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A HIGH-EFFICIENCY COLLECTION DEVICE FOR QUANTIFYING SEX PHEROMONE VOLATILIZED FROM FEMALE GLANDS AND SYNTHETIC SOURCES

T.C. BAKER, L.K. GASTON, M. MISTROT POPE, L.P.S. KUENEN, and R.S. VETTER

Division of Toxicology and Physiology, Department of Entomology
University of California, Riverside, California 92521

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Abstract—A high-efficiency collection device for sex pheromones volatized from forcibly extruded female glands is described. Filtered nitrogen gas is the carrier and glass wool the adsorbent. Small quantities of distilled carbon disulfide are used to rinse the glass wool. Recovery efficiency of synthetic compounds was usually 90–100%, and a mean of 2.4 ± 0.65 SD ng/min of (*Z*)-7-dodecenyl acetate was recovered in emissions from individual *Trichoplusia ni* (Hubner) glands.

Key Words—Volatilized pheromone, glass adsorption, *Trichoplusia ni*, cabbage looper, quantifying pheromone emission.

INTRODUCTION

Sex pheromone research has progressed steadily toward the point where quantification of component release rates from both natural and synthetic sources is essential. It is necessary not only during implementation programs, such as for formulation of disruptants, but also during the initial isolation and identification process. For instance, aldehydes sometimes can be found in abundance by aeration of glands but not in glandular extracts (Hill et al., 1975; Cross et al., 1976; Weatherston et al., 1971; Sanders and Weatherston, 1976), and their contribution to optimal behavioral responses can be underestimated or missed entirely. Although a variety of collection devices have been reported for quantifying synthetic compound emissions (see Weatherston et al., 1981), few can be used efficiently for female emissions

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because of: (1) extremely low quantities of emitted pheromone relative to background peaks from solvent, adsorbent, or extracted scales; (2) adsorption of pheromone onto female bodies, reducing recovery; and (3) degradation of pheromone through oxidation or body surface enzymes. Even for quantifying emissions from controlled-release formulations, it is difficult (Weatherston et al., 1981) to account for all the pheromone that has left an emission surface, i.e., to obtain a mass balance.

In a previous report (Baker et al., 1980), it was determined that glass surfaces were excellent adsorbers of pheromone, and also were easily desorbed of pheromone by solvent rinses. This static-air–glass adsorption method had several disadvantages, however, which limited its usefulness. First, in the collection chamber ca. 90% of collectable pheromone was lost due to adsorption onto calling females' bodies, and although it could be accounted for and used as a release rate correction factor, this loss seemed undesirable. Second, as with any collection device using air as a carrier, oxidation of labile compounds could readily occur and cause miscalculations and loss of otherwise recoverable materials such as aldehydes, when they are left on the adsorbing surface too long (Weatherston et al., 1981). These factors, plus the goal of having a more dynamic system capable of quantifying very low emission rates in a variety of flow regimes, prompted us to investigate further the use of glass as an adsorbent and to try to develop a system that would be useful for both female- and synthetic-emitted pheromone for collecting high-lability compounds with nearly 100% efficiency.

METHODS AND MATERIALS

General Procedure. The collection device is illustrated in Figure 1. Charcoal- and glass wool-filtered nitrogen (N_2) was introduced from a ground glass connector at an ambient temperature 21–23°C and a flow rate of 0.5 ml/sec ± 10%. Volatiles were adsorbed onto ca. 0.25 g of moderately packed glass wool, eluted with distilled carbon disulfide (CS_2) (ca. 1.5 ml in 0.5-ml aliquots), and an internal standard was added immediately. The solution was condensed to ca. 5 μl, using a Snyder column apparatus with a small Teflon boiling chip, and analyzed by gas–liquid chromatography (GLC) on a column of 10% XF-1150 (100–120 mesh Ch W, AW DMCS, 1.394 g, 1.0 m × 4.0 mm OD). Oven temperature was 150°C, and N_2 flow was 25 ml/min. All glassware, pipets, and syringes were rinsed thoroughly with distilled acetone and dried in an oven at 125°C between uses. GLC peak areas were estimated using peak height × retention time. (*Z*)-7-Dodecenyl acetate (*Z*7-12:Ac) and (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), the internal standards, were formulated gravimetrically, then serially diluted to the desired concentration.

