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ENDOGENOUS AND EXOGENOUS FACTORS AFFECTING PHEROMONE PRODUCTION AND RELEASE IN THE OMNIVOROUS LEAFROLLER MOTH, PLATYNOTA STULTANA (WALSINGHAM)

presented by

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has been accepted towards fulfillment
of the requirements for

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Major professor
Ring T. Carde

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# ENDOGENOUS AND EXOGENOUS FACTORS AFFECTING PHEROMONE PRODUCTION AND RELEASE IN THE OMNIVOROUS LEAFROLLER MOTH, *PLATYNOTA STULTANA* (WALSINGHAM)

By
Reginald Paul Webster

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# ABSTRACT

## ENDOGENOUS AND EXOGENOUS FACTORS AFFECTING PHEROMONE PRODUCTION AND RELEASE IN THE OMNIVOROUS LEAFROLLER MOTH, PLATYNOTA STULTANA (WALSINGHAM)

By

Reginald Paul Webster

Temperature modified the expression of female calling (behaviour associated with pheromone release) in *Platynota stultana* (Lepidoptera: Tortricidae). Absolute temperature levels and not necessarily a decrease in temperature appear to modulate the timing of calling by delimiting a specific time interval or gate for the calling period. The threshold for calling appears to be related to the level of an endogenous factor, current temperature, and photoperiod. The onset of calling advanced to earlier in the day with age. Maximal pheromone titre and maximal proportion mating occurred in moths on second day after emergence. Pheromone production is rhythmic, with maximal titre being reached near the onset of the calling gate at 14°C. Thus, females appear to be capable of emitting pheromone at an appropriate rate any time within the calling gate. Pheromone titre appears to reflect a time-dependent readiness to respond to a decrease in temperature.

Pheromone biosynthesis in *Platynota stultana* is not under direct nervous control by the brain. After mating, pheromone titre in the glands declined to non-detectable levels (<0.2 ng/♀) within 14 hr, calling ceased, and the first batch of eggs was laid within 20-24 hr. Decapitation

resulted in a similar decline in pheromone titre, but oviposition and calling did not occur. Severing ventral nerve cord during the pupal stage did not influence subsequent pheromone biosynthesis, suggesting that a humoral factor(s) originating from the head must be present to maintain pheromone in the gland. After mating this factor is likely eliminated.

A juvenile hormone analogue (ZR-512) applied exogenously to virgin females appeared to block pheromone biosynthesis, elicited oviposition comparable to mated females, and terminated calling within 48 hr. The juvenile hormone analogue was as effective as juvenile hormone I, II, and III, eliciting oviposition in virgin females.

To My Mother and Father,
Elsa and Andrew

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# INTRODUCTION

The omnivorous leafroller moth, Platynota stultana (Walsingham) infests a large variety of greenhouse and field crops in California, Arizona, and Mexico (Atkins et al., 1957). Host plants include alfalfa, aster, avocado, citrus, cotton, pigweed, red pepper, rose, walnut, and grape (Atkins et al., 1957; AliNiazee et al., 1970). P. stultana is now an important pest of grapes in California. Early in the season larvae of P. stultana feed on foliage and in later generations shift to grapes as berry size increases. Feeding by larvae breaks the skin of the berries, allowing yeast and fungi to initiate bunch rot and eventually cause destruction of the entire bunch (AliNiazee and Stafford, 1972).

Adult males locate virgin females using a sex attractant pheromone, as first demonstrated by AliNiazee and Stafford (1971). Hill and Roelofs (1975) later identified two major components of the pheromone.

Sex attractant pheromones provide a sensitive, species-specific means for population sampling, and are an important part of many pest management programs. The use of pheromones as communication disruptants for direct control of pest populations is now a reality. However, to more efficiently utilize pheromones as communication disruptants, a greater understanding of the behavioural and physiological events involved in the coordination of the sexual behaviours between sexes that culminate in mating is required.

The precise relationship between rhythmicities of female calling and pheromone titre on or in the pheromone gland has not been established definitively amongst Lepidoptera. Comparatively little is known of the neural, neuro-hormonal and neuro-humoral influences on pheromone production and release in moths. Most evidence establishing endocrine regulation of pheromone production in moths is fragmentary and circumstantial.

In this dissertation I examine in two chapters: (1) the relationship between rhythmicities of female calling and pheromone titre in gland, and the influence of temperature and age on these rhythmicities; and (2) the effect of mating and endocrine regulation of pheromone production and release in *P. stultana*.

# CHAPTER 1

# Relationship Between Pheromone Titre, Calling, and Age

# INTRODUCTION

In Lepidoptera the behavioural and physiological events involved in sexual behaviour usually are coordinated temporally so that they occur synchronously under optimal conditions in a heterogeneous and variable environment. The expression of daily rhythms of sexual behaviours, such as calling (the behaviour associated with pheromone release) and pheromone release, is usually endogenous and the timing of these rhythms is usually modified by exogenous environmental cues, typically photoperiod and temperature (Sower *et al.*, 1971; Cardé and Roelofs, 1973; Cardé *et al.*, 1975; Baker and Cardé, 1971; Castrovillo and Cardé, 1979). The relationship between calling and pheromone titre in or on gland surface has been investigated in several species (Shorey and Gaston, 1965; Brady and Smithwick, 1968; Nagota *et al.*, 1972; Sower *et al.*, 1972; Fatzinger, 1973; Nordlund and Brady, 1974; Coffelt *et al.*, 1978). Shorey and Gaston (1965), Brady and Smithwick (1968), Nagota *et al.* (1972), and Nordlund and Brady (1974) used bioassays of pheromone gland extracts to quantify pheromone titre in gland. However, only relative changes in titre can be quantified using bioassays. The precise relationship between rhythmicities of female calling and pheromone titre has not been definitively established among moths. We investigated the relationship between calling rhythmicity and pheromone titre on/in gland of *Platynota stultana* (Walsingham).

## MATERIALS AND METHODS

### A. Insect Culture

*P. stultana* from Parlier, California was maintained on a pinto bean diet (Shorey and Hale, 1965) at 24°C, 75% relative humidity, and a 16:8 light:dark photoperiod regime. Photophase and scotophase light intensity were *ca*. 2100 and 0.3 lux, respectively. Pupae were segregated by sex and adults were held in separate cages according to emergence dates and sex. All experiments were conducted at 24°C, 75% relative humidity, and identical 16:8 light:dark regime, unless otherwise specified.

### B. Gas-Liquid Chromatographic Analyses

Gas-liquid chromatographic (GLC) analyses were conducted with three 2 mm internal diameter glass columns; a 1.8 m long column packed with 3% OV-1 on Gas Chrom Q,· a 3.5 m long column packed with 10% XF-1150 on Chromosorb-W, and a 3.5 m long column packed with 20% OV-275 on Gas Chrom-RZ.

### C. Pheromone Titre Measurements

The sex pheromone of *P. stultana* consists of a 88:12 blend of (*E*) and (*Z*)-11-tetradecenyl acetate (E11-14:AC; Z11-14:Ac)(Hill and Roelofs, 1975). To quantify pheromone titre on/in the pheromone gland of virgin females and avoid contamination of the sample with spurious compounds, the optimal time for extraction was determined. Since no differences in ratios of the blend of pheromone components were found in female pheromone gland extracts at different times of day or on different days, only the E11-14:Ac pheromone component was quantified. In *Argyrotaenia velutinana* (Walker), little variation in pheromone component blend ratios was found in female pheromone gland extracts (Miller and Roelofs, 1980). Between 13.50 and 14.50 hr (24 hr decimal clock) the last 2 abdominal segments containing the pheromone gland of day-2 females (emerged on day 1) were excised and soaked in 20 µl $CS_2$ for 5, 10, 15, 30, or 60 sec. Each sample contained gland rinses of 5 females to insure sufficient material for accurate quantification. An internal standard, (Z,E)-7,11-hexadecadienyl acetate (10 ng/µl) in 20 µl $CS_2$ was added, and then the sample was stored at $-10^{\circ}$ until analyzed by GLC on OV-275. Extractable pheromone was removed within 10-15 sec (Figure 1). Extraction for 18 hr ($N = 5$) resulted in no significant increase in titre over a 15 sec rinse.

The ratio of E and Z11-14:Ac, as determined on XF-1150 using female extracts, was not different from that determined on OV-275. Analyses on OV-1 showed no corresponding alcohols (<0.1%) to complicate analyses on OV-275 or XF-1150. All subsequent determinations of pheromone titre were of the E11-14:Ac component obtained from 15 sec abdominal tip washes of samples of 5 females analyzed by GLC on OV-275 or XF-1150.

### D. Female Calling Observations

Single females were placed in 4 cm x 4 cm (35 ml) clear plastic cups with plastic lined cardboard lids. Females were observed during scotophase using a light with a Kodak Wrattan$^{R}$ filter No. 19 which eliminates light below 600 nm. The moths appeared to be insensitive to the light. A female was scored as calling if the pheromone gland was extruded and visible, or if abdomen was turned away from observer the female assumed the typical calling posture: wings elevated, legs extended, and abdomen raised above the substrate. There was individual variability in the extent of this behaviour. Near the end of calling (0.5-1.0 hr), calling behaviour became less pronounced, i.e., wings and abdomen were lowered toward substrate. Once the pheromone gland was either entirely retracted or typical resting posture was assumed (wings and abdomen

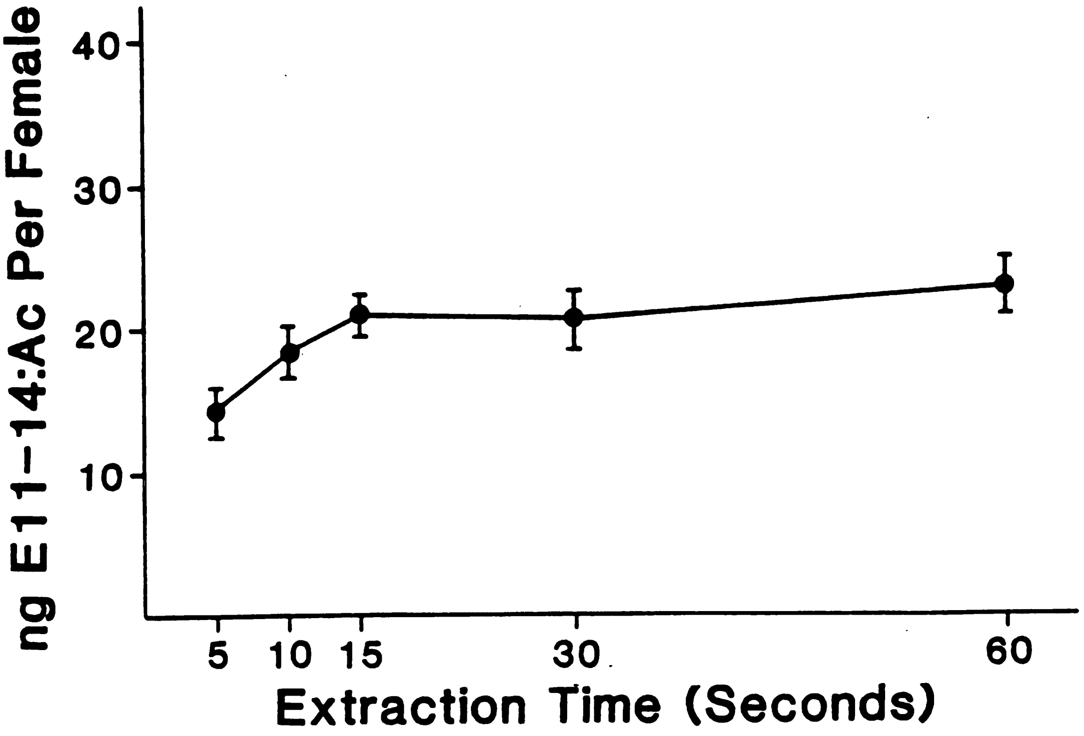


Figure 1. Quantity of the E11-14:Ac pheromone component obtained from different extraction times of abdominal tip washes of females. The quantity indicated is $\bar{x}$ ± SD obtained from 10 samples of 5 females.

lowered to substrate) calling was considered terminated. Photoperiod light intensities were identical to those used in rearing.

