Solid-Phase Microextraction Analysis of Static-Air Emissions of Ammonia, Methylamine, and Putrescine from a Lure for the Mexican Fruit Fly (*Anastrepha ludens*)

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A method was developed to analyze ammonia, methylamine, and putrescine emanating from a lure for the Mexican fruit fly (*Anastrepha ludens*). Lures were put into closed glass vessels containing 2% phosphoric acid to trap the basic volatiles. The phosphoric acid solution was then made basic, and solid-phase microextraction (SPME) was used to sample the dissolved chemicals and load them without solvent onto a thick-film gas chromatography column. Calculated emissions from the lures at 35 °C were 234−373 μg/h for ammonia, 25.8−61.3 μg/h for methylamine, and 15.0−19.4 ng/h for putrescine during the first 48 h after opening the tubes. 1-Pyrroline, a chemical not present in the lures by design, emanated at ∼20 ng/h. Flame thermionic detection aided analysis of putrescine and 1-pyrroline. Experiments with standards indicated that determination errors of ±50% could be expected due to inherent problems associated with the analytical methods.

Keywords: *Mexican fruit fly; Anastrepha ludens; lures; volatiles analysis; ammonia; methylamine; putrescine; 1-pyrroline; solid-phase microextraction*

INTRODUCTION

Robacker and Warfield (1993) developed an attractant (AMPu) for the Mexican fruit fly (*Anastrepha ludens* Loew) consisting of a 10:10:1 mixture of ammonium bicarbonate, methylamine hydrochloride, and putrescine. An agar formulation of this attractant with ammonium carbonate substituted for ammonium bicarbonate (the molar ratio of ammonia was not changed), was highly attractive to Mexican fruit flies when tested in a citrus orchard in plastic microcentrifuge tubes (Robacker, 1995).

Emissions from AMPu/agar lures were determined for several reasons. Preliminary tests indicated that the agar preparations partially dehydrated within 2 days in the field. Therefore, emissions from lures were measured to determine if they changed as lures aged from 1 to 2 days. Because lures were very attractive during the first 24 h (Robacker, 1995), it seemed important to determine the attractive emission rates. It was hoped that development of better formulations (i.e., formulations that emit the chemicals at constant rates for a period of 1−2 weeks) would be aided if attractive "target" rates were known and a controlled laboratory method was available to verify that these target rates had been achieved. Finally, Robacker and Warfield (1993) hypothesized that 1-pyrroline, a chemical that forms spontaneously from putrescine (Amoore et al., 1975) may be responsible for the observed attractiveness of putrescine in AMPu. Collection and quantification of emissions from AMPu/agar lures allowed us to determine how much 1-pyrroline emanated from the lures.

Emissions of ammonia, methylamine, and putrescine from the AMPu/agar lures were not measured previously because no satisfactory method was available. There are several reasons for this lack of methodology. First, these chemicals do not retain well on adsorbents, such as Super Q, that are commonly used to collect emissions from insect lures. Second, solvents used to extract these chemicals interfere with gas chromatography (GC) analysis, particularly of ammonia and methylamine, chemicals that elute near the solvents. Third, most GC columns and glass inserts adsorb small amounts of these chemicals, making trace analysis very difficult.

The purposes of this work were to develop and evaluate a method to collect and quantify volatiles such as ammonia and low molecular weight amines and to use the method to quantify emissions from AMPu/agar lures like those evaluated for attractiveness to Mexican fruit flies in field tests (Robacker, 1995). Four experiments were conducted. The first experiment was to construct calibration curves for ammonia, methylamine, and putrescine by solid-phase microextraction (SPME) sampling and GC analysis. SPME was chosen for this work because it allows solventless injection of analytes, a key point just discussed. Also, a high degree of linearity is possible with SPME quantification (Arthur et al., 1992b; MacGillivray et al., 1994). The second experiment was conducted to measure the accuracy of the analysis method using known quantities of the chemicals spiked into test samples. The third experiment was to determine emissions of the three chemicals from the AMPu/agar lures. Finally, the fourth experiment tested the effect of reabsorption of chemicals into a blank agar tube after emission from the AMPu/agar lure. This last experiment was done as a control to determine if chemicals that emanate from the AMPu/agar lures appreciably reabsorb into the lures under the

