

Rate and Periodicity of Pheromone Release from Individual Female Artichoke Plume Moths, *Platyptilia carduidactyla* (Lepidoptera: Pterophoridae)¹

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ABSTRACT The rate and periodicity of the emission of the sex pheromone [(Z)-11-hexadecenal] from the pheromone glands of individual female artichoke plume moths, *Platyptilia carduidactyla*, were quantified. The mean rate of volatilization of (Z)-11-hexadecenal from the glands of the moths peaked at 1.52 ± 0.41 (SE) ng/min during the scotophase, and was at low levels during the entire photophase. This periodicity in emission rate paralleled the behavioral periodicity of calling by females, indicating a close coordination of the biochemical and behavioral aspects of pheromone release. The highest recorded emission rate from a female *P. carduidactyla* was 5.1 ng/min. No other pheromone-like compounds were detected in these pheromone collections.

(Z)-11-Hexadecenal was identified as the sex pheromone of the artichoke plume moth, *Platyptilia carduidactyla* (Riley), by Klun et al. (1981). These authors concluded that, if other long-range pheromonal components were present, they constituted less than 1.25% of the pheromonal blend. Since its identification, this synthetic sex pheromone has shown promise as an aid in the control of this species which is the most serious pest of artichokes (Haynes et al. 1981), and it is currently being used for monitoring populations and disrupting mating.

A modification of the pheromone collection technique developed by Baker et al. (1981) was utilized to quantify several aspects of the chemical communication system of *P. carduidactyla*. First, the emission rate of (Z)-11-hexadecenal from sex pheromone glands was determined. Second, the relationship between the periodicity of pheromone release from forcibly extruded pheromone glands and the periodicity of calling behavior was established. Third, the volatiles emitted by females were examined for traces of other sex pheromone components.

Materials and Methods

Female *P. carduidactyla* used in this study were reared from field-collected artichoke buds that contained larvae. Infested artichokes from Castroville, Calif., were held at 10°C in bins housed in a dark "cold" room. Last-instar larvae were collected as they emerged from these artichokes. Pupae were separated by sex, and female pupae were housed in a Percival environmental chamber (temperature 20°C; LD 14:10).

Individual 2- to 10-day-old virgin females were inserted abdomen-first into a 3.0-mm (ID) glass tube which had a 0.9-mm hole at the distal end (Fig. 1). A low-pressure vacuum was used to pull the female to the distal end, and a pipe cleaner was used to apply pressure to the head of the female, resulting in eversion of the ovi-

positor and the associated bilobed sex pheromone gland. Since the pheromone gland was forcibly extruded, volatilized pheromone could be detected (if released at a rate greater than 0.01 ng/min) even during periods when females did not actively call. The tube was inserted ovipositor-end-first through a Teflon-coated gas-liquid chromatography (GLC) septum (a 3.0-mm hole had been cut in the septum) into a modified version of the pheromone collection device described by Baker et al. (1981). Volatiles emitted from the gland's surface were collected for 10 min onto ca. 10 mg of glass wool. Some females could still walk after this procedure if they were removed promptly and carefully from the glass extrusion tube. An internal standard [either 3.0 ng of (Z)-11-tetradecenal or 5.0 ng of octadecenal] was added to the glass wool before it and the inside of the collector were rinsed with ca. 200 μ l of CS₂. This volume of solvent was then reduced to approximately 1 μ l (for capillary columns) or 6 μ l (for packed columns).

Analyses were made on a Varian 3700 gas chromatograph equipped with a hydrogen flame detector, a Hewlett-Packard 3380A integrator, and a Honeywell Elektronik 196 chart recorder. Injections were made onto two types of columns in the study of periodicity of pheromone release: (1) SP2330 glass capillary column (direct injection; injector temperature 180°C; temperature program run 90 to 150°C at 60°C/min with 1-min hold at 90°C; He flow rate = 100 cm/sec; N₂ make-up gas flow rate = 25 ml/min; column dimensions 30 m by 0.25 mm ID; df = 0.22 μ m), and (2) Silar 10C packed glass column (4.706g of 10% Silar 10C on acid-washed 100 to 120-mesh Chromasorb W; glass column 3 m by 4 mm (OD); oven temperature 180°C; N₂ flow rate = 30 ml/min). In addition, to examine the emitted volatiles more thoroughly for sex pheromone-like compounds, some injections were made onto a 80% SE52/20% BBBT glass capillary column (direct injection; column temperature = 180°C; He flow rate = 45 cm/sec; N₂ make-up gas flow rate = 25 ml/min; column dimensions 30 m by 0.25 mm ID; df = 0.3 μ m) and a 2% SF-96 packed column (on 100-120 Chromasorb W AcW-DMCS; oven temperature 170°C; N₂ flow rate = 30 ml/min; glass

