# Inhibition of pheromone biosynthesis and mating in the stored product moth, *Plodia interpunctella*

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*Abstract*

Moths depend on chemical communication for mating. In most cases it is the female that produces a blend of chemicals which are attractive to conspecific males. The process of sexual communication can be regarded as 5 stages in which interference could lead to the disruption of mating behavior.

(1) The production of a blend of specific chemicals typical for the species making it possible for the males to locate the female
(2) The timely production and release of the sex-pheromone blend during active periods of the day, an event which is under endogenous control in the female
(3) The perception of this blend by the sensitive sensory mechanism of the male
(4) The receptivity level of the female to mating so that copulation may proceed.
(5) After mating the female moth becomes non-receptive, production of pheromone is terminated and ovipositional behavior is initiated

Our extensive investigations, concerning the timely production, release and termination of phermomone biosynthesis of *Plodia interpunctella*, showed that octopamine can significantly inhibit pheromone production. Negative regulation of pheromonotropic activity occurs at the membrane receptor level by the interaction of an aminergic ( octopaminergic ) receptor with a pheromonotropic receptor. In addition, studies were conducted concerning the termination of pheromone production after mating A significant negative correlation was observed between the duration time of coitus and pheromone titres after mating. *P interpunctella* females stop calling immediately after separation of mating pairs. Pheromone titres drop significantly during the following hours in the scotophase and during the second night only 30% of the females will mate During the following nights mating is still suppressed. Research into the mechanisms underlying these processes will enlighten the possible pathways for the disruption and manipulation of sexual behavior and thereby allow the development of novel strategies for moth control.

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## Introduction

*Plodia interpunctella* females, as most other species of moths, produce sex pheromones to attract conspecific males. The major pheromone component of *P. interpunctella* was identified as Z,E 9,12 tetradecadienyl acetate (ZETA) (Kuwahara et al., 1971; Brady et al., 1971). Rhythmicity of pheromone biosynthesis was shown to be under the control of a neuropeptide (Pheromone Biosynthesis Activating Neuropeptide, PBAN) (Rafaeli & Gileadi, 1995a) In most species of moths, successful mating results in the termination of pheromone biosynthesis and/or a decrease in receptivity (Truman & Riddiford, 1974; Webster & Carde, 1984; Raina 1989; Foster, 1993; Raina et al., 1994; Kingan et al , 1995; Ramaswamy et al , 1996; Ando et al., 1996) These studies suggested that inhibitory factors, inhibiting either the action or the release of PBAN, may be introduced during copulation resulting in the reduced pheromone titres and/or receptivity. In some species the loss of receptivity following mating is only temporary whereas in others it is permanent

It has been suggested that mating may suppress pheromone titers and/or receptivity by either physical or humoral mechanisms The presence of sperm or spermatophore's in the females's reproductive system may stimulate stretch receptors which exert a neuronal pheromonostatic effect on the pheromone gland (Giebultowicz et al , 1990) or the transfer of a factor by the male may exerts a humoral pheromonostatic response in the female (Kingan et al., 1995). A pheromonostatic peptide has been identified in the noctuid moth, *Helicoverpa zea*, from the accessory sex glands of males (Kingan et al , 1995). However it should be noted that a synthetic form of this peptide has not been successfully shown to inhibit pheromone production as yet. This pheromonostatic effect however was dependent on an intact ventral nerve cord thus involving neural centers in the inhibitory response (Kingan et al , 1995). The involvement of neural centers have also been implicated in the Tortricid mated female moth, *Epiphyas postvittana* (Foster, 1993) however, in another

tortıcıd moth, *Planotortrix octo* (Foster and Roelofs, 1994) a humoral factor was ımplıcated. In *Helıcoverpa armigera* we have shown that octopamıne, whıch may act as neurotransmıtter as well as neurohormone, ınhıbıts pheromone productıon when ınjected to ıntact ınsects or applied *in vitro* to pheromone glands (Rafaelı & Gileadı, 1995b, 1996; Rafaeli et al., 1997). Thus, ın the few species studied dıfferent mechanısms seem to be ımplicated in the mechanısm of pheromonostasıs.

