CALLING BEHAVIOUR OF THE CAROB MOTH, *ECTOMYELOIS CERATONIAE* (ZELLER) (LEPIDOPTERA: PYRALIDAE), LABORATORY AND FIELD EXPERIMENTS

Mahmoud Soufbaf Sarjami*, Ghadir Nouri Ghanbalani, Hossein Goldansaz*** and Rasoul Asghari Zakaria******

* Plant Protection Dep., College of Agriculture, Mohaghegh Ardebili University, Ardebil, IRAN. mhsoofsar@yahoo.com
** Plant Protection Dep., College of Agriculture, Mohaghegh Ardebili University, Ardebil, IRAN.
*** Plant Protection Dep., Tehran University, Karaj, IRAN.
**** Plant Protection Dep., College of Agriculture, Mohaghegh Ardebili University, Ardebil, IRAN.

[**Sarjami, M. S., Ghanbalani, G. N., Goldansaz, H. & Zakaria, R. A.** 2009. Calling behaviour of the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), laboratory and field experiments. Munis Entomology & Zoology, 4 (2): 472-485]

ABSTRACT: The calling behaviour of virgin female of carob moth, *Ectomyelois ceratoniae* (Zeller) (Lep.:Pyralidae) was studied at three different constant temperatures under laboratory conditions. Most virgin females called during the first and fewer during second scotophase after emergence, regardless of the temperature. Moths maintained at 30°C started calling significantly later in the scotophase than those maintained at 20 or 25°C. At all temperature regimes, the mean onset time of calling (MOTC) advanced from about 453rd to 345th minute after the onset of the scotophase, and the mean time spent calling (MTSC) increased by >30 minutes over the 8 days. Cohorts of females were also observed at two different periods in middle summer-late summer (early August and early September 2006) in the field to examine the effect of fluctuating abiotic conditions (temperature, wind velocity) and age on calling behaviour. All females started calling from eclosion day on. Calling started from the 370 to 280 minutes of scotophase depending on the age of the females and the mean time spent calling (MTSC) increased by >100 minutes over the 8 days. Age-related changes in the mean onset time of calling and the mean time spent calling were much less evident under field conditions, due to the inhibitory effects of low temperatures and high winds on female calling activity.

KEY WORDS: calling behaviour, *Ectomyelois ceratoniae*, carob moth, and pomegranate.

The carob moth, *Ectomyelois ceratoniae* (Zeller) (Lep.:Pyralidae), is the key pest of many crops worldwide. It inflicts a great deal of damage to the crops annually and is of concern to growers as few insecticides are available for controlling this pest (Vetter et al. 1997). Fortunately chemical control of the pest has not practiced and currently nonchemical methods of control is used and recommended. One of the probable control methods that merit consideration is the use of insect sex pheromone. Synthetic sex pheromone in Lepidoptera has been extensively used for monitoring, timing spray and controlling methods (e.g. lure & kill, mass trapping or mating disruption) (Eiras, 2000 and references therein). Little information is available regarding behaviour of the carob moth (Vetter et al. 1997). The only detailed information on the calling behaviour of *Ectomyelois ceratoniae* (as a date pest) is available from the work of R.S.Vetter in United States, who studied the effect of age and photoperiod. However, a species' pheromone communication system can vary between populations in different geographical regions (Noldus and Potting, 1990). Apart from chemical variation, adaptation to different environmental conditions and host plants might also have led to behavioral variation between populations in different areas (Noldus and Potting, 1990). Therefore this research was conducted to appreciate the pheromone biology of the carob moth under three constant temperature conditions (20, 25, 30°C) in the laboratory and under variable field conditions.

METHODS AND MATERIALS

Insects

Moths were originally collected from pomegranate (*Punica granatum* L.) orchards in the Gherdefaramarz (29°48´-33°30´N, 52°45´-56°30´E) of Yazd province, Iran in 2006 and maintained on dry pistachios diet, local cultivar "Nokhodu" at 29±1°C, 75±5% RH, under a LD 16:8 photoperiod regime.