Calling rhythms were observed from day 1 (day of adult emergence) to day 6. Individual females were placed in plastic cups (containing 1 cm x 1 cm cotton wicks soaked with 5% sucrose solution) at 12.00 hr (3-5 hr after emergence) and observed for calling at hourly intervals between 15.00 and 04.00 hr and 3 hr intervals during rest of period, each day through day 6. Cotton wicks were replaced on alternate days.

### E. Observations on Calling Gate

Several species of moths exhibit an ability for daily adjustments of calling rhythm to ambient temperature (Batiste, 1970; Sower *et al*., 1971; Sanders and Lucuik, 1972; Cardé *et al*., 1975; Castrovillo and Cardé, 1979; Baker and Cardé, 1979). A rapid decrease in temperature induces calling within several minutes if the temperature is decreased during the gate for calling, the gate being the time interval during which calling can be induced by a change in temperature. Decreasing the temperature before the gate results in a latent period before calling commences. By decreasing the temperature at different times the timing of the calling gate can be determined. Calling gates were determined for day-1, -2, and -3 females. Moths were placed in plastic cups at 10.00 hr. Groups of moths (20) were removed from an environmental cabinet (24°) and placed at various times in a walk-in-chamber at 14°. A control group of moths was maintained at 24°. The experiment was replicated 3 times. A Bailey BAT-4 amplifying thermometer with a MT-3 microprobe accurate to 0.5° was used to determine rate of temperature decrease within the plastic cups. Mean time of onset of calling, calling latency (time between the decrease in temperature and the onset of calling), and end of calling were determined for each group of moths.

### F. Mating

Male and female pairs were placed in 2.5 cm x 3 cm (40 ml) clear plastic vials with cotton stoppers 6 hr prior to scotophase. Beginning at 18.00 hr (2 hr prior to initiation of scotophase) at 0.5 hr intervals to 03.00 hr those individuals *in copula* and calling were recorded for day-1 through day-6 females. Only day-2 males were used. Twenty moths were used for each of the 5 replicates using a completely randomized design. Mean onset of mating and proportion that mated were determined for each age group of females.

## RESULTS

### A. Pheromone Titre Measurements

We first determined if pheromone titre changed with age as in *A. velutinana* (Miller and Roelofs, 1977). Titre was determined at 19.50 hr (1-2 hr before calling began) for day-1 through day-7 moths (Figure 2). Pheromone titre reached maximal levels on day 2 (28.5 ng/♀), then progressively decreased to day 7 (1.8 ng/♀).

A subsequent study examined possible periodicity of pheromone titre on/in gland surface in relation to calling periodicity during the first 6 days post-emergence (Figure 3). Titre was determined at 3 hr intervals from 09.00 hr on day 1 (0-3 hr after emergence) to 09.00 hr on day 7. Between 18.00 and 24.00 hr titre was determined at 1.5 hr intervals. Six replications were done using a completely randomized design. Only 1.5 ng/♀ of E11-14:Ac was present on the gland surface on day 1 at 09.00 hr 0-3 hr after emergence, then there was a progressive increase in titre to 17.7 ng/♀ at 22.50 hr, when the first individuals began to call

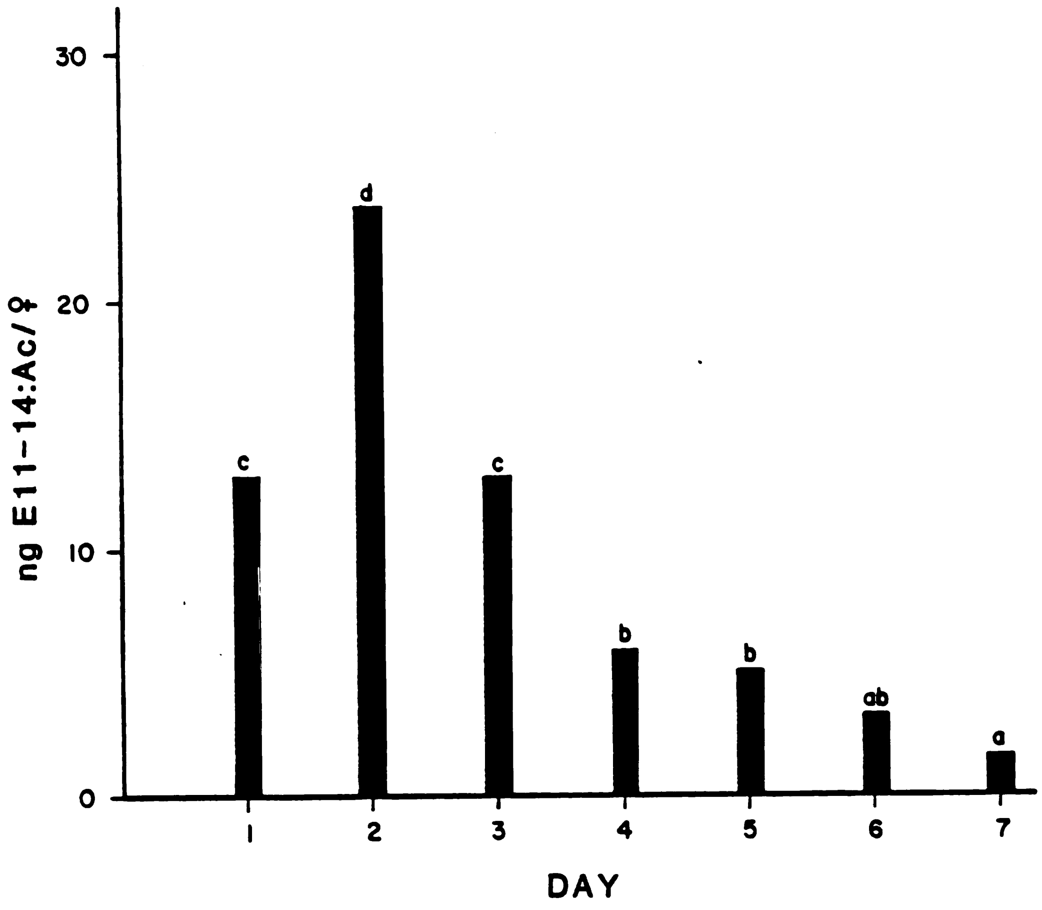


Figure 2. Titre of E11-14:Ac pheromone component obtained from 15 sec abdominal tip washes of day-1 through day-7 females at 19.50 hr. Means are from 6 samples of 5 females. Bars with same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

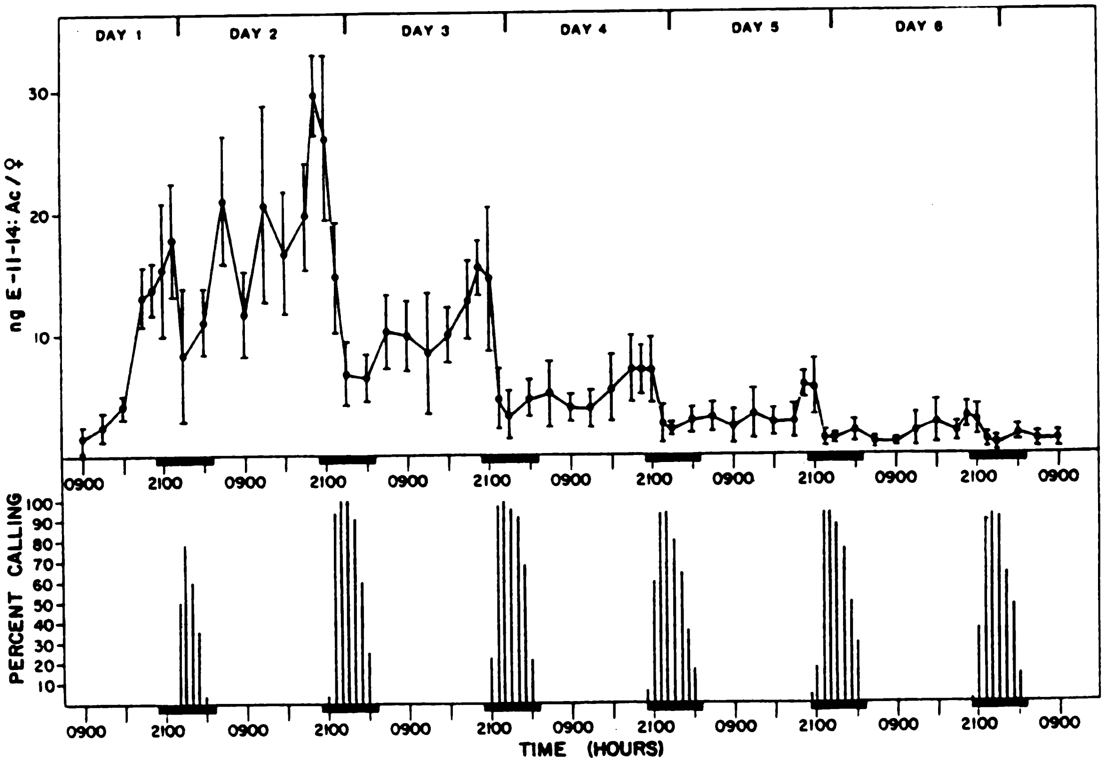


Figure 3. (Top) Titre of E11-14:Ac pheromone component obtained from 15 sec abdominal tip washes of females over 6 days. The quantity is the x̄ ± SD obtained from 6 samples of 5 females. (Bottom) The calling rhythm of virgin females held under identical conditions at 24° (N = 100).

(Figure 3). Titre of E11-14:Ac then decreased to 8.2 ng/♀ by 24.00 hr. On day 2 there was no significant increase in pheromone titre between 03.00 and 18.00 hr. Between 18.00 and 19.50 hr pheromone content increased significantly from 19.7 to 29.6 ng/♀ and remained at this level to 21.00 hr (27.00 ng/♀) which is ca. 1.0 hr prior to the onset of calling. Once calling commenced (22.20 hr), titre dropped abruptly to 14.2 ng and 6.8 ng/♀ at 22.50 and 24.00 hr, respectively. On subsequent days a similar rhythm of pheromone titre was observed, with no significant change in titre between 03.00 hr (after calling ceased) and 15.00 hr. Maximal titre of E11-14:Ac usually was reached by 19.50 hr and each day titre decreased after calling began.

These observations suggest a daily rhythm of pheromone production in *P. stultana*. However, much sample variability in titre of E11-14:Ac was found, even though each sample contained pheromone rinses of 5 females. Because sample variability may have masked subtle changes in titre, we re-examined pheromone titre changes for day-2 and -3 moths with 24 and 23 replicates, respectively. Samples were taken at same times as in previous experiment, except for an additional sample at 16.50 hr (Figure 4). On days 2 and 3 pheromone titre remained the same between 03.00 and 09.00 hr. Titre then increased, reaching maximal levels at 15.00 and 19.50 hr on days 2 and 3, respectively. On both days, the increase in titre appeared to level off at 15.00 hr, then increased to slightly higher levels between 18.00 and 19.50 hr (Figure 4). Once calling commenced (22.20 hr on day 2; 21.80 hr on day 3) titre decreased abruptly.