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Table 1. Determination Error of Known Concentrations of Ammonia, Methylamine, and Putrescine Added to Emissions-Trapping Solution in Collection Vials and Calculated by the Experimental Method

	ammonia (mg/mL)			methylamine (mg/mL)			putrescine (μg/mL)		
expt[a]	actual	calcd	error (%)	actual	calcd	error (%)	actual	calcd	error (%)
1a	15.0	13.2	−12	2.5	2.6	5	0.92	0.86	−7
1b	30.0	25.2	−16	5.0	3.9	−22	1.8	1.3	−28
2a	2.0	2.1	5	0.20	0.21	3	1.00	0.83	−17
2b	8.0	6.6	−18	0.80	0.70	−12	4.0	5.6	39
3a	2.0	1.8	−8	0.20	0.22	9	1.0	1.1	6
3b	8.0	6.2	−22	0.80	0.84	5	4.0	4.9	22
3c	16.5	12.2	−26	2.7	3.8	39	1.0	0.72	−28
3c	16.5	10.2	−38	2.7	4.0	47	1.0	0.50	−50
3c	16.5	9.6	−42	2.7	3.8	41	1.0	0.46	−54
3d	33.0	18.2	−45	5.4	7.2	33	2.0	1.4	−32
mean ($n = 10$)	−	−	−22 ± 5.0	−	−	15 ± 7.5	−	−	−15 ± 10

[a] Experiment 1, solutions analyzed immediately after putting them into collection vials; experiment 2, solutions put into collection vials overnight before analysis; experiment 3, solutions put into collection vials with agar tubes overnight before analysis; letters following experiment numbers refer to different concentrations tested.

static-air conditions of these tests, resulting in calculated emissions that underestimate actual levels.

MATERIALS AND METHODS

Chemicals. Ammonium carbonate, isopropylamine, putrescine, and 2,5-dimethylpyrazine were obtained from Aldrich Chemical Company (Milwaukee, WI). Methylamine hydrochloride and trimethylamine hydrochloride were obtained from Sigma Chemical Company (St. Louis, MO). All chemicals were at least 98% pure. Phosphoric acid (85% aqueous, HPLC grade) and sodium hydroxide (electrolytic pellets, 98%) were obtained from Fisher Scientific (Fair Lawn, NJ).

Chemical Analysis Method. All quantitative chemical analyses were conducted by the following method. A SPME device (Supelco, Inc., Bellefonte, PA) containing a polydimethylsiloxane-coated (100 μm) fiber was used to sample the chemicals in tapered 0.1-mL glass Varian-type autosampler vials (Alltech Associates, Inc., Deerfield, IL; cat. no. 9482). The assembly needle was inserted into 0.18 mL of the calibration solutions through the septum in the screw cap on the 0.1 mL vials (actual internal volume, 0.34 mL). The fiber was exposed in the solutions for 10 min at laboratory temperatures (21−23 °C). Preliminary tests indicated that a 10 min adsorption time was sufficient to ensure reproducibility. The fiber was then inserted immediately into an on-column injector port of a Shimadzu GC-17A (Shimadzu Scientific Instruments, Inc., Columbia, MD) for thermal desorption for 2 min at 210 °C. The analytical column was a DB-1 capillary column (J&W Scientific, Folsom, CA; 60 m, fused silica, 0.32 mm i.d., 5-μm film). The GC operating conditions were as follows: injector temperature, 210 °C; detector temperature, 220 °C; helium carrier linear velocity, 30 cm/s for 70 °C analyses and 20 cm/s for 140 °C analyses; on-column injection; column temperature, 70 °C isothermal for ammonia, methylamine, trimethylamine, and isopropylamine, and 140 °C isothermal for putrescine and 2,5-dimethylpyrazine. Because the inner diameter of the analytical column was smaller than the diameter of the SPME fiber, on-column injection was accomplished by inserting the fiber into a 10-cm length of deactivated fused silica (0.53 mm; Supelco) in the injection port connected to the analytical column by a GlasSeal connector (Supelco). Flame ionization detection (FID) was used for ammonia, methylamine, trimethylamine, and isopropylamine, and flame thermionic detection (FTD-17, Shimadzu) for putrescine and 2,5-dimethylpyrazine. GC peak heights were quantified with Millennium 2010 Chromatography Manager software (Waters Corporation, Milford, MA).

