemicals were centered at about 100 times the most attractive concentrations observed in cage-top bioassays. 1-Pyrroline was not very attractive at any concentration in cage-top bioassays. The concentrations of 1-pyrroline chosen for testing were based in part on literature that indicated it is a primary odor to human olfaction and thus could be detected at concentrations much lower than for similar chemicals (Amoore et al., 1975). Furthermore, Jang et al. (1994) showed that 1-pyrroline elicited attraction of Mediterranean fruit flies at concentrations much lower than those of other pheromone components. Test concentrations of 3-pyrroline were similar to those of 1-pyrroline.

Orchard testing procedures were the same as those used for chemicals tested in McPhail traps. Four replications over time were conducted for dimethylamine hydrochloride ($N = 16$ replicates of each treatment), 2 for ethanolamine ($N = 8$), 16 for 1-pyrroline in aqueous formulation ($N = 64$), 10 for 1-pyrroline in agar ($N = 40$), and 16 for 3-pyrroline ($N = 64$).

Two-way ANOVA testing effects of bait and test day (replication over

time) was conducted for each orchard experiment for captures of both males and females using SuperANOVA (Abacus Concepts, 1989). For the purpose of these analyses, the four blocks in the orchard were treated as trap-bait replications. Means separations were conducted by Fisher's protected least significant difference method. A two-way ANOVA was conducted for the test of 1-pyrroline–AMPu combinations in agar to determine if the combinations attracted males and females in different relative numbers than were attracted by AMPu alone. Data from blank traps were not used in this analysis of trap bait by sex interaction.

RESULTS

Laboratory Bioassays. All chemicals were significantly more attractive than solvent controls at one or more test quantities (Table 1). The most attractive chemicals were 3-pyrroline and spermidine, which were about twice as attractive as controls. 1-Pyrroline and indole 3-acetic acid were the least attractive chemicals.

Orchard Tests. Except for the experiments involving 1-pyrroline and 3-pyrroline, the general outcome of these experiments was that combinations of test chemicals with AMPu were less attractive than AMPu alone. Ammonium bicarbonate–AMPu combinations were much less attractive than AMPu alone (males: $F = 3.0$; $df = 5,18$; $P < 0.05$; and females: $F = 4.5$; $df = 5,18$; $P < 0.01$) (Figure 1A). Combinations of AMPu with the higher concentrations of dimethylamine hydrochloride, ethylamine, isoamylamine, 2,5-dimethylpyrazine, pyrrolidine, and ethanolamine generally captured fewer flies, especially

TABLE 1. MEAN COUNTS OF *A. ludens* AT SOLVENT BLANKS OR TEST CHEMICALS USING CAGE-TOP BIOASSAYS

	10 ng		100 ng		1 μg		10 μg		100 μg	
	Test	Solvent	Test	Solvent	Test	Solvent	Test	Solvent	Test	Solvent
1-Pyrroline	27.2	24.3	22.5	22.9	22.2	24.0	29.2[a]	26.1		
3-Pyrroline	17.3	18.0	16.3	13.6	19.0[a]	12.7	32.9[a]	16.5		
2-(Methylamino) ethanol	10.2	12.6	12.5	10.3	14.6	14.0	16.5[a]	10.8		
Spermidine	11.4	13.3	15.7	11.6	19.2[a]	11.9	20.7[a]	10.7		
Spermine			15.0	16.8	18.2	18.0	21.5	17.4	27.2[a]	16.7
Indole 3-acetic acid			13.4	14.6	18.2	17.1	17.9	16.8	17.5[a]	13.1

[a]The mean response to a chemical was significantly different from the response to a solvent control by paired t tests ($P < 0.05$).

