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Lepidoptera: Tortricidae

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ORIENTAL FRUIT MOTH[1] PHEROMONE COMPONENT EMISSION RATES MEASURED AFTER COLLECTION BY GLASS-SURFACE ADSORPTION[2]

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Abstract—We collected and quantified both synthetic and natural Oriental fruit moth [*Grapholitha molesta* (Busck)] sex pheromone components in 250-ml round-bottom flasks by hexane rinsing of compounds adsorbed on the glass walls. This simple procedure collected 100% of the pheromone evaporated inside the flask and was useful for quantifying both nanogram and microgram amounts of emitted pheromone. Emission rates of (*Z*)-8-dodecenyl acetate from 1000, 100, and 10 μg-loaded rubber septa were 219, 12, and 1.2 ng/hr, respectively. *G. molesta* females emitted this component at 3.2 ng/hr, close to the emission rate from a 10 μg-loaded septum, which evokes "long-range" and "close-range" behaviors in the male. The corresponding alcohol, (*Z*)-8-dodecenyl alcohol, was emitted from rubber septa ca. 3 times faster than the acetate.

Key Words—Oriental fruit moth, *Grapholitha molesta*, pheromone release rate, airborne collection, (*Z*)-8-dodecenyl acetate, (*Z*)-8-dodecenyl alcohol.

INTRODUCTION

An important problem in studies of insect olfaction is determination of the quality and quantity of an airborne odor. For insect pheromone research using synthetic chemicals, the problem often has been avoided by referring only to the chemical quantities applied to a release surface, leaving the

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emission rates from the surface undetermined. Methods of measuring pheromone component emission rates have included: (1) extraction of the release substrate to determine the quantity remaining after an elapsed time (Kuhr et al., 1972; Maitlen et al., 1976); (2) visual inspection of quantity remaining in hollow fibers after an elapsed time (Brooks et al., 1977); (3) collection and quantification of airborne components using total condensation (Browne et al., 1974); and (4) adsorption on charcoal (Keller et al., 1964), lanolin (Brady and Smithwick, 1968), triglycerides (Roller et al., 1968), and gas–liquid chromatographic (GLC) solid supports such as Porapak Q (Byrne et al., 1975).

Measuring emission rates from live insects is often difficult due to the small quantities emitted and the presence of nonpheromone components and impurities. These become predominant at high analytical sensitivities and interfere with quantification. For minor components, the problem is even more acute. Identification (Cardé et al., 1979) of the minor pheromone components of the Oriental fruit moth, *Grapholitha molesta* (Busck) was accomplished using pheromone collected by rinsing the glass holding vessels with solvent, a method similar to that of Weatherston et al. (1971). We report here on the ease and efficiency of the glass-surface adsorption technique for quantifying emission rates of both synthetic and female-emitted *G. molesta* sex pheromone components.

METHODS AND MATERIALS

The glass-surface adsorption procedure simply involved placing the pheromone source, either a rubber septum dispenser or moths, into a 250-ml, 24/40 ground glass joint, round-bottomed flask, which was then sealed with a ground glass stopper. After a predetermined time, the flask was opened, the dispenser or moths removed, and the flask washed with small quantities of redistilled hexane. The quantities of pheromone components present in the solvent rinse were determined by GLC using an internal standard.

Chemicals. All quantifications were performed on a Packard model 7300 series GLC equipped with an H_2 flame-ionization detector operated isothermally at 170° C with an N_2 flow rate of 30 ml/min. Peak height times retention time was the integration method used. The internal standard, (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), contained less than 1% of the *E* isomer as measured on a 2-mm × 1.8-m glass 10% XF-1150 [on Chromosorb DMCS AWW (100/120 mesh)] GLC column and was greater than 99% free of other volatile impurities. The (*Z*)-8-dodecenyl acetate (*Z*8-12:Ac) was purified on a 10% silver nitrate liquid chromatography column, and it contained no detectable quantities of either (*E*)-8-dodecenyl acetate (*E*8-12:Ac) or any 12-carbon alcohols as checked on a 2-mm × 1.8-m 3% OV-1

[on Gas-Chrom-Q (100/120 mesh)] and XF-1150 column. Other volatile impurities were less than 0.1%. The (*Z*)-8-dodecenyl alcohol (*Z*8–12:OH) was prepared by saponification of the above-purified *Z*8–12:Ac. After work-up, it contained no detectable amounts of the *E* isomer as checked on XF-1150 and no detectable quantities of any 12-carbon acetates or other volatile impurities on OV-1.