Laboratory Experiments

For the calling study, eggs were obtained from females, and after the incubation period, larvae were transferred to pistachios and maintained in the described rearing conditions in growth chambers. At least 3 days before eclosion, pupae were separated by sex and females were individually placed in cylindrical plastic transparent cages (12cm height × 6cm diameter) (the tops were covered with gauze) and then held at 29±1°C for the duration of pupal period. Upon emergence, virgin females provided with a 10% sugar solution and then were separated into three groups with one group set up in each temperature regime. Each group of virgin females was placed in growth chambers (20, 25, 30°C). 30, 55, and 45 virgin females were observed for all three replications for 20, 25, and 30°C, respectively in laboratory. Observations were made from eclosion day onwards as a first scotophase to the 8th scotophase. In the field experiments, 15 and 18 females were observed in early August and early September, respectively. In all experiments, females were observed each night at 15 min intervals all through 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 was recorded as having called for 30 min. If she was calling at one observation and not at the next, she was recorded as having called for 15 min. (Goldansaz and Mc Neil. 2003). The calling posture of the female carob moth is curling abdomen between wings.

Field Experiments

Two series of field experiments were conducted in pomegranate orchards of Gherdefaramarz district of Yazd province in early August and early September 2006, using females produced from the laboratory culture. Individual cages containing of virgin females were hanged out from pomegranate trees branches (15 and 17 cages for each experiment respectively). These cages were equipped with a rooflike cover as a sticky layer. This layer prevents:

1-Females to capture by predators (such as ants that attack resting females),

2-Direct radiation on cages (these were major mortality factors in our preliminary observations). Observations were made every 15 min 2h after sunset to 30 min after sunrise on each night for 8 days to determine the mean age of first calling, the mean onset time of calling and the mean time spent calling. Preliminary observations had shown that calling did not occur in first 2 h of scotophase and terminated in early morning. Temperature measurements beside the trees were taken coincident with each observation period and wind speed measurements were taken from Yazd Meteorology Center by 1 km distance from experiment plot.

Statistical Analysis

Analysis of variance, followed by a least significant different test, was used for the mean age of calling for the first time data in the laboratory experiments. The data for the mean onset time of calling and mean time spent calling from all experiments were subjected to ANOVA. In the field experiments the effects of wind speed and ambient temperature on the proportion of calling females throughout the night were examined using a multiple regression analysis on transformed (logit) data (JMP6 (SAS product, 2005)).

RESULTS

The mean age of calling for the first time was not affected by temperature under laboratory conditions, with females at 30°C calling for the first time at a not significantly older age than those at the lower temperatures(Fig.1). This parameter did not vary between two field tests and all females initiated calling from eclosion day at both early August and early September.

In our experiments *E. ceratoniae* ″calling″ did not weaken with age and became intensive on the 1-3rd day from the emergence and more intensive on 4-6th day and most intensive on 7-8th day (Fig.2a), in other word MTSC was significantly different between days 1-3, 4-6 and 7-8. The mean onset time of calling, MOTC (Fig.2b) occurred significantly earlier at consecutive days of calling and the significant temperature×age interaction resulted from the earlier onset of calling of females during 4th day and later onset on 7th day of calling (Table 1, Fig.2b). The mean time spent calling, MTSC (Fig.2a) increased significantly on consecutive days of calling. There was also a significant effect of temperature on the mean time spent calling (Table 1), with females at 25°C calling more than those at lower and upper temperatures (Fig.2a).

