ROLES OF PUTRESCINE AND 1-PYRROLINE IN ATTRACTIVENESS OF TECHNICAL-GRADE PUTRESCINE TO THE MEXICAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

DAVID C. ROBACKER
Crop Quality and Fruit Insects Research, USDA, Agricultural Research Service
Kika de la Garza Suptropical Agricultural Research Center, 2413 E. Highway 83, Building 200, Weslaco, TX 78596

ABSTRACT

Laboratory experiments were conducted to determine if 1-pyrroline, present as a contaminant of putrescine, was responsible for the observed attractiveness of putrescine to the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). Technical-grade putrescine contained 0.025% 1-pyrroline measured by gas chromatography. Putrescine purified by high performance liquid chromatography contained 0.0000053% 1-pyrroline, constituting a 99.98% reduction compared with technical-grade putrescine. Purified putrescine was more attractive than technical-grade putrescine over a range of concentrations. The amount of 1-pyrroline found in technical-grade putrescine was attractive, but less so than technical-grade putrescine at 2 concentrations. Either purified putrescine or an amount of 1-pyrroline equivalent to that in technical putrescine could substitute for technical putrescine in combinations with 2 other attractive chemicals, ammonium bicarbonate and methylamine HCl. Results indicate that putrescine more so than 1-pyrroline accounts for the attractiveness of technical putrescine but that either chemical enhances the attractiveness of ammonia and methylamine about equally.

Key Words: *Anastrepha ludens*, attractants, lures, putrescine, 1-pyrroline

RESUMEN

Experimentos de laboratorio fueron conducidos para determinar si 1-pirrolina, la cual está presente como un contaminante en la putrescina, fue responsable de la atracción a la putrescina observada para la mosca mejicana de las frutas, *Anastrepha ludens* (Loew) (Diptera: Tephritidae). La putrescina de grado técnico tuvo un contenido de 1-pirrolina de 0.025%, medida por cromatografía de gases. La putrescina purificada por cromatografía liquida de alto rendimiento tuvo un contenido de 0.0000053% de 1-pirrolina, constituyendo una reducción de 99.98% en comparación con la putrescina de grado técnico. La putrescina purificada fue más atractiva que la putrescina de grado técnico sobre una gama de concentraciones. La cantidad de 1-pirrolina encontrada en la putrescina de grado técnico fue atractiva, pero menos que la putrescina de grado técnico a 2 concentraciones. Ya sea la putrescina purificada o una cantidad de 1-pirrolina equivalente a la que se encuentra en la putrescina técnica pudiera sustituir la putrescina técnica en combinaciones con 2 otros químicos atractivos, el bicarbonato de amonio y el HCl metilamino. Los resultados indican que la putrescina mas que la 1-pirrolina explica la atracción de la putrescina técnica ya que cualquier químico aumenta la atracción del amonio y el metilamino casi igualmente.

Independent research conducted by three different groups has implicated putrescine as an important attractant for fruit flies. Wakabayashi & Cunningham (1991) first reported putrescine as a component of a four-chemical mixture attractive to the melon fly, *Bactrocera cucurbitae* (Coquillett). Robacker & Warfield (1993) provided evidence that putrescine was attractive to the Mexican fruit fly, *Anastrepha ludens* (Loew), both by itself and as part of a three-chemical mixture. Finally, Heath et al. (1995) demonstrated that a combination of ammonium acetate and putrescine was more attractive than ammonium acetate by itself to the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Mexican fruit fly.

1-Pyrroline was identified as a component of male-produced pheromone of the Mediterranean fruit fly (Baker et al. 1985) and was shown to be slightly attractive to sexually active female Mediterranean fruit flies, and to synergize attractiveness of other pheromone components (Jang et al. 1994). Robacker & Bartelt (1997) found 1-pyrroline in volatiles produced by bacterial fermentations attractive to the Mexican fruit fly, and Robacker et al. (1997) demonstrated that 1-pyrroline not only was attractive to the Mexican fruit fly but also synergized attractiveness of a mixture of ammonium carbonate, methylamine HCl, and putrescine.

