

## Calling behavior of the female carob moth, *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) under cycling and constant temperatures in laboratory

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**Abstract:** The calling behavior of virgin carob moth females, *Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae) was evaluated under two different thermal regimes, cycling and constant temperatures under laboratory condition. The period of calling activity under cycling temperature was prolonged and the total period of calling activity of an individual female was increased. Although moths maintained under cycling temperature started calling significantly later for the first time in the scotophase than those maintained at constant temperature of 25 °C, the calling activity at cycling temperature was more than at 25 °C. At two temperature regimes, the mean onset time of calling (MOTC) advanced from about 441 to 189.5<sup>th</sup> min after the onset of the scotophase, and the mean time spent calling (MTSC) increased by > 120 min during eight days. MTSC and MOTC for cycling thermal regime were 96.8 ± 6.5 and 275.9 ± 9.3 min respectively. We assume that the extension of the calling period under cycling temperature might be due to potential similarities of the temperature regime to natural thermal fluctuations which could lead to an increased proportion of females mated; apart from its importance in efficacy of sex pheromone extraction under laboratory conditions.

**Keywords:** calling behaviour, carob moth, cycling temperature

### Introduction

The *Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae), is one of the key pests of pomegranates and it inflicts great damage to this crop annually. It is of concern to growers because few insecticides are available for its control (Vetter *et al.*, 1997). Fortunately, chemical control of the pest has not become a widespread strategy in pomegranates, and now nonchemical methods are being recommended. Use of insect sex pheromone is one of the probable control methods that motivates both management experts and farmers nowadays.

Synthetic sex pheromone in the Lepidoptera has been widely used for monitoring, timing of spray and controlling methods (e.g. lure & kill, mass trapping or mating disruption) (Eiras, 2000 and references therein). Females of every moth species use chemical communication for searching mate, release their sex pheromone in a distinct time window and calling occurs at any combination of time and temperature within a calling window (Webster, 1988). Taking into consideration the time of sexual activity in moths, it is known that great majority of females, release their sex pheromone and copulate during the scotophase. However, some species call during the light period, soon after dawn or late in the afternoon, with one species *Lymantria dispar* (L.) being active during the entire light period (Mozuraitis and Buda, 2006).

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The effect of temperature on the timing of pheromone release in moths depends on the time of the day in which a particular species is active. Contrary to diurnal species, lower temperatures stimulate calling in most of nocturnal species, when females are held under different constant temperature regimes since the time of emergence or experience the cycling temperatures at some time following emergence (McNeil, 1991; Delisle, 1992; West and Bowers, 1994; Mozuraitis and Buda, 2006). Indeed, to study the chemical structure of compounds which are used in male-female moth relationships, these chemicals should be collected during female calling periods. For such investigations, however, the diurnal rhythms of calling as well as the environmental conditions approving of such activities must be known. Some detailed information is available from the work of Vetter *et al.*, (1997) in United States, who studied the effect of age and photoperiod on calling behavior of the carob moth (as a date pest); indeed, we previously studied the effect of age, temperature and wind velocity on carob moth calling behavior as a pomegranate pest (Soofbaf *et al.*, 2007; Soufbaf *et al.*, 2009), and more recently Ziaaddini *et al.*, (2010) have shown the effect of geographical variation on the calling behavior of the carob moth in Iran. As demonstrated formerly, a species' pheromone communication system can vary among populations in different geographical areas apart from chemical variation (Noldus and Potting, 1990). Data obtained during our previous studies on calling of virgin *E. ceratoniae* females have revealed that pheromone release behaviour of this pyralid was more sustainable under constant 25 °C temperature regime compared with two other constant regimes including 20, and 30 °C and was qualitatively and quantitatively different under constant temperature regime compared with the natural conditions with fluctuating factors (Soofbaf *et al.*, 2007; Soufbaf *et al.*, 2009). Therefore, the aim of the current study was to investigate the pattern of pheromone release behaviour of virgin *E. ceratoniae* females under constant and cycling temperature regimes as well as to obtain information on temperature conditions optimal for calling activity. These information could be used in sex pheromone collection through laboratory trials

to collect pheromones from virgin females at proper times and under suitable thermal regime with more success. Pheromones could be practical in many areas of research in entomology via well-known pheromone strategies such as mating disruption, mass trapping, monitoring and lure and kill in IPM programs to facilitate the insect pest control approaches.