Females placed in the field in early August (mean daytime temperature: 26.24°C) called for the first time at the same age (0 day) that the other cohort started 29 days later, when the mean daily temperature was 23.79°C . All females initiated calling from eclosion day at both early August and early September. The age-related changes in the mean onset time of calling and the mean time spent calling seen in the laboratory (Fig.2) were less evident under the field conditions (Fig.3). This was due to marked day- to- day variations associated with changing climatic conditions, and is reflected by significant cohort×age interactions (Table 2). In both field experiments, the best regression models (in the proportion of females calling) included temperature, time of night and wind speed (Tables 3, 4).The relationship between wind and time of night present in early August (Table 3) and in early September (Table 4). There was a significant opposite relationship between calling and wind speed in both experiment (Table 3 and 4). For interpretation we require wind tunnel data that has not been studied or published by any authors to date. An examination of the temporal patterns of the proportion of females calling on specific days provided some insights into the manner in which different abiotic factors may influence calling behaviour. For example, the best part of the cohort called for ≈ 3 h on 9 August, when the air temperature was very steady and wind speeds decreased to ≈ 0 m/s in 5th hour of night. Mean and maximum wind speed was 0.335, 1.9 m/s respectively (Fig. 4a). In contrast, on 11 August, females called for only ≈ 45 minutes. At this time the mean and maximum wind speed was 1.6, 3.65 m/s respectively. On 11 August, majority of calling occurred during 345 to 390 min. after sunset and a significant high wind speed (mean of wind speed = 3.16 m/s) inhibited calling in the earlier minutes (240-330 min) of night (Fig. 4c). In other words, all calling behaviour was delayed until 330th minute of night, coinciding with a < 3.5 m/s wind speed, however calling resumed right away under the declined wind speed. In the models (Tables 3 and 4), time of night was a significant parameter. Clearly, there was a significant time^2×temperature interaction in early September (Table 4), but it is possible that this was due to the changes in some other abiotic factors that were not measured in this study. On 8th September an increase in wind speed from 0 to >2 m/s at late night (530-570 min.) influenced females, and all calling behaviour stopped at late night (Fig.5) contrary to patterns generally observed in other nights. No clear fluctuation in temperatures was seen in all nights in both field experiments, and an equal trend was seen, that is temperature decreased gradually to morning. Mean of temperature decrease in first experiment was 5.24°C and in second trial was 7°C. High wind speeds at earlier minutes of night on three successive days in September (9, 10, 11 Sep.) (Fig.6a, b, c) inhibited calling to start at 240-300 min. but on 12 September still air in this period (Mean wind speed = 0.2875 m/s) allowed calling window to transfer to earlier times (240th min. after sunset) (Fig. 6d) and to extend to almost 280 min. (The greater calling window on 6th night of calling).

Pattern of Calling Behaviour

The pattern of calling behaviour of virgin *E.ceratoniae* females was the same during the scotophase. Shorter periods of pheromone release behaviour (up to 90 min.) prevailed during the activity under laboratory conditions, but longer (up to 150 min.) periods were dominant during the activity under the natural conditions.

DISCUSSION

E. ceratoniae has been considered a crepuscular and nocturnal moth. Our data indicates that late night is a typical pheromone release period for the carob moth, however there was only one peak of calling, suddenly declined to zero by appearance the photophase, contrary 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 observed early in the morning, under laboratory conditions, and these peaks are concealed in nature by low morning temperatures (Mozuraitis et al., 2006). Also in our field experiments some natural conditions, especially low temperature at late night suppressed the calling window. Our data showed that females stopped calling during change period from darkness to light, indicating that light might be an important signal to stop 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*. This finding is in accord with the patterns generally reported for the Lepidoptera species (McNeil, 1991). Virgin females modified their calling patterns in response to temperature with some limitations. Females at 25ºC called more than those called at lower and upper temperatures. In other words, this temperature regime relatively may be an appropriate thermal condition for pheromone release by this species. In our experiments *E.ceratoniae* ″calling″ did not weaken with age and became intensive on successive days (Fig. 2a), contrary with the patterns generally reported for most pyralids (Karalius and Buda, 1995). Once pheromone emission has been initiated, both mean onset time of calling and mean time spent calling of *E.ceratoniae* changes as a function of age at all temperatures, although the differences are more pronounced under the constant laboratory conditions than under variable field conditions. These changes 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 rival with younger conspecifics. 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). From 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 *E.ceratoniae* females is synchronous that have been reported for many moth species (Mazomenos et al. 2002). In these species pheromone production occurs during the period where 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). Age and temperature are two of the many factors affecting the occurrence of calling behaviour of female insects (Howse et al. 1998).

There are limited research papers in which the calling behaviour of females under natural conditions is compared with the calling behaviour under constant temperatures. Females of species *Choristoneura rosaceana* 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 a cooler periodically changing temperature programmes (in the range of 9-17 °C) than under constant regimes at 15°C (Delisle, 1992).

Under natural conditions, the calling periods of females of *Lambdina fiscellaria fiscellaria* (Guenee) were longer during cooler nights with temperature ranges of 5-8°C than during warmer nights with temperature ranges of 10-14°C (West and Bowers, 1994). In our field experiments temperature differences between 8 nights were not so clear and great to change the calling patterns, but in a given night the calling periods were longer than a given scotophase in laboratory constant temperature regimes.