### B. Female Calling Observations

Individual females were observed for time of onset of calling from day 1 through day 6 (Table 1). Females were observed beginning at 18.00

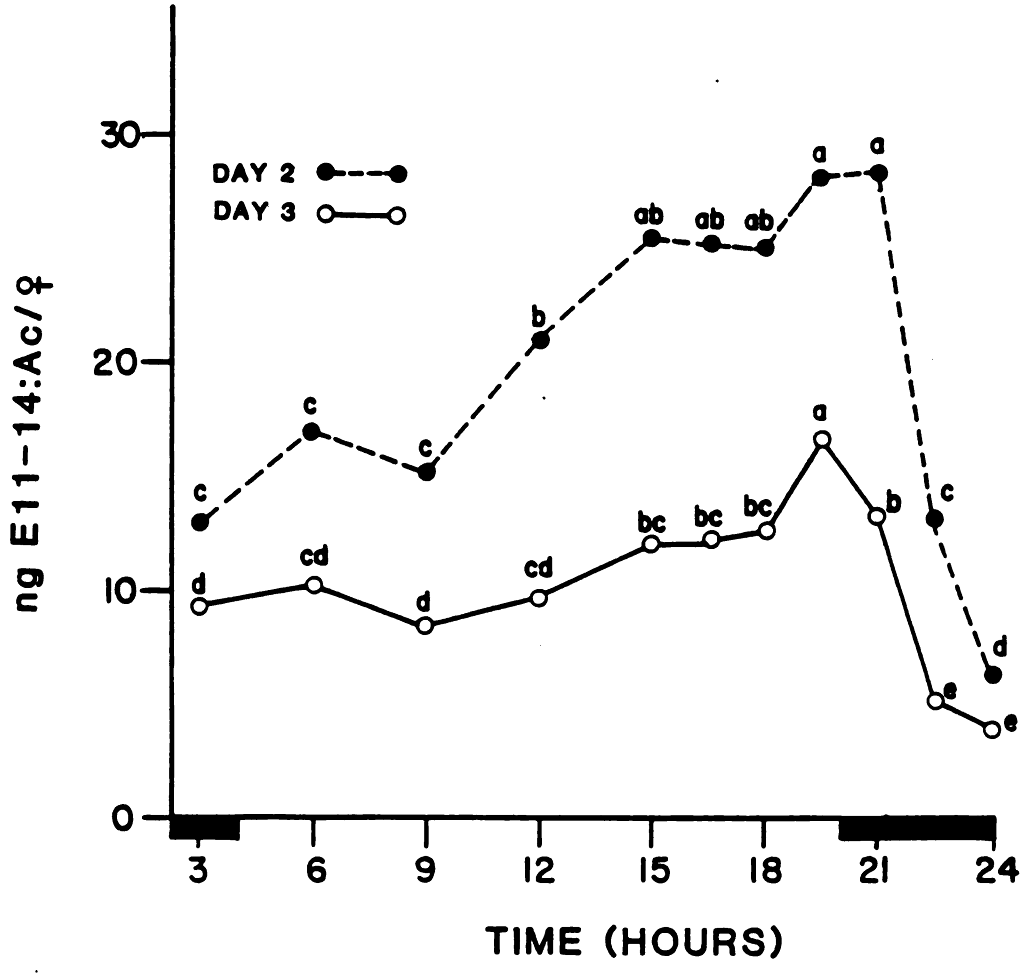


Figure 4. Titre of E11-14:Ac pheromone component obtained from 15 sec abdominal tip washes of day-2 and -3 females at different times. Each point is $\bar{x}$ titre obtained from 24 and 23 samples of 5 day-2 and -3 females, respectively. Points on each line with the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

Table 1. Mean time of onset of calling, mating, and percent mating of female *Platynota stultana* at different ages. For each column means and percentages followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test. In columns 2 and 3 five replicates of 20 moths each were used and treatment differences were analyzed using means of each replicate.

| Age (day) | $\bar{x}$ Time of onset of calling (N=76) | $\bar{x}$ Time of onset of mating (N=100) | Percent mating (N=100) |
|---|---|---|---|
| 1 | 23.6 a ± 0.8 | 23.3 a | 44.0 b |
| 2 | 22.2 b ± 0.4 | 21.9 b | 74.0 a |
| 3 | 21.8 c ± 0.3 | 21.7 b | 50.2 b |
| 4 | 21.4 d ± 0.3 | 21.6 b | 57.0 b |
| 5 | 21.2 e ± 0.5 | 21.4 b | 40.0 b |
| 6 | 21.1 e ± 0.5 | 21.7 b | 52.0 b |

hr at 0.5 hr intervals until all individuals had initiated calling for at least 1.0 hr. On day 1, females began calling between 22.50 and 01.00 hr ($\bar{x}$ = 23.60 hr) and all females eventually called. On the following days the mean time of the onset of calling became progressively earlier until day 5. Some individuals on days 5 and 6 began calling at 20.00 hr. After day 1, most females (≥90%) initiated calling within 1.0 hr of each other.

## C. Observations on Calling Gate

Groups of day-1 moths were cooled from $24^{o}$ to $14^{o}$ at 1 hr intervals, between 15.00 to 22.00 hr (Figure 5). Calling could not be induced before 15.00 hr in day-1 moths. For day-2 and -3 moths decreases in temperature were initiated at 12.00 hr and ended at 20.00 and 19.00 hr, respectively (Figures 7 and 8). Individuals calling were observed at 0.5 hr intervals beginning 0.5 hr after the transfer to colder temperature. After the decrease in temperature began, temperature decreased within the cups from $24^{o}$ to $16^{o}$ in 3.5 min and to $14^{o}$ in 8.0 min (N = 5).

Only 17% of day-1 females initiated calling after a decrease in temperature at 15.00 hr, with mean onset of calling at 18.30 hr and a calling latency of 3.30 hr (Figures 6 and 8). Thereafter, the percentage initiating calling increased (60% at 18.00 hr; 82% at 22.00 hr) and calling latency decreased each time temperature was decreased. From 18.00 hr onward no significant change in calling latency occurred. The calling gate for day-1 females, therefore, began at ca. 18.00 hr.

For day-2 and -3 moths ≥95% of females initiated calling at all times temperature was decreased. At 12.00 hr $\bar{x}$ onsets of calling were 16.70 and 17.40 hr and calling latencies were 4.70 and 5.40 hr for day-2 and -3 moths, respectively (Figures 6, 7, and 8). Calling latency decreased on subsequent times temperature was dropped until 16.00 hr

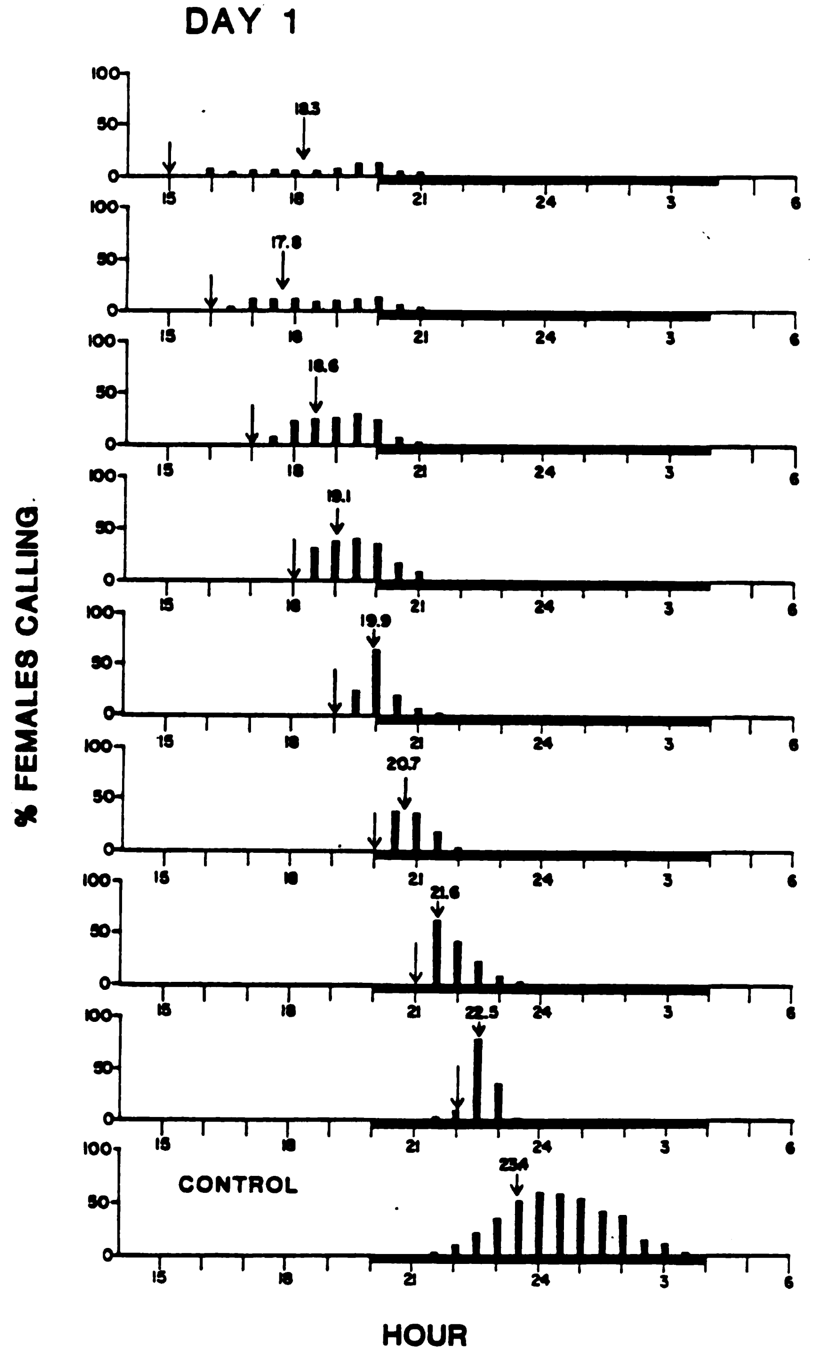


Figure 5. Effect of decreases in temperature on calling of day-1 females. Arrows on x axis indicate times of decreases in temperature ($24^{\circ}$ to $14^{\circ}$). Numbers above arrows denote $\bar{x}$ hour of onset of calling. Mean onset of calling was calculated using time of onset of calling after termination of initial calling bouts. Bars represent mean percent of 3 replicates of 20 moths.

