

COMPOSITION, QUANTIFICATION, AND PERIODICITY OF SEX PHEROMONE VOLATILES FROM INDIVIDUAL *HELIOTHIS ZEA* FEMALES

M. MISTROT POPE, L. K. GASTON and T. C. BAKER

Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside,
CA 92521, U.S.A.

(Received 14 February 1984; revised 23 May 1984)

Abstract—The airborne volatiles emitted from individual female *Heliothis zea* (Boddie) pheromone glands were collected by adsorption onto glass wool, analyzed, and quantified on an SP-2330 capillary GLC column. All of the compounds previously reported from gland washes, 16:Ald, Z7-16:Ald, Z9-16:Ald and Z11-16:Ald, were found in the volatile emissions. The forcibly extruded female *H. zea* pheromone glands exhibited a periodicity of pheromone release: maximal pheromone emission occurred between 1 and 2 h and the minimal pheromone emission between 5 and 21 h after the onset of scotophase.

Key Word Index: Sex pheromone, *Heliothis zea*, periodicity of emission

INTRODUCTION

(Z)-11-Hexadecenal (Z11-16:Ald) was first identified from female *Heliothis zea* by Roelofs *et al.* (1974). Klun *et al.* (1980) detected the presence of three additional compounds in pheromone gland washes of *H. zea*: (Z)-7-hexadecenal (Z7-16:Ald), (Z)-9-hexadecenal ((Z9-16:Ald), and hexadecenal (16:Ald). It has been shown, however, that compounds rinsed from the pheromone gland of an insect are not always found in the volatile emissions from that gland (Pope *et al.*, 1982) or are found in significantly different proportions than in rinses (Hill *et al.*, 1975; Sanders and Weatherston, 1976). Using the nitrogen stream-glass wool apparatus for collecting emitted pheromone described by Baker *et al.* (1981), it was our intention to determine the emission rates and ratios of the compounds previously found in *H. zea* pheromone gland washes, and also to study the effect of photoperiod on the emission of these compounds.

MATERIALS AND METHODS

Larvae of *H. zea* were raised on a pinto bean media modified from that described by Shorey and Hale (1965). Pupae were separated by sex and placed in separate environmentally controlled chambers ($25 \pm 1^\circ\text{C}$, 30–70% r.h.) on a 14:10 light:dark photoperiod. Adults had continuous access to an 8% sucrose solution. Females used in this study were 1–6-days old and were prepared for collection as described by Baker *et al.* (1981) and Pope *et al.* (1982), with the exception that EDTA- Na_4 (ethylenediaminetetraacetate) was not injected into the females. Females were immobilized by chilling them for a few minutes at -20°C , then their abdomens were ligated with fine string. A ligated abdomen was placed into the collector and pressure was applied by tightening a screw until the ovipositor and gland were

fully extended into the nitrogen stream (Pope *et al.*, 1982). Collection time for each gland was 10 min, with a nitrogen flow rate through the collector of 120 ml/min. No breakthrough of pheromone adsorbed onto the glass wool would have occurred in 10 min (Baker *et al.*, 1981). (Z)-11-Tetradecenal (Z11-14:Ald) [2.85 ng] was added to the glass wool as an internal standard after the pheromone collection was completed, before rinsing the glass wool with 200 μl of CS_2 . The sample was evaporated under a gentle stream of nitrogen to ca. 10 μl .

Samples were analyzed on a Varian Model 3740 gas chromatograph equipped with an SP-2330 glass capillary column using direct injection and temperature-programming from 90°C (1 min hold) to 150°C at $60^\circ\text{C}/\text{min}$. Both a Honeywell Elektronik 196 strip chart recorder and a Hewlett-Packard 3380A integrator recorded the peaks. The retention times (\pm SE) relative to Z11-14:Ald of synthetic Z11-16:Ald, 16:Ald, Z7-16:Ald, and Z9-16:Ald were 1.541 ± 0.0024 , 1.291 ± 0.0012 , 1.480 ± 0.0026 and 1.497 ± 0.0026 , respectively. The relative retention times of the four compounds collected from female *H. zea* were 1.547 ± 0.0015 , 1.295 ± 0.0015 , 1.487 ± 0.0005 and 1.504 ± 0.0005 . Blank collections confirmed the presence of no detectable peaks in the regions where pheromone compounds were found, but 2 peaks caused by solvent impurities did sometimes appear. These did not interfere with quantification of the 4 compounds (Fig. 1).

RESULTS

Quantification of individual female *H. zea* pheromone gland volatiles throughout the photoperiod indicated that the females of this species have both maximal and minimal periods of pheromone release. The maximal period of pheromone release occurred between 1 and 2 h after the onset of scotophase (Fig.