In the second field experiment, due to irrigation of the orchard (where first experiment had been done), cages placement and observations were limited to marginal trees and closer to orchard walls, therefore wind speed effect in these areas was less evident, probably due to less coverage of wind on areas in question. Further laboratory experimentation and field testing should take our result into account. It should be noted that to increase the efficacy 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 extend the total duration of the calling activity of the females by keeping temperatures about 25°C during the scotophase and in the final quarter of 8^{th} scotophase.

ACKNOWLEDGEMENTS

We thank the department of plant protection, university of Tehran for laboratory rearing assistance.

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Table 1. Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Ectomyelois ceratoniae* females as a function of calling age under different constant temperature conditions in the laboratory.

	effect	d.f.	*F*-value	*P*
Mean onset time of calling	Block	2	0.0446	-
	Temperature	2	5.5461	0.0702
	Age	7	10.8806	0.0000
	Temperature × age	14	3.1784	0.0045
Mean time spent calling	Block	2	3.9097	0.1145
	Temperature	2	20.0665	0.0082
	Age	7	6.4354	0.0001
	Temperature × age	14	2.6920	0.0125

Table 2. Analysis of variance of the mean onset time of calling and mean time spent calling by virgin *Ectomyelois ceratoniae* females as a function of calling age under field conditions at two different periods in summer 2006.

	effect	d.f.	*F*-value	*P*
Mean onset time of calling	Cohort	1	1.9907	0.1774
	Age	7	13.2064	0.0000
	Cohort × age	7	4.5256	0.0002
		1	0.5656	-
Mean time spent calling	Cohort	7	2.0594	0.0538
	Age	7	4.8896	0.0001
	Cohort × age			

