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Effect of age on female calling pattern inlegume spotted pod borer *Maruca vitrata* (Lepidoptera: Crambidae)

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Abstract
The legume spotted pod borer, *Maruca vitrata* is a serious pantropical insect pest of grain legumes. Experiments were conducted at NBAIR, Bangalore. Female calling studies on *M. vitrata* were investigated at 27±2°C with 65-70% of relative humidity and photoperiod of 12L: 12D. Calling behaviour of virgin females of *M. vitrata* observed from one to eight days. Females started calling from 2nd hour of scotophase (7pm) and terminated at 3am (scotophase). The mean percentage of calling was maximum at 6th hour of scotophase in 3 day old moths with 44%. None of the females called during first hour of scotophase. The results revealed that age of insects determine the female calling pattern of *M. vitrata*. These studies provide valuable information for indentation of sex pheromone in insects.

Keywords: *Maruca vitrata*, legume spotted pod borer, female calling pattern, pheromone

Introduction
The Legume spotted pod borer, *Maruca vitrata* (Lepidoptera: Pyralidae) is an important insect pest of grain legumes in the tropics and subtropics.*Maruca vitrata* is the scientific name of Cowpea spotted pod borer synonymously called as *Maruca testulalis*.The legume spotted pod borer, *Maruca vitrata* (F.) is an important one because of its extensive distribution, wide host range, and destructiveness (Taylor, 1967) [13]. Larval infestation can occur at all stages of crop. They generally feed on the young flower buds and bore into pods thus protects the larvae from natural enemies and insecticides. The infested pod with bored holes plugged with excreta render the pods unmarketable and leads to considerable yield loss up to 20-80%.
Even though pesticides control the pest, in cowpea limited and high frequency of insecticide applicationresulted in drastic reduction of natural enemies and increased resistance to insecticides (Ekesi, 1999) [5]. Hence, alternative method of pest management has to be developed which helps in timely application of insecticides. Very few studies had been undertaken to know the basic biology of *Maruca vitrata*, so the knowledge of calling of *M. vitrata* provides valuable information for evolving tractable unified pest management approaches such as monitoring and timely application of insecticides(Downham,2004).In Lepidoptera calling happens in a precise period of the day, which is important for initiating semiochemical-based pest monitoring and management.

Material and methods
Maruca vitrata larvae were collected from infestedflowers and young pods of cowpea. Larvae were maintained in rearing room at the temperature of 27±2°C and relative humidity of 65-70%, photoperiod of 12L: 12D. Larvae were reared individually to prevent cannibalism by keeping them in transparent plastic containers of 7 cm diameter and 12 cm height size with absorptive paper. Early instar larvae provided with flowers and flower buds of cowpea as food. Later instars were fed with pods, flower and flower buds. Waste, excreta and other extraneous material were timely removed. After pupation, pupae were separated based on genital characters and kept in separate boxes.

Female calling behaviour
Female moths of different ages viz, oneto eight-day old were confined to a transparent cylindrical plastic container of 12.5 cm diameter; 24 cm height size individually and provided with ventilation. Moths were provided with 10% honey soaked cotton ball as food. The calling of female moths of 1-8 day old were observed throughout scotophase (lights off at 18:00 and on at 06:00 h) within every 20minutes. interval. Females moths extending the ovipositor beyond the abdominal tip overlying the pheromone gland scored as calling females (Fig.1).

Light was provided using LED 3w lamp with red cellophane during scotophase. ANOVA was used to analyze the results.

Results

Calling behaviour

Calling pattern of one to eight day old moths were observed. Calling was initiated during 2nd hour of scotophase and reached maximum at 6th hour of scotophase in third day old moths**.** Calling females of *Maruca vitrata* were correlated with their age. There was no calling observed during thefirst hour of scotophase irrespective of the moth age. Single calling peak was observed at sixth hour for the moths of all age groups except for 3 days old where additional peak of calling at 6th and 8th hour of scotophase were observed (Fig 2c).