## Materials and Methods

### Insects

Moths were collected from pomegranate (*Punica granatum* L.) orchards located in Gherdefaramarz (29°48'–33°30' N, 52°45'–56°30' E) of Yazd province, Iran in 2006 and maintained on dry pistachio diet, local cultivar "Nokhodu" at  $29 \pm 1$  °C,  $75 \pm 5\%$  RH, under a photoperiod of 16L: 8 D h in a growth chamber in the entomology laboratory of Mohaghegh Ardabili University, Ardabil province, Iran. Moths were reared in a wooden cage (70 × 70 × 100 cm) and eggs were collected in white plastic containers (20cm diameter × 10cm height) in which females were released individually. Females were fed on 10% honey solution during either rearing in wooden cage or through oviposition period in containers.

### Laboratory experiments

In order to study on calling behavior, eggs were obtained from females, and after incubation period, larvae were transferred on pistachio and maintained in previously described condition in growth chamber. At least 3 days before eclosion, pupae were separated by sex and females were placed individually in transparent plastic containers (7 × 4 cm) (the top of containers was covered with gauze) and then held at  $29 \pm 1$  °C during pupal period. After emergence, virgin females were fed on 10% sugar solution and then they were separated in two groups. Each group of virgin females was placed in growth chambers at cycling and 25 °C during the 2<sup>nd</sup>–3<sup>rd</sup> hour of scotophase. Cycling temperature was set up manually in a germinator starting from 30 °C at the beginning of scotophase. Six changes were done in temperature during scotophase from 30 to 5 °C as five degrees of Celsius was decreased per each 3h. One temperature change was done at

the beginning of photophase (5 to 30 °C). In this study, observations were made from day of eclosion as the first scotophase. In all experiments females were observed at 15 min time intervals throughout the scotophase, using a torch covered with two layers of tissue paper and a red parafilm layer (Turgeon & McNeil, 1983). If a female was calling on two consecutive observations, she would be recorded as having calling for 30 min. If she was calling at one observation and not at the next, she would be recorded as having calling for 15 min. (Goldansaz and McNeil 2003).

### Statistical analysis

Multivariate general linear model was used to determine statistically significant difference between the duration of the calling period and the daily onset times of calling (in minutes after the beginning of the scotophase), under the effects of fixed set of factors including age and temperature at 0.05% probability level (SAS, 2005).

### Results

Virgin females extended their ovipositor behind their body by holding their abdomen vertically to show their calling posture (Fig. 1). The calling behavior of females followed similar temporal patterns at two examined temperatures (Figs. 2a, b), but the values of MTSC and MOTC for cycling temperature were significantly higher and occurred sooner (respectively) than in the constant temperature. In current experiments, carob moth's "calling" did not weaken with age and became intensive on successive days from the emergence to 8<sup>th</sup> day. Mean onset time of calling (Fig. 2b) significantly occurred earlier at successive days of calling. Mean time spent calling (Fig. 2a) significantly increased on successive days of calling, resulting more from an increase in the duration of individual calling bouts than from an increase in the number of bouts (data not shown).

### Calling behavior under constant temperature

At constant temperature, 25 °C, carob moth females started calling within 6 h of the beginning

of the scotophase, with a peak of activity recorded in 8<sup>th</sup> h after the light off, when 76.8% of the individuals were active. The calling window for the species was about 2 h per day (Fig. 3a). The total period of calling activity of an individual female lasted for  $77.4 \pm 3.5$  min per day.

### Calling behavior under the cycling temperature

The calling behavior of the virgin females was significantly different between the cycling and constant temperature regimes, with a peak of calling activity, occurring at the end of the scotophase (6<sup>th</sup> h) (Fig. 3b). The pheromone release started  $275.9 \pm 9.3$  min after the onset of the scotophase, i.e. much earlier than under the constant temperature conditions ( $405.2 \pm 2.9$  min) (Table 2). The total calling period of the individual female lasted  $96.8 \pm 6.5$  min per day that was significantly more than this value under the constant temperature regime ( $77.4 \pm 3.5$  min) (Table 2). Both age and temperature had significant effects on both evaluated calling parameters including MTSC and MOTC (Table 2). Nearly 57% of the females were active during the peak period of calling, and this proportion of the active females differed in comparison with the one observed during activity peak under constant temperature regime. Each female called 1.72 bouts on an average. Low activity, with 7–14% of the females calling was registered at the time 2.5 to 5<sup>th</sup> h from the beginning of the scotophase (Fig. 3b). Two hours before the end of the scotophase, the calling activity of the females started to increase once more (Fig. 3b). The peak of the calling activity was detected in 57% of the females while, observed calling activity had been continuing to the highest at constant temperature during the same period of photophase.