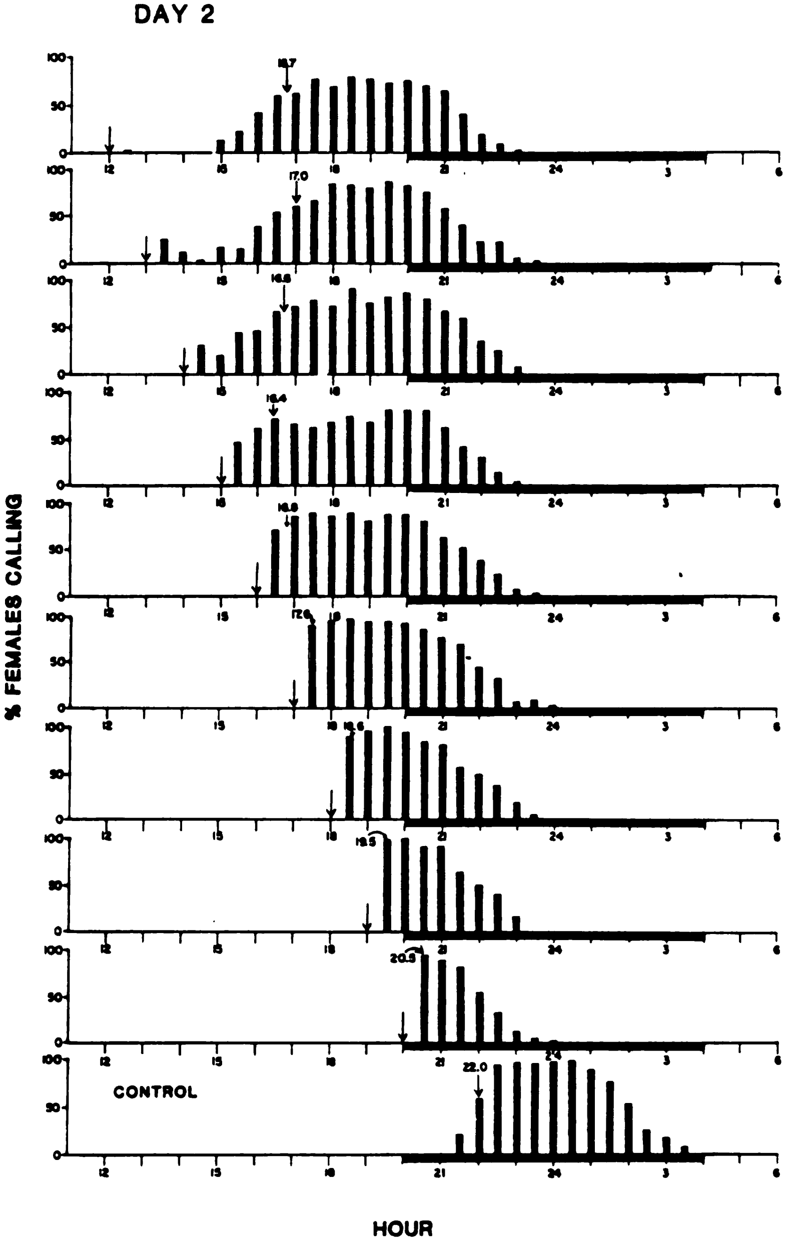


Figure 6. Effect of decreases in temperature on calling of day-2 females. Arrows on x axis indicate times of temperature decreases ($24^{\circ}$ to $14^{\circ}$). Numbers above arrows denote $\bar{x}$ hour of onset of calling. Mean onset of calling was calculated using time of onset of calling after termination of initial calling bouts. Bars represent mean percent of 3 replicates of 20 moths.

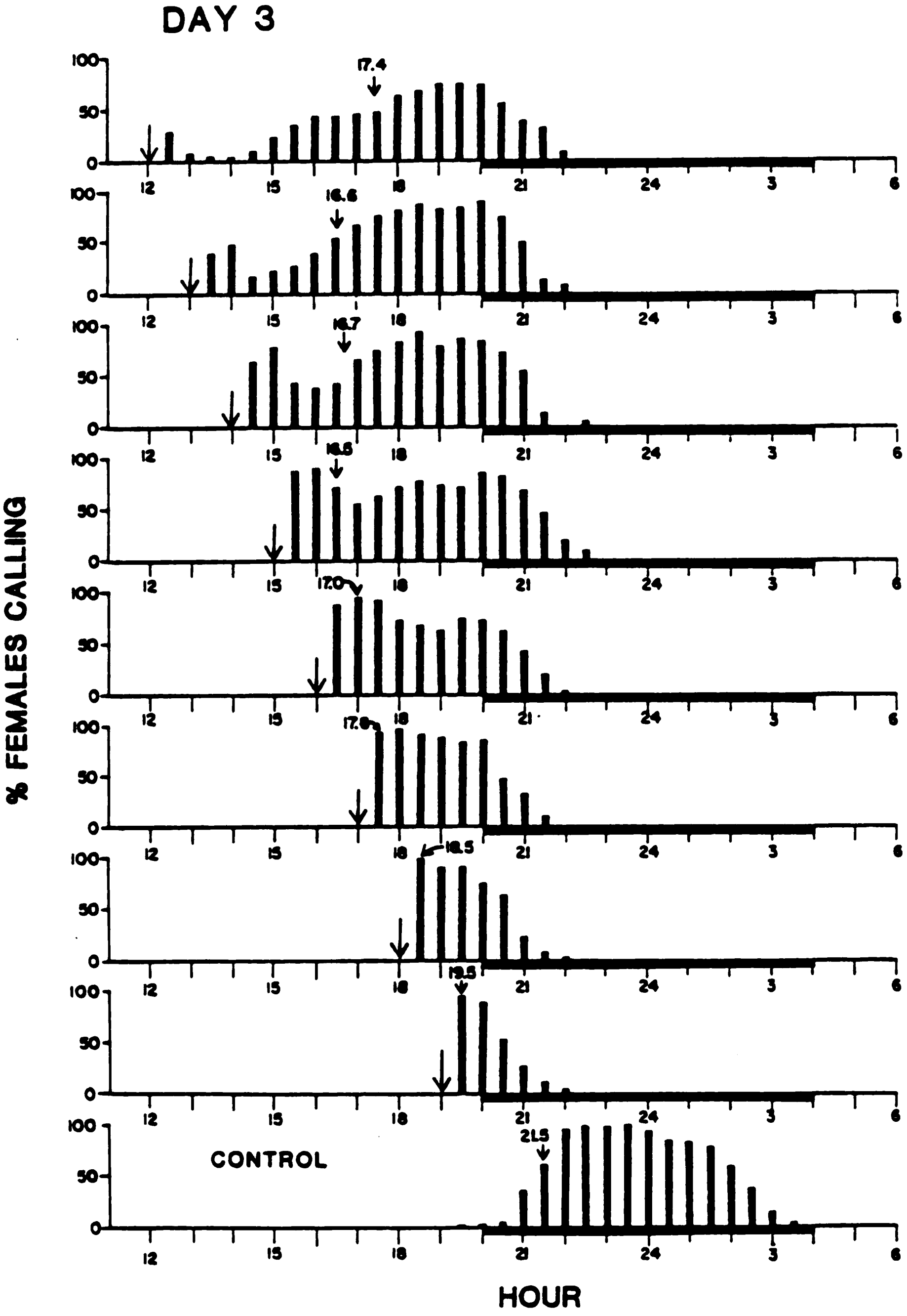


Figure 7. Effect of decreases in temperature on calling of day-3 females. Arrows on x axis indicate times of temperature decreases (24° to 14°). Numbers above arrows denote $\bar{x}$ hour of onset of calling. Mean onset of calling was calculated using time of onset of calling after termination of initial calling bouts. Bars represent mean percent of 3 replicates of 20 moths.

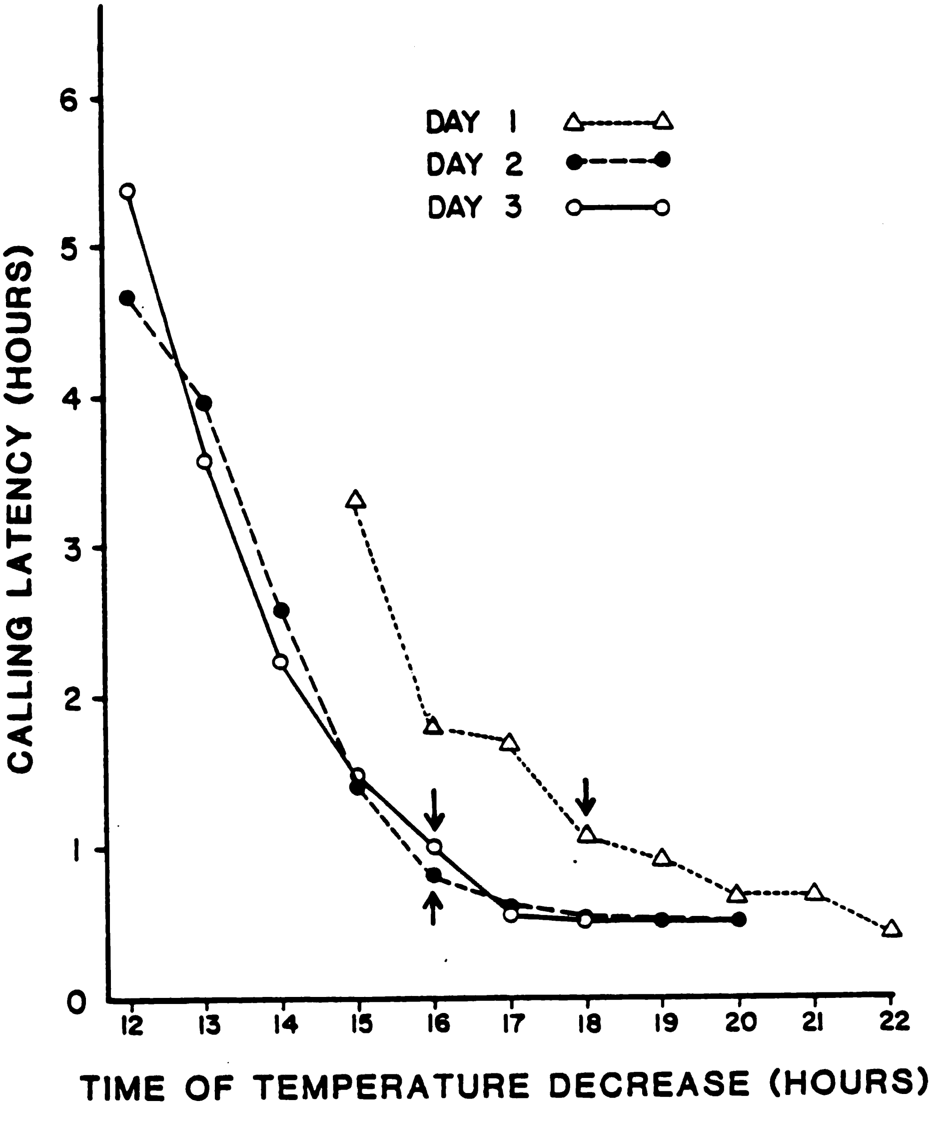


Figure 8. Calling latencies of day-1, -2, and -3 females after decreases in temperature (24° to 14°) at different times. Arrows denote beginning of calling gates. Calling latencies, starting at onset of calling gate, were not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test. Calling latency was calculated using time of onset of calling after termination of initial calling bouts. Each point represents the $\bar{x}$ calling latency from 3 replicates of 20 moths.

for day-2 and -3 moths. Thereafter, calling latencies were ≤0.5 hr. The calling gate for day-2 and -3 females began at _ca_. 16.00 hrs. In order to determine more precisely calling latency during the calling gate, observations were made every 2 min starting 2 min after a decrease in temperature at 19.00 hr for day-3 moths. Mean calling latency was 10 min (0.17 hr) and ranged from 2 to 18 min (N = 49).

Between 12.00 and 16.00 hr, some day-2 and -3 females initiated calling within 0.5 hr after the decrease in temperature, called between 0.5 and 1.0 hr, then ceased calling (Figures 6 and 8; Table 2). These individuals resumed calling within 1.0 to 4.5 hr. Thereafter, progressively fewer individuals exhibited these initial calling bouts after the decrease in temperature and instead, called continuously once temperature was decreased. The maximum proportion of initial calling bouts of day-2 and -3 moths occurred after a decrease in temperature at 13.00 hr. The decreases in temperature elicited a greater proportion of the initial calling bouts in day-3 moths (Table 2). Calling latency was determined for the initial calling bouts of day-3 moths when temperature was decreased at 13.00 hr. Observations were made every 2 min beginning 2 min after the decrease in temperature. For those moths that exhibited an initial calling bout (24 out of 50) mean calling latency was 17 min (0.28 hr) and ranged from 10 to 26 min.

