ATTRACTION OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE) TO VOLATILES FROM AVIAN FECAL MATERIAL

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ABSTRACT

Flight tunnel bioassays confirmed attraction of female Caribbean fruit flies, *Anastrepha suspensa* (Loew), to volatiles from aqueous solutions of avian fecal material and methanol extracts of avian fecal material. Attraction was highest to freshly prepared and 72-h-old solutions of crude material. In direct comparisons between aqueous solutions of crude material and weight-equivalent amounts of methanol extract, more females were captured in response to volatiles from crude material in tests of 0-, 24- and 72-h-old solutions. Ammonia release rate was greater from the crude material than from the methanol extract in tests of 0-, 24- and 48-h-old solutions, The greatest amount (± sd) of ammonia was released from freshly prepared aqueous solutions of crude material (777 ± 250 µg/h from 75 mg of crude material) but dropped within 24 h (288 ± 96 µg/h from 75 mg of crude material) and then stayed close to that level. The greatest amount of ammonia released from methanol extracts was obtained from freshly prepared solutions (229 ± 70 µg/h from 75 mg crude material weight equivalent), also dropped within 24 h (98 ± 12 µg/h from 75 mg crude material weight equivalent) and then stayed fairly constant. Numbers of flies captured by either solution were directly correlated with ammonia release within the first 48 h of testing only, indicating that ammonia was partially or wholly responsible for attraction to the crude material during the first 48 h of testing. An increase in capture of females by volatiles from avian fecal material after 72 h in aqueous solution, which was observed in all tests, indicates that some chemical(s), other than ammonia, remain to be identified that are involved in fruit fly attraction.

Key Words: Caribbean fruit fly, attractants, ammonia, avian fecal material

RESUMEN

Los bioensayos en túneles de vuelo confirmaron la atracción de las hembras de la mosca frutera del Caribe, *Anastrepha suspensa* (Loew), por volátiles de soluciones acuosas de material fecal de aves y por extractos en metanol del mismo material. La atracción fue más alta por las soluciones de material crudo frescas y de 72 horas de preparadas. En comparaciones directas entre las soluciones acuosas de material crudo y las cantidades equivalentes en pesos de extracto de metanol, más hembras fueron capturadas en repuesta a volátiles de material crudo en pruebas con soluciones de 0, 24 y 72 horas de edad. La tasa de liberación de amonio fue mayor en el material crudo que en el extracto de metanol en pruebas de 0, 24 y 48 horas. La mayor cantidad (± sd) de amonio fue liberada de las soluciones acuosas frescas de material crudo (777 ± 250 µg a partir de 75 mg de material crudo), pero cayó dentro de las 24 horas (288 ± 96 µg/h a partir de 75 mg de material crudo) y entonces permaneció cercana a ese

nivel. La mayor cantidad de amonio liberado de los extractos de metanol fue obtenida de soluciones frescas (229 ± 70 µg/h a partir de 75 mg de material crudo por equivalente en peso), también cayó en 24 horas (98 ± 13 µg/h a partir de 75 mg material crudo por equivalente en peso) y entonces permaneció medianamente constante. Los números de moscas capturados directamente en cualquier solución estuvieron directamente correlacionados con la liberación de amonio dentro de las primeras 48 horas de prueba solamente, indicando que el amonio fue parcial o totalmente responsable de la atracción hacia el material crudo durante las primeras 48 horas de prueba. Un aumento en la captura de las hembras por los volátiles de material fecal de aves en solución acuosa después de las 72 horas, el cual fue observado en todos los tests, indica que algunas sustancias, diferentes del amonio, están pendientes de ser identificadas como envueltas en la atracción de moscas fruteras.

Many adult insects require protein meals to ensure reproductive success. This requirement has been the basis for the successful use of protein-based liquid baits for detecting adults of pest Tephritidae (Anonymous 1989). McPhail traps, which are bell-shaped glass traps with a water reservoir (Newell 1936), baited with torula yeast (TY)-borax pellets (ERA Int., Freeport, NY) are currently used for detection and delineation of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in Florida (Anonymous 1989). These traps have low efficiency (Calkins et al. 1984) and improved lures are needed for these and other pest Tephritidae currently monitored with protein-baited traps (Calkins 1993). Food-based synthetic attractants have been developed for the Mexican fruit fly, *Anastrepha ludens* (Loew), and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Robacker & Warfield 1993, Heath et al. 1995, Robacker 1995). These synthetic attractants, which include ammonia in combination with 1,4 diaminobutane (putrescine), are based on volatiles emitted from liquid protein baits. Adult tephritids have been observed feeding on plant exudates, rotting fruits, decaying insects and bird dung (Christenson & Foote 1960), substances that provide sources of protein. Identification of volatile chemicals from natural food sources, such as bird dung, may provide additional components that could improve the effectiveness of the food-based synthetic attractants.