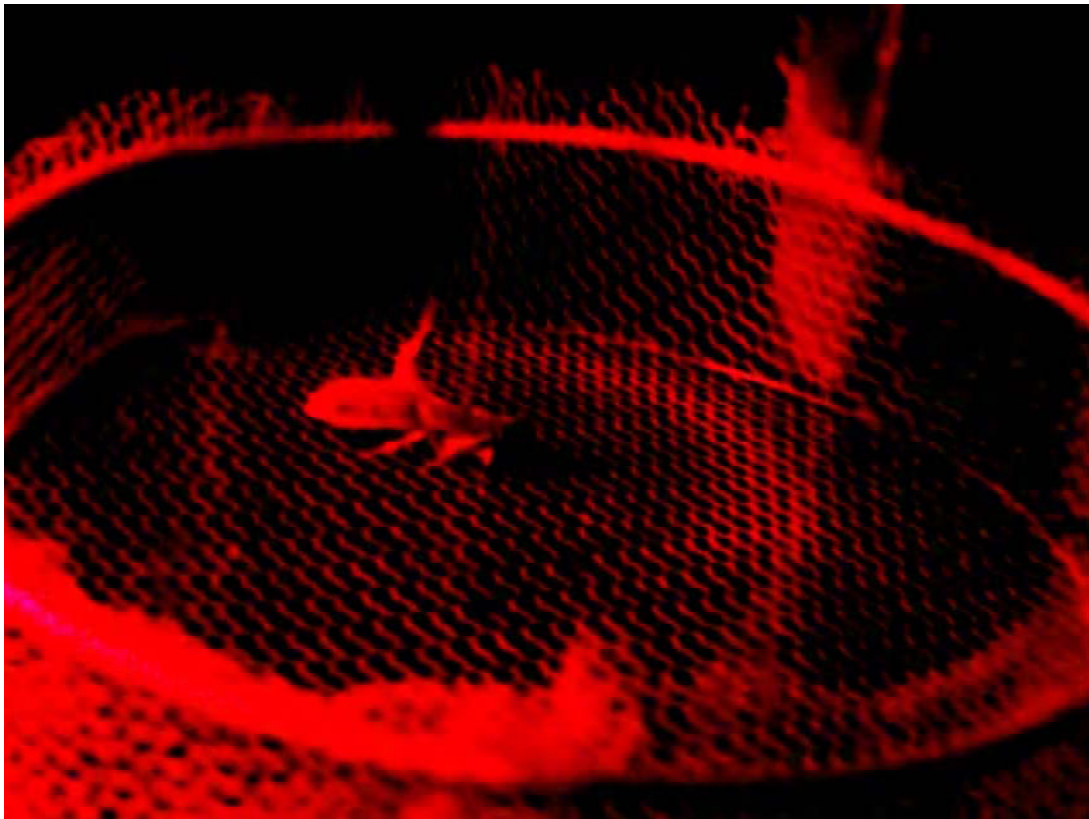
**Table 1** The mean onset time of calling (MOTC) and mean time spent calling (MTSC) by virgin *Ectomyelois ceratoniae* females as a function of calling age under different temperature conditions in the laboratory.

Temperature	n	MTSC (mean $\pm$ SE)	MOTC (mean $\pm$ SE)
Cycling	80	$96.8 \pm 6.5$	$275.9 \pm 9.3$
Constant (25 °C)	80	$77.4 \pm 3.5$	$405.2 \pm 2.9$