females, than AMPu alone (Figures 1–4). ANOVAs were significant for captures of females in the test of dimethylamine hydrochloride in McPhail traps ($F = 4.7$; $df = 5,41$; $P < 0.01$), males in the ethylamine experiment ($F = 2.4$; $df = 5,41$; $P < 0.05$), females in the 2,5-dimethylpyrazine experiment ($F = 3.0$; $df = 5,156$; $P < 0.05$), males in the pyrrolidine experiment ($F = 3.7$; $df = 5,41$; $P < 0.05$), males and females in the test of dimethylamine hydrochloride in agar formulations (males: $F = 5.6$; $df = 7,117$; $P < 0.001$; and females: $F = 7.8$; $df = 7,117$; $P < 0.001$), and males and females in the ethanolamine experiment (males: $F = 5.6$; $df = 7,55$; $P < 0.001$; and females: $F = 7.1$; $df = 7,55$; $P < 0.001$). In some cases such as ethanolamine, no individual combinations were significantly less attractive than AMPu alone despite large ANOVA F values. Traps baited with dimethylamine hydrochloride alone or ethanolamine alone (Figure 4) were not significantly more attractive than blank traps. Ammonium bicarbonate, ethylamine, isoamylamine, 2,5-dimethylpyrazine, and pyrrolidine were not tested alone.

Combinations of 1-pyrroline with AMPu were significantly more attractive to flies than AMPu alone. This was true whether 1-pyrroline was tested in a separate tube from the AMPu (Figure 5A) or mixed into the AMPu/agar formulation (Figure 5B). Traps baited with combinations of AMPu and 1-pyrroline in separate tubes captured 53% more males and 48% more females than AMPu-alone traps, summed over all 1-pyrroline concentrations. Likewise, traps baited with combinations of AMPu and 1-pyrroline mixed in agar in the same tube captured 77% more males and 37% more females than AMPu-alone traps. The relative attractiveness of the combinations to males and females was not significantly different from the relative attractiveness of AMPu alone (bait × sex interaction from the experiment in which 1-pyrroline was mixed with AMPu in agar: $F = 0.004$; $df = 1,316$; $P = 0.95$). ANOVAs were highly significant for both males and females in the experiment with separate tubes (males: $F = 6.3$; $df = 7,489$; $P < 0.0001$; and females: $F = 13.2$; $df = 7,489$; $P < 0.0001$), and in the experiment with 1-pyrroline mixed into the agar (males: $F = 13.3$; $df = 4,186$; $P < 0.001$; and females: $F = 15.1$; $df = 4,186$; $P < 0.001$). Traps baited with 1-pyrroline alone were not significantly more attractive than blank traps.

Combinations of 3-pyrroline and AMPu were not significantly more attractive than AMPu alone (Figure 6), although these combinations captured 10% more males and 12% more females than AMPu alone. Traps baited with 3-pyrroline alone were not significantly more attractive than blank traps.

DISCUSSION

Previous research with *A. ludens* has demonstrated that combinations of attractants from different attractant systems generally are less attractive than the

more potent of the two attractants alone (Robacker, 1993). In this context, an attractant system is defined as a group of chemicals, receptors, and central nervous system centers that function together in producing behavior related to some biological function. Two examples were combinations of pheromones (sexual behavior system) with host-fruit odor (sugar-feeding system), in which combinations were less attractive than pheromone alone to sexually active female flies (Robacker and Garcia, 1990), and host-fruit odor with bacterial odor (protein-feeding system), in which combinations were less attractive than host-fruit odor to sugar-hungry flies (Robacker, 1991). However, combining attractive chemicals from the same system, such as hexanol and ethyl hexanoate identified from host-fruit volatiles, usually has additive effects on attractiveness (Robacker et al., 1990). The combinations used in this study would seem to be of the latter type because all the chemicals tested, including AMPu, are attractive to sugar-fed, protein-starved flies (Robacker and Warfield, 1993; Robacker and Flath, 1995). Nevertheless, many of the combinations of these chemicals with AMPu were less attractive than AMPu alone.

The explanation for the very low attractiveness of the AMPu/ammonium bicarbonate combinations may be as simple as irritation caused by high concentrations of ammonia on various receptor types. One possible explanation for the low attractiveness of the other combinations could be competition for the same olfactory receptor. This seems likely for dimethylamine and ethylamine, which may compete for the same receptor sites as methylamine. The same explanation was proposed for two pheromone components, (*Z*)-3-nonenol and (*Z*,*Z*)-3,6-nonadienol, each of which synergized the effect of a third pheromone component, but the first alcohol inhibited the effect of the latter when they were present together (Robacker and Hart, 1985). A similar hypothesis can be offered for some of the other chemicals, although their structures differ enough from the AMPu chemicals that other explanations may be more appropriate.