Calibration Curves. Calibration curves for ammonia, methylamine, trimethylamine, putrescine, and 2,5-dimethylpyrazine were constructed from six calibration solutions containing various concentrations of the chemicals in 2% phosphoric acid. Trimethylamine and 2,5-dimethylpyrazine were included because they were used as surrogate standards in volatiles collections from AMPu/agar lures. Concentrations ranged in steps of 2X from 1−32 mg/mL, 0.1−3.2 mg/mL, 0.05−1.6 mg/mL, 0.5−16 μg/mL and 0.25−8 μg/mL, respectively, for the five chemicals. Isopropylamine was added to each solution as an internal standard at a concentration of 0.4 mg/mL. The pH of solutions was adjusted to 11 with saturated, aqueous sodium hydroxide before analysis. Volume changes due to added sodium hydroxide were taken into account in determining final concentrations of chemicals.

Analyses were as described under Chemical Analysis Method. Five replications each were conducted for 70 and 140 °C analyses of the set of six calibration solutions, with each replication on a different day. Calibration curves were constructed with the Simple Regression procedure of SuperANOVA (Abacus Concepts, 1989) and the Curve-Fit option of Cricket Graph 1.3.2 (Cricket Software, 1986−89).

Analysis of Known Amounts of Ammonia, Methylamine, and Putrescine. Three experiments were conducted to determine the accuracy of the analytical methods. In the first, known amounts of ammonia, methylamine and putrescine (see Table 1 for concentrations), from the same stock solutions used to prepare the calibration solutions, were put into 20-mL glass vials with 0.5 mL of aqueous 2% phosphoric acid containing trimethylamine hydrochloride at 0.33 mg/mL and 2,5-dimethylpyrazine at 1 μg/mL. The phosphoric acid solution, with trimethylamine and 2,5-dimethylpyrazine included as surrogate standards for 70 and 140 °C analyses, respectively, was put into the vials because it was used as an emissions-trapping solution for AMPu/agar lures in experiments described in the next section. The solutions were removed immediately from the 20-mL vials for analysis. This experiment tested accuracy of the SPME analysis method. In the second experiment, solutions were left in the closed vials for 24 h at 35 °C before analysis to test the ability of the trapping solution to hold onto the chemicals that are dissolved in it for 1 day. In the third experiment, a 1.9-mL microcentrifuge tube containing 1% agar at pH 6.5 was put into the vials (tube oriented open end upward) for 24 h at 35 °C. This experiment tested whether agar tubes would affect quantification of the chemicals in the trapping solution.

Solutions were spiked with isopropylamine at 0.4 mg/mL before GC analysis. Analyses were conducted as just described. Calibration curves to quantify chemicals in the vials were constructed each day from two assays of each of the two calibration solutions with the lowest and highest concentrations of the chemicals. These control experiments with known concentrations of chemicals were conducted following preliminary collections and analyses of volatiles from AMPu/agar lures so that concentrations could be chosen below, near, and above those calculated in volatiles collections from the AMPu/agar lures.

Collection and Quantification of Emissions from AMPu/Agar Lures. AMPu/agar lures were made as described previously (Robacker, 1995). Briefly, an aqueous solution of ammonium carbonate, methylamine hydrochloride, and putrescine was mixed with hot agar (Bacto Agar, Difco Laboratories, Detroit, MI) solution in 1.9 mL microcentrifuge tubes. Final concentrations in AMPu/agar lure tubes were: am-

monium carbonate, 60 mg/mL; methylamine hydrochloride, 100 mg/mL; putrescine, 10 mg/mL; and agar, 1%. The pH of the AMPu/agar matrix was 8.5−8.8.

For collection of volatiles, caps were removed from AMPu/agar lure tubes, and each tube was placed upright into a 20-mL glass vial with a screw top. Vials were charged with 0.5 mL of emissions-trapping solution and left for 24 h at 35 °C. The 35 °C temperature was chosen to approximate daytime temperatures in citrus orchards in Texas where the lures had proven attractive to Mexican fruit flies. After 24 h, trapping solutions were removed, vials were rinsed with water, and another 0.5 mL of emissions-trapping solution was added to vials along with the same AMPu/agar lures. Volatiles were collected from the AMPu/agar lures for another 24 h. The pH of each emissions collection was adjusted to 11.0 with aqueous sodium hydroxide for GC analysis by SPME as already described.