Determiniation of Breakthrough Time. Ninety nanograms of each of seven model pheromone compounds, decyl acetate (10:Ac), decyl alcohol

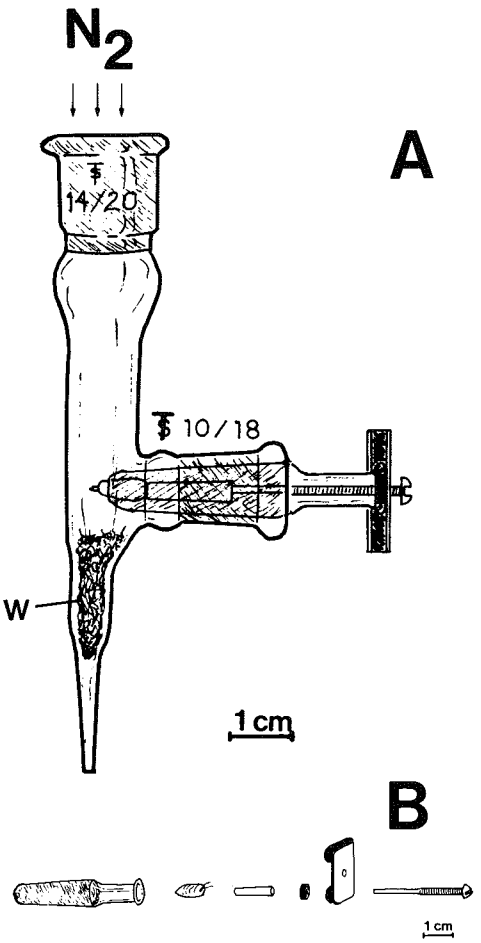

FIG. 1. (A) The one-receptacle collection device. Charcoal- and glass-wool-filtered N_2 gas at 0.5 ml/sec flows through the lumen and passes over pheromone emitter such as (pictured) a forcibly extruded female gland held in an extrusion tube. A volume of packed glass wool (W) then adsorbs the pheromone. (B) Expanded view of the extrusion tube components, with (from left to right) the extrusion tube, ligated abdomen with gland unextruded, Teflon plunger, GLC-conditioned silicone rubber gasket, steel clip, and screw for exerting pressure.

(10:OH), dodecyl acetate (12:Ac), (*Z*)-9-tetradecenal (*Z*9–14:ALD), tetradecyl acetate (14:Ac), (*Z*)-11-hexadecenal (*Z*11–16:ALD), and hexadecyl acetate (16:Ac), were injected onto the tip of an artificial, glass gland (a modified gland extrusion tube, Figure 1B), in 9 μl hexane. The gland was placed within 10 sec of impregnation into the collection device with the N_2 stream already flowing. Compounds "breaking through" the glass wool

collection area were captured in 20-min fractions in glass capillaries cooled by dry ice and connected by Teflon to the distal end of the apparatus. The capillaries were rinsed with ca. 30 μl CS_2, and 50 ng internal standard was added immediately. Pressure was equalized in this system by drawing a vacuum on the end of each capillary to match the N_2 flow. The glass wool and glass gland were rinsed with CS_2 as above, and 50 ng internal standard was added immediately. The total quantity collected from glass wool, gland, and breakthrough fractions was compared to the amount introduced into the collector. The latter was determined by injecting the gland with compounds as above, waiting ca. 10 sec to mimic time normally elapsed before insertion into the collector, then washing the gland surface immediately with CS_2 and adding internal standard.