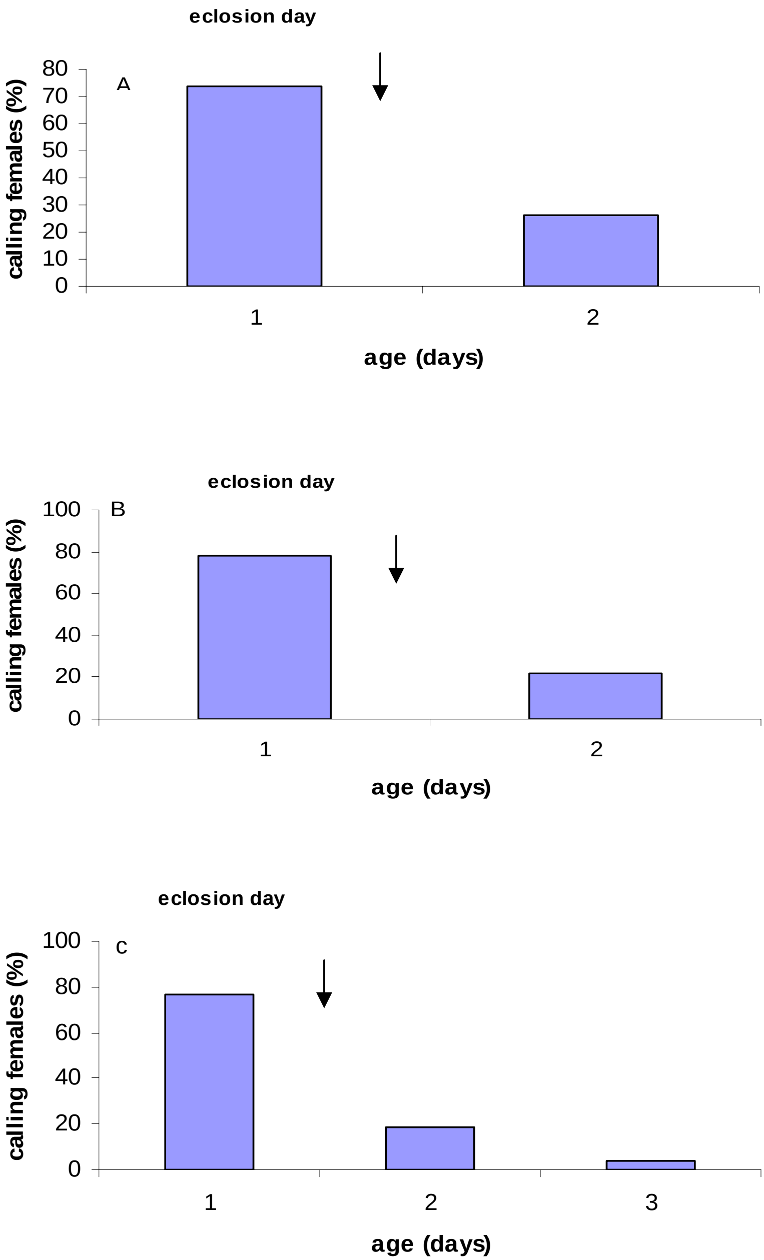


Fig. 1. The age (days after eclosion) at which virgin *Ectomyelois ceratoniae* called for the first time at three different constant temperature regimes: (A) 20 °C, (B) 25 ° C, and (C) 30 ° C.

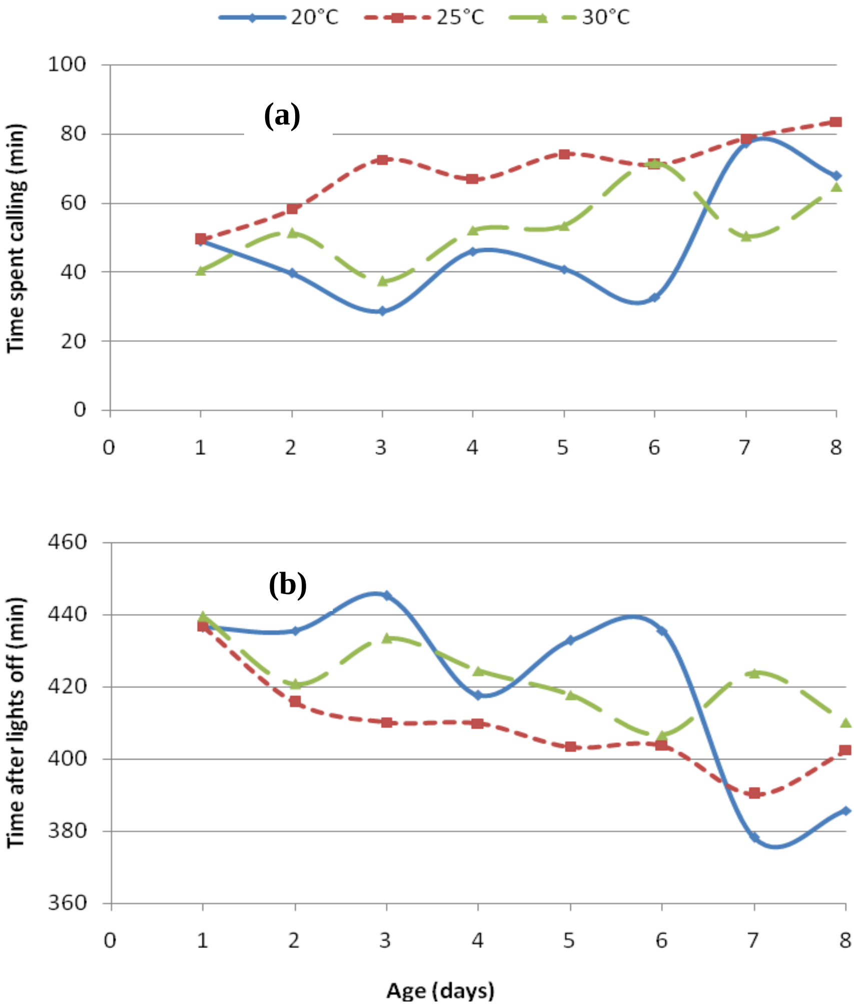


Fig.2. (a) Mean time spent calling (minutes) and (b) mean onset time of calling (minutes after lights-off signal) of virgin *E. ceratoniae* as a function of calling age at 20, 25, and 30 ° C.