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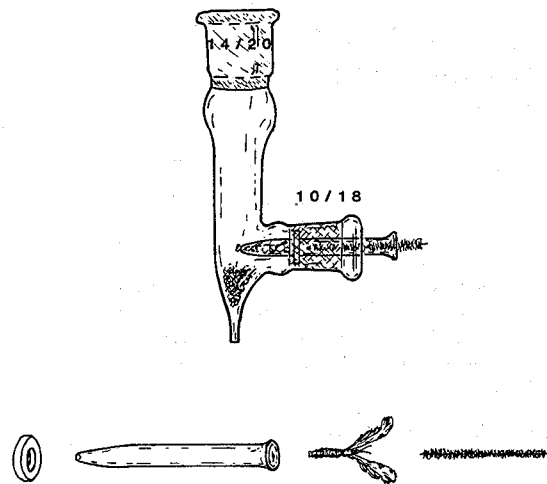


FIG. 1. Pheromone collection device. A female is inserted into the extrusion tube (bottom), and her ovipositor (and associated sex pheromone gland) is everted. The extrusion tube is then inserted into the collector (top) through a Teflon-coated GLC septum. Nitrogen gas flows through the lumen of the collector at a rate of 2 ml/sec, and over the extruded pheromone gland. A volume of glass wool in the end of the collector adsorbs the pheromone. The collector is 6 cm long.

column 2 m by 4 mm OD). Other procedures used in the quantification of pheromone volatiles were described by Pope et al. (1982).

To observe the periodicity of calling behavior, females were individually transferred ca. 1h before scotophase to clear plastic containers (5.8 by 5.8 by 10.7 cm) which contained a wire-mesh screen (7.5 by 10.0 cm; mesh size 0.7 by 0.7 cm). Females would crawl up onto this screen and call. At the start of a scotophase, these females were taken in the plastic cages to another environmental chamber housed in a dark room. The temperature in this chamber was kept at a constant 20°C. The light intensity at the center of this chamber was ca. 0.1 lux during the scotophase. Female moths were observed 1h before scotophase began, at 30-min intervals for its duration, and at 11, 12, 13, 14, 15, 18, 21, and 23 h. after the initiation of a scotophase. Observations were aided by an Iota Cam fiber optic system (set on the lowest setting). The light-emitting end of the flexible fiber optic cable was covered with a filter that transmitted only wavelengths of light above 600 nm (Kodak Wratten filter no. 29). This red light did not stimulate any observable changes in the females' behavior.

Results and Discussion

Some collections of volatiles from extruded pheromone glands of female *P. carduidactyla* contained only one detectable peak, and this compound had a retention time identical to that of synthetic (Z)-11-hexadecenal on the SP2330 capillary column (Fig. 2a). Other collections contained no detectable (Z)-11-hexadecenal, but most of these had no other peaks above noise level within the range of retention times that were recorded (Fig. 2b).