Amoore et al. (1975) reported that technical-grade putrescine contains a small amount of

1-pyrroline that is produced by spontaneous oxidation of putrescine. The presence of 1-pyrroline is easily verifiable by gas chromatography. Because everyone who 'demonstrated' the attractiveness of putrescine to fruit flies used technical putrescine, it was never actually proven that putrescine itself, without 1-pyrroline present as a contaminant, is attractive to fruit flies. Three facts that suggest the possibility that 1-pyrroline is responsible for the observed attractiveness of technical putrescine are: 1) 1-pyrroline is attractive to fruit flies at low concentrations (Jang et al. 1994; Robacker et al. 2000); 2) emissions of putrescine and 1-pyrroline were equal from a lure that contained technical putrescine by design and 1-pyrroline only as a contaminant of technical putrescine (Robacker & Bartelt 1996); and 3) 1-pyrroline is responsible for the perceived odor of technical putrescine in human olfaction (Amoore et al. 1975), a fact that has significance here because of mounting evidence of similarities in neural functioning in humans and insects (Hildebrand & Shepherd 1997).

The purpose of this work was to determine whether putrescine or 1-pyrroline is responsible for the reported attractiveness of technical putrescine to fruit flies. Our method was to remove 1-pyrroline from technical putrescine and test attractiveness of a range of concentrations of purified putrescine to the Mexican fruit fly. Attractiveness of 1-pyrroline at concentrations equal to those present in various technical putrescine solutions was also tested. Finally, attractiveness of mixtures of ammonium bicarbonate and methylamine HCl with either purified putrescine or 1-pyrroline was tested to determine if purified putrescine and 1-pyrroline could substitute for technical putrescine.

Materials and Methods

Chemistry Methods

Technical-grade putrescine was purified by high performance liquid chromatography (HPLC) using a Waters 717plus Autosampler and 600E Multisolvent Delivery System with 60F Pump (Waters Corp., Milford, MA). The system was controlled by a NEC Image 466es computer with Millennium™ 2010 Chromatography Manager Software. The HPLC column was a Waters µ-Bondapak C18 (3.9 mm × 30 cm, 125 A, 10 µm particle size). Mobile phase was water at 1 ml/min. Technical-grade putrescine (98%; Sigma Chemical Co., St. Louis, MO) was dissolved in water (100 mg/ml), and 200 µl was injected. Detection of putrescine eluting from the column was done by measuring pH elevation of the eluant with a pH meter (Fisher Accumet Model 805, Fisher Scientific Co., Pittsburgh, PA) and by gas chromatographic (GC) analysis of fractions (see below). Both purified putrescine and technical putrescine were kept at -20°C when not in use.

Sampling of 1-pyrroline and putrescine for GC quantitation was done by solid-phase microextraction (SPME) using a polydimethylsiloxane fiber (100 µm coating) (Supelco, Inc., Bellefonte, PA). Introduction of chemicals from the SPME fiber onto the GC was by thermal desorption for 0.5 min into a 10 cm deactivated fused silica retention gap (0.53 mm ID) in an on-column injector at 210°C. The retention gap was connected to the analytical column by a GlasSeal connector (Supelco). A DB-1 column (60 m × 0.32 ID, 5 µm film) (J & W Scientific, Folsom, CA) was used in a Shimadzu GC-17A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD) with flame ionization (FID) and flame thermionic (FTD) (Model FTD-17) detectors, and a cool on-column injector.

1-Pyrroline was synthesized by acid hydrolysis of 4-aminobutyraldehyde diethylacetal (Aldrich Chemical Co., Inc., Milwaukee, WI) using the method of Schopf & Oechler (1936). 1-Pyrroline reaction product was kept at -20(C when not in use. The concentration of 1-pyrroline in the reaction product was determined using a calibration curve prepared with pyrrolidine (99%, Sigma). Pyrrolidine solutions and 1-pyrroline reaction product dilutions were adjusted to pH >12 with NaOH. For GC analysis, the SPME fiber was inserted through a septum into the headspace above 1 ml of the solutions in sealed 4 ml vials at room temperature for 5 min. Analyses were at 100°C and carrier gas (He) linear velocity of 30 cm/sec. Detection was by FID.