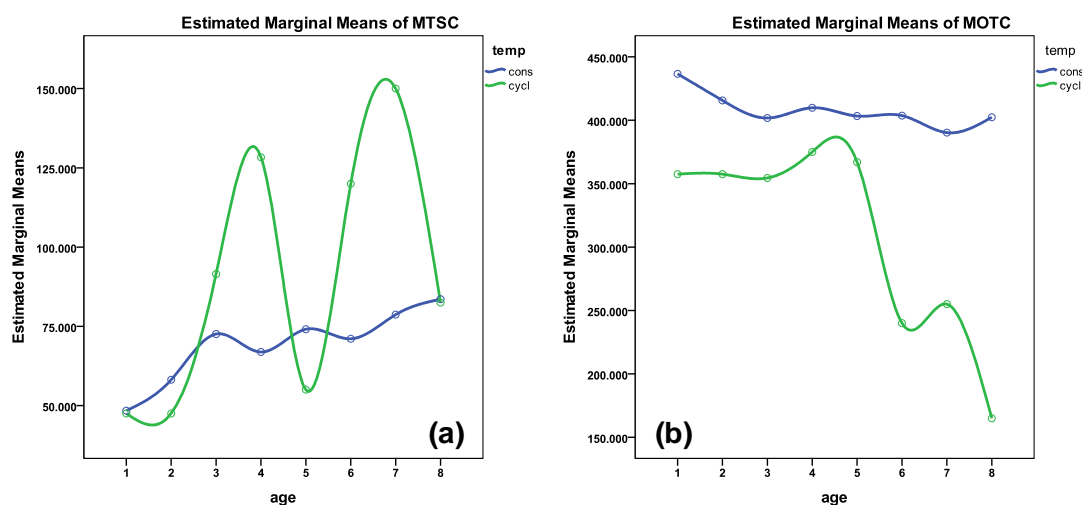
**Table 2** Analysis of the mean onset time of calling (MOTC) and mean time spent calling (MTSC) by virgin *Ectomyelois ceratoniae* females as a function of calling age under different temperature conditions in the laboratory. A multivariate general linear model approach was used to assay changes in MTSC and MOTC between temperatures with age of females.

Source	Dependent variable	Type III sum of square	df	F
Model	MTSC (model R <sup>2</sup> = 0.713)	16224.164	15	6.123***
	MOTC (model R <sup>2</sup> = 0.966)	105647.267	15	60.538***
Temperature	MTSC	2674.954	1	15.144**
	MOTC	58820.356	1	505.581***
Age	MTSC	11246.262	7	9.096**
	MOTC	38247.492	7	46.964***
Age × Temperature	MTSC	6392.853	7	5.170**
	MOTC	28073.682	7	34.472***
error	MTSC	2826.177	16	
	MOTC	1861.473	16	

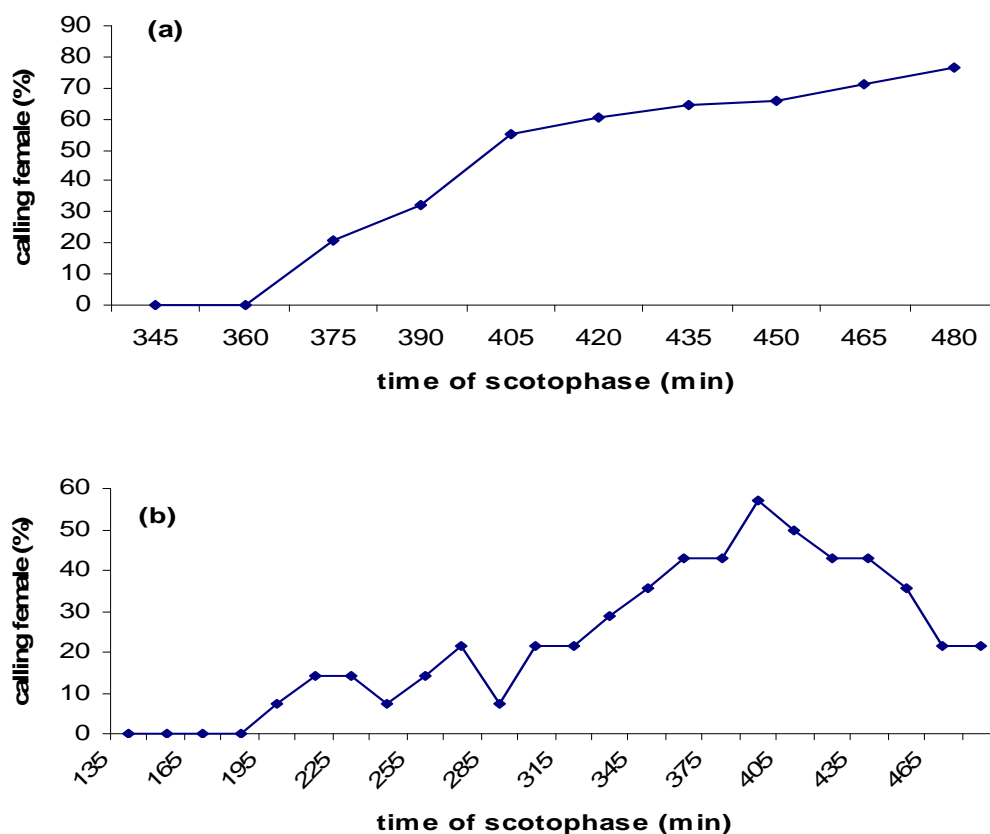
The only significant main effects and their interactions showed here. \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001.