Pheromone titre was determined for day-3 moths that had an initial calling bout and those that did not after a decrease in temperature at 13.00 hr. Females were observed continuously after the decrease in temperature began. As soon as calling commenced those moths were placed in an environmental cabinet at 24°C. This resulted in immediate cessation of calling. At 13.50 hrs, 0.5 hr after initiation of the decrease in

Table 2. Percent of female *Platynota stultana* with initial calling bouts after decrease in temperature. Only those individuals that terminated calling for at least 0.5 hr then resumed calling were used in calculating the percent calling. For each column percentages followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test. Three replicates of 20 moths were used.

| Time of Temperature Decrease | % Females with Initial Calling Bout After Decrease in Temperature | | |
|---|---|---|---|
| | Day 1 | Day 2 | Day 3 |
| 12:00 | -- | 1.7 a | 27.7 b |
| 13:00 | -- | 25.0 c | 54.4 c |
| 14:00 | -- | 15.0 b | 38.3 b |
| 15:00 | 0.0 | 13.3 b | 29.8 b |
| 16:00 | 0.0 | 1.7 a | 8.3 a |
| 17:00 | 0.0 | 0.0 a | 0.0 a |
| 18:00 | 0.0 | 0.0 a | 0.0 a |
| 19:00 | 0.0 | 0.0 a | 0.0 a |
| 20:00 | 0.0 | 0.0 a | -- |
| 21:00 | 0.0 | -- | -- |
| 22:00 | 0.0 | -- | -- |

temperature, moths that had not called were also placed in the environmental cabinet. Samples of individual female pheromone gland rinses were then taken, alternating between those that called and those that did not. Mean pheromone titre of those that called was 9.73 ng/♀ and ranged from 1.5 to 26.5 ng/♀ (N = 28). For those that did not initiate calling immediately after the temperature decrease, mean titre was 7.7 ng/♀ and ranged from 0.7 to 23.1 ng/♀ (N = 28). The means were not significantly different.

## D. Effect of Temperature on Termination of Calling

In day-2 and -3 moths a decrease in temperature (24° to 14°) after 15.00 hr elicited calling within 0.5 hr. However, regardless of when decreases in temperature began, $\bar{x}$ time of termination of calling occurred at about the same time (Figures 6 and 7, Table 3). This resulted in a progressive shortening of calling period as time of decrease in temperature was delayed. This suggests that temperature regulated when calling was terminated in P. stultana and that calling may be terminated by a decrease in temperature after 21.00 hr in day-2 moths. (Figure 6). Therefore, we decreased temperature (24° to 14°) in day-2 moths at 21.00, 22.00, and 23.00 hr. Calling was observed every 0.5 hr beginning at 18.00 hr, ending at 04.00 hr (Figure 9). Normally $\bar{x}$ time of onset calling of day-2 moths is ca. 22.00 hr at 24°. In this experiment $\bar{x}$ time of onset of calling in the control group maintained at 24° was 21.20 hr and the $\bar{x}$ time of end of calling was at 01.70 hr. In the control group all females had initiated calling by 22.00 hr. When temperature was decreased at 21.00 hr all individuals initiated calling by 21.50 hr and the $\bar{x}$ end of calling was at 21.90 hr. A decrease in temperature at 22.00 hr elicited a 58% reduction in calling within 0.5 hr. When temperature was decreased

Table 3. Mean time of termination of calling for day-1, -2, and -3 *Platynota stultana* females. For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test. Three replicates of 20 moths. Treatment differences were analyzed using means of each replicate.

| Time of Temperature Decrease | $\bar{x}$ Time of Termination of Calling | | |
|---|---|---|---|
| | Day 1 | Day 2 | Day 3 |
| 12:00 | --- | 21.1 d | 20.6 bc |
| 13:00 | --- | 21.2 d | 20.7 b |
| 14:00 | --- | 21.3 cd | 20.9 b |
| 15:00 | 20.2 d | 21.2 d | 20.6 bc |
| 16:00 | 19.3 d | 21.3 cd | 20.2 d |
| 17:00 | 19.8 d | 21.7 bc | 20.2 cd |
| 18:00 | 20.0 d | 21.8 b | 20.3 cd |
| 19:00 | 20.2 d | 22.0 b | 20.5 bcd |
| 20:00 | 21.0 cd | 21.8 b | --- |
| 21:00 | 22.2 bc | --- | --- |
| 22:00 | 22.7 b | --- | --- |
| Control | 01.3 a | 01.3 a | 01.8 a |

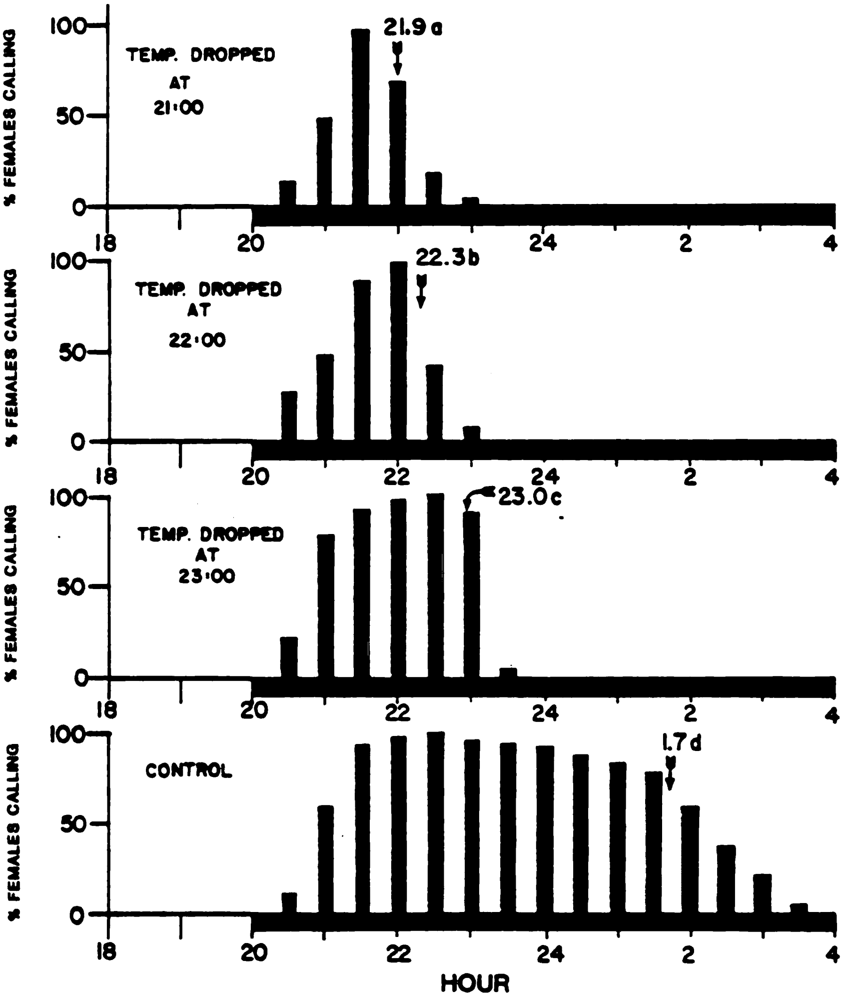


Figure 9. Effect of decreases in temperature on termination of calling of day-2 females. Numbers above arrows denote $\bar{x}$ hour of onset of calling. Bars represent mean percent of 3 replicates of 20 moths.

at 23.00 hr, a 95% reduction in calling was induced within 0.5 hr after the decrease in temperature.

E. Mating

Mating occurred within 0.5 hr after initiation of calling for all age groups of females. The $\bar{x}$ time of onset of mating for day-1 moths was 1.4 hr later than day-2 and 1.9 hr later than day-5 females (Table 1). The $\bar{x}$ time of onset of mating was not significantly different for day 2-6 females. Day-2 females, which contained the greatest quantity of pheromone, had the greatest percentage mating of any age group.

## DISCUSSION

Temperature modified the expression of calling in female *P. stultana*. Decreasing temperature from 24° to 14° advanced the onset of calling up to 5 hr. A decrease in temperature was followed by a rapid initiation of calling (within 0.5 hr) only when the temperature shift occurred within a particular time interval or gate, which at 14° began about 5 hr prior to onset of calling at 24°. If the decrease in temperature occurred before this gate, there was a latent period before calling commenced. *A. velutinana*, *Holomelina immaculata* (Reakirt) (Cardé *et al*., 1975), and *Grapholitha molesta* (Busck) (Baker and Cardé, 1979) exhibited similar responses to temperature decreases. When the decreases in temperature occurred before the calling gate, calling began between 16.00 and 17.00 hr in day-2 and -3 *P. stultana*. This suggests that calling was not induced by the decrease in temperature itself, but rather calling initiation may result from an interaction of the current temperature and hour of photoperiod. In *G. molesta* the temperature level had a major effect on inducing calling whether the level was reached by an increase or decrease in temperature (Baker and Cardé, 1979). Baker and Cardé (1979) suggested that a temperature decrease increases early calling by lifting inhibition rather than by inducement. Higher temperatures may suppress calling by delaying its expression to later in the photoperiod. A temperature decrease removes calling suppression, allowing expression of calling rhythm earlier in the photoperiod.

In _P_. _stultana_ a shift in temperature from 24° to 14° preceding $\bar{x}$ onset of calling at 24° and $\bar{x}$ end of calling at 14° elicited calling within 10 min. If the shift from 24° to 14° followed $\bar{x}$ end of calling at 14°, those females still calling terminated calling within 0.5 hr of the shift. However, regardless of when the shift from 24° to 14° came, if the change occurred prior to $\bar{x}$ end of calling at 14°, calling ended at about the same time (between 21.00 and 22.00 hr). This suggests that in _P_. _stultana_ the termination of calling results from an interaction of the current temperature and hour of photoperiod, rather than the decrease in temperature alone. It appears that the current temperature and photoperiod modulate calling by delimiting a specific time interval or gate for the calling period. At a given temperature the time of onset and end of calling are specified, and thus at each temperature a different time interval or gate for calling is specified. Should the temperature increase (or decrease) calling will begin, end, or continue depending on the gate delimited by the current temperature. In _Synanthedon_ _pictipes_ (Grote & Robinson) and _G_. _molesta_ there are upper and lower temperature limits above or below which calling will not occur (Gorsuch _et_ _al_, 1975; Baker and Cardé, _et_ _al_., 1979). Thus, the absolute limits of how far the calling period will be advanced into photophase by low temperatures or delayed into scotophase by high temperatures will probably be partially governed by the upper and lower temperature thresholds for calling in P. stultana.