A sound understandıng of the factors affectıng normal mating behavior of an ınsect ıs essential to optımıze the practical application of semiochemicals for luring the ınsects to traps and/or for disruptıng theır matıng. The present study was desıgned to investigate matıng behavıor and suppression of pheromone biosynthesıs ın the stored product moth *Plodia interpunctella*.

## Materials & Methods

### Insect culture

*P. interpunctella* were reared on a dıet contaınıng 80% ground wheat, 10% glycerın 5% brewer's yeast and 5% honey in a 14·10 L·D regıme at 28 − 30℃ and 70% r. h. Wandering stage larvae were allowed to pupate ın separate glass vıals plugged with cottonwool. Emerged adults were sexed and aged and maintained ın ısolatıon for calling and mating behavioral studıes.

### Behavioral observations: calling, mating and receptivity

Behavıoral studıes were carrıed out ın a culture room under the 14 10 L D photoperıodıc regime. Durıng the scotophase hours observations were carrıed out usıng a dım red light. Daıly callıng behavıor was noted durıng the photophase and scotophase hours and the number of females wıth extruded glands were recorded using 5 − 10 groups of 4 − 5 females/group. Mating behavior at dıfferent tımes of the photoperıod was observed after the introductıon of naive, 1 day old males into the glass vials contaınıng vırgın females of varıous ages. The tıme of coıtus and separatıon of pairs was recorded. Mated females were frozen ın groups of 3 − 4 at −80℃, at varıous tımes after matıng, for subsequent pheromone gland extractıon. Matıng was verıfıed postmortem by the presence of a spermatophore. Each female group was identıfıed so that the time of coitus and separatıon could be correlated to pheromone tıtres. For each mated female group frozen for subsequent pheromone extraction a virgın female group was also frozen to be used as a control for pheromone tıtre determinatıons of vırgın females under the same condıtıons. Receptivıty was examıned by repeating the above mating experiments usıng once-mated females.

### Determinationf of pheromone titers

Ovıpositor tıps were removed and extracted for 15 mın ın 10μl hexane contaınıng 25ng tıdecanyl acetate as ınternal standard The extracts were separated on a Tracor gas chromatograph wıth a splıtless ınjector, and a flame ıonızation detector on a 30m DB225 fused silica capıllary column (ı. d. 0.25mm) (Alltech, USA) usıng the Schımadzu Chromatography Computer Program to calculate tıtres according to the ınternal standard method. The followıng condıtıons were used: Inıtıal temperatures of 120℃ were held for 4 mın then ıncreased to 270℃ at 10℃/mın and held at 250℃ for 5 mın The detector temperature was held at 300℃ and column inlet at 280℃. Nıtrogen was used as carrier gas at flow pressure of 22 psı. Each extract contaıned a pool of 3 − 4 ovipositor tips and values were normalızed to sıngle female equıvalents

### Pheromone gland cultures *in vitro*

*De novo* pheromone productıon was monitored using dıssected ovıposıtor tips as previously reported for *H. armigera* (Soroker and Rafaelı, 1989). Ovipositor tips were removed from one-day old females during the 10th hour of the photophase. They were transected along the dorsal sıde and the ınternal tissues were removed and cleared. The resultant tıssue was incubated ın physıologıcal salıne contaınıng 0.25μCı [1-14C]-acetate (NEN, USA) ın the presence or absence of Hez-PBAN (Peninsula Lab., USA) and octopamıne (Sıgma, USA) After 3 hours the glands were extracted ın hexane and the amount of radıoactıvity was measured usıng a lıquıd scıntıllation counter (Kontron) as reported previously (Soroker and Rafaelı, 1989; Rafaeli et al., 1990; Rafaelı et al., 1997).

### Statistics

Statıstıcal analysıs was performed usıng a Statview 4.01 package on a Macintosh computer usıng Students T-test, analysis of varıance and sımple regression.