**Figure 1** Calling posture of virgin *Ectomyelois ceratoniae* female under laboratory conditions (Photographer: M. Soufbaf, 2007).



**Figure 2** (a) Mean time spent calling (MTSC) (minutes) and (b) the mean onset time of calling (MOTC) (minutes after lights-off signal) of virgin *Ectomyelois ceratoniae* as a function of calling age at cycling regime (mean: 15 °C), and 25 °C. temp, cons and cycl are the abbreviations for temperature, constant and cyclic respectively.



**Figure 3** (a) The broad peak of calling activity of virgin *Ectomyelois ceratoniae* at constant temperature (25 °C) and (b) peaks of calling activity at cycling regime.

## Discussion

The pheromone release posture of carob moth females (Fig. 1) is very similar to that of other species, *Phyllonorycter junoniella* (Zeller) (Mozuraitis *et al.*, 2006), *Ph. ulmifoliella* (Hübner) (Mozuraitis *et al.*, 1997), *Ph. blancardella* (Fabricius) (Mozuraitis *et al.*, 1999), *Ph. Acerifoliella* (Zeller), and *Ph. heegeriella* (Zeller) (Mozuraitis *et al.*, 2000), *Ph. emberizaepenella* (Bouché) (Mozuraitis *et al.*, 2002) and also is similar to some pyralids, such as *Anagasta kuehniella* (Z.) (Traynier, 1968) and *Plodia interpunctella* (Hb.) (Brady and Smithwich, 1968). Delisle (1992) reported that females of species *Choristoneura rosaceana* (Harris) showed no significant difference in the mean time spent calling under a constant 20 °C temperature regime and under thermocycle (in the range from 12 to 25 °C), but the corresponding activity was shorter under colder periodically changing temperature programs (in the range of 9-17 °C) than under constant regimes at 15 °C. Under natural conditions, the calling periods of females of *Lambdina fiscellaria fiscellaria* (Guenée) were longer during cool nights with temperature ranges of 5-8 °C than during warmer nights with temperature ranges of 10-14 °C (West and Bowers, 1994). It is also known that the calling period under different constant temperature regimes is longer under cool regimes than under warm ones in *Argyrotaenia velutinana* (Walker), (Card'e *et al.*, 1975), *Platynota stultana* (Webster, 1988) and *Mamestra configurata* (Walker) (Gerber and Howlader, 1987), but another pattern is found in *Platyptilia carduidactyla* (Riley) (Haynes and Birch, 1984). Carob moth has been considered a crepuscular and nocturnal moth. Our data indicate that late night is a typical pheromone release period for carob moth, however there was only one peak of calling, suddenly declined to zero by appearance of the photophase, in contrast with some moth species, e.g. in the sesiids *Paranthrene tabaniformis kungesana* (Rott.) and *Synanthedon tipuliformis* (Cl.) (Mozuraitis

*et al.*, 2006). In these species, it has been suggested that the small peaks of calling activity were observed early in the morning under laboratory conditions, and these peaks are suppressed in natural conditions by low morning temperatures (Mozuraitis *et al.*, 2006). Our previous study showed that females stopped calling during transition period from darkness to light, indicating that light might be an important cue to stop calling (Soofbaf *et al.*, 2007; Soufbaf *et al.*, 2009). Webster (1988) demonstrated that temperature changes could induce a second peak of calling activity on the same day in *Platynota stultana* (Walsingham) females. He showed that this tortricid has a distinct time frame or gate for calling at each temperature within appropriate range and that a decrease or increase in temperature may or may not result in a second peak of calling activity on the same day, depending on the hour of the photoperiod that the change occurs. In our experiments only some small peaks were observed before major peak of calling (2 h before photophase) and this second peak of calling activity was not observed under cycling thermal regime; but two parameters inside calling behavior including MTSC and MOTC differed from other thermal regimes significantly. Our data showed that females started to call during darkness, indicating that darkness might be an important cue to initiate calling. Our results clearly indicate that patterns of calling in *E.ceratoniae* vary with age. The age at which females initiate calling for the first time is not temperature dependent in *E.ceratoniae* (Soofbaf *et al.*, 2007; Soufbaf *et al.*, 2009). This finding is in agreement with the patterns generally reported for Lepidoptera species (McNeil, 1991). Females at cycling temperature called more than those at constant temperatures. In other words the cycling temperature regime may be an appropriate thermal condition for release of pheromone by this species. However further experiments are needed to get more detailed data characterizing the stimulatory effect of lower temperature on calling of females and to which extent this behavior might be extended. In other