Decreases in temperature prior to the calling gate at 14° of day-2 and -3 moths elicited short, initial calling bouts (0.5-1.0 hr in duration) in some individuals. The calling bouts began shortly (6-20 min, $\bar{x} = 13$ min) after the lower temperature was attained. These individuals resumed calling near the onset of the calling gate at 14°. When the decrease in temperature came near the commencement of the calling gate, most individuals called continuously for several hours. The initiation of the short calling bouts does not appear to be a simple nervous response to a decrease in temperature because calling did not begin until after a lower temperature (14°) was attained. Furthermore, the proportion of these initial calling bouts varied with time of day, suggesting a changing threshold for calling. The threshold for calling may be related to the presence or level of an endogenous factor(s) (probably a hormone), in addition to current temperature and photoperiod. Furthermore, the level of this factor required to elicit calling may be different from that required to maintain calling. In Antheraea polyphemus (Cramer) the intrinsic neurosecretory cells of the corpora cardiaca appear to secrete a hormone in response to a nervous signal from the brain, that elicits calling. Blood from calling females induced calling in non-calling individuals. This hormone presumably acts on the female nervous system to trigger calling behaviour (Riddiford, 1974). In P. stultana release of the factor may be regulated by photoperiod and temperature. Temperature may determine, in part, the gate for the release of this factor.

The onset of calling periods of P. stultana advanced with age. The mechanism underlying this shift is not clear, but may be related to the shift from 18.00 to 16.00 hr of the calling gates and the increase in the proportion of moths that exhibited initial calling bouts from day 1 to 3. In other Lepidoptera the onset of calling or mating have been shown to advance with age (Hirano and Muramoto, 1976; Swier et al., 1977; Kanno, 1979). Kanno (1979) suggested that by calling earlier, older females increase their chance of mating by being the first to attract males. In P. stultana maximum pheromone titre occurred on day 2, then declined. Maximal proportion of mating also occurred with day-2 moths. In a field test using traps baited with virgin females, day-2 females were most attractive, followed by day-3, -1, and -4 females; day-6 females elicited little trap catch (AliNiazee and Stafford, 1971). Any unmated individuals older than 4 days might therefore be at a competitive disadvantage and would have to call earlier to compete with younger moths. The greater propensity of older moths (day-3) to exhibit initial calling bouts after a decrease in temperature may also be important in their ability to compete with younger individuals.

Mating usually occurred within 0.5 hr of onset of calling in P. stultana. Individuals that did not mate during first hour of calling did not mate that night. This suggests that pheromone release rate may be highest immediately after initiation of calling. Indeed, a rapid decline in titre ensued shortly after calling onset. In Trichoplusia ni (Hübner) pheromone release rate declined exponentially after onset of calling (Bjostad et al., 1980), probably due to evaporation from the gland surface (Sower et al., 1972). However, until actual emission is studied in P. stultana, the precise relationship between rhythmicity of female calling, pheromone titre, and emission cannot be established definitively.

In P. stultana lower temperatures advance the onset of calling. A decrease in temperature elicits calling within several minutes if it occurs within the gate for the lower temperature. These females should be capable of emitting pheromone at an appropriate rate any time within the gate. Therefore, the quantity of available pheromone should be at a high level throughout the gate. Indeed, maximal quantity of E11-14:Ac was reached on day 2 and 3 at *ca*. 15.00 hr, which was close to the onset of the calling gate at 14°. In *P. stultana* pheromone titre appears to reflect a time-dependent readiness to call if temperature is decreased.

Pheromone production appears to be rhythmic in *P. stultana*. Each day, once calling commenced, pheromone titre dropped abruptly. When calling ended (*ca*. 01.50 hr), little change in titre occurred until after 09.00 hr, then titre increased until 15.00 or 18.00 hr, prior to the initiation of calling at 24° (*c.a*. 22.00 hr). Although pheromone production appears to be rhythmic, it may be difficult to establish that it is regulated by an endogenous oscillator, because continuous light and dark conditions do not suppress calling behaviour in *P. stultana*. Few species of Lepidoptera have been shown to exhibit daily rhythmicity of pheromone titre. A daily rhythm of titre was not found in *T. ni* (Shorey and Gaston, 1965; Sower *et al*., 1972). Whole pheromone gland content (626 ng/♀) and surface content (48 ng) remained relatively constant throughout a 24 hr period (Sower *et al*., 1972). *T. ni* calls in a series of bouts ($\bar{x} = 20$ min) during scotophase and appears to rely on biosynthesis and a large reservoir of pheromone within the gland to replace that lost to evaporation from the gland surface (Sower *et al*., 1972; Bjostad *et al*., 1980). In *Plodia interpunctella* (Hübner) and *Ephestia cautella* (Walker) the rhythm of pheromone titre from surface washes of glands was synchronous with calling rhythm, titre being highest during calling (Coffelt _et_ _al_., 1978). Whole gland content was not examined. In _P_. _stultana_ all pheromone was extracted from the gland within 15 sec. This species appears to rely on biosynthesis prior to and possibly during calling to replace pheromone lost during previous calling bouts. However, it would be necessary to determine gland content before and after a release period to examine critically the relationship between amount contained and amount released.

# CHAPTER 2

## The Effect of Mating, Juvenile Hormone, and a Juvenile Hormone Analogue on Pheromone Titre, Calling, and Oviposition

# INTRODUCTION

Evidence establishing the role of endocrine factors regulating pheromone production in moths is fragmentary and circumstantial (Cardé and Webster, 1981). In tissue culture juvenile hormone (JH) was essential for maintaining the integrity of the pheromone gland cells of *Diatraea saccharalis* (F.) (White *et al*., 1972). However, JH was not necessary in tissue culture for pheromone production in *Plodia interpunctella* (Hübner) (Srinivasan *et al*., 1979), which suggests that pheromone production is not under direct nervous control by the brain. Most studies with Lepidoptera have centered on endocrine regulation of reproductive behaviours, such as calling, mating, and oviposition (Riddiford, 1974; Riddiford and Ashenhurst, 1973; Riddiford and Williams, 1971; Truman and Riddiford, 1971). In some insect groups such as bark beetles (scolytidae) and cockroaches, endocrine regulation of pheromone production and reproductive behaviours is well established (Barth, 1961; Barth and Lester, 1973; Borden *et al*., 1969; Hughes and Renwick, 1977; Truman and Riddiford, 1974).

Brady and Smithwick (1968), Hagan and Brady (1981), and Shorey *et al*. (1968) have shown that pheromone production is unaffected by mating in several moths in which females are polygamous. However, little is known of the effect of mating on pheromone titre in lepidopterous species such as *Platynota stultana* (Walsingham) and *Hyalophora cecropia* (L.), which mate only once and terminate calling after mating (Truman and Riddiford, 1971; see Chapter 1). The cyclical changes in pheromone titre in P. stultana suggest neural or neuroendocrine regulation of pheromone production (see Chapter 1). The effect of mating on pheromone titre and the possible endocrine regulation of pheromone production and reproductive behaviour in P. stultana are reported here.

## MATERIALS AND METHODS

### A. Insect Culture

P. stultana from Parlier, California was maintained on a pinto bean diet (Shorey and Hale, 1965) at 24°C, 75% relative humidity and a 16:8 light:dark regime. Photophase and scotophase light intensities were 2100 and 0.3 lux, respectively. Pupae were segregated by sex and adults were held in separate cages according to date of emergence and sex. Experiments were conducted at 24°C, 75% relative humidity and 16:8 light:dark regime.

### B. Gas-Liquid Chromatographic Analyses

Gas-liquid chromatographic (GLC) analyses were conducted with three 2 mm internal diameter glass columns: a 3.5 m long column with 10% XF-1150 on Chromosorb-W, a 3.5 m long column with 20% OV-275 on Gas Chrom-RZ, and a 1.8 m long column with 3% OV-1 on Gas Chrom-Q.

### C. Pheromone Titre Measurements

The sex pheromone of P. stultana consists of an 88:12 blend of (E) and (Z)-11-tetradecenyl acetate (E and Z11-14:Ac) (Hill and Roelofs, 1975). No differences in ratios of pheromone components were found in female tip extracts at different times of day or on different days (see page 2). Therefore, only the E11-14:Ac pheromone component was quantified. There were no differences between the ratios of E and Z11-14:Ac from female extract as determined on XF-1150 and OV-275.

Analyses on OV-1 showed no corresponding alcohols (<0.1%) to complicate analysis of E11-14:Ac on OV-275 or XF-1150. Extractable pheromone was removed within 10-15 sec (see page 3). An internal standard, (_Z_, _E_)-7, 11-hexadecadinyl acetate (10 ng/µl) was added to all samples which then were stored at -10° until GLC analysis. Determinations of pheromone titre were of the E11-14:Ac component obtained from 15 sec abdominal tip washes of samples of 1, 2, or 5 females analyzed by GLC on OV-275 or XF-1150.

## D. Behavioural Observations

All moths, unless otherwise specified, were observed and maintained individually in 4 cm x 4 cm (35 ml) clear plastic cups with plastic-lined cardboard lids. During scotophase, moths were observed using a light with a Kodak Wratten[R] filter No. 19 which eliminates light below 600 nm. The moths appeared to be insensitive to the light. Photoperiod light intensities were identical to those used in rearing.

1. _Calling_. Females were scored as calling if the pheromone gland was visible and extruded or if the female assumed the typical calling posture: wings elevated, legs extended, and abdomen raised above substrate.

2. _Mating_. Male-female pairs of day-2 moths (emerged on day-1) were placed in 2.5 cm x 8 cm (40 ml) clear plastic vials with cotton stoppers 6 hr prior to scotophase. Beginning at 18.00 hr (24 hr decimal clock), 2 hr prior to onset of scotophase, individuals _in copula_ and calling were recorded at 0.5 hr intervals to 03.00 hr.

## E. Operations

1. _Decapitation_. Adult females were immobilized on ice and decapitated using a fine pair of iris scissors. In day-2 and older

moths no bleeding ensued after removal of the head. As some bleeding occurred in newly emerged moths (0-3 hrs after eclosion), the wound was sealed with paraffin. The moths lived 6-10 days after decapitation.

2. Transection of ventral nerve cord. One to 2 day-old pupae were anesthetized under $CO_2$ and the ventral nerve cord was severed between abdominal segments 7 and 8 using a microscalpel. The wound was sealed with paraffin. For sham-operated insects, a similar incision was made on the right lateral portion of abdomen between abdominal segments 7 and 8. Mortality of operated- and sham-operated pupae was *ca*. 10%. Pheromone titre was determined prior to onset of calling at 21.00 hr for individual day-2 moths. Other similarily operated moths were observed at 22.00, 22.50, and 23.00 hr for calling.

3. Topical applications. JH I, II, and III (Sigma Chemical Co., St. Louis, Missouri, USA) and the Juvenile hormone analogue (JHA), ZR-512 (Zoecon Corp., Palo Alto, CA, USA) were dissolved in acetone and applied topically in 1 µl acetone. The compounds were applied to the venter of abdominal segment 5 with a microapplicator after the moths were immobilized on ice.

## RESULTS

### A. Effect of Mating on Pheromone Titre and Calling

Most females mated within 0.5 hr after initiation of calling. The majority of moths were mated by 22.00 hr and no individuals mated after 24.00 hr (Figure 10, top). Moths remained *in copula* 1 to 1.5 hr. Females did not call after mating.