Recovery Efficiency. Efficiency was measured not only in the breakthrough study, but also in two separate experiments with different emission quantities. Nine μl of a nine-component solution [the seven compounds used above, plus hexadecyl alcohol (16:OH) and (*Z*)-11-hexadecenyl alcohol (*Z*11-16:OH)], were applied with a syringe to the tip of the glass gland. Solution concentrations of 10 ng/μl and 1 μg/μl of each component in CS_2 were used to determine the system's efficiency of recovery as well as the evaporation rates from the glass gland for each component. The volatiles carried by the N_2 were adsorbed by the glass wool and subsequently eluted with CS_2. Fifty ng and 5 μg of internal standard, respectively, were added. Quantities recovered from glass wool and gland were compared to quantities actually introduced into the collector, as above, for each replicate, and percent recovery of each compound was calculated.

Pheromone Collection from Female Moths. Trichoplusia ni (Hübner) females in their fourth scotophase were placed in a freezer (−20°C) for 5–10 min. Abdominal scales were removed with a gentle vacuum. A ligature was tied about the abdomen near the thorax and the abdomen then severed just anterior to the ligature. The abdomen was placed in the gland extrusion tube (Figure 1B) so that the distal end protruded through the small opening in the tube. A cylindrical Teflon plunger was placed behind the abdomen and a silicone rubber gasket placed at the opening of the tube (Figure 1B). A machine screw was inserted through a steel clip and the rubber gasket. Pressure was applied behind the Teflon plunger with the screw to extrude the gland. Five-minute collections were made using the one-receptacle collection apparatus. Sixty-minute collections were made using a four-receptacle apparatus similar to the device in Figure 1 except that the four receptacles opened into the N_2 stream at 90° angles to each other. At the end of the collection period, the glass wool was eluted with CS_2 and 50 ng 14:Ac added as an internal standard. Each collection was condensed and analyzed as described above.

RESULTS AND DISCUSSION

The recovery efficiency of nanogram quantities of model pheromone compounds was nearly 100% (Tables 1 and 2). The glass wool adsorbed and retained for 2 hr nearly all the materials except 10:Ac without appreciable breakthrough (Table 1). Importantly, the two aldehydes suffered no apparent oxidation or other degradation in either the 2- or 1-hr (Table 2) collections as evidenced by their high rate of recovery. Compounds of greater molecular weight were collected in increasing quantities on the glass gland and, consequently, in decreasing quantities from the glass wool, as compared to lower-molecular-weight compounds. The recovery of microgram quantities also appeared to be nearly 100%, although only lower-molecular-weight compounds such as 10:OH and 12:Ac had been volatilized and collected from the glass wool in μg amounts after 1 hr.

Collection of pheromone from ligated *T. ni* female abdomens appeared quite successful. For the first 5 min of gland extrusion a mean of 2.4 ($\pm$ 0.65 SD) ng/min *Z*7–12:Ac and 0.25 ($\pm$ 0.07 SD) ng/min 12:Ac was collected. The *Z*7–12:Ac quantity is somewhat lower than that reported by Bjostad et al. (1980) from intact, calling females using Porapak-Q® as the adsorbent in an air stream. They collected a mean of 21.8 ($\pm$ 3.7 SD) ng/min *Z*7–12:Ac. One reason for the discrepancy between the two studies may be the large difference in flow rates over the gland. Their rate, 37.5 ml/sec, was nearly 100-fold greater than ours and could have substantially increased volatilization of pheromone from the gland surface. Also, unlike their technique which collected pheromone during a female's initial gland extrusion, our method collected from females already calling for an unknown period of time. Alternatively, the differences may reflect a reduced ability of ligated female abdomens to produce pheromone compared to intact females. If this is so, an adaptation of our system could be used for intact females, in which a vacuum just matching N_2 flow is drawn on the end of the apparatus to equalize pressure, eliminating the need for a sealed receptacle for abdomens.