For preparation of various solutions for bioassay, it was necessary to quantify putrescine in HPLC purifications of technical putrescine, and 1-pyrroline both in technical putrescine and in putrescine purified by HPLC. Concentrations of putrescine in HPLC collections were determined using a FID calibration curve constructed from several concentrations of technical putrescine. The concentration of 1-pyrroline in a 10 mg/ml aqueous solution of technical putrescine was determined using a FID calibration curve for pyrrolidine in 10 mg/ml aqueous solutions of technical putrescine. The pyrrolidine calibration solutions were prepared in solutions containing putrescine to compensate for effects of putrescine on SPME analysis of 1-pyrroline (Robacker & Bartelt 1996). The concentration of 1-pyrroline in purified putrescine was determined by comparing FTD peak areas of 1-pyrroline in purified putrescine (HPLC fractions, approximately 2-5 mg/ml of putrescine), with 1-pyrroline areas measured using previously determined concentrations of 1-pyrroline in dilutions of technical putrescine. FTD was used for 1-pyrroline in purified putrescine because concentrations were low and FTD is 35× more sensitive than FID to 1-pyrroline. All solu-

tions were adjusted to pH >12 for SPME samplings. For GC analyses, the SPME fiber was inserted through a septum into 2 ml of the solutions in sealed 4 ml vials at room temperature for 5 min. GC analyses of putrescine were at 180°C and analyses of 1-pyrroline were at 100°C. Linear velocity of He carrier gas was 30 cm/sec.

Various concentrations of technical putrescine, purified putrescine, and 1-pyrroline were prepared in water at pH 9, to determine attractiveness of purified putrescine and 1-pyrroline relative to attractiveness of technical putrescine containing equivalent amounts of putrescine and 1-pyrroline. Test solutions were prepared by dilution of stock solutions each day bioassays were performed. Before preparations, the stock solution of purified putrescine was checked for purity by GC. Concentration of 1-pyrroline in purified putrescine stock solution did not increase for the duration of these experiments. Also, the stock solution of 1-pyrroline was calibrated by GC each day before test solutions were made. The concentration of 1-pyrroline in the stock solution decreased by about 10% over the course of these experiments.

Because putrescine is known to oxidize to 1-pyrroline, an experiment was conducted to determine if 1-pyrroline that formed during bioassay of purified putrescine could be sufficient to affect attraction of flies to the filter papers. Ten microliters of a technical putrescine solution (1 mg/ml in water, pH 9) were put onto a 2×2 cm piece of filter paper inside a 4 ml vial with a cap containing a septum. Headspace inside the vial was sampled for 1 min by SPME by inserting the fiber through the septum. A vial with technical putrescine was sampled once, either immediately after the putrescine was applied to the paper or 10 min later, then discarded. 1-Pyrroline was analyzed by GC-FTD at 100°C using the Shimadzu instrument, DB-1 column and procedure described above.

Insects and Test Conditions

Flies were from a culture that originated from yellow chapote fruit (*Sargentia greggii* S. Wats.) collected in Nuevo Leon, Mexico, in 1997. Flies were sugar-fed and protein-starved since eclosion, because this physiological state facilitates attraction to AMPu, a lure that contains putrescine (Robacker 1998). Flies were tested when 2-12 days old. Within this range, age does not affect attraction to bacterial odors that contain 1-pyrroline and other attractive amines (Robacker & Garcia 1993). Laboratory conditions for holding and testing flies were 22 ± 2°C, 50 ± 20% relative humidity, and photophase from 0630 to 1930 h.

Bioassay Method

Attractiveness of test chemicals and mixtures was evaluated using cage-top bioassays as described in Robacker & Warfield (1993). Briefly, the bioassay was conducted by placing 2 filter paper triangles containing test chemicals or mixtures (treatment papers) and 2 papers containing water or control mixtures (control papers) on the top of an insect cage ($30 \times 30 \times 30$ cm) and counting the number of flies beneath each paper once each minute for 10 min. The 2 treatment papers were positioned diagonally across from each other on the cage top as were the 2 control papers. The filter papers were raised 5 mm above the cage top using plastic rings. Each bioassay cage contained 180-200 flies (sex ratio approximately 1:1). A cage of flies was used once per day for up to 3 days before it was discarded.