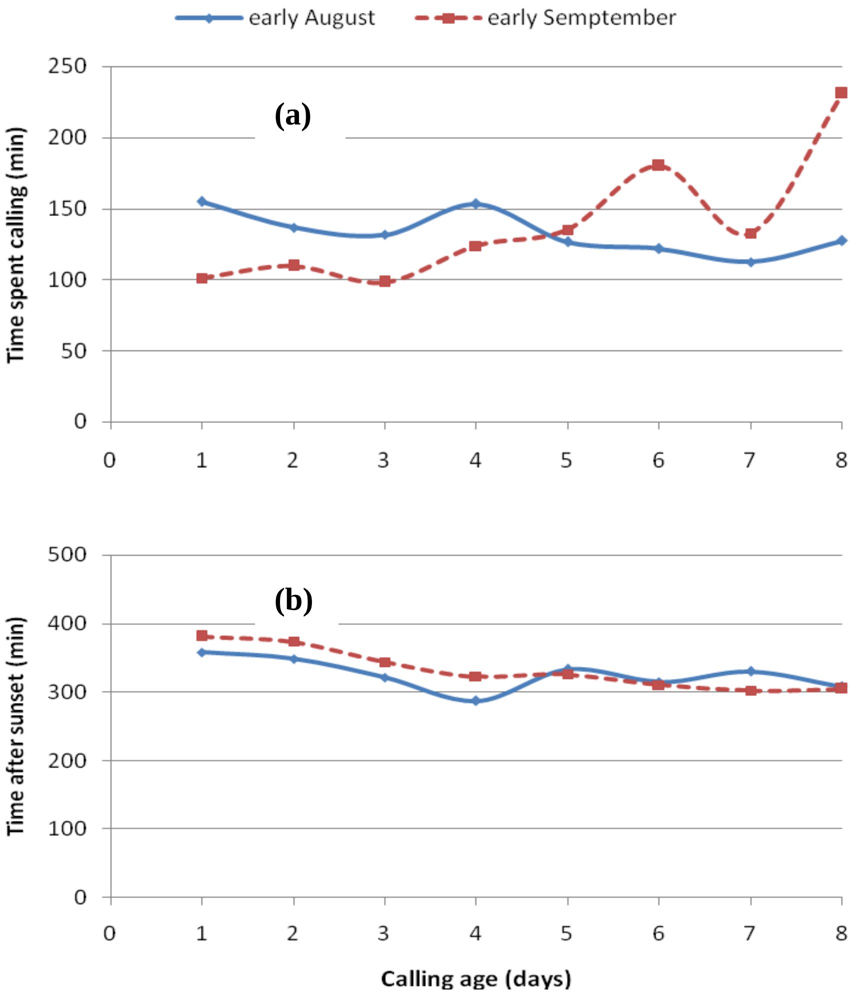


Fig.3. (a) Mean time spent calling (minutes) and (b) mean onset time of calling (minutes after sunset) of virgin *Ectomyelois ceratoniae* as a function of calling age under field conditions in early August and early September 2006.

Table 3. Regression analysis of the proportion of virgin *E .ceratoniae* calling throughout the scotophase under field conditions from 7 to 14 August 2006 as a function of time of night, temperature, and wind speed.

Factor	d.f.	PE*	SE	*t*	*P*
Intercept	1	27.217732	11.09879	2.45	0.0341
Time of night	1	-0.028442	0.008594	-3.31	0.0079
Wind	1	-2.267366	0.720398	-3.15	0.0104
Temperature	1	-0.286532	0.31421	-0.91	0.3833
Time^2	1	0.0001777	0.000245	0.73	0.4847
Time^3	1	3.7131e-7	8.571e-7	0.43	0.6741
Wind^2	1	2.5658159	2.052906	1.25	0.2398
Wind^3	1	2.9726479	0.72744	4.09	0.0022
Temperature^2	1	0.2155203	0.609069	0.35	0.7308
Wind × time	1	0.0498296	0.035101	1.42	0.1861
Temperature × time	1	0.0078448	0.021275	0.37	0.7200
Wind × time^2	1	-0.000258	0.00013	-1.99	0.0751
Temperature × time^2	1	1.3514e-5	2.928e-5	0.46	0.6543

*parameter estimate

Table 4. Regression analysis of the proportion of virgin *E. ceratoniae* calling throughout the scotophase under field conditions from 6 to 13 September 2006 as a function of time of night, temperature, and wind speed.