Changes in pheromone titre were determined after mating and in virgin females (Figure 10, bottom). Pheromone titre was determined in

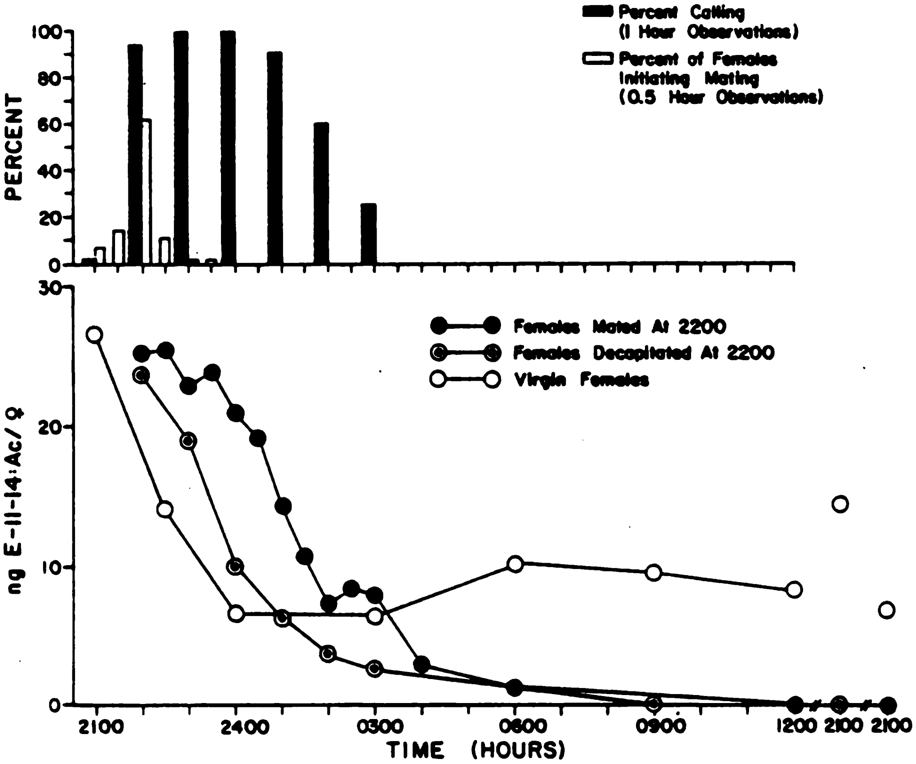


Figure 10. (Top) Calling and mating periodicity of day-2 females of *Platynota stultana* (N = 100). (Bottom) Titre of E11-14:Ac pheromone component obtained from 15 sec abdominal tip washes of day-2 females mated at 22.00 hr, decapitated at 22.00 hr, or virgin. Each point is $\bar{x}$ titre obtained from 6 samples of 5 females.

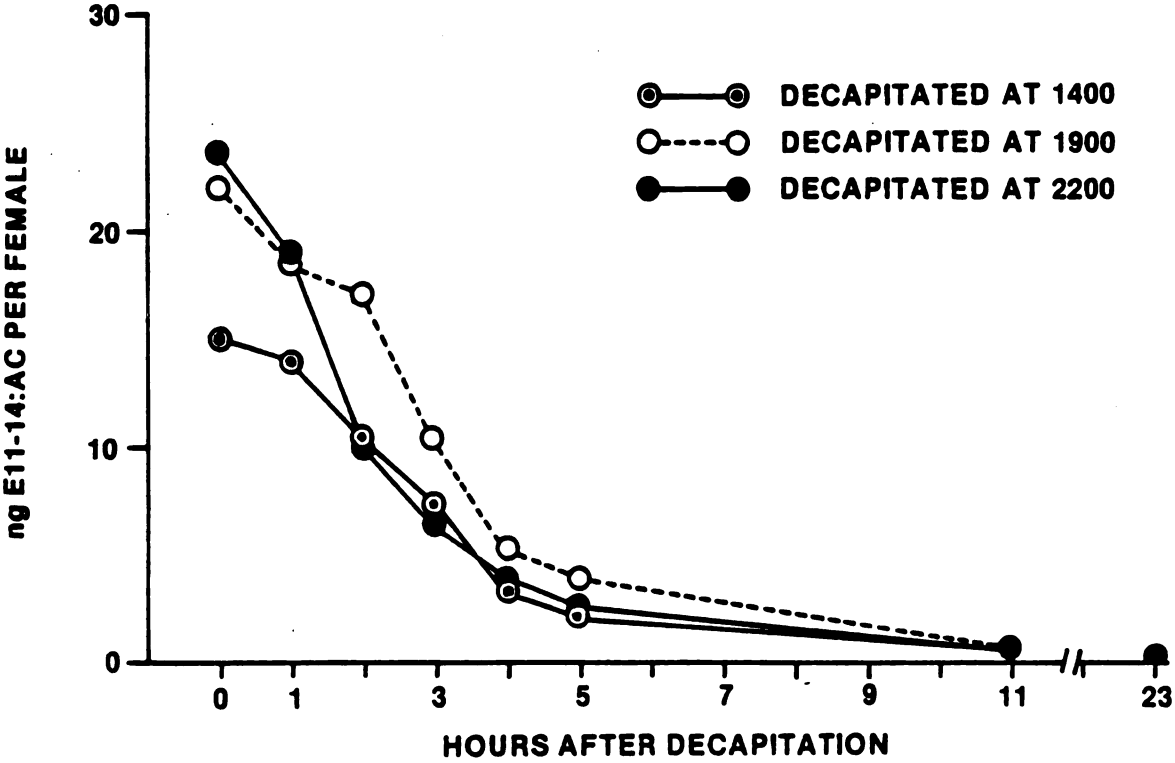
individuals that were in copula at 22.00 hr. Pheromone titre decreased from 22.2 ng/♀ at 22.00 hr to 7.3 ng/♀ at 02.00 hr. Only 1.3 ng/♀ was present at 06.00 hr and no detectable quantities (<0.2 ng/♀) of pheromone were present 14 and 47 hr after mating. In virgin females titre decreased from 27.0 ng/♀ at 21.00 hr to 6.8 ng/♀ at 24.00 hr and then remained near this level until 12.00 hr (8.3 ng/♀). The decrease in pheromone titre in virgin females was probably, in part, the result of evaporation from the gland surface (see page 25) while the decline in mated females may be the result of enzymatic degradation of pheromone because these individuals did not call.

## B. Effect of Decapitation on Pheromone Titre and Calling

Virgin females were decapitated and subsequent changes in pheromone titre were determined to see if a factor(s) from the head influences pheromone titre (Figures 10 and 11). After decapitation pheromone titre declined to <5.0 ng/♀ in 5 hr and <0.5 ng/♀ in 11 hr, regardless of the time of decapitation. No decapitated individuals called. The decrease in pheromone titre was very similar to the decline after mating (Figure 10). This finding is suggestive of enzymatic degradation of pheromone and of neural, neuro-humoral, or neuro-hormonal regulation of pheromone production.

## C. Effect of Severing Ventral Nerve Cord on Pheromone Titre and Calling

Severing the ventral nerve cord during the pupal stage did not appear to affect either pheromone titre or calling behaviour of adult P. stultana. There were no significant differences (at the 5% level, Student-Newman-Keuls' multiple range test) in $\bar{x}$ titre between control (16.1 ± 11.1 SD ng/♀), $CO_2$-anesthetized (26.5 ± 11.5 ng/♀), sham-operated (20.0 ± 10.4 ng/♀), or operated (10.8 ± 5.7 ng/♀) groups of



<u>Figure 11</u>. Titre of E11-14:Ac pheromone component obtained from 15 sec abdominal tip washes of day-2 females decapitated at 14.00, 19.00, and 22.00 hr. Each point is $\bar{x}$ titre obtained from 6 samples of 5 females.

moths (N = 8). All individuals of the control, $CO_2$-anesthetized, sham-operated, and operated groups of moths called. These findings suggest that pheromone production is not under direct nervous control by the brain.

## D. Effect of Exogenous JHA on Pheromone Titre in Decapitated Females

Because JH is required for pheromone production in several insects (Borden *et al*., 1969; Hughes and Renwick, 1977; Barth and Lester, 1973; Truman and Riddiford, 1974), the influence of the JHA, ZR-512 on pheromone titre of decapitated virgin females was investigated. A 10 μg dose of topically applied JHA had no influence on the decline of pheromone titre in decapitated moths (Table 4). Pheromone disappeared from the glands of decapitated moths when JHA was applied 0 or 12 hr after decapitation. JHA did not maintain or restore pheromone in decapitated females.

## E. Effect of Exogenous JHA, JH I, II, and III on Behaviour and Pheromone Titre of Intact Moths

1. *Effect of JHA on mated and virgin females*. For JH or a JHA to be effective in maintaining or restoring pheromone titre it may be necessary for the moth to be intact. JHA was applied topically to females 1 to 10 min after uncoupling. JHA did not maintain or increase pheromone titre in mated females (Table 5). There were no significant differences in titre 24 hr after treatment between JHA-treated and untreated virgin females, or between JHA-treated and untreated mated females.

2. *Effect of JHA on oviposition by virgin females*. Mated *P. stultana* lay the first batch of eggs (50-200) 20-24 hr after copulation, usually close to the onset of scotophase, and lay a total of *ca*. 300

Table 4. Effect of exogenous JHA (ZR-512) on titre of the E11-14:Ac pheromone component of virgin decapitated females. Day-1 and -2 moths decapitated at 09.00 and 21.00 hr, respectively. Acetone and JHA were topically applied 0.0 or 12.0 hr after decapitation. Each value is $\bar{x}$ titre obtained from 10 samples of 2 females taken 24 hr after topical application. For each column, means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| | ng E11-14:Ac/♀ | | |
|---|---|---|---|
| Treatment | Day-1<br>0 hr | Day-2<br>0 hr | Day-2<br>12 hr |
| Unoperated | 15.1 b | 7.2 b | 9.2 b |
| Decapitated | 0.0 a | 0.0 a | 0.0 a |
| Decapitated + Acetone | 0.0 a | 0.0 a | 0.0 a |
| Decapitated + 10 µg JHA | 0.0 a | 0.0 a | 0.0 a |

Table 5. Effect of exogenous JHA on titre of the E11-14:Ac pheromone component and oviposition by virgin and mated females. Topical applications were made between 11.00 and 11.50 hr to day-2 moths. Each value is $\bar{x}$ titre obtained from 10 individual females taken 24 hr after topical application. For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| Treatment | ng E11-14:Ac/♀ | $\bar{x}$ No. eggs/♀ |
|---|---|---|
| Virgin | 9.7 a | 1.5 a |
| Virgin + 10 µg JHA | 9.2 a | 29.4 b |
| Mated | 0.1 b | 107.0 c |
| Mated + Acetone | 0.0 b | 104.0 c |
| Mated + 10 µg JHA | 0.2 b | 93.9 c |

eggs. Similar values were reported by Atkins *et al*. (1957). Virgin females normally do not oviposit more than 5-10 eggs during the first 4 days after emergence and <70 eggs by 8 days after emergence (Table 6). However, within 20 to 24 hr after topical application of 10 μg of JHA virgin females laid 29.4 eggs/♀ (Table 5).

Exogenously applied JHA was effective in inducing oviposition in virgin females at doses of 0.1 to 100 μg (Table 6). A 0.1 μg dose was only slightly less active than doses of 1.0, 10, or 100 μg in inducing oviposition in virgin females, but the higher doses induced oviposition more rapidly. The total number of eggs laid was similar at the doses tested and comparable to the number normally laid by mated females. Most eggs were laid within 48 hr after treatment with JHA. Untreated and acetone-treated moths laid significantly fewer eggs with most oviposition occurring 3 to 6 days after treatment.

3. *Effect of JH I, II, and III and JHA on oviposition*. The activity of exogenously applied JH I, II, and III and JHA in inducing oviposition and their effect on calling in virgin females was compared (Table 7). The JHA was as effective as JH I, II, and III in inducing oviposition in virgin females (at the dose tested). The total number of eggs laid was similar in the JH I, II, and III and JhA treatments. However, JH I caused earlier oviposition than JH II and III and JHA. JH I-treated moths laid the greatest number of eggs by day 4. As with mated moths, most oviposition occurred 20-24 hr after treatment, close to the beginning of scotophase. JH I, II, III and JHA reduced the proportion calling from ≥ 73.3% on day-3 to 0.0-13.3% on day 4, 48 hr after treatment (Table 7). Prior to day 4 most individuals (>70%) called in all treatments.