Adults of C. *capitata* and the apple maggot, *Rhagoletis pomonella* (Walsh), feed on bird dung in the field (Hendrichs & Hendrichs 1990, Hendrichs & Prokopy 1990). Droppings that were held for 24-48 h before testing were more attractive than droppings that were tested before 24 or after 48-72 h, and avian fecal material was more attractive than liquid protein bait in field cage trials (Prokopy et al. 1992, Prokopy et al. 1993a). We report herein the results of laboratory trials that were conducted to evaluate the attraction of Caribbean fruit fly females to volatiles from avian fecal material. Change in attractiveness of avian fecal material over a 4 d period and attraction of flies to volatiles from avian fecal material partially purified by solvent extraction were also tested. Ammonia is one of the volatile chemicals produced by avian fecal material (e.g., Beard & Sands 1973), and ammonia is a known attractant for fruit flies (reviewed in Econompoulos 1989). Therefore, the role of ammonia in attraction was investigated by measuring the release rate of ammonia and correlating female preference with ammonia release from avian fecal material and from methanol extractions.

Material and Methods

Caribbean fruit flies used in this study were obtained as pupae from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in

Gainesville, Florida. Flies were given water and adult food (3:1 mixture of refined cane sugar:brewer's yeast), and were maintained in screen cages (30× 30 × 30 cm) in a laboratory with a photoperiod of 12:12 (L:D) h at room temperature and ambient humidity. Females were 4-10 d post-eclosion and were protein-starved for 24 h before testing.

Avian fecal material was obtained as droppings from housed chickens (Prokopy et al. 1993a). Droppings were collected within 24 h of deposition and placed in storage at 4°C. Fecal material was removed from storage and incubated at room temperature and ambient relative humidity for 24 h before use. This incubation time was found to be important for attractiveness (Prokopy et al. 1993a). Fecal material was tested as aqueous solutions of crude material (mg/ml) and as aqueous solutions of methanol extract (µl/ml) in tap water. Aqueous solutions were used to prevent desiccation of the sample during the bioassay. Previous studies indicated that only water and methanol extracts of crude material retained biological activity for apple maggot attraction (B. D. D., C. R. L. & R. J. P., unpublished data). Methanol extracts of the avian fecal material were made by mixing two parts methanol (volume) to one part crude material (weight). After mixing for 20 min, the particulate material was removed by filtration through a 70-100 µ (micron) sintered glass filter. The filtrate was concentrated to 50% of the original volume under vacuum. Weight equivalents (1 mg/µl) were used for comparisons with crude materials. Fresh solutions were made for each replicate, and solutions were tested over a 4-d-time period (0, 24, 48 and 72 h after preparation) to test for change in attractiveness over time.

Bioassays were conducted using a two-choice volatile attractant bioassay system (Heath et al. 1993). The test insects were released in a flight tunnel (122 × 30.5 × 30.5 cm plexiglass chamber). Two horizontally-mounted traps, with orange sticky paper (Atlanta Paste and Glue, Brooklyn, NY) on the front to retain responding flies, were suspended inside the tunnel. Test substrates (100 ml) were placed in narrow mouth flasks (500 ml), and entrained volatiles from the test substrate were introduced into the bioassay test chamber. Volatiles from the test substrates were vented through the flight tunnels for at least one hour before addition of flies to allow volatile release to stabilize. There were 20-25 females tested per bioassay, and numbers of flies per trap were recorded after approximately 20 h.