Factor	d.f.	PE*	SE	*t*	*P*
Intercept	1	8.1086602	1.874041	4.33	0.0007
Time of night	1	-0.006729	0.001386	-4.86	0.0003
Wind	1	0.1906338	0.149756	1.27	0.2238
Temperature	1	-0.055824	0.063877	-0.87	0.3969
Time^2	1	-0.0001	1.289e-5	-7.78	<.0001
Time^3	1	6.1463e-7	1.308e-7	4.70	0.0003
Wind^3	1	-0.317954	0.142048	-2.24	0.0420
Temperature^2	1	0.0018732	0.023197	0.08	0.9368
Wind × time	1	-0.003416	0.001583	-2.16	0.0488
Wind × time^2	1	3.9042e-6	1.653e-5	0.24	0.8167
Temperature × time^2	1	1.2654e-5	5.399e-6	2.34	0.0344

*parameter estimate

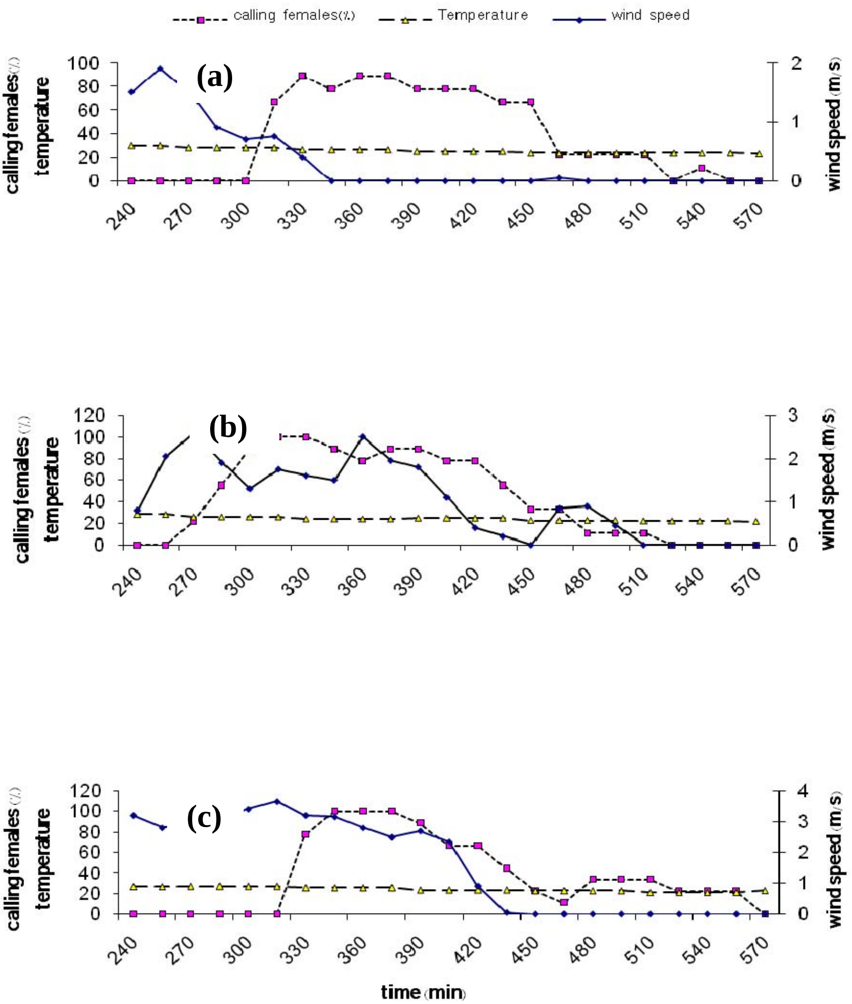


Fig.4. Variability in the proportion of virgin *Ectomyelois ceratoniae* calling throughout the night, as a function of temperature and wind speed on (a) 9, (b) 10, and (c) 11 August 2006.