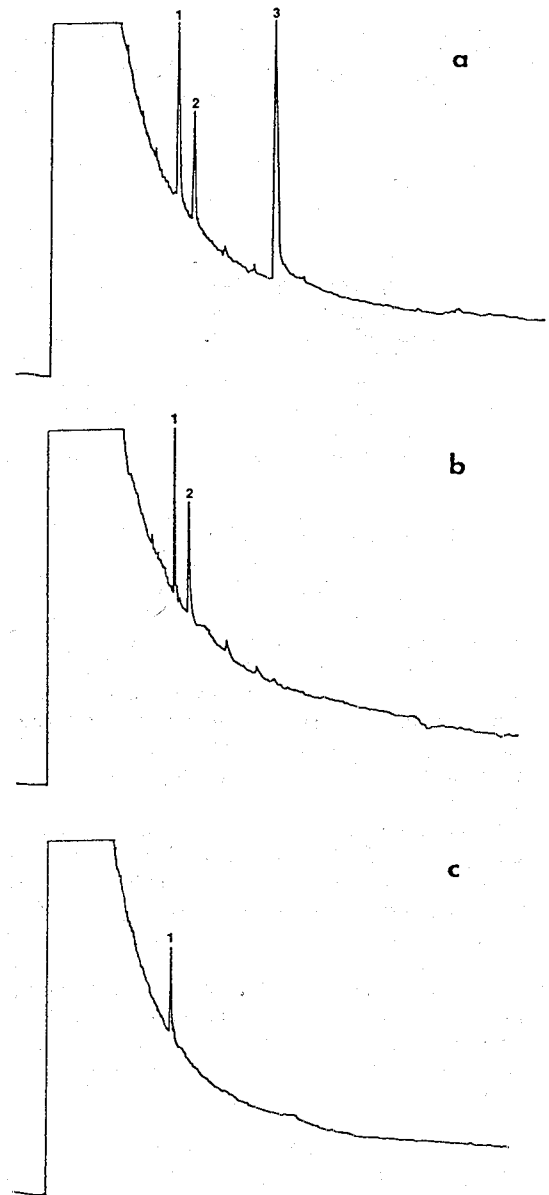


FIG. 2. GLC chromatograms from injections onto an SP2330 glass capillary column, including: (a) a 10-min collection from a female that emitted (Z)-11-hexadecenal [peak 3 = (Z)-11-hexadecenal]; (b) a 10-min collection from a female that emitted no detectable (Z)-11-hexadecenal [peak 2 = (Z)-11-tetradecenal, our internal standard]; and (c) concentrated solvent CS₂ (peak 1 = solvent contaminant). The visible descending slope of the solvent front covers a range of retention times that includes those of most sex pheromone-like compounds.

The recorded descending slope of the solvent front was visible from ca. 4 min after injection and the recording was stopped at ca. 25 min. This range of retention times includes most sex pheromone-like molecules, including: dodecanal, dodecyl acetate, tetradecanal, tetradecyl ace-

tate, hexadecanal, hexadecyl acetate, hexadecanal, hexadecanol, octadecanal, and the mono- and diunsaturated (nonconjugated) derivatives of these molecules. Collections from females that emitted more than 10 ng of (Z)-11-hexadecenal in the 10 min collection period were examined more closely for other compounds that potentially could be secondary pheromonal components. Other than solvent contaminants, there was no evidence of any consistent peaks in these collections (the limitation of our system would not allow us to detect a compound if it were present at less than 0.1 ng or ca. 1% of the amount of (Z)-11-hexadecenal released by these maximally releasing females). In addition, collections analyzed on SE52/BBBT ($n = 12$) and SF-96 ($n = 4$) columns showed no signs of consistent additional peaks that may have overlapped with (Z)-11-hexadecenal on the SP2330 capillary column. No other components were found in the regions of either internal standard. Thus, we could detect no evidence of secondary pheromonal components in the volatiles actually emitted from sex pheromone glands of *P. carduidactyla*, substantiating the results of Klun et al. (1981) from ovipositor washes.

The emission rate of (Z)-11-hexadecenal from extruded pheromone glands showed a distinct diel periodicity, reaching a maximum between 2 and 6 h after the initiation of scotophase (Fig. 3a). The average pheromone emission rate was 1.52 ± 0.409 (SE) ng/min during this 4-h period. During the last 2 h of scotophase and the entire photophase, the emission rate of (Z)-11-hexadecenal was much lower, averaging between 0.02 and 0.09 ng/min (1 to 6% of the average emission rate during the peak period.) This distinct periodicity in the emission of (Z)-11-hexadecenal from extruded pheromone glands indicates that there is a diel periodicity in the biosynthesis of the sex pheromone or in the transportation of the pheromone to the surface of the gland.

The periodicity of calling (i.e., the eversion of the sex pheromone gland by the female) paralleled the periodicity of pheromone release from forcibly extruded pheromone glands (Fig. 3). More than 50% of all females tested were calling during the period between 2 and 6.5 h after the initiation of scotophase. Of the females, 85% were calling at 3 h after lights-off. No females were observed to call during the last 1.5 h of scotophase or during the entire photophase.

A well-defined periodicity to calling behavior, like that found in *P. carduidactyla*, appears to be widespread in the Lepidoptera that use long-range chemical communication (e.g., Baker and Cardé 1979, Castrovillo and Cardé 1979, Sower et al. 1971). Charlton and Cardé (1982) documented a diel periodicity in the quantity of pheromone released by female *Lymantria dispar* (L.) and a similar periodicity to calling behavior. In this species the periodicities of pheromone release and calling were not as discrete as in *P. carduidactyla*. Morse et al. (1982) demonstrated that release of pheromone by *Choristoneura fumiferana* (Clemens) peaked during the scotophase. The similarity of the temporal profiles of emission rate of (Z)-11-hexadecenal and calling behavior demonstrates the tight regulation and coordination of biochemical and behavioral aspects of pheromone release