Table 6. Effect of different doses of exogenous JHA on $\bar{x}$ number of eggs laid by virgin females. Topical applications were made between 21.00 and 22.00 hr to day-2 females. Eggs were counted at 22.00 hr, 1, 2, 3, 4, and 6 days after treatment (N = 10). For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| | $\bar{x}$ No. Eggs/♀ | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Day 1 | Day 2 | Day 3 | Day 4 | Day 6 | Total |
| µg JHA | | | | | | |
| $10^{-1}$ | 26.6 b | 94.2 b | 123.4 | 4.6 | 54.0 | 302.8 b |
| $10^{0}$ | 52.4 c | 149.3 bc | 113.5 | 26.7 | 49.4 | 391.3 b |
| $10^{1}$ | 57.7 c | 166.2 c | 83.3 | 13.2 | 8.3 | 325.7 b |
| $10^{2}$ | 71.5 c | 104.1 bc | 54.6 | 27.0 | 12.7 | 269.9 b |
| Acetone | 1.5 a | 12.9 a | 64.4 | 17.5 | 22.0 | 118.3 a |
| Control | 4.7 a | 2.0 a | 15.3 | 10.9 | 31.1 | 64.0 a |

Table 7. Effect of exogenous JH I, II, III and JHA on oviposition and calling of virgin females. Topical applications were made between 19.00 and 20.00 hr to day-2 moths. Calling was observed at 22.00, 23.00, and 24.00 hr. Eggs were counted at 09.00 hr (N = 15). For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| Treatment | $\bar{x}$ No. Eggs/♀ | | | | Percent Calling | | |
|---|---|---|---|---|---|---|---|
| (10 µg) | Day 3 | Day 4 | Day 5 | Total | Day 2 | Day 3 | Day 4 |
| JH I | 0.0 | 140.3d | 25.6a | 165.9b | 73.3 | 80.0 | 6.7 |
| JH II | 0.0 | 69.6bc | 43.9ab | 113.5b | 93.3 | 73.3 | 0.0 |
| JH III | 0.0 | 48.5b | 81.9b | 130.4b | 86.7 | 86.7 | 13.3 |
| JHA | 0.0 | 101.1c | 67.1b | 168.2b | 100.0 | 73.3 | 13.3 |
| Acetone | 0.0 | 1.0a | 13.2a | 14.2a | 86.7 | 93.3 | 80.0 |
| Control | 0.0 | 2.7a | 13.6a | 16.3a | 93.3 | 100.0 | 93.3 |

4. Effect of JHA on pheromone titre. The influence of the JH I, II, III and JHA on behaviour was most pronounced 48 hr after treatment. Therefore, changes in titre after treatment with JHA were examined after longer intervals. Exogenous treatment of JHA significantly reduced pheromone titre in virgin females treated on day 1 and 2 (Tables 8 and 9). Pheromone titre was 3.1 and 1.0 ng/♀ 60 and 84 hr after treatment of day-1 moths, respectively (Table 8). This compares with 9.2 and 5.2 ng/♀ amongst controls. Females treated on day 2 exhibited a reduction in titre in response to exogenous JHA similar to day-1 treated moths (Table 9).

The quantity of pheromone present at 36 hr after treatment (15.3 ng/♀) in moths treated on day 1 was comparable to the amount (ca. 13 ng/♀) found at the end of the first calling period of untreated day-1 moths (see page 8). In moths treated on day 2 the quantity of pheromone present in the gland 24 hr after treatment (5.4 ng/♀) was comparable to that normally present at the end of the previous calling period of untreated moths on day 2 (9.1 ng/♀). Because the moths treated on day 1 and 2 had one calling period before the above samples were taken (Table 7), pheromone loss may have been due to evaporation from the gland surface during calling rather than to degradation. Therefore, exogenous JHA may interfere with biosynthesis of new pheromone. At 72 hr after treatment, pheromone titre had not significantly decreased from the quantity present at 48 hr. Because few individuals called 48 hr after treatment with JHA (Table 7), little new pheromone was likely synthesized. As in previous experiments, JHA elicited oviposition in virgin females (Tables 8 and 9).

Table 8. Effect of exogenous JHA on titre of the E11-14:Ac pheromone component and oviposition by day-1 treated virgin females. Topical applications were made between 10.00 and 11.00 hr to day-1 (0-3 hr-old moths). Samples and eggs counted at 21.00 hr (prior to onset of calling) 36, 60, and 84 hr after topical applications (N = 10). For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| | ng E11-14:Ac/♀ | | | $\bar{x}$ No. Eggs/♀ | | |
|---|---|---|---|---|---|---|
| Treatment | 36 hr | 60 hr | 84 hr | 36 hr | 60 hr | 84 hr |
| Control | 25.2b | 9.2b | 5.2b | 0.3a | 8.6a | 11.0a |
| Acetone | 21.6ab | 11.2b | 4.7b | 0.0a | 5.3a | 20.0a |
| 10 μg JHA | 15.3a | 3.1a | 1.0a | 43.0b | 229.4b | 241.0b |

Table 9. Effect of exogenous JHA on titre of the E11-14:Ac pheromone component and oviposition by day-2-treated virgin females. Topical applications were made between 10.00 and 11.00 hr to day-2 moths. Samples taken at 21.00 hr (prior to onset of calling) 24, 48, and 72 hr after topical applications (N = 10). For each column means followed by the same letter are not significantly different at the 5% level according to Student-Newman-Keuls' multiple range test.

| | ng E11-14:Ac/♀ | | | $\bar{x}$ No. Eggs/♀ |
|---|---|---|---|---|
| Treatment | 24 hr | 48 hr | 72 hr | 72 hr |
| Control | 15.7a | 6.8a | 4.5a | 27.7a |
| Acetone | 11.0b | 6.2a | 4.5a | 36.1a |
| 10 μg JHA | 5.4c | 0.3b | 0.7b | 324.8b |

# DISCUSSION

Pheromone production in *P. stultana* does not appear to be under direct nervous control by the brain. Cutting the ventral nerve cord during the pupal stage did not prevent pheromone production. In other insects, such as bark beetles (Borden *et al.*, 1969; Hughes and Renwick, 1977) and cockroaches (Barth, 1961; Barth and Lester, 1973; Truman and Riddiford, 1974) JH is necessary for pheromone production. Exogenously applied JH III induced pheromone synthesis in *Ips paraconfusus* (Borden *et al.*, 1969). Hughes and Renwick (1977) suggested that gut extension after feeding in *I. paraconfusus* removed neural inhibition of JH production by the corpora allata (C.A.) and then the JH mediated brain hormone release which in turn stimulated pheromone production. In several species of cockroaches cyclic changes in levels of JH released by the C.A. influences onset of sexual behaviour and pheromone production (Barth and Lester, 1973; Truman and Riddiford, 1974). However, in the silkmoths *Antheraea polyphemus* (Cramer) and *H. cecropia*, the C.A. had no apparent function in calling and oviposition behaviour (Riddiford and Williams, 1971; Truman and Riddiford, 1971). In *D. saccharalis* JH was required in tissue culture to prevent cellular disintegration (White *et al.*, 1972). In *P. interpunctella* pheromone production in tissue culture occurred without the presence of JH (Srinivasan, *et al.*, 1979). In *P. stultana* exogenously applied JHA interfered with, rather than stimulated pheromone production and is probably not involved in new pheromone production.

Mating in *P. stultana* resulted in the termination of calling and in the gradual elimination of pheromone from the pheromone glands (presumably by enzymatic degradation). In species that mate only once, such as *P. stultana*, maintenance of pheromone and calling behaviour after mating is not essential and thus, pheromone degradation and termination of calling was not unexpected. However, in species that mate more than once, production of pheromone and calling after mating is essential. In *Trichoplusia ni* (Hübner) and several species of Noctuidae, and *P. interpunctella* (Pyralidae) which typically mate more than once, pheromone titre was unaffected by mating (Shorey, 1963; Shorey *et al.*, 1968; Brady and Smithwick, 1968; Hagan and Brady, 1981). Mating caused a significant reduction in calling in *T. ni* and *P. interpunctella*, but did not eliminate calling entirely (Brady and Smithwick, 1968; Hagan and Brady, 1981).

Decapitation resulted in elimination of pheromone in *P. stultana*, presumably by enzymatic degradation. Because cutting the ventral nerve cord had no effect on pheromone biosynthesis, pheromone loss must not be under direct nervous control by the brain. It appears that a factor originating from the head which inhibits pheromone loss must be present to maintain pheromone in the gland. After mating (or decapitation) this factor is either eliminated or its activity is inhibited, allowing pheromone to be lost or degraded.

In *P. stultana* and *H. cecropia* oviposition occurs shortly (within 24 hr) after mating and normally does not occur in virgin females. In *H. cecropia* the switch to mated behaviour is triggered by reception of sperm (Truman and Riddiford, 1971). Females mated to castrated males exhibited an oviposition pattern similar to that of virgin females. It was not determined if the sperm alone could elicit normal mated behaviour, because the accessory glands were not removed. In _P. interpunctella_ and _T. ni_ viable sperm and male accessory gland secretion was required for maximal oviposition (Karpenko and North, 1973; Lum and Brady, 1973; and Lum and Flaherty, 1970). In _H. cecropia_ the sperm (and possibly the accessory gland secretion) cause the bursa copulatrix to release a humoral factor that in turn elicits oviposition and terminates calling (Riddiford and Ashenhurst, 1973). However, the switch from virgin to mated behaviour occurs only if the cc-ca complex is present and connected to the brain (Truman and Riddiford, 1971). The bursa factor probably acts through the central nervous system rather than directly on the corpora cardiaca and the C.A. does not appear to play a role in calling and oviposition in _H. cecropia_ (Riddiford and Williams, 1971; Truman and Riddiford, 1971).

In _H. cecropia_ JH is sequestered from circulating hemolymph, and also synthesized _de novo_ in and stored by the accessory sex glands (Shirk _et al._, 1980). During mating, JH along with other seminal material, is transferred to the bursa copulatrix of the female. Shirk _et al._ (1980) did not demonstrate a function for the transferred JH. In _P. stultana_, JH I, II, and III and JHA induced oviposition behaviour comparable to that observed in mated females, and terminated calling within 48 hr. Oviposition was induced only in intact moths, and thus the exogenous JH likely acts through the central nervous system to elicit oviposition. JH may be involved in the switch from virgin to mated behaviour in _P. stultana_. In light of the present results, it is possible that exogenous JH transferred in the seminal fluid may be involved in the switch from virgin to mated behaviour. However, confirmation of this role must await further studies on the effects of exogenous JH on reproductive behaviour in *H. cecropia* and studies of possible JH transfer from male to female *P. stultana* during mating.

# LIST OF REFERENCES

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# APPENDIX 1.1

## Voucher Specimen Data

Page 1 of 1 Pages

| | | |
|---|---|---|
| | Museum where deposited | Michigan State University |
| | Other | |
| Number of: | Adults ♂ | 5 |
| | Adults ♀ | 5 |
| | Pupae | |
| | Nymphs | |
| | Larvae | |
| | Eggs | |

| Species or other taxon | Label data for specimens collected or used and deposited |
|---|---|
| *Platynota stultana* (Walsingham) | Laboratory culture obtained from Parlier, CA |

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Reginald P. Webster

Date October 8, 1981

Voucher No. 1981- 2

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

Date