## Results & Discussion

### Diel periodicity of calling behavior and pheromone production in virgin females

Newly emerged virgin females begın callıng behavıor one hour before the onset of scotophase and call throughout the scotophase (Fıg 1). Older females (2 − 4 day old) commence callıng behavıor earlıer (5 hours before the onset of scotophase). Pheromone tıtres peak at the onset of scotophase at all ages but reach maxımum levels ın 2-day old females (Fig 1). Sımilar age effects have been reported for other species (West et al., 1984; Howlander & Gerber, 1986) although not in all moths (Swier et al., 1977; Turgeon & McNeıl, 1982; Kou & Chow, 1987). This

phenomenon has been regarded by some workers as an adaptive process: Older females may increase their probability of being located by males by calling earlier and for longer periods. However, their pheromone titres are lower as they grow older. This does indicate that separate endogenous control mechanisms for calling behavior and pheromone production exist and therefore there may also be a separate mechanism for the suppression of these two independent processes.

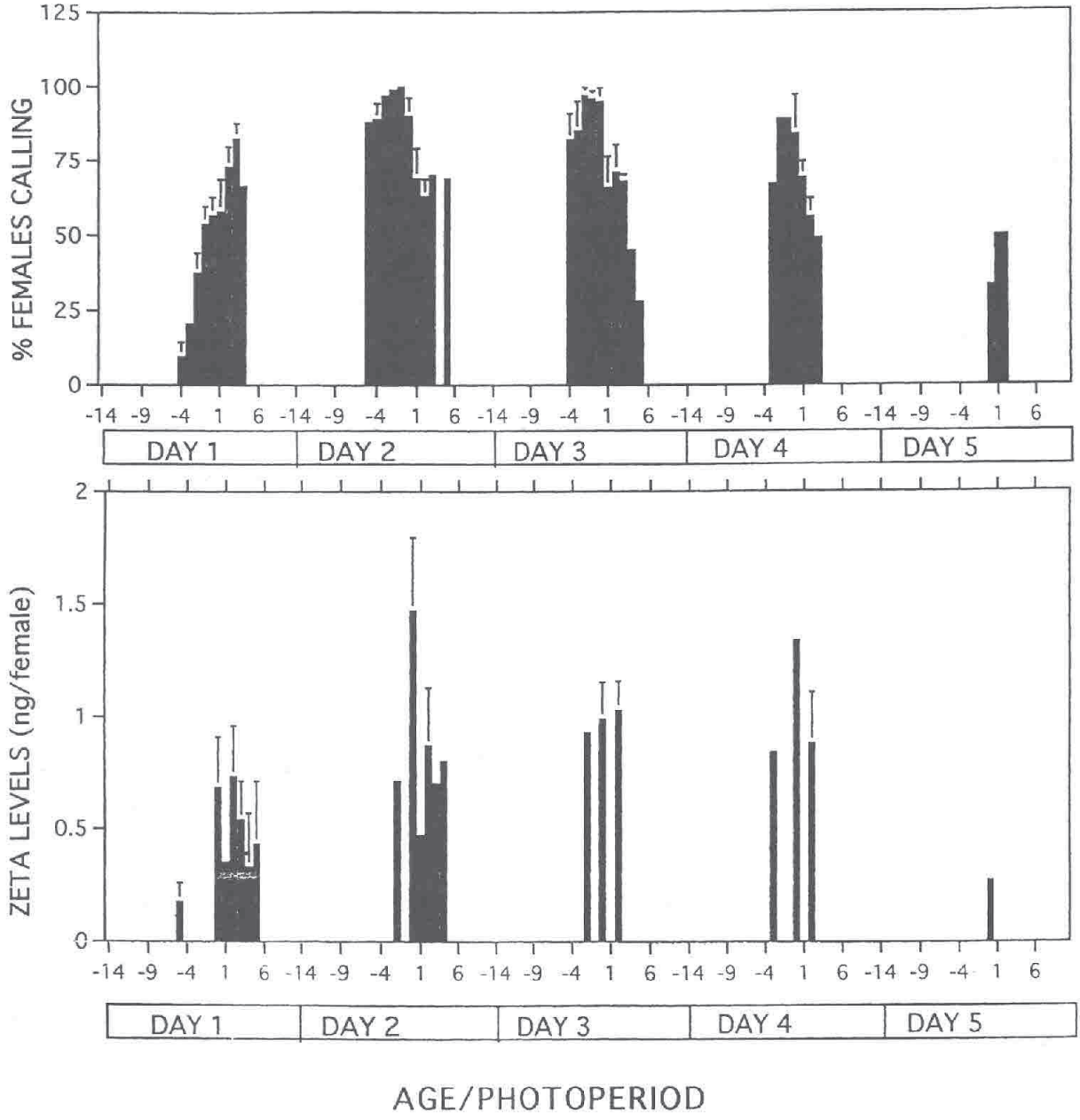


**Fig. 1.** Diel periodicity of calling behavior (top) and ZETA titres (below). Histograms represent the means ± SEM of at least 10 observations and 5 titre determinations respectively.

## Pheromonostatic effect of octopamine

Pheromonotropic activity was tested by using isolated ovipositor tips in physiological saline as described in the methods section. PBAN induced a significant increase in pheromone biosynthesis but in the presence of the aminergic neuromodulator/neurotransmitter, octopamine, a significant inhibition of pheromone production was observed (Table 1). These results corroborate our findings on octopaminergic inhibition of pheromone biosynthesis in another moth species