investigations, temperatures were increased or decreased at various times before or during a calling period in an effort to understand the pathways or mechanisms by which temperature changes can modify the calling process (including the duration of calling) and how such changes in temperature, depending on the time at which they occur, may extend or shorten the calling period (Baker and Cardé, 1979; Castrovillo and Cardé, 1979; Webster and Cardé, 1982; Delisle and McNeil, 1987; Gerber and Howlader, 1987; Mozuraitis *et al.*, 2006). In our experiments, carob moth "calling" did not weaken with age and became intensive on successive days, contrary to the patterns generally reported for most pyralids (Karalius and Buda, 1995). These changes, which result in an increasingly greater calling window over successive days of pheromone emission, have been reported in a large number of Lepidoptera (McNeil, 1991). Swier *et al.*, (1977) suggested that these changes may increase the probability of an ageing virgin female attracting a mate, when competing with younger con-specifics. This idea is supported by work on the oblique banded leafroller, *Choristoneura rosaceana*, in which younger females had higher pheromone titer (Delisle & Royer, 1994) and under field conditions attracted many more males than did older conspecifics (Delisle, 1992). After the results of field experiments (that males were captured during calling period each night) (data not shown), it can be concluded that calling behaviour and pheromone production of carob moth females are synchronous as have been reported for many moth species (Mazomenos *et al.*, 2002). In these species pheromone production occurs during the period when females are calling and releasing pheromone (Mazomenos, *et al.*, 2002). Most virgin females called for the first time during eclosion day. This is contrary to the patterns generally reported for many moth species (e.g. *Mamestra configurata*, Howlader and Gerber, 1986). To increase the efficiency of sex pheromone collection under laboratory conditions by solid phase micro extraction

method, which is usually carried out for about 3 h, it is suitable to prolong the total duration of the calling activity of the females by arranging cycling temperature conditions and maintaining low temperatures (about 16 °C) both during the scotophase and in the late part of 4<sup>th</sup> scotophase. A question, however, remains unanswered: are the titers of pheromone under different temperature regimes equal? The answer to this question can help us in making decision on the time (age of moth and time of day) of pheromone extraction under laboratory conditions.

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## فعالیت فراخوانی ماده باکره کرم گلوگاه انار تحت دماهای ثابت و چرخه‌ای در آزمایشگاه

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**چکیده:** فعالیت فراخوانی ماده‌های باکره کرم گلوگاه انار (*Ectomyelois ceratoniae* (Zeller) (Lep.: Pyralidae) تحت دو رژیم مختلف دمایی شامل دماهای ثابت و چرخه‌ای در شرایط آزمایشگاهی مورد مطالعه قرار گرفت. مدت فعالیت فراخوانی و کل زمان فعالیت فراخوانی یک ماده انفرادی در دمای چرخه‌ای افزایش نشان داد. اگرچه شب‌پره‌های نگهداری شده در دمای چرخه‌ای فراخوانی را برای اولین بار به‌طور معنی‌داری در زمان دیرتری در دوره تاریکی شروع کردند ولی مقدار این فعالیت در این دما نسبت به دمای ۲۵ درجه سلسیوس بیشتر بود. میانگین زمان شروع فراخوانی (MOTC) در دو رژیم دمایی از دقیقه ۴۴۱ به دقیقه ۱۸۹/۵ بعد از شروع دوره تاریکی پیشرفت نموده و میانگین مدت فراخوانی (MTSC) به مقداری بیش از ۱۲۰ دقیقه در طول ۸ روز افزایش یافت. MTSC و MOTC برای رژیم دمای چرخه‌ای به ترتیب  $96/8 \pm 6/5$  و  $275/9 \pm 9/3$  دقیقه بود. به نظر می‌رسد که افزایش فعالیت فراخوانی در دمای چرخه‌ای، صرف‌نظر از اهمیت آن در کارایی استخراج فرومون جنسی در شرایط آزمایشگاهی، ممکن است به دلیل شباهت‌های بالقوه موجود بین رژیم دمایی آزمایشی و نوسانات دمایی طبیعی باشد که می‌تواند باعث افزایش نسبت ماده‌های جفت‌گیری کرده شود.

**واژگان کلیدی:** رفتار فراخوانی، کرم خرنوب، دمای چرخه‌ای