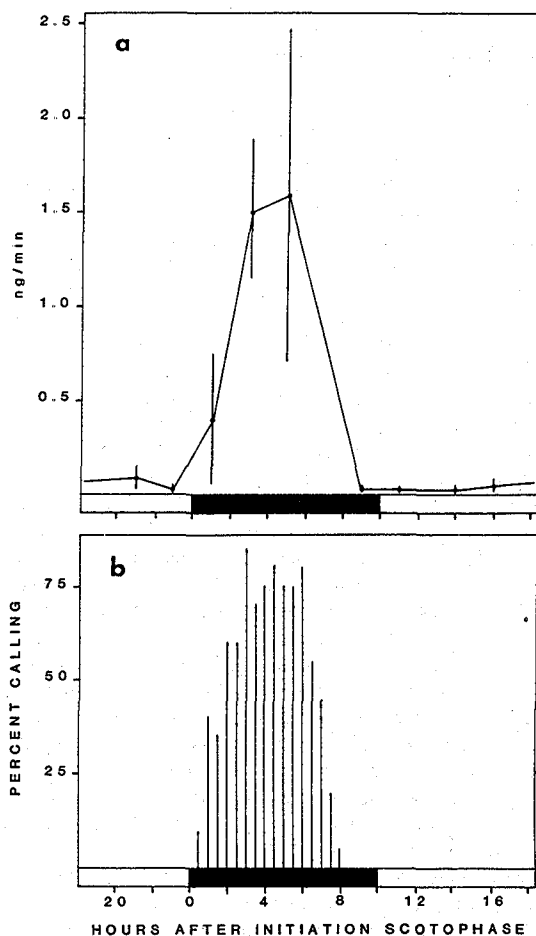


FIG. 3. Comparison of the periodicity of (a) pheromone emission and (b) calling behavior in a photoperiod of LD 14:10 (temperature = 20°C). (a) $n = 10, 11, 4, 7, 8, 10, 5, 11,$ and 13 for 0-2, 2-4, 4-6, 8-10, 10-12, 13-15, 15-16, 20-22, and 22-24 h after the initiation of scotophase, respectively. Vertical bars represent SE. (b) $n = 20$ for each time interval.

in *P. carduidactyla*. Similarly, Pope et al. (1982, and unpublished data) documented a discrete periodicity to pheromone emission from pheromone glands that coincided with mating activity periodicities in *Heliothis virescens* (F.) and *Heliothis zea* (Boddie), respectively. Coffelt et al. (1978) found that relatively large amounts of pheromone were present in the sex pheromone glands of *Plodia interpunctella* (Hübner) during a period when 90% of females were calling. In these species, there is strong evidence that the amount of pheromone available for chemical communication cycles in synchrony with the behavioral periodicity of calling.

To maximize the effectiveness of disruptant formulations of pheromone and minimize their cost, it is important to establish the relationship between the release rate of pheromone from synthetic pheromone sources and the percent disruption of mating. Field experimentation to accomplish this goal are inherently difficult to

design as well as costly, especially when several orders of magnitude of release rate of pheromone have to be tested. Experience with an effective emission rate of the pheromone of one species will not necessarily translate into adequate mating disruption in another species, since known emission rates span several orders of magnitude. Baker et al. (1980) estimated that female *Grapholitha molesta* (Busck) release 0.05 ng of their major pheromonal component, (Z)-8-dodecenyl acetate, per min. Female *Pectinophora gossypiella* (Saunders) release on the average 0.1 ng of (Z,Z)-7,11-hexadecadienyl acetate per min (Haynes et al. unpublished data). Gypsy moths, *L. dispar*, emit 0.2 ng of disparlure per min during the peak emission period (Charlton and Cardé 1982). Even in closely related species, there is a considerable difference in the release rate of the same major component: the release rate of *H. virescens* peaks at ca. 3.3 ng/min (Pope et al. 1982), whereas *H. zea*'s rate peaks at less than 1 ng/min (Pope et al., unpublished data). Female cabbage looper moths, *Trichoplusia ni* (Hübner), release up to 49 ng of (Z)-7-dodecenyl acetate per min (mean = 23 ng/min) (Bjostad et al. 1980). The pheromonal release rate from extruded glands of female *P. carduidactyla* falls into an intermediate category, averaging 1.52 ± 0.409 (SE) ng/min during the peak calling period. The highest release rate measured was 5.11 ng/min. We believe that these values establish a well-defined reference point that could prove to be useful in tailoring the design of the pheromone elution system and establishing a minimum release rate necessary for each point source to be competitive with a female. In artichoke fields in coastal California, the artichoke plume moth calls at a time of day when temperatures usually range from ca. 8 to 15°C. Release of sufficient quantities of synthetic pheromone at these cool temperatures may necessitate some modification of the current pheromonal technology, but recognizing these potential problems early may alleviate costly errors. In any case, the periodicity of pheromone release, the composition of the pheromonal blend, and the quantities of pheromone a female *P. carduidactyla* is capable of releasing are important dimensions to the chemical communication system, and thus knowledge of these factors may lead to more effective use of the artichoke plume moth's pheromone in agriculture.

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