In one-day-old female moths, maximum calling occurred during 6thhour (11:00 pm) with 14.58 per cent and least being during 2ndhour (7:00pm) of scotophase. However, In second day old moths, maximum mean percentage was recorded during 6th hour with 31.67 per cent, and least being 7.5 per cent at 2nd hour of scotophase (Fig 2). In three-day-old moths, the peak calling occurred during 6th hour with 43.75per cent, which was on par with 8th hour of scotophase whereas minimum percentage of calling recorded during 2nd hour of scotophase with 7.5 per cent (Fig. 2a). In fourth day old moths, maximum calling with 26.25 per cent was recorded during 6th hour of scotophase and minimum percent of 5.42 in 2nd hour of scotophase (Fig.2b).Calling behaviour of five-day-old moths was recorded maximum during 6th hour of scoto phase with 16.67per cent, which was on par with 7th hour and least percentage recorded during 2nd hour with 1.67 per cent (Fig 3a). In 6-day-old female moth's maximum calling occurred with 11.47 per cent at 6th hour of scotophase and least being 2.5 per cent during 2nd hour of scotophase (Fig.3b). The calling percentage drastically reduced in seven-day-old females with 5.4 per cent during 6th hour of scoto phase and 1 per cent at 2nd hour of scoto phase (Fig.4a). For the eight-day-old females, it was 2.9 per cent during 6th hour and 1.67 per cent during 4th hour of scotophase (Fig 4b). Females of *Maruca vitrata* showed clear calling sequence where the percentage of calling moderately increased in younger females *i.e*.15 and 30 per cent for one and two day old moths respectively. Among all the age groups, highest peak of calling with 44 per cent was observed in 3-day-old moths. There was gradual reduction from fourth, fifth, sixth, seventh and eighth day old moths (26.25; 16.67; 11.67,Similar trend of calling were recorded in all age groups of moths. There was minimum percent of calling in initial hours and gradually increase in proceeding hours. However, there was a decrease after 7th hour of scoto phase.

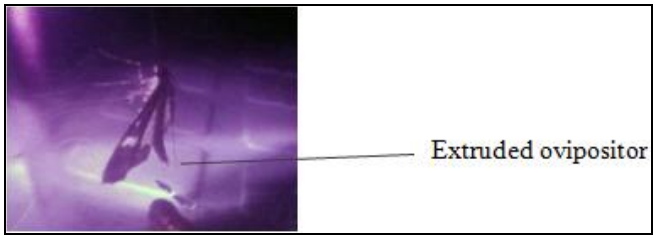

Fig 1: Calling female extruding its ovipositor

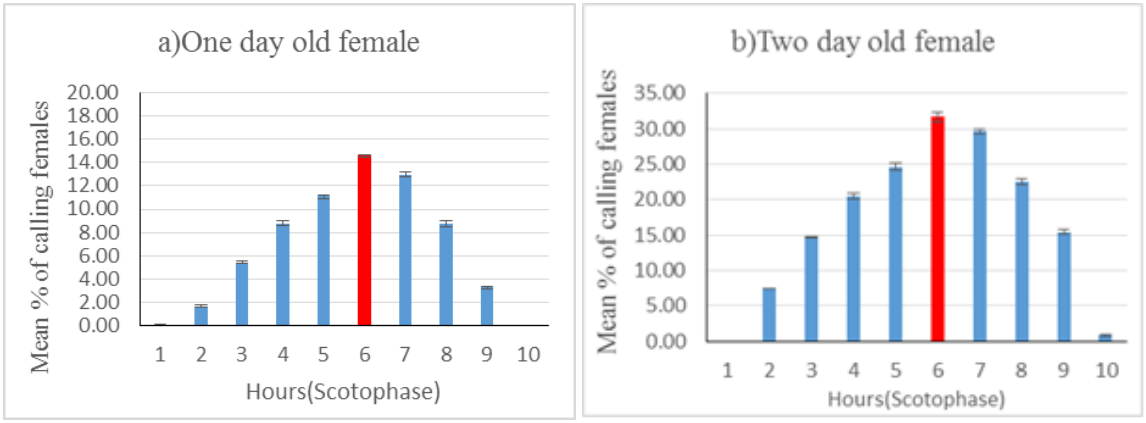

Fig 2: Calling pattern of one-day old (a) and two-day old (b) female moths of *M. vitrata*