Bioassays of Putrescine and 1-Pyrroline Individually

The purpose of these tests was to determine if amounts of purified putrescine and 1-pyrroline equivalent to those in technical putrescine could account for the attractiveness of technical putrescine. Four quantities of technical putrescine and equivalent quantities of purified putrescine and 1-pyrroline were tested. Amounts tested are shown in Tables 1-2. Each bioassay evaluated one quantity of one test chemical against water as the control. Samples of test chemicals or water were applied to filter papers in 10 µl aliquots.

Two experiments were conducted, one to test purified putrescine and the other 1-pyrroline. In the first experiment, the 4 quantities of purified putrescine were tested along with the 4 quantities of technical putrescine. The second experiment was conducted the same way using 1-pyrroline instead of purified putrescine.

Bioassays of Combinations

The purpose of these tests was to determine if purified putrescine and/or 1-pyrroline could substitute for technical putrescine in the attractive mixture of ammonium bicarbonate, methylamine HCl and technical putrescine (AMPu) published by Robacker & Warfield (1993). The method was to compare attractiveness of combinations of ammonium bicarbonate and methylamine HCl with either technical putrescine, purified putrescine or 1-pyrroline. In all bioassays, 10 µl of a solution of ammonium bicarbonate (>99%, Sigma) and methylamine HCl (>99%, Sigma) in water at pH 9, was applied both to the treatment papers and the control papers, resulting in 10 µg of each chemical on all papers. In addition, treatment papers received 10 µl of one of the following, each in water at pH 9: 1 µg of technical putrescine; 1 µg of purified putrescine; 0.25 ng of 1-pyrroline; or 0.30 pg of 1-pyrroline. The first 1-pyrroline amount, 0.25 ng, is equivalent to the amount found in 1 µg of technical putrescine. The latter 1-pyrroline amount, 0.30 pg, was intended to be equivalent to the amount in 1 µg of

purified putrescine, but was approximately 6× higher due to miscalculation. Testing of the actual equivalent amount of 1-pyrroline was deemed unnecessary based on bioassay results with 0.30 pg. Control papers received an additional 10 µl of water. The additional 10 µl aliquots put onto treatment and control papers were placed near to, but not overlapping, the 10 µl of ammonium bicarbonate and methylamine HCl solution.

The combinations were tested in a series of 3 experiments. In each experiment, bioassays of technical putrescine combinations were paired with bioassays of combinations containing one of the other 3 chemicals (Table 3).

Test of Observer Bias and/or pH

An experiment was conducted to test observer bias and/or effect of pH on bioassay results. The additional experiment was conducted like the other combination experiments except that water at pH 9, the same pH as all bioassay test solutions in previous experiments, was applied to treatment papers instead of a test chemical. In this last experiment, the observer was unaware that the test chemical was water.

Statistical Analysis of Bioassays

Bioassay count differences were obtained by subtracting the total count at the control papers from the total count at the treatment papers. Paired t-tests were performed to determine attractiveness of chemicals or combinations on treatment papers relative to control papers by testing whether bioassay count differences were significantly different from 0. Student's t-tests or analyses of variance (ANOVA) were used to determine attractiveness of various chemicals or combinations relative to other chemicals or combinations by testing whether their bioassay count differences were significantly different from each other. SuperANOVA (Abacus Concepts 1989) was used to perform ANOVA's.

Results and Discussion

HPLC Purification of Putrescine

Peak elution of putrescine from the HPLC column was between 7-9 min but elution occurred over at least 30 min. No attempt was made to improve column efficiency by addition of stabilizers to the mobile phase because I wanted the purified putrescine in water only. Concentrations of putrescine in 2 HPLC collections were 2.2 and 5.3 mg/ml (1 determination of each).

Concentrations of 1-Pyrroline in Putrescine

The mean concentration of 1-pyrroline in a 10 mg/ml solution of technical putrescine was calculated as 2.5 ± 0.12 (SE) µg/ml (n = 3 determinations). This is approximately 0.025% w/w relative to putrescine. The mean concentration of 1-pyrroline measured in 2 concentrations of HPLC purified putrescine (2.2 and 5.3 mg/ml) was calculated as 0.20 ± 0.015 ng/ml (n = 2 determinations, one for each putrescine concentration), or 0.0000053% w/w. The reduction of 1-pyrroline in purified putrescine relative to technical putrescine was 99.98%.