(Rafaeli and Gileadi, 1995b). Octopamine has been implicated in many insect specific physiological systems as a neuromodulator and/or neurotransmitter. Several aminergic receptor types and subtypes have been described (Evans, 1981) thus ascribing specific functions to these compounds In our previous extensive studies concerning the signal transductory mechanisms as a result of octopamine we have concluded (Rafaeli and Gileadi, 1995b; 1996) that octopamine inhibits the production of intracellular cAMP, an action which is diffrent from the octopamine receptor Type II responsible for the stimulation of intracellular cAMP levels. We are presently screening several aminergic analogs in order to characterize the specific binding characteristics of this novel octopaminergic receptor and develop specific mimics for this function.

**Table 1.** Effect of octopamine on pheromone production by pheromone gland cultures *in vitro*.

| Treatment | *De novo* pheromone production (cpm/pheromone gland) |
|---|---|
| CONTROL (saline alone) | 181.4±29 |
| PBAN(0.5pmol/10μl) | 784.8±155.6 |
| PBAN(0.5pmol/10μl + OCTOPAMINE($10^{-3}$M) | 272.2±47.6 |

## Effect of mating on pheromone production and calling behavior

Significant differences in pheromone titres of mated females were observed when mating occurred during the hours of peak pheromone production (Table 2).

**Table 2.** Effect of mating on pheromone titres of day 1 females.

| Photoperiod (hours in scotophase) | ZETA titres (ng/female) (means ± SEM[1]) | |
|---|---|---|
| | Virgin | Mated |
| 0 | 0.117±0.061 | 0.182±0 093 |
| 1 | 0.35±0 155 | 0.046±0 027*[2] |
| 2 | 0.731±0.234 | 0.221±0.129* |
| 3 | 0.538±0.172 | 0.196±0.075* |
| 4 | 0.271±0.204 | 0.361±0.361 |
| 5 | 0.425±0.247 | 0.399±0.096 |

[1]at least 5 replicates of pooled ovipositor tip-extracts(3 – 4 female equivalents) for each determination

[2]*denotes a significant difference from virgin female titres ($p < 0.05$)

*P. interpunctella* females stop calling immediately after separation of mating pairs (Table 3). Pheromone titres drop significantly 2 hours after mating and remain low during the following hours of the scotophase (Table 3). Observations of once mated females during the following nights showed that phermone titres remain significantly lower than virgin females and less than 20% females call Re-matings occur in only 30% of the mated females (Table 3).