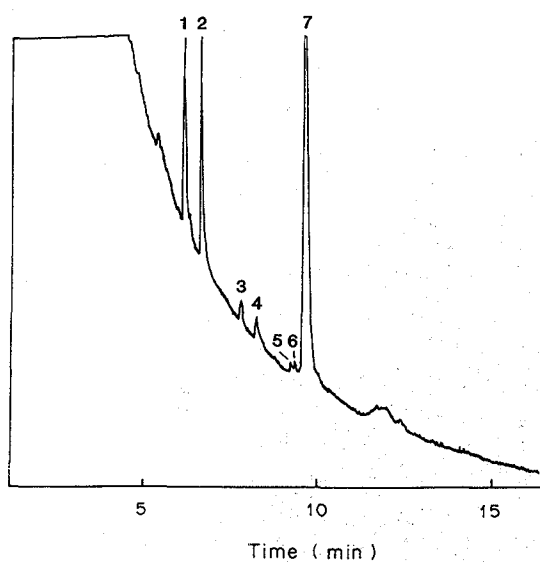


Fig. 1. SP-2330 chromatogram of a 10-min female *H. zea* volatile collection where Z7-16:Ald (peak 5) and Z9-16:Ald (peak 6) are present in detectable quantities along with 16:Ald (peak 4) and Z11-16:Ald (peak 7). Peak 2 is the internal standard, Z11-16:Ald, and Peaks 1 and 3 are solvent contaminants.

2). During this period, the major compound emitted, Z11-16:Ald, was released at an average rate (\pm SE) of 0.89 ng/min (\pm 0.25; $N = 13$), and was released in detectable amounts by 100% of the females. Of the other three compounds, only 16:Ald could be quantified with any certainty, with an average release rate (\pm SE) of 0.07 ng/min (\pm 0.02; $N = 13$). Detectable amounts of 16:Ald were released by 77% of the females during the period of maximal emission. The mean per cent compositions during the period of maximal pheromone release for Z11-16:Ald and 16:Ald were 93.5 and 6.5% respectively (Table 1).

During the maximal period of pheromone release, only 3 of the 13 maximal collections made from females contained detectable quantities of Z7-16:Ald and Z9-16:Ald. The release rate (\pm SE) of each compound was 0.02 ng/min (\pm 0.003) and 0.03 ng/min (\pm 0.01), respectively, and these three collections were associated with a relatively high release rate of Z11-16:Ald (> 1.2 ng/min). Thus the Z7-16:Ald and Z9-16:Ald were present in a 1:1 ratio, each comprising ca. 1% of the total emission (Fig. 1).

The period of minimal pheromone release occurred

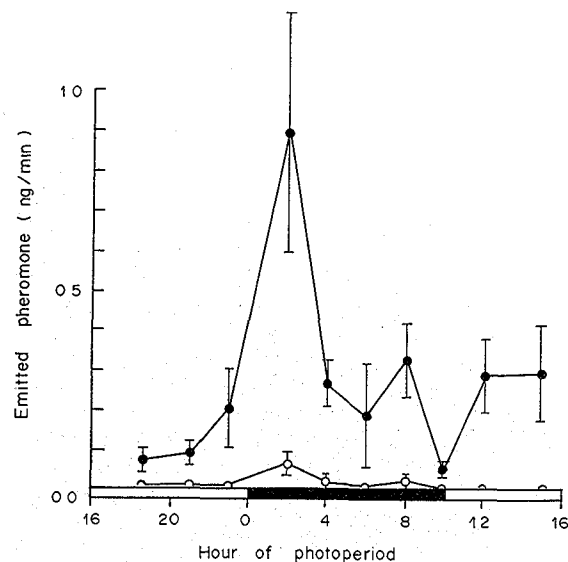


Fig. 2. Periodicity of pheromone emission from female *H. zea* on a 14:10 light:dark photoregime. The darkened area on the X-axis indicates the scotophase. $N = 14, 10, 13, 8, 4, 6, 2, 3, 4$, and 5 for hours 20-21, 22-0, 1-2, 3-4, 5-6, 7-8, 9-10, 11-13, 14-16 and 17-19, respectively. The solid circles represent Z11-16:Ald and the open circles represent 16:Ald. Bars around the means indicate \pm SE.

between 5 and 21 h after the onset of scotophase (Fig. 2). (The hours 22 through 0 and hours 3 to 4 were hours of rapidly increasing and decreasing pheromone release, respectively, and were therefore not included in either the maximal or minimal periods of release.) The mean release rates (\pm SE) and corresponding per cent compositions of Z11-16:Ald and 16:Ald during this period were 0.17 ± 0.03 ng/min (94.4%) and 0.01 ± 0.002 ng/min (5.6%), respectively (Table 1). Ninety-two per cent of the females released detectable amounts of Z11-16:Ald during this period while only 18% released detectable amounts of 16:Ald.