Formation of 1-Pyrroline from Technical Putrescine in Vials

1-Pyrroline peak areas obtained from samples 10 min after application of technical putrescine solution onto filter papers in vials were 96 ± 2.2% (mean ± SE, n = 3 replications) as great as those sampled immediately. This indicates that the amount of 1-pyrroline in the vials did not increase during the 10 min test. Note that this does not prove that 1-pyrroline did not form from putrescine in the vial. It is possible that 1-pyrroline both formed and degraded inside the vial. However, the result indicates that no net increase in the amount of 1-pyrroline should occur from oxidation of purified putrescine during bioassays.

Attractiveness of Putrescine and 1-Pyrroline

Technical putrescine and HPLC purified putrescine were significantly more attractive than water at the 1 and 10 µg test quantities, and purified putrescine was also significantly more attractive than water at the 0.1 µg quantity (smallest $t = 3.4$, df = 11, $P < 0.01$, for 0.1 µg purified putrescine vs. water) (Table 1). There were no significant differences between responses to technical putrescine and purified putrescine at any of the 4 test quantities by *t*-tests. ANOVA also indicated that purified putrescine and technical putrescine did not differ in attractiveness, summed over all concentrations. However, an ANOVA that included data from only the 3 highest test quantities showed that purified putrescine was significantly more attractive than technical putrescine ($F = 6.4$; df = 1,50; $P < 0.05$). The results suggest that something removed from technical putrescine by purification, perhaps 1-pyrroline, inhibited attractiveness of putrescine.

Technical putrescine and 1-pyrroline were significantly more attractive than water at the 2 highest technical putrescine test quantities and the 3 highest 1-pyrroline quantities (smallest $t = 3.1$, df = 11, $P < 0.05$, for 25 pg of 1-pyrroline vs. water) (Table 2). There were no significant differences between responses to technical putrescine and 1-pyrroline at any of the 4 equivalent test quantities. ANOVA also indicated that 1-pyrroline and technical putrescine did not differ in attractiveness, summed over all concentrations.

TABLE 1. ATTRACTIVENESS OF TECHNICAL-GRADE PUTRESCINE AND PURIFIED PUTRESCINE TO MEXICAN FRUIT FLIES IN CAGE-TOP BIOASSAYS.[1]

Test chemical applied to T paper[2]	Total putrescine	Total 1-pyrroline	Mean ± SE Count at T	Mean ± SE Count at C[3]	Ratio T/C
Technical putrescine	0.010 µg	2.5 pg	51.2 ± 4.6	46.0 ± 5.8	1.11
Purified putrescine	0.010 µg	0.53 fg	56.4 ± 4.2	49.9 ± 5.0	1.13
Technical putrescine	0.10 µg	25 pg	57.8[4] ± 8.3	51.5 ± 4.6	1.12
Purified putrescine	0.10 µg	5.3 fg	63.9[4,5] ± 4.2	50.2 ± 4.0	1.27
Technical putrescine	1.0 µg	250 pg	77.8[4,5] ± 9.0	54.8 ± 6.8	1.42
Purified putrescine	1.0 µg	53 fg	75.3[4,5] ± 6.1	48.4 ± 4.6	1.56
Technical putrescine	10 µg	2.5 ng	71.3[4,5] ± 3.1	52.7 ± 4.9	1.35
Purified putrescine	10 µg	530 fg	80.2[4,5] ± 5.1	51.5 ± 4.0	1.56

[1]Six replications of the experiment were conducted. Each replication included 2 bioassays of each purified putrescine quantity and 1 of each technical putrescine quantity, conducted in random order.
[2]T (treatment) paper contains amounts of test chemicals shown in table.
[3]C (control) paper contains water.
[4]Purified putrescine was more attractive than technical putrescine by ANOVA including only the highest 3 test quantities.
[5]The count at T was significantly greater than the count at C by paired t-test ($P < 0.05$).

An ANOVA that included data from only the 2 highest test quantities showed that technical putrescine was significantly more attractive than 1-pyrroline (F = 4.3; df = 1,33; $P < 0.05$). The results show that 1-pyrroline does not completely account for the attractiveness of technical putrescine, at least at some concentrations.