**Table 3.** Effect of mating on calling behavior, pheromone titres and female receptivity

| Time after mating (hours) | % females calling (means ± SEM[1]) | ZETA titres (ng/female) (means ± SEM[2]) | % females receptive (means ± SEM[1]) |
|---|---|---|---|
| 0 | 90±6.2 | 0.87±0.26 | 70.7±0.5 |
| 2 | 0±0 | 0.088±0.001 | 0±0 |
| 24 | 10±6.7 | 0.4±0.046 | 31.5±6.1 |
| 48 | 17.6±3.6 | 0.44±0.136 | 17.6±0.6 |
| 72 | 2.8±0.5 | 0±0 | 5.9±4.2 |

[1] at least 5 replicates of group observations (4 – 5 females/group)

[2] at least 5 replicates of pooled ovipositor tip-extracts (3 – 4 female equivalents) for each determination

## Correlation between time in coitus and pheromone levels

A significant negative correlation was observed between the duration time of coitus and pheromone titres after mating (Fig. 2) No correlation was observed between the photoperiod at which mating occurred and the subsequent suppression of pheromone titres (data not shown). This significant correlation may indicate that suppression may be due to the transfer of a pheromonostatic factor from the male to the female during coitus. The longer the period the more likely is the success for a strong and permanent suppression of pheromone biosynthesis. We are presently characterizing this mating factor. Further studies will extend out knowledge and enable the synthesis of active analogs which may be used for permanent suppression of the reproductive potential of the pest species.

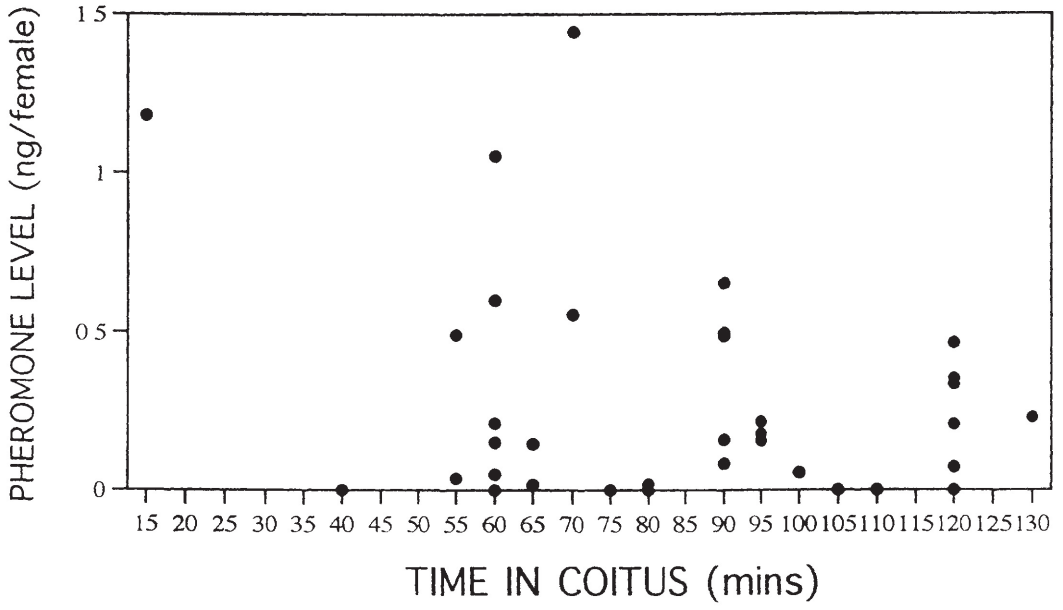


**Fig.2.** Negative correlation between time in-coitus and remaining pheromone levels in extracts of mated female ovipositor tips (Simple regression analysis: $R = 0.51$; $p = 0.05$).

Research into the mechanisms underlying both these inhibitory processes (octopaminergic and male-transfer) will enlighten the possible pathways for the disruption and manipulation of sexual behavior and thereby allow the development of novel strategies for moth control which may be integrated into an IPM program

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