Parent stock solutions for these studies were prepared as follows: 10 μl of neat *Z*11–14:Ac and *Z*8–12:Ac, weighing 8.8 and 8.9 mg, respectively, were placed in 1 ml hexane. Their ratio on GLC was 51.2:48.8; hence, we concluded starting quantities were identical. Stock solutions of *Z*11–14:Ac (1 μg/μl) and of *Z*8–12:Ac (10 μg/μl) in hexane were formulated gravimetrically. *Z*8–12:OH was added to the *Z*8–12:Ac solution to make a 1:1 mixture, whose ratio measured 50.5:49.5 on OV-1.

Effects of Flask Chilling and the Number of Hexane Rinses on Recovery. All GLC analyses were performed on OV-1. One μl of solution containing 10 μg *Z*8–12:Ac and *Z*8–12:OH was applied by a Hamilton 10 μl syringe to each of six 1.5-cm-diam microscope glass cover slips. Ten seconds after application, each slip was placed in a 250-ml round-bottom flask, taking care to prevent any contact with the application surface, and the flasks were stoppered. The neat compounds were allowed to evaporate for at least 12 hr, at which time no material was visible on the slips. Then three flasks were chilled for ca. 5 min at −20° C, while the other three remained at 23° C. Flasks were opened, 10 ml of hexane were added immediately, and the hexane was swirled continually in the reclosed flasks for 30 sec and removed by pipette. Five μg (in 5 μl solution) of the internal standard were added. Two separate rinses followed, treated identically to the original rinse. Each rinse solution was then evaporated to less than 1 ml. For GLC analyses, a 50-μl aliquot was evaporated to dryness under nitrogen, and a few microliters of CS_2 were added immediately.

Collection Efficiency. Applying 1 μl solution containing 10 μg *Z*8–12:Ac and *Z*8–12:OH to cover slips resulted in unacceptable variation in the quantity applied, always less than the expected 10 μg, possibly due to droplet evaporation or creeping up the syringe needle. To minimize such variation, larger volumes of a more dilute (1 μg/μl) solution were used. Five μg were applied to each of 5 cover slips which, after 10 sec, were placed and sealed within flasks. After 3–5 hr at 25° C, each flask was opened, the cover slip removed and bathed for 5 min in 10 ml hexane, and the flask rinsed with two 10-ml hexane rinses. Five μg (5 μl) of the internal standard were added into the first hexane rinse and the second rinse was combined with the first. Five μg of internal standard were also added immediately into the cover slip bath.

To measure the quantity actually applied to each slip, 5 μg of *Z*8–12:Ac and *Z*8–12:OH were applied. After 10 sec, each slip was bathed in ca. 10 ml of hexane, and 5 μg internal standard added immediately.

Rate of Emission from Rubber Septum Dispensers. Using disposable glass micropipettes, rubber septum dispensers (A. H. Thomas Corp., 5 × 9 mm sleeve type) were impregnated with either 1000, 100, or 10 μg each of *Z*8–12: Ac plus *Z*8–12: OH in 100, 10, and 10 μl hexane, respectively. After ca. 12 hr of aging in an exhaust hood at 22° C, the 1000-μg septa were placed singly on their sides in the bottom of sealed flasks at 25° C for 3 hr; the 100 and 10-μg septa were treated the same way for 16 hr. They were then removed and the flasks rinsed twice with 10 ml hexane to which 5 μl internal standard (50 ng) were added.

Measurement of Z8–12: Ac and Z8–12: OH Adsorbed onto Moths. Fifty noncalling females were placed in each flask for 3 hr with a 1000 μg-loaded septum and for 16 hr with a 100 μg-loaded septum. The flask was chilled for 5 min at −20° C, and then the septum and immobilized females were removed. Two 10-ml hexane rinses with 50 ng of added internal standard were then pipetted from the flask and filtered through a small plug of glass wool to remove scales. The filtrate was rotoevaporated and concentrated for GLC analysis.