Attractiveness of Putrescine and 1-Pyrroline with Ammonium Bicarbonate and Methylamine HCl

Combinations of ammonium bicarbonate and methylamine HCl with 1µmg of either technical putrescine or purified putrescine were significantly more attractive than ammonium bicarbonate and methylamine HCl alone (t = 3.8, df = 19, $P < 0.01$, for each chemical) (Table 3, Pair 1). Moreover, both chemicals increased the attractiveness of the combinations equally. These results indicate that purified putrescine can account for the increase in attractiveness that occurs when technical putrescine is added to a mixture of ammonium bicarbonate and methylamine HCl.

Combinations with either 1 µg of technical putrescine or 0.25 ng of 1-pyrroline, the amount present in 1 µg of technical putrescine, were also significantly more attractive than combinations without these chemicals (smaller t = 2.5, df = 23, $P < 0.05$ for 1-pyrroline) (Table 3, Pair 2). Again, both chemicals increased the attractiveness of the combinations equally. These results indicate that the amount of 1-pyrroline equal to that in technical putrescine can account for the increase in attractiveness that occurs when technical putre-

TABLE 2. ATTRACTIVENESS OF TECHNICAL-GRADE PUTRESCINE AND 1-PYRROLINE TO MEXICAN FRUIT FLIES IN CAGE-TOP BIOASSAYS.[1]

Test chemical applied to T paper[2]	Total putrescine	Total 1-pyrroline	Mean ± SE Count at T	Mean ± SE Count at C[3]	Ratio T/C
Technical putrescine	0.010 µg	2.5 pg	45.8 ± 5.6	43.2 ± 6.7	1.06
1-pyrroline	nd[4]	2.5 pg	45.0 ± 3.5	45.7 ± 3.9	0.98
Technical putrescine	0.10 µg	25 pg	51.7 ± 7.3	45.2 ± 6.0	1.14
1-pyrroline	nd[4]	25 pg	50.6[5] ± 3.5	43.8 ± 3.0	1.15
Technical putrescine	1.0 µg	250 pg	66.0[5,6] ± 8.4	43.8 ± 5.2	1.51
1-pyrroline	nd[4]	250 pg	53.2[5,6] ± 4.0	42.0 ± 2.9	1.27
Technical putrescine	10 µg	2.5 ng	74.3[5,6] ± 7.6	40.8 ± 8.3	1.82
1-pyrroline	nd[4]	2.5 ng	63.7[5,6] ± 5.6	43.8 ± 4.0	1.45

[1]Six replications of the experiment were conducted. Each replication included 2 bioassays of each 1-pyrroline quantity and 1 of each technical putrescine quantity, conducted in random order.
[2]T (treatment) paper contains amounts of test chemicals shown in table.
[3]C (control) paper contains water.
[4]nd, not detected by GC/FTD.
[5]The count at T was significantly greater than the count at C by paired t-test ($P < 0.05$).
[6]Technical putrescine was more attractive than 1-pyrroline by ANOVA including only the highest 2 test quantities.

TABLE 3. ATTRACTIVENESS OF MIXTURES CONTAINING AMMONIUM BICARBONATE, METHYLAMINE HCl AND EITHER TECHNICAL-GRADE PUTRESCINE, PURIFIED PUTRESCINE OR 1-PYRROLINE TO MEXICAN FRUIT FLIES IN CAGE-TOP BIOASSAYS.

Pair[1]	Test chemical applied to T paper[2]	Total putrescine	Total 1-pyrroline	Mean ± SE Count at T	Mean ± SE Count at C[3]	Ratio T/C
1	technical putrescine	1.0 µg	0.25 ng	99.2[4] ± 3.7	84.4 ± 4.4	1.18
	purified putrescine	1.0 µg	0.053 pg	102.0[4] ± 4.2	86.2 ± 3.5	1.18
2	technical putrescine	1.0 µg	0.25 ng	112.2[4] ± 4.1	95.5 ± 3.8	1.17
	1-pyrroline	nd[5]	0.25 ng	114.4[4] ± 4.6	100.3 ± 3.5	1.14
3	technical putrescine	1.0 µg	0.25 ng	109.2[4,6] ± 4.8	88.9 ± 4.4	1.23
	1-pyrroline	nd[5]	0.30 pg	92.1 ± 3.7	87.4 ± 3.7	1.05
4	technical putrescine	1.0 µg	0.25 ng	110.7[4,6] ± 2.7	90.9 ± 3.4	1.22
	water (pH 9)	nd[5]	nd[5]	93.1 ± 1.9	89.7 ± 2.9	1.04