Preliminary tests, which evaluated a range of two-fold dilutions (6, 3, 1.5, 0.75 and 0.38 mg/ml) of crude material in tap water, were used to determine appropriate concentrations for the bioassays. These tests indicated that 0.38-1.5 mg/ml was the optimal range for fruit fly capture in the laboratory bioassay. Capture decreased at concentrations greater than 1.5 mg/ml, indicating that test volatiles were repellent at these higher concentrations. Three experiments were conducted to evaluate attractiveness of avian fecal material for *A. suspensa*. In experiment 1, females were exposed to volatiles from 0.38, 0.75 or 1.5 mg/ml of aqueous solutions of crude material or a water blank with 4, 3 and 3 replicates, respectively, of each concentration. In experiment 2, females were exposed to volatiles from 0.38 or 1.5 µl/ml methanol extract in water or a water blank with an equivalent amount of methanol, with 5 replicates of each concentration. In experiment 3, females were given the choice of aqueous solutions of crude material and methanol extract (0.75 mg/ml and 0.75 µl/ml), and there were 13 replicates. In the last 4 replicates of experiment 3, release rates of ammonia from the test substrates were determined each day before the substrate was used in the bioassay. Ammonia release rates were determined using an ammonia-specific ion-selective electrochemical probe (Orion, Boston, MA) following the procedure of Heath et al. (1995).

Number of flies captured by test substrate versus blank and by crude material versus extract were analyzed by two sample *t*-tests (Proc TTEST, SAS Institute, 1985). Effects of other factors were analyzed using a mixed model with interaction (Proc

GLM, SAS Institute 1985). These factors included test solution concentration and time period. Data were assessed by the Box-Cox procedure (Box et al. 1978) and were square-root (x + 0.5) transformed to stabilize the variance before analyses. Correlations between ammonia release rate and number of females trapped for each time period were tested using Proc CORR (SAS Institute 1985).

Results and Discussion

More flies responded to the test solution than to the associated blank in all tests in experiments 1 and 2 (Table 1). Concentration of test substrate had no effect on fly capture, so data from all concentrations were grouped. Separate analyses were conducted on the effect of time period (age of test substrate) on response to crude material and to methanol extract. For these analyses, number responding to the control subtracted from number responding to test substrate, and the difference was used as the response variable (Table 1). Time period affected capture in response to volatiles from crude material (F = 3.15; df = 3, 36; P = 0.0366), but not from methanol extract. The highest capture was obtained with volatiles from fresh solutions and from 72-h-old solutions of crude material.

In experiment 3, more females were captured by crude material for all but the 48-h old test substrates (Table 2). Average ammonia release rates from the test substrates in the last 4 replicates are given in Table 3. The highest amount of ammonia was obtained from the freshly prepared crude material. There was a 37% drop in release rate within 24 h of testing, and ammonia remained at that level throughout the remainder of the study. Ammonia release from the crude material was higher than release from the methanol extract for the first 48 h testing. After 72 h, although the release rate of ammonia from crude avian fecal material was still higher on average, the ammonia release was more variable among the samples and the difference was not significant. Numbers of flies trapped were correlated with ammonia release rate for 0-h (r = 0.69, P = 0.05) and 24-h (r = 0.63, P = 0.05) old test substrates. The number of flies trapped was also indirectly related to presence of methanol, which could indicate that the methanol was repellent. There was methanol in the extract test solution that was not in the crude material test solution. However, the amount of methanol was small (75 µl in 100 ml of water), and preliminary tests indicated that, if anything, the small amount of methanol was attractive.

No correlation between ammonia release and number of flies trapped was found in tests with 48-and 72-h-old substrates. Although there was a significant difference in ammonia released from crude material and methanol extracts in 48-h-old test substrates (Table 3), there was no difference in fly capture (Table 2). The reverse was observed in tests of 72-h-old test substrates, that is, that although there was no difference in ammonia release rates, more flies were captured in response to volatiles from the crude material. Thus, it appears that some attractive chemicals other than ammonia are released from the crude material after 72 h in aqueous solution, Prokopy et al. (1993a, 1993b) found that reducing microbial activity by the addition of antibiotics to avian fecal material reduced attractiveness to fruit flies. The 72 h time lag observed in our studies may indicate that microorganisms, which are utilizing breakdown products from earlier microbial action, may be responsible for the production of these late-appearing volatile chemicals.

The results of this study confirm that volatile chemicals released from avian fecal material are attractive to female Caribbean fruit flies. Ammonia was released in high amounts and there was a direct correlation between ammonia release from and capture of female flies by freshly prepared aqueous solutions of avian fecal material. Thus, ammonia appears to be partially or wholly responsible for fruit fly attraction to

TABLE 1. AVERAGE ± SD NUMBER OF FEMALE *ANASTREPHA SUSPENSA* TRAPPED IN LABORATORY BIOASSAYS OF RESPONSE TO VOLATILES FROM AQUEOUS FORMULATIONS OF TEST SUBSTRATE VERSUS CONTROL IN TESTS CONDUCTED OVER A FOUR DAY TIME PERIOD (FRESHLY PREPARED SOLUTIONS TESTED DURING 0 H).