During analyses of emissions from AMPu/agar lures, an unknown peak with the same retention time as 1-pyrroline was observed by FTD. This peak was analyzed by GC-MS to determine its identity. GC-MS analyses were conducted with a Hewlett Packard 5890 GC with a HP 5970 mass selective detector (electron energy = 70 eV). Mass spectral data were acquired over a mass range of 20−80 amu, at 7 scans/s. Injection was by SPME as described for analyses of ammonia, methylamine, and putrescine. SPME sampling was done by inserting the fiber through a septum into the headspace above 0.5 mL of an AMPu/agar lure emissions collection in a 2-mL vial for 30 min. The analytical column was a DB-1 (30 m, 0.32 mm i.d., 5 μm film). The linear velocity of the helium carrier gas was 20 cm/s. The column oven temperature was 35 °C for 1 min, then programmed at 25 °C/min to 220 °C.

Standard 1-pyrroline for GC-MS was synthesized by acid hydrolysis of 4-aminobutyraldehyde diethyl acetal according to methods of Schopf and Oechler (1936). A 50-mL conical flask containing a magnetic stirbar was cooled in an ice-water bath and charged with 0.36 g of 4-aminobutyraldehyde diethyl acetal (Aldrich, 90%) and 4 mL of 2 N hydrochloric acid (Fisher). The reaction mixture was stirred for 25 min before 6 mL of 1.2 M potassium carbonate (J. T. Baker Chemical Company, Phillipsburg, NJ) were stirred into the flask. The concentration of 1-pyrroline in this product and in AMPu emissions was determined with a calibration curve for pyrrolidine (Sigma, 99%).

Reabsorption of Emissions by Agar. This experiment was conducted to determine the tendency of the chemicals emitted from the AMPu/agar lures to be reabsorbed by the agar, a phenomenon that would be less likely in the field where air currents probably carry the chemicals away from the lures before they can reabsorb. A tube containing only 1% agar (pH = 6.5) and an AMPu/agar lure tube were put into a 50-mL round-bottomed flask with a glass stopper. The two tubes were placed parallel to each other inside the flask with their open ends adjacent. The tubes were oriented at ~45° with their open ends higher than their closed ends. The purpose of the 1% agar tube was to provide an agar surface for the chemicals to absorb. In concurrent tests, volatiles were collected from AMPu/agar lures in 50 mL flasks without 1% agar tubes. Flasks contained 0.5 mL of emissions-trapping solution as usual. The flasks were kept for 24 h at 35 °C before analysis of the trapping solutions as before. The hypothesis was that the trapping solution from the flask with the 1% agar tube would contain less of the chemicals if agar was reabsorbing a significant amount of the chemicals after they had volatilized.

RESULTS AND DISCUSSION

Calibration Curves. Sampling error for repeated analyses of chemicals at any one concentration of the calibration solutions was very small in most cases. The two cases with the highest sampling error (in terms of coefficient of variation) were the lowest (mean FTD response ± SD = 1.34 ± 0.32 mV) and highest (21.3 ± 3.7 mV) concentrations of putrescine.

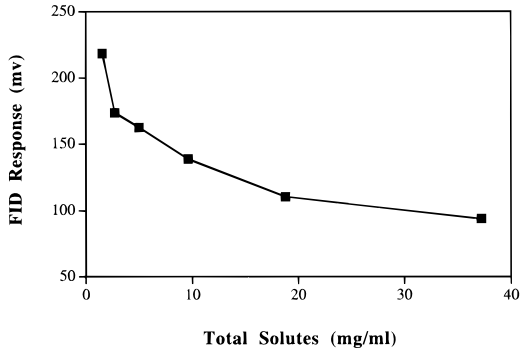

Figure 1. FID response to 0.4 mg/mL of isopropylamine in solutions containing different concentrations of total solutes (ammonia + methylamine + trimethylamine + isopropylamine + putrescine + 2,5-dimethylpyrazine) at pH 11.0.