[1]Replications: Pair 1, 20; Pair 2, 24; Pair 3, 36; Pair 4, 18.
[2]T (treatment) paper contains 10 µg of ammonium bicarbonate, 10 µg of methylamine HCl, and amounts shown in the table of technical putrescine, purified putrescine, and 1-pyrroline.
[3]C (control) paper contains 10 µg of ammonium bicarbonate and 10 µg of methylamine HCl.
[4]The count at T was significantly greater than the count at C by paired t-test ($P < 0.05$).
[5]nd, not detected by GC/FTD.
[6]The T-C count difference was significantly greater for combinations with technical putrescine than with 1-pyrroline or water, respectively.

scine is added to a mixture of ammonium bicarbonate and methylamine HCl.

The combination with 0.30 pg of 1-pyrroline, an amount greater than that present in 1 µg of purified putrescine but less than that in 1 µg of technical putrescine, was not significantly more attractive than ammonium bicarbonate and methylamine HCl alone (Table 3, Pair 3). As in previous experiments, the combination with 1 µg of technical putrescine was significantly more attractive than ammonium bicarbonate and methylamine HCl alone ($t = 6.6$, df = 35, $P < 0.001$). The combination containing technical putrescine was significantly more attractive than the combination containing 1-pyrroline ($t = 3.6$, df = 34, $P < 0.001$). These results indicate that the amount of 1-pyrroline in purified putrescine does not account for the increase in attractiveness that occurs when purified putrescine is added to a mixture of ammonium bicarbonate and methylamine HCl (Experiment 3).

Effect of Observer Bias and pH

Combinations in which water at pH 9 was substituted for a test chemical were not more attractive than ammonium bicarbonate and methylamine HCl alone (Table 3, Pair 4). As in previous experiments, the combination with 1 µg of technical putrescine was significantly more attractive than ammonium bicarbonate and methylamine HCl alone ($t = 5.2$, df = 17, $P < 0.001$). These results indicate that observer bias and pH of test solutions were not significant problems in this work. However, the 4% increase in counts at combinations with water at pH 9 gives an indication that some positive bias may be present in the results.

Relative Attractiveness of Putrescine and 1-Pyrroline in Technical Putrescine

This research indicates that attractiveness of technical putrescine is a function of both putrescine and 1-pyrroline. Both purified putrescine and 1-pyrroline were individually attractive at concentrations equivalent to those in technical putrescine. Despite the finding that each is attractive, 2 lines of evidence indicate that putrescine plays a bigger role than 1-pyrroline in attractiveness of technical putrescine. First, putrescine purified of most 1-pyrroline was more attractive than technical putrescine at some concentrations (Table 1), suggesting that 1-pyrroline may inhibit attractiveness of putrescine in technical putrescine. Second, technical putrescine was more attractive than 1-pyrroline at some concentrations equivalent to those in technical putrescine (Table 2), indicating that 1-pyrroline cannot account for all of the attractiveness of technical putrescine. However, experiments also showed that combinations of ammonia and methylamine with either technical putrescine, purified putrescine or 1-pyrroline were equally attractive (Table 3). Thus, under some conditions such as in the combinations tested here and in the absence of putrescine, 1-pyrroline can substitute for putrescine.

Neural Reception

The evidence that both putrescine and 1-pyrroline are attractive individually, that technical putrescine is less attractive than the putrescine it contains, and that 1-pyrroline can substitute for putrescine in certain combinations with other chemicals, is difficult to reconcile to traditional models of odorant reception. Actually, the results

are not surprising if the 2 chemicals share the same receptor neuron. In this model, the neuron can accept either chemical in the absence of the other, responds better to putrescine, and has a higher threshold response to putrescine when 1-pyrroline also binds. This is a tenable hypothesis based on recent findings that a single insect or mammalian olfactory receptor neuron can accept and respond to numerous similar and sometimes dissimilar odorants (Lemon and Getz 1999, Laurent 1999). The model does not preclude the possibility that additional receptor neuron types that bind one or both chemicals, perhaps at different threshold response levels, also are present on the antenna.

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