Rate of Pheromone Emission from Calling Females. Eleven groups of 50 *G. molesta* females (3–11 days old) were placed in each flask 2 hr before lights off, ca. 1 hr after the onset of calling (Baker and Cardé, 1979a). Every 15 min, 20 randomly chosen females were observed in each flask for evidence of "calling" posture (wings elevated and abdomen raised from the substrate), ovipositor extrusion, or both. Thus, total time of female calling was estimated. At the end of the calling period, the flasks were chilled at −20° C for ca. 5 min to immobilize the females, which then were removed. Pheromone yield was determined as described in the previous section.

RESULTS

Effects of Flask Chilling and Number of Hexane Rinses on Recovery. Of the total recovered pheromone components, 95–97% was collected during the first hexane rinse, 2–4% during the second rinse, and 0–1% during the third (Table 1). Two rinses collected at least 99% of the pheromone adsorbed on the glass walls. A small percentage of alcohol and acetate ($\bar{X}$ = 0.6% ± 0.19 SD; n = 6) was recovered from the glass cover slips. Chilling the flask immediately before the first rinse did not increase the collection of pheromone. Only about 6 μg of *Z*8–12: Ac and 5 μg *Z*8–12: OH were collected out of the 10 μg supposedly applied to the slip. However, further investigation involving bathing slips in hexane immediately after pheromone application showed that only about 6 μg of acetate were actually applied to the slips. Some of the 1 μl of solution may have been deposited on the outside of the syringe needle. Thus, in subsequent applications, we applied pheromone in 5 μl of solution to

TABLE 1. EFFECTS OF CHILLING OF FLASKS (n = 3) AND NUMBER OF HEXANE RINSES UPON RECOVERY OF PHEROMONE COMPONENTS: Ac, Z8-12:Ac; OH, Z8-12:OH

	Mean percentage recovered (± SD) Rinses						Mean recovered (μg, ±SD)	
	First		Second		Third			
Flask treatment	Ac	OH	Ac	OH	Ac	OH	Ac	OH
Chilled	96 (±0.00)	96 (±0.02)	3 (±0.00)	3 (±0.02)	1 (±0.00)	0 (±0.00)	6.1 (±0.8)	4.7 (±0.1)
Unchilled	95 (±0.18)	96 (±0.00)	4 (±0.17)	3 (±0.00)	1 (±0.00)	0 (±0.00)	5.8 (±1.2)	4.8 (±0.7)

reduce pheromone loss. Less Z8-12:OH than Z8-12:Ac was recovered from both chilled and unchilled flasks. Possibly more of the alcohol than acetate either volatilized from the cover slip before it was sealed in the flask or evaporated during sample preparation for GLC analyses. The alcohol has a lower molecular weight than the acetate and volatilizes more quickly.

Collection Efficiency. As calculated, 100% of the alcohol and 103% of the acetate introduced into the flasks on the cover slips was recovered by rinsing the flasks' glass walls (Table 2). The quantities actually introduced into the flasks (3.9 μg Z8-12:OH and 4.4 μg Z8-12:Ac) again were slightly less than the expected 5 μg supposedly applied to the slips, and again the amount of alcohol lost appeared greater than the acetate (Table 2). Nevertheless, the glass-surface adsorption system was 100% efficient at recovering volatilized *G. molesta* pheromone.

Rate of Emission from Rubber Septum Dispensers. Z8-12:OH was emitted 2–3 times faster than Z8-12:Ac at three different septum dosages (Table 3). For both the alcohol and acetate, a 10-fold increase in septum loading produced at least a 10-fold increase in emission rate.

TABLE 2. COLLECTION EFFICIENCY OF PHEROMONE COMPONENTS IN GLASS FLASKS

	Average Z8-12:Ac (μg ± SD)	Average Z8-12:OH (μg ± SD)
Quantity introduced (n = 10)	4.4 ± 0.4	3.9 ± 0.4
Quantity recovered (n = 5)		
From flask	4.4 ± 0.5	3.8 ± 0.7
From cover slip	0.1 ± 0.2	0.1 ± 0.1
Total	4.5 ± 0.4	3.9 ± 0.5
Efficiency	103%	100%

TABLE 3. MEAN RELEASE RATES (ng/hr) OF *Grapholitha molesta* PHEROMONE COMPONENTS FROM RUBBER SEPTA