DISCUSSION

All four of the compounds previously identified from pheromone gland washes (Klun *et al.*, 1980) were detected in the peromone emissions of female *H. zea*, although Z7-16:Ald and Z9-16:Ald were difficult to detect. When all four compounds were present in quantifiable amounts ($N = 3$), their composition percentages (90.3% Z11-16:Ald, 7.0%

Table 1. Compounds found in volatile emissions from female *H. zea*

	Emitted volatiles (ng/min \pm SE)*		% Composition		% Emitting compound	
	Max† $n = 13$	Min‡ $n = 38$	Max† $n = 13$	Min‡ $n = 38$	Max† $n = 13$	Min‡ $n = 38$
Z11-16:Ald	0.89 ± 0.25	0.17 ± 0.03	92.71	94.4	100	92
16:Ald	0.07 ± 0.02	0.01 ± 0.002	7.29	5.56	77	18
Z7-16:Ald§	0.02 ± 0.003	—	1.20	—	23	0
Z9-16:Ald§	0.03 ± 0.01	—	1.44	—	23	0

*Compounds which were below the lower limit of detection (0.01 ng/min) were counted as 0.

†Maximal period of volatile emission, 1-2 h after the onset of scotophase.

‡Minimal period of volatile emission, 5-21 h after the onset of scotophase.

§ $n = 3$ for determinations made for Z7-16:Ald and Z9-16:Ald.

16:Ald, 1.2% Z7-16:Ald and 1.4% Z9-16:Ald) were quite similar to those found in gland washes (92.4% Z11-16:Ald, 4.4% 16:Ald, 1.1% Z7-16:Ald, and 1.7% Z9-16:Ald; Klun *et al.*, 1980). For *H. virescens* the per cent composition of the six aldehyde compounds collected in both emissions (Pope *et al.*, 1982) and washes (Klun *et al.*, 1980) were less similar; the 14:Alds were emitted at higher ratios than they were found in the gland, and (Z)-11-hexadecenyl alcohol, ca. 3% of the gland wash, was not emitted in detectable amounts. Thus there appears to be good correspondence between the composition of gland extracts and emissions for compounds of identical chain lengths and functionality (Pope *et al.*, 1982; this study), but poorer correspondence when these molecular differences are present, such as between acetates and aldehydes (Hill *et al.*, 1975; Sanders and Weatherston, 1976; Silk *et al.*, 1982) and alcohols and aldehydes (Pope *et al.*, 1982).

Of the four compounds identified from gland washes, only two, Z11-16:Ald and Z9-16:Ald, appear to be pheromone components. Z11-16:Ald by itself can sometimes elicit upwind flight in a wind tunnel (Vetter and Baker, 1983) and result in low capture levels of males in traps (Roelofs *et al.*, 1974), but the addition of Z9-16:Ald to Z11-16:Ald significantly increases the number of males flying all the way to the source (Vetter and Baker, 1983) and the numbers captured in traps (Sparks *et al.*, 1979; Hartstack *et al.*, 1980). Z7-16:Ald and 16:Ald admixed with Z11-16:Ald and Z9-16:Ald at various rates and ratios failed to elevate upwind flight or courtship responses in the wind tunnel (Vetter and Baker, 1983) and in most trapping experiments failed to increase capture levels above those caused by the mixture of Z11-16:Ald and Z9-16:Ald (Sparks *et al.*, 1979; Hartstack *et al.*, 1980). Thus, although 16:Ald is emitted by the majority of maximally emitting female *H. zea* and comprises 6.5% of the blend, it seems to be behaviourally inert. The 16:Ald emitted by female *H. virescens*, on the other hand, as part of the pheromone blend causes increased close-range source location and hairpencil extrusions.

It is interesting that although Z9-16:Ald is critical for complete male *H. zea* flight to the source, it was emitted in quantifiable amounts by so few females. For the major component, Z11-16:Ald, the emission rates from maximally emitting female *H. zea* were ca. four times lower than for *H. virescens* (0.89 ng/min for *H. zea*, 3.26 ng/min for *H. virescens*; Pope *et al.*, 1982) and low emission rates automatically make more difficult the detection of compounds such as Z9-16:Ald that are emitted as 1% or less of the blend. In general, trace amounts (1% or less) of compounds that have significant behavioural activity as part of a pheromone blend present a challenge to pheromone researchers, even when using gland extracts. Increasing the duration of collection from female *H. zea* from 10 to 30 min proved unsuccessful in collecting more Z7-16:Ald and Z9-16:Ald, because in *H. zea* and *H. virescens*, most of the pheromone is emitted in the first 10 min of forcible gland extrusion (unpublished data). In other species the forcibly extruded gland continues to emit pheromone at constant rates for a much longer period (unpublished data).