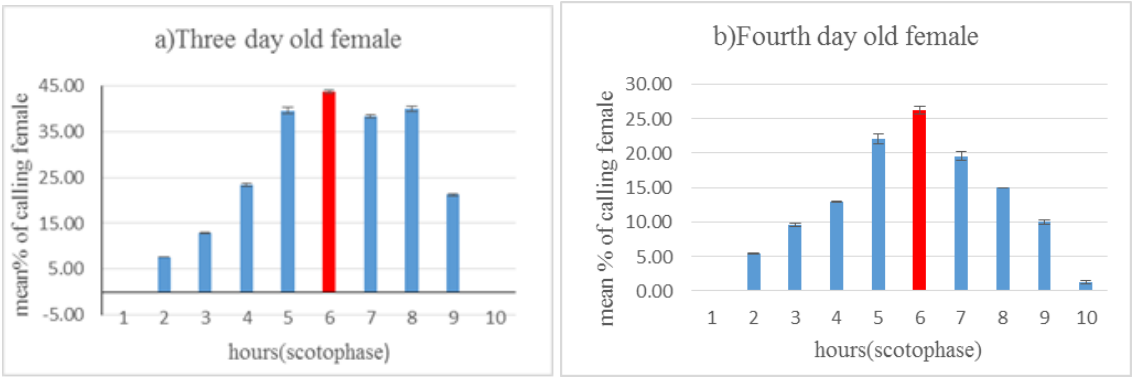

Fig 3: Calling pattern of three-day old (a) and four-day old (b) female moths of *M. vitrata*

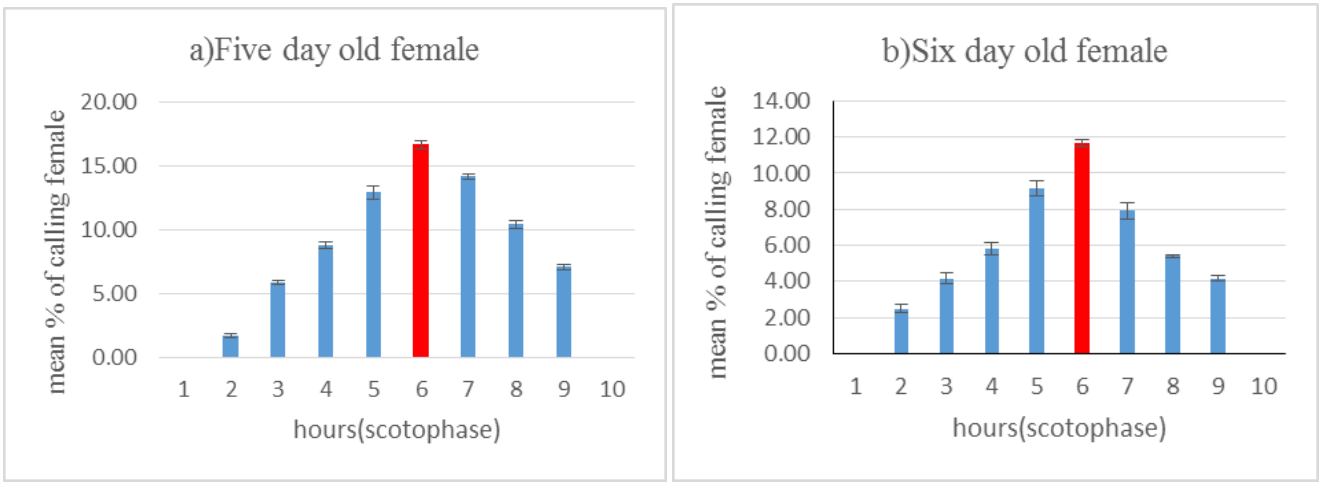

Fig 4: Calling pattern of Five-day old (a) and Six-day old (b) female moths of *M. vitrata*