Calibration curves were generally linear. Linear regression R^2 values were: ammonia, 0.98; methylamine, 0.99; trimethylamine, 0.98; putrescine, 0.92; and 2,5-dimethylpyrazine, 0.99. However, curvature was evident in the regressions for ammonia, trimethylamine, and putrescine. Curvilinear regression equations were calculated for the latter three chemicals. Curvilinear regression (3rd order polynomial) accounted for more variability than linear regression, resulting in R^2 values of 0.996, 0.995, and 0.955 for ammonia, trimethylamine, and putrescine, respectively. Because of time constraints in producing six-point calibration curves each day, two-point linear regression curves were constructed with only the lowest and highest concentrations of the calibration standards and compared with the curvilinear regression equations that used all six calibration solutions. These two-point linear regression curves differed from the six-point curvilinear regression curves by −30% for ammonia (i.e., the linear curve underestimated the concentration on the curvilinear curve), +7% for trimethylamine, and −48% for putrescine at points on the respective curves that differed the most. Most relevant to the current work (as will be discussed later), the two-point linear curves differed from the six-point curvilinear curves by −29% for ammonia and −15% for putrescine at concentrations of the respective chemicals that were similar to those actually analyzed in emissions collections from the AMPu/agar lures. Assuming that the curvilinear equations predicted with 100% accuracy, an argument that certainly is not true, then errors in calculating ammonia and putrescine concentrations from the two-point linear regression curves would be −29% and −15%, respectively.

A second factor that affects quantification is related to the total concentration of solutes in the vials. The amount of isopropylamine adsorbed by SPME fibers (as shown by FID response) from calibration solutions containing the same concentration of isopropylamine decreased as the concentration of total solutes increased (see Figure 1). A similar finding was reported in which amounts of several analytes adsorbed by SPME fibers decreased markedly when the concentration of methanol in the solutions increased from 1 to 3% (Arthur et al., 1992a). This phenomenon is not a "salting out" effect. Arthur et al. (1992a) showed that increasing ionic strength with salt results in increased adsorption by the SPME fibers. Demonstration that this factor influences errors in determination of ammonia and putrescine will be discussed in the next section.

These results indicate that some error should be inherent in SPME/GC quantification in this work. After consideration of errors caused by differences in concen-

trations of total solutes (as demonstrated with isopropylamine), errors associated with linear versus curvilinear regressions, and time constraints in producing six-point curvilinear calibration curves each day, we decided to use the simpler two-point linear calibration curves for all analyses.

Analysis of Knowns. Empirical determinations of known amounts of ammonia, methylamine, and putrescine in solutions containing varying amounts of total solutes, without using the surrogate standards trimethylamine and 2,5-dimethylpyrazine or the internal standard isopropylamine to correct the calculations, indicated that errors of ±50% are possible for the three chemicals in a single analysis (Table 1). Concentrations of ammonia and putrescine calculated from the calibration regression equations usually were lower than actual concentrations, whereas those of methylamine usually were greater than actual. Within an experiment, the highest errors were associated with solutions that contained the highest concentrations of total solutes. For example, errors were higher for solutions 1b, 2b, and 3d than for 1a, 2a, and 3a, respectively. Paired *t* tests comparing errors (absolute values) from solutions 1a with 1b, 2a with 2b, 3a with 3b, 3b with 3c, and 3c with 3d were conducted to test this assertion for each of the three chemicals. The test was significant for ammonia ($t = 5.9$; df = 4, $p < 0.01$), but tests were not significant for the other two chemicals. Whether chemicals were analyzed immediately or left in collection vials for 24 h may also have affected error. For example, error was higher for solutions 3c and 3d that were held 24 h in collection vials than for solutions 1a and 1b (actual concentrations similar to 3c and 3d) that were analyzed immediately after they were put into collection vials. The *t* tests comparing errors (absolute values) from solutions 1a and 1b with those from 3c and 3d were significant for ammonia ($t = 3.3$; df = 4; $p < 0.05$) and methylamine ($t = 3.9$; df = 4; $p < 0.05$), but not for putrescine. Whether or not an agar tube was present in vials in which solutions were held for 24 h had no apparent effect on determination error. Specifically, error was about the same for the pairs of equimolar solutions 2a and 3a, and 2b and 3b, even though 3a and 3b were held with agar tubes and 2a and 3a were not. Mean errors, calculated over the three experiments combined, ranged between 15 and −22% for the three chemicals (Table 1).

The use of linear regression instead of curvilinear regression does not account for the observed errors shown in Table 1. The errors are, instead, more highly correlated to the total concentration of solutes. To illustrate this, errors for ammonia were highest at 33 mg/mL (Table 1) and errors for putrescine were highest in experiment 3 at 1−2 μg/mL, concentrations in each case near where linear and curvilinear curves would intersect.