The forcible extrusion of glands at various hours of

the photoperiod to capture emitted volatiles may be a good way to monitor and compare the biosynthesis and transport of pheromone to the gland surface in a variety of species. In female *H. zea* and *H. virescens*, these processes appear to have a distinct periodicity, and it would be interesting to learn whether this holds true only for aldehyde-emitting species. It does for artichoke plume moth females (*Platyptilia carduidactyla*) which emit Z11-16:Ald (Haynes *et al.*, 1983), and thus more acetate- and alcohol-emitting species need to be looked at in this same way.

REFERENCES

- Baker T. C., Gaston L. K., Pope M. M., Kuenen L. P. S. and Vetter R. S. (1981) A high efficiency collection device for quantifying sex pheromone volatilized from female glands and synthetic sources. *J. chem. Ecol.* **7**, 961-968.
- Hill A. S., Cardé R. T., Kido H. and Roelofs W. L. (1975) Sex pheromone of the orange tortrix moth, *Argyrotaenia citrana* (Lepidoptera: Tortricidae). *J. chem. Ecol.* **1**, 215-224.
- Hartstack A. W. Jr, Lopez J. D., Klun J. A., Witz J. A., Shaver T. N. and Plimmer J. R. (1980) New trap designs and pheromone bait formulation for *Heliothis*. *Proc. Belt. Prod. Res. Conf.* pp 132-136.
- Haynes K. F., Gaston L. K., Pope M. M. and Baker T. C. (1983) Rate and periodicity of pheromone release from individual female artichoke plume moths, *Platyptilia carduidactyla* (Lepidoptera; Pterophoridae). *Envir. Ent.* **12**, 1597-1600.
- Klun J. A., Bierl-Leonhardt B. A., Plimmer J. R., Sparks A. N., Primiani M., Chapman O. L., Lepone G. and Lee G. H. (1980) Sex pheromone chemistry of the female tobacco budworm moth, *Heliothis virescens*. *J. chem. Ecol.* **6**, 177-183.
- Klun J. A., Plimmer J. R., Bierl-Leonhardt B. A., Sparks A. N., Primiani M., Chapman O. L., Lee G. H. and Lepone G. (1980) Sex pheromone chemistry of the female corn earworm moth, *Heliothis zea*. *J. chem. Ecol.* **6**, 165-175.
- Pope M. M., Gaston L. K. and Baker T. C. (1982) Composition, quantification, and periodicity of sex pheromone gland volatiles from individual *Heliothis virescens* females. *J. chem. Ecol.* **8**, 1043-1055.
- Roelofs W. L., Hill A. S., Cardé R. T. and Baker T. C. (1974) Sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. *Life Sci.* **14**, 1555-1562.
- Sanders C. J. and Weatherston J. (1976) Sex pheromone of the eastern spruce budworm (Lepidoptera: Tortricidae): Optimum blend of *trans*- and *cis*-11-tetradecenal. *Can. Ent.* **108**, 1285-1290.
- Shorey H. H. and Hale R. L. (1965) Mass rearing of the larvae of nine noctuid species on a simple artificial diet. *J. econ. Ent.* **58**, 522-524.
- Silk P. J., Wiesner C. J., Tan S. H., Ross R. J. and Grant G. G. (1982) Sex pheromone chemistry of the western spruce budworm *Choristoneura occidentalis* Free. *J. Chem. Ecol.* **8**, 351-362.
- Sparks A. N., Carpenter J. E., Klun J. A. and Mullinix B. G. (1979) Field responses of male *Heliothis zea* (Boddie) to pheromonal stimuli and trap design. *J. Ga ent. Soc.* **14**, 318-325.
- Vetter R. S. and Baker T. C. (1983) Behavioral responses of male *Heliothis virescens* in a sustained flight wind tunnel to combinations of the 7 compounds identified from the female sex gland. **9**, 747-759.
- Vetter R. S. and Baker T. C. (1984) Behavioral responses of male *Heliothis zea* in a sustained flight wind tunnel to combinations of the 4 compounds identified from the female sex gland. *J. chem. Ecol.* **10**, 193-202.