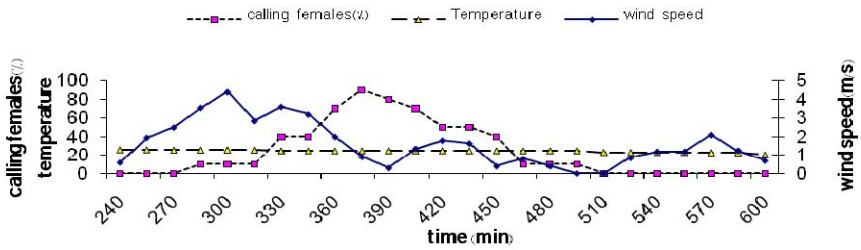
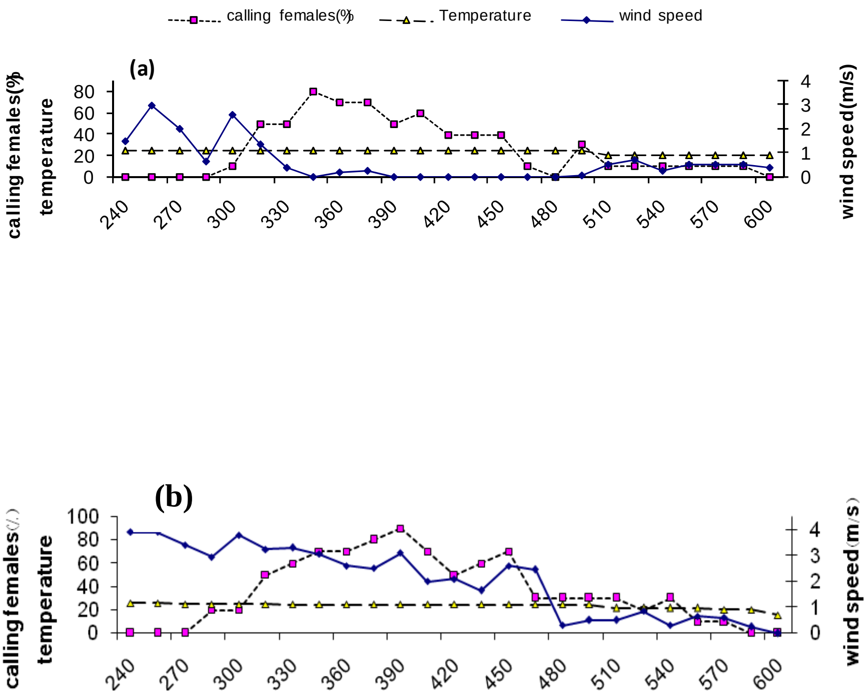


Fig.5. Variability in the proportion of virgin *Ectomyelois ceratoniae* calling throughout the night on 8 September 2006.



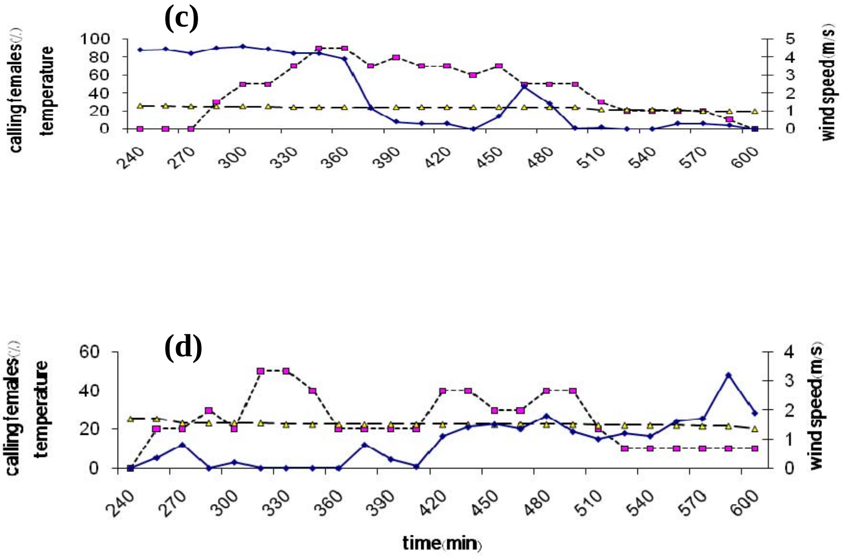


Fig.6. Variability in the proportion of virgin *Ectomyelois ceratoniae* calling throughout the night, as a function of temperature and wind speed on (a) 9, (b) 10, (c) 11, and (d) 12 September 2006.