	Quantity impregnated		
Compound	1000μg (n = 4)	100 μg (n = 7)	10 μg (n = 3)
Z8-12:Ac	219 ± 69 (SD)	12 ± 4	1.2 ± 0.3
Z8-12:OH	664 ± 202	33 ± 10	2.8 ± 1.4

Percentage Adsorption onto Moths. The presence of 50 female *G. molesta* in each flask reduced by 90% the amount of Z8-12:Ac that would have been recovered in both the 1000- and 100-μg septum flasks (Table 4). Also, Z8-12:OH recovery was reduced by 75 and 93% in the 1000- and 100-μg septum flasks, respectively. The lost material likely was adsorbed onto the moths' bodies or perhaps some was transferred to the tarsi as the females walked on the glass. The acetate loss was inexplicably greater than that of the alcohol. Obviously the presence of females in the flask caused substantial losses in pheromone recovery. These rates of loss were then used in calculating rate of pheromone emission by females confined in the flasks.

Rate of Pheromone Emission by Calling Females. GLC tracings of female effluvia from flask rinses were fairly clean and dominated by the 12-carbon acetates and alcohols as well as the internal standard, Z11-14:Ac (Figure 1). A mean of 24.9 ng Z8-12:Ac (ca. 7% *E* (Cardé et al., 1979) and 10.5 ng Z8-12:OH (ca. 10% *n*-dodecanol) (Cardé et al., 1979) were collected over the 2-hr period (Table 5). Only ca. 36 out of the 50 females in each flask

TABLE 4. LOSS OF RECOVERABLE PHEROMONE DUE TO ADSORPTION ONTO 50 NONCALLING FEMALES IN THE FLASK DURING EMISSION OF SYNTHETIC COMPONENTS FROM RUBBER SEPTA

	Septum loading			
	Z8-12:Ac		Z8-12:OH	
	1000μg[a]	100 μg[b]	1000 μg[a]	100 μg[b]
Amount collected without moths (μg)	0.66 ± 0.20 (SD) (n = 4)	0.19 ± 0.06 (n = 7)	2.00 ± 0.60 (n = 4)	0.53 ± 0.15 (n = 7)
Amount collected with moths (μg)	0.07 ± 0.03 (n = 4)	0.02 ± 0.01 (n = 6)	0.51 ± 0.15 (n = 4)	0.09 ± 0.04 (n = 6)
Reduction	90%	90%	75%	83%

[a]Release time 3 hr.
[b]Release time 16 hr.

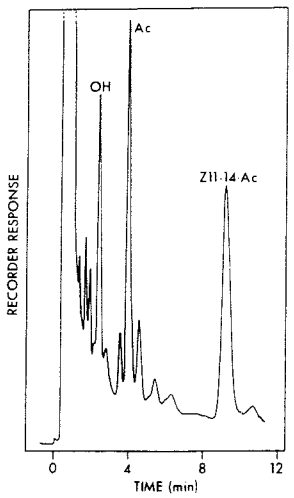

FIG. 1. GLC tracing (OV-1 column) of glass-adsorbed pheromone components from calling *G. molesta* females: OH, 12-carbon alcohol region; Ac, 12-carbon acetate region; *Z*11–14: Ac, the internal standard, having a relative quantity of 50 ng. Packard model 7300 series GLC equipped with an H_2 flame-ionization detector was operated isothermally at 170° C. N_2 flow rate was 30 ml/min.

($\bar{X} = 71\% \pm 0.11$ SD; $n = 11$) appeared to be emitting pheromone at any time during the collection period. After corrections for pheromone loss due to adsorption onto the 50 females' bodies and for percentage of dodecanol and (*E*)-8-dodecenyl acetate, the estimated mean *Z*8–12: Ac emission rate per calling female was 3.2 ng/hr. Mean *Z*8–12: OH emission rate was 0.7 ng/hr.

DISCUSSION

The glass-surface adsorption technique was simple, convenient, and 100% efficient for collection of microscale quantities. Quantities from a few

TABLE 5. EMISSIONS OF *Z*8–12: Ac AND *Z*8–12: OH FROM CALLING *Grapholitha molesta* FEMALES

	*Z*8–12: Ac	*Z*8–12: OH
Collected from $\bar{X}$ of 36 calling females/flask in 2 hr (ng)	23.2 ± 10.4 (SD)	8.4 ± 3.1
$\bar{X}$ ng/hr/calling female	0.32 ± 0.14	0.12 ± 0.04
Corrected emission rate (ng/hr/♀), accounting for adsorption on moths	3.2	0.7

nanograms to a few micrograms also were easily collected and quantified; with a clean solvent, background GLC peaks were few and small and did not hinder measurements of the peaks of interest. This system appears well-suited for measuring emission rates of synthetic pheromone from different substrates without wind flow.