The advantages of the N_2–glass wool device are many, not the least of which is the obviation of extensive precollection treatment to clean up the adsorbent such as Porapak-Q (Byrne et al., 1975). Because small quantities of solvent are used and the glass wool is free of impurities, interfering background GLC peaks are reduced, and nanogram quantities can be quantified. Therefore, quantification of pheromone emitted from individual females can be performed for many lepidopterous species. A further advantage is that the glass wool can be used repeatedly hundreds of times because during each rinsing it is stripped of all compounds. Scales are not shed into the glass wool, and, therefore, are not extracted to add to background impurities, and the pheromone does not have a chance to readsorb onto

TABLE 1. BREAKTHROUGH TIMES AND RECOVERY EFFICIENCY OF 7 MODEL PHEROMONE COMPOUNDS USING N_2–GLASS WOOL COLLECTING DEVICE WITH GLASS "GLAND" LOADED WITH 90 NG OF EACH COMPOUND[a]

	$\bar{X}$ percent compound recovered (± SD) ($N = 6$)							
			Breakthrough					
	From glass wool	From glass gland	0–20 min	20–40 min	40–60 min	60–80 min	80–100 min	100–120 min	Total (%)
10:Ac	80 ± 34	0 ± 0	10 ± 13	3 ± 1	7 ± 4	6 ± 3	6 ± 3	14 ± 10	127
10:OH	82 ± 25	1 ± 3	0 ± 0	0 ± 0	0 ± 0	4 ± 6	4 ± 6	1 ± 3	89
12:Ac	89 ± 10	0 ± 0	1 ± 1	1 ± 1	0 ± 0	1 ± 2	1 ± 2	2 ± 2	95
Z9–14:ALD	76 ± 11	11 ± 4	0 ± 0	0 ± 0	0 ± 0	1 ± 2	1 ± 2	2 ± 3	91
14:Ac	74 ± 12	22 ± 11	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	96
Z11–16:ALD	56 ± 11	30 ± 12	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	86
16:Ac	26 ± 4	72 ± 6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	98

[a]Collection time: 2 hr. N_2 flow at 0.5 ml/sec. The percent recovery is as a percent of compound actually introduced into the apparatus measured by controls.

TABLE 2. PERCENT COMPOUND RECOVERED FROM COLLECTING DEVICE DURING 1-HR COLLECTION BEGINNING WITH EITHER 90 NG OR 9 μG EACH COMPOUND, NO BREAKTHROUGH COLLECTION MADE

	$\bar{X}$ percent compound recovered[a]					
	90 ng			9 μg		
	From glass wool	From glass gland	Total (%)	From glass wool	From glass gland	Total (%)
10:Ac	85 ± 44	0 ± 1	85	6 ± 3	63 ± 8	69
10:OH	94 ± 13	0 ± 0	94	16 ± 3	76 ± 9	92
12:Ac	92 ± 9	4 ± 3	96	8 ± 2	97 ± 5	105
Z9-14:ALD	78 ± 21	13 ± 5	91	4 ± 1	97 ± 4	101
14:Ac	52 ± 21	41 ± 24	93	2 ± 2	103 ± 7	105
Z11-16:ALD	37 ± 19	45 ± 28	82	1 ± 1	102 ± 6	103
16:Ac	12 ± 8	80 ± 21	92	0 ± 0	101 ± 9	101
16:OH	0 ± 0	73 ± 12	73	0 ± 0	105 ± 13	105
Z11-16:OH	0 ± 0	85 ± 26	85	0 ± 0	93 ± 6	93

[a] $N = 4$ for 90 ng, $N = 3$ for 9 μg.

females' bodies as in some other systems (Baker et al., 1980). Most importantly, more labile compounds such as aldehydes do not oxidize in the N_2 stream, and hence collection and quantification of these compounds can proceed for long periods without jeopardizing the high efficiency. A device using filtered air and glass beads instead of glass wool has recently been described for quantifying emission rates of synthetic pheromone from controlled release formulations (Weatherston et al., 1981).

Our four-receptacle device allows for collections from four females at once and newly extruded glands can be continually rotated into the device to replace used glands. Thus, for those species emitting at very low rates or for collection of minor components even in high-rate emitters, mass collection and quantification can be performed more easily. In addition, flow rate may be varied to study its effects on emission rate, a characteristic lacking in a static-air–glass adsorption device.

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