	Time Period (h)			
Test Substrate	0	24	48	72
Crude material	10.1 ± 0.7*	7.3 ± 0.8*	8.5 ± 0.8*	11.7 ± 1.1*
Water control	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.5	1.0 ± 0.5
Crude material minus control	8.7 ± 2.5	5.9 ± 2.6	7.0 ± 3.4	10.7 ± 4.4
Methanol extract	9.5 ± 1.0*	10.0 ± 0.7*	9.7 ± 1.0*	8.5 ± 0.9*
Methanol/water control	2.7 ± 0.5	2.3 ± 0.6	1.2 ± 0.2	1.9 ± 0.4
Methanol extract minus control	6.8 ± 3.7	7.7 ± 3.3	8.5 ± 2.9	6.6 ± 2.8

*Significant difference in number trapped by treatment odor source and paired control odor source (*t*-test, $P < 0.001$).

TABLE 2. AVERAGE ± SD NUMBER OF FEMALE *ANASTREPHA SUSPENSA* TRAPPED IN LABORATORY BIOASSAYS OF RESPONSE TO VOLATILES FROM AQUEOUS FORMULATIONS OF AVIAN FECAL MATERIAL VERSUS METHANOL EXTRACT OF AVIAN FECAL MATERIAL.

Time period (h)	Number of females trapped		*t*-test comparisons		
	Avian fecal	Methanol extract	*t*	df	*P*
0	7.0 ± 2.82	3.9 ± 2.06	3.17	24	0.0044
24	8.1 ± 2.88	4.3 ± 1.70	4.14	24	0.0004
48	6.8 ± 3.98	6.1 ± 2.61	0.47	24	ns*
72	7.1 ± 2.72	4.5 ± 2.63	2.49	24	0.0201

*not significant.

TABLE 3. AVERAGE ± SD RELEASE RATE OF AMMONIA FROM AQUEOUS SOLUTIONS OF AVIAN FECAL MATERIAL VERSUS METHANOL EXTRACT (MG EQUIVALENTS) OF AVIAN FECAL MATERIAL. AMMONIA MEASUREMENTS WERE MADE AT THE START OF THE TIME PERIOD.

Time period (h)	Ammonia release rate (µg/h)		*t*-test comparisons		
	Avian fecal	Methanol extract	*t*	*df*	*P*
0	778 ± 250	229 ± 70	4.22	6	0.0055
24	288 ± 96	98 ± 12	4.37	8	0.0024
48	250 ± 108	93 ± 30	3.12	8	0.0142
72	244 ± 232	93 ± 41	1.27	6	ns*

*not significant.

fresh bird dung. Methanol extracts maintained some activity, but were less attractive than equal amounts of the crude material and did not show an increase in attractiveness with aging as was observed with crude material. It is hypothesized that microbial activity in the aqueous solutions of avian fecal material is responsible for the production of the late-appearing attractive compounds. Methanol extracts may not provide substrates needed for this microbial activity. Volatiles from microorganisms found associated with fruit flies or larval-infested fruit have been shown to be attractive to various fruit flies (e.g., Courtice & Drew 1983, Jang & Nishijima 1990, Robacker et al. 1991, MacCollom et al. 1992). Studies on the microbial profile of avian fecal material over this time period are underway to identify microorganisms that may be responsible for production of additional chemical attractants.

ACKNOWLEDGMENTS

The authors thank Ara Manukian, Charles P. Powell (USDA/ARS, Gainesville, FL), John Howell, Yasmin Cardoza, Shayla Emran (Univ. of Florida, Gainesville) and

Shaun Smith (Jacksonville, FL) for technical assistance; and John Sivinski (USDA/ARS, Gainesville, FL), Michael Hennessey (USDA/ARS, Miami, FL), David Robacker (USDA/ARS, Weslaco, TX) and Roger Vargas (USDA/ARS, Honolulu, HI) for critically reviewing an earlier draft of this manuscript. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement of recommendation for its use by the USDA. This research was funded, in part, by USDA SEA CRG 8700564.

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