The technique was also effective for collecting and quantifying the pheromone emitted by *G. molesta* females which will call in still air. However, a substantial correction factor had to be introduced to account for the pheromone lost by adsorption onto the females' bodies, a phenomenon which should be considered with any collection technique.

Many studies have verified the necessity for using airborne- rather than gland-collected pheromone; essential components, especially aldehydes, often appear in substantial quantities only in airborne samples, and not gland extracts (Hill et al., 1975; Cross et al., 1976; Sanders and Weatherston, 1976; Weatherston et al., 1971). Also, when the emission rates of components from females are known, multicompound emission rates and ratios can be manipulated either to match those of the female for monitoring purposes or exceed them for disruption of communication. The emission rates from *G. molesta* females and rubber septum dispensers agree remarkably well. Septa loaded with 10 and 100 μg *Z*8–12: Ac emitted this compound at 1.2 and 12 ng/hr, respectively. The optimum *Z*8–12: Ac septa loading (as part of a blend with *Z*8–12: OH and *E*8–12: Ac) for full behavioral response in wind tunnel assays and trap capture in the field is ca. 10–100 μg (Baker and Cardé, 1979b). By comparison, female emission rate of *Z*8–12: Ac was estimated at 3.2 ng/hr, a figure within the range of septa which elicit optimal behavioral responses.

At the three septum loadings tested, *Z*8–12: OH was emitted 2–3 times faster than an equal amount of *Z*8–12: Ac. Half-life calculations of *Z*7–12: Ac (35 days) (Butler and McDonough, 1979) and *Z*7–12: OH (12.5 days) (McDonough, personal communication) on rubber septa also indicate ca. 3 times faster release rate for the alcohol than the corresponding acetate. Females emit ca. 22% as much *Z*8–12: OH as the acetate (Table 5), and so to mimic the female-emitted *Z*8–12: OH percentage a 7–11% loading of *Z*8–12: OH to *Z*8–12: Ac would be needed on a septum. In fact, a 1–10% *Z*8–12: OH septum ratio was behaviorally optimum (Baker and Cardé, 1979b). Thus, the female-emitted percentage of *Z*8–12: OH agrees well with that of the synthetic from rubber. Our 664 ng/hr release rate estimate of 1000 μg *Z*8–12: OH on a rubber septum is in approximate agreement with half-life release rate calculations for 1000 μg of another 12-carbon alcohol, (*E*)-8, (*E*)-10-dodecadienyl alcohol, on rubber: 790 ng/hr (Maitlen et al., 1976). However, the calculated half-life release rate of 1000 μg *Z*7–12: Ac on rubber septa (Butler and McDonough, 1979) is 600 ng/hr, about three times that of *Z*8–12: Ac in our study.

To optimize the accuracy and sensitivity of the glass-surface collection technique, the following precautions should be noted: (1) the rinse solvent should be as free as possible of impurities; (2) when impregnating a release substrate with starting quantities in a solution, a large volume of dilute rather than small volume of concentrated solution should be applied to minimize loss during application; (3) when objects with large surface areas (such as a group of female moths) are placed in the flask, the quantities lost due to prerinse removal of these objects should be calculated. With regard to 2 (above), Vick et al., (1978) found glass flask rinsing to be only about 50% efficient at collecting *Sitotroga cerealella* (Olivier) pheromone components, but they inferred, rather than measured, the quantity of pheromone applied to glass slides in 0.9–1.2 μl of solvent. From our experience, applying the pheromone in 1 μl of solution can result in a 40–50% loss before or during application, possibly due to droplet evaporation on the syringe needle. We minimized such losses by using a more dilute solution and applying a greater volume (5 μl) to the slide.

Glass-surface adsorption is a deceptively simple collection system. Pheromone so collected can be rapidly quantified, even in microgram and nanogram quantities, while generally avoiding contamination with spurious compounds often encountered with organic collecting agents.

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