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Reproductive status of areca nut white grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae)

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Abstract
Investigations on the reproductive status of emerged adult beetles of *Leucopholis lepidophora* was studied in laboratory and field conditions. In laboratory condition ovarian development of *L. lepidophora* was studied by dissecting of different stages of female beetles at regular intervals. Number of eggs in the lateral and common oviduct was counted. As females become older, the number of eggs in oviducts decreased. Based on the fat body and developmental state of their ovaries females were graded into four groups *viz.*, Virgin females, gravid females, laying females and spent females. Field collections were made at weekly intervals and collected beetles were dissected to distinguish them as a virgin, gravid, laying and spent based on laboratory grading. The present findings revealed that most of the female emerged from the soil are virgin with well-developed ovaries. Most of the emerged females were virgin (51.62%), followed by spent (24.55%), gravid (16.61%) and laying females (7.22%). The responses of male beetles of *L. lepidophora* to traps baited with different grades of adult female beetles were studied as a suggestion of the pheromone production. Virgin female baited trap effectively attracts male beetles of 22.8±13.85 (Mean±SD) compare to Mated and laying females. These results strongly indicated that virgin female beetles secrete sex pheromone to attract males, as long as they are virgin in the state. This finding has implication for the management of areca nut white grub through isolation, synthesis and field evaluation of sex pheromone that can be used for mass trapping under field condition.

Keywords: *Leucopholis lepidophora*, virgin, laying females, gravid females, sex pheromone

Introduction
Arecanut is the important commercial crop grown in southwestern region and North Eastern regions of India. Arecanut is attacked by several pest and diseases throughout the year. Among them, white grubs feed on the roots and causing high yield losses [1]. Arecanut roots were infested by three important species of white grubs in Western Ghats and coastal regions of Karnataka and Kerala. Dominant species of white grubs are *Leucopholis lepidophora* mainly distributed in the hilly areas of India [3, 2] whereas, *Leucopholis burmeisteri* and *Leucopholis coneophora* distributed throughout the costal areas of Karnataka and Kerala. The third instar grubs feed on tender roots of areca nut and cause damage and expressing symptoms like yellowing of leaves, stem tapering, reduced length of internodes, reduced vigour, nut fall leads to a reduction in yield and ultimately death of the palm [4]. Kalleshwaraswamy *et al.* [2] reported that *L. lepidophora* caused 27.86-36.97 per cent damage to areca nut with a yield loss of 39.79–41.60 per cent in different districts of Western Ghats of Karnataka. Third instar grubs feeding on tips of tender roots of areca nut. High population density it denuded the entire base of the bole and six to eight grubs are enough to kill palms [5].
Further, the affected palms loose anchorage due to loss of roots and slight jerk to the palm they topple down [6]. Several integrated management strategies have been adopted to tackle the menace of different species of areca white grubs [1, 4]. This includes collection of larvae by digging soil, application of bio control agents and insecticides to soil, flooding with insecticidal application, Collection of adult beetles during their emergence and collection of male beetles through female-baited traps [1, 7-13].
Male and female beetles emerge from the soil for mating at dusk from 18:30 to 19:30 hours from June to October [4, 2, 13]. Sex ratio in *L. lepidophora* was female biased [2, 13]. Adult beetles of *L. lepidophora* exhibited protandrous type (males emerging before females) of emergence pattern during the emergence period [13]. Our previous studies have indicated that a female beetle secretes sex pheromone for that males are attracted. Once emerged, the calling

female secrete pheromone, for that numerous males are attracted and hover around the female. Understanding the reproductive status of the emerged female adult beetles is essential for incorporating integrated pest management practices. Therefore, in-depth ecological studies regarding this are needed in the management of white grubs. Hence, this study was planned to find out information on the reproductive status of adult beetles.

Materials and Methods

Reproductive status of *L. lepidophora* in laboratory condition

A laboratory culture was established in 2014. A day old female beetle emerged from pupa was allowed to mate with a male beetle by caging for three days. Each female was separated and allowed to oviposit in a plastic container (17cm ht. X 13 cm dia.) filled with soil. Forty such beetles of eight groups comprising five females in each container were maintained. Those eight groups were dissected at 5, 10, 15, 20, 25, 30, 35 and 40 days. The elytra and hind wings were removed and the reproductive system was taken out using forceps. A number of eggs present in common and lateral oviducts were counted and also the number of eggs laid in different day intervals was recorded. Fat body content was assessed visually. Based on the fat body content and developmental state of their ovaries and reproductive status was assessed and females were divided into different groups. Similarly, at above-mentioned intervals, five unmated females were dissected and a morphological difference in the reproductive system was studied.

Reproductive status of *L. lepidophora* in field condition

During adult emergence period, female beetles were hand collected by close monitoring by two persons in an areca nut garden using head torches from 18:00 to 21:00 hrs in an area of 1 acre for two consecutive years (June-2015 to September-2015 and June-2016 to September-2016). This experiment was done in areca growers field Gulukoppa (Hosanagara taluk, Shivamogga district; 13°52´ N; 75°12´ E, 692 msl). The emerging adult beetles were collected by using shuttle badminton racket, by using a ladder of 10 feet and also from various plants, where they found resting. Collected beetles were transferred through plastic containers to the laboratory for further studies. Collected samples of each individual were sexed, based on the tibial spur. Females were dissected to assess whether they are virgin, gravid, laying female or spent females.