Correction of the results with surrogate standards trimethylamine and 2,5-dimethylpyrazine contained in the emissions-trapping solution, or the internal standard isopropylamine added to solutions immediately before GC analysis, did not improve accuracy of determinations. The effect of total concentration of solutes on quantification by SPME, and to a lesser extent the imperfect linearity of calibration curves, may have affected quantification in such a manner that simple adjustments based on surrogate/internal standards were ineffective.

Table 2. Emissions (Mean ± SEM) of Ammonia, Methylamine, Putrescine, and 1-Pyrroline from AMPu/Agar Lures at 35 °C in 20 mL Vials for Periods of 0−24 and 24−48 h after Lures Were Taken from Refrigeration and Opened[a]

	emissions	
chemical	0−24 h	24−48 h
ammonia	274 ± 12.0 μg/h	234 ± 14.1 μg/h
methylamine	25.8 ± 2.1 μg/h	27.8 ± 0.8 μg/h
putrescine	15.0 ± 8.7 ng/h	19.4 ± 8.3 ng/h
1-pyrroline	19.8 ± 0.9 ng/h	19.6 ± 1.5 ng/h

[a] Emissions of each chemical from AMPu lures at collection times 0−24 or 24−48 h were not significantly different by *t* tests ($n = 5$ replications).

Table 3. Emissions (Mean ± SEM) of Ammonia, Methylamine, and Putrescine from AMPu/Agar Lures at 35 °C Collected in 50-mL Flasks with or without a Second Tube Containing Only Agar[a]

chemical	AMPu lure alone	AMPu lure + agar tube
ammonia	373 ± 18.6 μg/h	350 ± 23.1 μg/h
methylamine	61.3 ± 3.2 μg/h	51.0 ± 7.0 μg/h
putrescine	17.0 ± 4.4 ng/h	15.3 ± 4.4 ng/h

[a] Emissions of each chemical from AMPu lures in flasks with or without agar tubes were not significantly different by *t* tests ($n = 3$ replications).

Quantification of Emissions from AMPu/Agar Tubes. Calculated emission rates of ammonia, methylamine, and putrescine from AMPu/agar lures are presented in Table 2. The three chemicals emitted at the same rates during day 2 (24−48 h) as during day 1 (0−24 h). This result suggests that emission rates were relatively constant over the 2 days. Emission of ammonia was ~10 times higher than emission of methylamine and >10 000 times higher than emission of putrescine.

1-Pyrroline was identified in AMPu/agar emissions on the basis of GC retention time and mass spectrum identical to synthesized 1-pyrroline. The 1-pyrroline probably formed by spontaneous oxidation of putrescine, as suggested by Amoore et al. (1975). 1-Pyrroline was much less evident in calibration solutions or controls in which putrescine was left for 24 h in vials with trapping solution at 35 °C. This result indicates that the 1-pyrroline was not formed from putrescine while in the trapping solution but formed in the lure and was emitted into the air. Emission rates were the same on both days of emissions collections and about the same as emission rates of putrescine (Table 2). Whether or not 1-pyrroline contributes to the attractiveness of the AMPu/agar lures is not known. 1-Pyrroline has been identified as a pheromone of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann; Baker et al., 1985; Jang et al., 1989) and its attractiveness to *A. ludens* is under investigation.

Reabsorption of Emissions by Agar. Calculated emissions from AMPu/agar lures were not significantly affected by presence in the collection flask of a second tube containing 1% agar at pH 6.5 (Table 3). Thus, reabsorption of the volatilized chemicals by the AMPu/agar lures is negligible. Therefore, the chemicals that volatilize from the AMPu/agar lures primarily end up in the emissions-trapping solution.

Calculated emissions of ammonia and methylamine in this experiment were considerably higher than those in the previous experiment (Table 2). Calibration curves used to quantify emissions were similar in the two experiments. The discrepancy was due either to

differences in emissions inside 50-mL flasks versus 20-mL vials, or to differences between the AMPu/agar lures prepared for the two experiments.

Accuracy of Emission Rates. The surrogate standards, trimethylamine and 2,5-dimethylpyrazine, and the internal standard, isopropylamine, were not used to correct the calculated emission rates because such corrections did not improve the accuracy of determinations of known concentrations of the three chemicals. Also, no correction was made for reabsorption of emissions into the agar medium from which they volatilized because this appeared to be unimportant (Table 3).