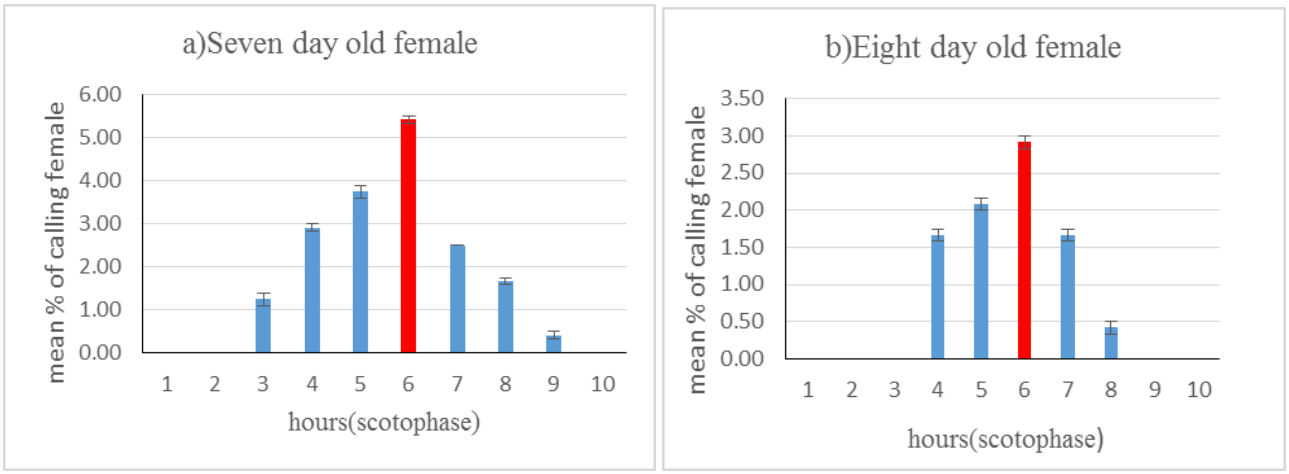

Fig 5: Calling pattern of Seven-day old (a) and Eight-day old (b) female moths of *M. vitrata*

Discussion

In lepidopteran, the calling behaviour, male courtship and mating behaviour are dependent on age and regulated by a circadian rhythm that is usually influenced by externalfactors like temperature and photoperiod and certain physiological factors (Turgeon and McNeil, 1982; Snir *et al*.,1986; Kou and Chow, 1987) [14, 11, 9]. During calling period *M. vitrata* exhibit different position such as curved abdomen pressed to substrate and standing in horizontal position and frequent antennal movements which was found similar in study of (Batista-Pereira *et al*., 2004) [3] where there is presence of different positions in *C. vestigialis* females during calling behaviour is which is also typical for several species of moths such as *Thyrinteina arnobia* (Lepidoptera: Geometridae) and *Hydraecia micacea* (Lepidoptera: Noctuidae) (West *et al*., 1984) [15]. The protrusion of the ovipositor to expose the cuticle overlying the pheromone gland is the typical sign of the calling position as observed in other lepidopterans (Turgeon and McNeil 1982; West *et al*., 1984; Howlader and Gerber, 1986) [14, 15, 6] which occurred in present studies too. The female calling, courtship and mating of most nocturnal moths occurred during Scotophase and very few of them reported on latter phase of the photophase and scotophase, which is in contradict with present studies were calling behaviour is restricted to scoto phase only. It was observed that female moths of *M. vitrata* quickly bend their abdomen downwards before initiating the calling behaviour but maintain its abdomen horizontal to substrate surface in later hours and expand their wings and gives pressure to the pheromone gland reported in previous studies of (Ambrogi and Zarbin, 2008) [1]. The calling females where less in later days compare to early days. In present we found that age of female moths had significant influence on calling pattern. Third day old females presented highest mean percentage of calling than younger females. Similar results were obtained for some lepidopteran species, such as *Agrotis ipsilon* (Lepidoptera: Noctuidae) (Swier *et al*. 1977) [12], *H. micacea* (West *et al*. 1984) [15], and *Chilo suppressalis* (Lepidoptera: Pyralidae) (Kanno, 1979) [8]. This greater amount of calling with age can be correlated with reproductive maturity (Swier *et al*. 1977) [12]. As seen in Fig.2 maximum calling activity occurs during 6th hour of scoto phase in 3rd day old female moths. This result suggests that the specific time to extract sex pheromone glands should be between sixth and eighth hours of scoto phase. These facts can be used in identification of sex pheromone, deciding the trap installation time, and other methods on semio chemical based pest management.

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