Combinations of 1-pyrroline with AMPu were more attractive than AMPu alone even though 1-pyrroline was nearly unattractive by itself. 1-Pyrroline is the only semiochemical studied to date that is almost unattractive by itself but that synergizes attractiveness of another set of chemicals that are attractive to Mexican fruit flies engaged in food-finding behavior.

1-Pyrroline appears to be a rather ubiquitous naturally occurring chemical as its odor is present in many products of plant and animal origin, probably as a result of enzymatic degradation of certain amino acids and spontaneous oxidation of various amino acid metabolites (Amoore et al., 1975). 1-Pyrroline is important to human olfaction as a primary odor (Amoore et al., 1975), and it has been identified as an attractive component of male-produced volatiles of the Mediterranean fruit fly (Baker et al., 1985). Interestingly, Jang et al. (1994) reported that the attractiveness to sexually active female Mediterranean fruit flies of 1-pyrroline by itself is small, but it synergizes the attractiveness of other pheromonal components.

Neither 3-pyrroline nor pyrrolidine performed the same way as 1-pyrroline despite structural similarities. Both were considerably more attractive than 1-pyrroline when tested alone, and neither significantly increased the attractiveness of AMPu in orchard tests. Pyrrolidine actually decreased the attractiveness of AMPu, a result similar to one observed when pyrrolidine decreased the attractiveness of a more complex mixture of amines and acids in laboratory experiments (Robacker and Warfield, 1993). The results suggest that the imine (−N=CH−) functionality of 1-pyrroline may be a critical molecular feature in olfactory reception. However, the low pK_a (6.7) of 1-pyrroline (Amoore et al., 1975) may also account for its greater attractiveness than the strongly basic 3-pyrroline (pK_a 10.5) (Amoore et al., 1975) because at pH 8.8 (pH at which 3-pyrroline was tested) it would exist mostly in the ionized, nonvolatile form. In contrast, 1-pyrroline (tested at pH 8.8 or higher) would exist almost entirely in the nonionized, volatile form. Thus, the concentration of 1-pyrroline evolving from the preparations should be greater than that of 3-pyrroline at a given concentration in solution. Pyrrolidine, like 3-pyrroline, is a strong base (pK_a 11.0) (The Merck Index, 1983) and would be mostly in the ionized, nonvolatile form at pH 8.8 at which it was tested. However, pyrrolidine was repellent in the largely ionized form and increasing its concentration in the air probably would only increase its repellency.

AMPu has already proven as attractive as *Torula* yeast to Mexican fruit flies in citrus orchard tests (Robacker, 1995). Results of this work suggest that attractiveness of AMPu could be enhanced by addition of 1-pyrroline. Although 1-pyrroline has been reported as unstable in solution (Nomura et al., 1977), 1-pyrroline trimer in neat form is stable and evolves 1-pyrroline monomer as an evaporation product (Baker et al., 1992). Consequently, it may be possible to develop a formulation of AMPu and 1-pyrroline trimer that would emit the four attractive components at stable rates for periods of perhaps a month or more.

Acknowledgments—We thank Maura Rodriguez and Cyndi Rodriguez for technical assistance; Sammy Ingle for insects; and USDA-APHIS (Mission, Texas) for irradiation of pupae. We also thank Ken Hagen (Professor of Entomology, Emeritis, University of California, Berkeley) for discussion during the developmental phase of the work, and Dr. Chang-Joo Lee (USDA-ARS, Insect Chemical Ecology Laboratory, Beltsville, Maryland) for useful criticism of the manuscript. This work was partially supported by a grant from the Citrus Research Board (Visalia, California, Project 58-6204-4-038). Use of a product brand in this work does not constitute an endorsement by the USDA.

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