Emission rates for ammonia, methylamine, and putrescine, shown in Table 2, were calculated from concentrations in trapping solutions estimated to be ~11 mg/mL of ammonia, 1.2 mg/mL of methylamine, and 0.8 μg/mL of putrescine. These concentrations are comparable to calculated concentrations in control solution 3c for ammonia and putrescine and 3b for methylamine (Table 1). If determination error in these analyses is most dependent on total concentration of solutes as was just discussed, then solution 3c is the most appropriate control solution for determination of error for all three chemicals. Solution 3c determination errors were −35, 42, and −44% for ammonia, methylamine, and putrescine, respectively. Thus, actual emissions of ammonia and putrescine were probably higher than calculated emissions, and the actual emission of methylamine was probably lower than calculated.

Although volatilization rates of the chemicals in static air probably are not as great as would occur in moving air such as in field conditions, it is noteworthy that Heath et al. (1995) obtained rates of ammonia emission from ammonium acetate lures similar to those reported here from AMPu/agar lures. They reported ammonia emission ranging from 80 to 200 μg/h for their medium dosage and from 300 to 500 μg/h for their high dosage lures. Because both the ammonium acetate and the AMPu/agar lures were optimized for maximum attractiveness, it is likely that ammonia emissions from the two lures are similar in the field. Thus, available evidence indicates that emission rates of AMPu components in the closed system described here were not much different from rates of emission of components from ammonium acetate lures determined in moving air.

Theory of the Volatile-Collection and Analysis Method. The theory of this closed-system method of volatiles collection is that ammonia, methylamine, and putrescine will volatilize at reliable rates from the AMPu/agar lures and be quantitatively transferred to a trapping solution. The purpose of the phosphoric acid was to trap the volatile ammonia and amines by converting them into nonvolatile salts. Because formation of free amines [pK_as of ammonia and aliphatic amines = 9−11 (March 1968)] is much less at the low pH of the trapping solution (pH = 1.8) than at the higher pH of the agar medium (pH = 8.5−8.8), the effect of the phosphoric acid should be to create a one-way transfer of amines volatilizing from the top of the tube to the trapping solution. Heath et al. (1995) recently used a similar acid solution to trap airborne ammonia emitted from ammonium acetate fruit fly lures.

The SPME analysis method was chosen for two reasons. First, SPME was developed specifically to analyze organic chemicals in water (Belardi and Pawliszyn, 1989; Zhang and Pawliszyn, 1993). Second, the polydimethylsiloxane fiber does not appreciably adsorb water, resulting in solventless injection of the solutes onto the GC column. This solventless injection is extremely important for ammonia and methylamine that would co-elute with most solvents, making their analysis difficult by GC. Other important factors in the success of this work were the use of on-column injection and a thick-film (5 μm) GC column. Previous research had indicated that these measures were necessary to prevent adsorption of trace quantities of ammonia and low molecular weight amines on active sites in the GC system (Robacker and Flath, 1995).

The reason this "closed system" volatile trapping method was adopted is that standard methods in which air was passed over AMPu lures and through adsorbents, such as Super Q, did not yield satisfactory results. Similarly, Robacker et al. (1993) were unable to collect attractive components volatilizing from bacterial cultures using Tenax GC, Porapak Q or Super Q. The attractive components were later identified as ammonia and mostly low molecular weight amines by SPME (Robacker and Flath, 1995).

Our method was developed specifically to analyze ammonia and water-soluble amines emanating from AMPu/agar lures, but should also work for other types of chemicals and lures. Undoubtedly, the SPME technique that we present here will be modified and improved in future studies. For example, emissions could be collected directly as they volatilize from insect lures, either in static air or in moving air. This direct collection would eliminate the need to trap the chemicals on adsorbents such as Super Q, or in our case in phosphoric acid solution. In this work we did not try this direct-sampling method because of a perceived, but untested, need to concentrate the putrescine to levels that could be detected by our GC methods. It may also be possible to improve the accuracy of the quantification by standardizing the samples so that effects such as that demonstrated with isoproplyamine in solutions containing different total concentrations of solutes could be neutralized. In the current study we adjusted all of the solutions to pH 11 but did not attempt to equalize the ionic strength or concentration of total solutes in the solutions.

To our knowledge, this is the first attempt to use SPME to analyze volatiles from an insect lure. We hope that our work will serve as a basis for further innovations in analysis of semiochemicals emanating from insects, plants, and artificial substrates.

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