The attraction of males by virgin, mated and laying females in the field

The experiment was conducted with three sets of 25 female beetles. First set composed freshly emerged female beetles in the field. Whereas, the second set included mated females. Some mated females which were brought to the laboratory for egg laying were included in the third set. These beetles were maintained individually in plastic containers with soil. The trap was constructed with a bucket trap with a small plastic box (5 cm dia. × 6 cm ht.) and funnel. In which all three sets of beetles were placed separately and used as bait to attract male beetles. A total of 15 traps were used for this experiment. Traps were placed in the arecanut garden at 18:30 hrs and recovered on the next day early morning. Each trap test had a total of 25 replications which were conducted on 1st, 6th, 19th and 24th July 2016.

Results and Discussion

Reproductive status of *L. lepidophora* in laboratory condition

Laboratory mated females of *L. lepidophora* were dissected, developmental state of their ovaries was observed and the reproductive status was assessed. In mated females, the number of mature eggs (those present in lateral and common oviduct) varied with age (Table 1). After 25th day, the number of eggs found in oviducts was nil. As females become older, the number of eggs in oviducts was decreased. During the experimental period, the mean number of mature eggs found from 5 to 25 days of age were 23.4±10.76 at 5th day and 2±4.47 at the 25th day. Based on the fat body and developmental stage of their ovaries, females were divided into three groups as below:

Grade 1: Virgin female: Females with developed ovaries (reproductively active) and abdominal cavity with full of fat bodies (Plate 1).

Grade 2: Gravid females: Both mature and immature eggs almost equal in proportion with abdomen fully filled with fat bodies; eggs in the lateral and common oviduct were 5 to 32 and age of female was 5 to 10 days (Plate 2).

Grade 3: Laying female: Less number of eggs in the ovariole and less fat body content; eggs in the lateral and common oviduct were 1 to 32 and age of female was 10 to 25 days (Plate 3).

Grade 4: Spent: No eggs in the ovariole. Less or no fat body in the abdomen; No eggs in the lateral and common oviduct age of females is 25 days or more (Plate 4).

A number of eggs laid by white grub *L. lepidophora* in the laboratory before dissection to understanding reproductive status are represented in Table 2. Egg production in females occurred continuously and was found to vary with age. Egg laying by young mated female started at the age of 10 days. The mean number of eggs laid by these females during their total egg-laying period was 12.8±7.19 (Classified as grade 2). More number of eggs was laid in the age of 20 to 25 days on an average of 14.8±7.66 and 15.2±14.34, respectively (Table 2) (Classified as grade 3). After 25 days no eggs were found in oviducts and classified as grade 4.The number of eggs laid by individual female varied. It could be due to variation in the number of ovaries present in the individual female. Female beetles laid eggs more than once in clutches of different sizes at a different time interval. This result confirms that there will be continuous development of eggs from 10 to 25 days which is in accordance with the egg-laying of ground beetles in several small clutches [14]. This grading technique was used to categorize the field collected female beetles.

Reproductive status of *L. lepidophora* in field condition

Based on the groups made from laboratory study, the reproductive status of field-collected female beetle was assessed. During 2015, the highest percentage of virgin beetles were collected and ranged from 7.69-80 per cent followed by spent (10-80%) and gravid females (8-50%). Percentage of laying females collected was very low (6.67-40%). A total of 150 females were collected in 13 weeks from June 1st week to September 1st week. Among these, 46.67% were virgin females followed by spent females (22.67%), gravid females (20%) and laying females (8%) (Table 3). Peak emergence of virgin females was observed in the

beginning to mid-season of emergence period from June to July. The peak of gravid females was found in July. Whereas, most of the females were virgin (28.57-100%) with an average of 55.73 per cent followed by spent females (7.14-86.67%) with an average of 25.95 per cent. Per cent of gravid and laying females caught were 12.21% and 6.11%, respectively (Table 4). This means most female beetles hand collected were virgin females and reproductively highly active and produce sex pheromone to attract male beetles.

The results showed that maximum percentages of the hand collected females were virgin followed by spent, gravid and laying females. This indicated that mating occurs after emergence during June and they go back to the soil and start laying eggs. The virgin females were reproductively active and immediately attained the calling posture and secrete sex pheromone and attract adult males for copulation. The present findings are in accordance with studies on females of Japanese beetle, *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae). The virgin females had well-developed ovaries [15, 16]. The well-developed ovaries in scarabaeid beetles are linked to sex pheromone secretion attracting male beetles [17]. Their study with Japanese beetle, *D. ishigakiensis* indicated that adult females do not emerge from the soil until ovaries mature [16]. At the end of the adult emergence period (September), most of the emerged beetles were spent rather than virgin, gravid or laying female. Virgin females were more at the beginning of the emergence period (June) and the number of spent females was higher at the end of the adult emergence period (September). Fewer emergences of spent females were noticed in mid-season of adult emergence period.

The attraction of males by the virgin, mated and laying females in the field

An average of 22.8±13.85 (Mean±SD) male beetles were attracted by virgin females per week. However, no male beetle was attracted to mated and laying females of *L. lepidophora* (Table 5).

This is the first study where in comparison of attraction of males by different grades of females were tested. Similarly, Ventura *et al.* [18] reported that young virgin females attract more number of male beetles compared to mated females. A large number of male beetles were captured by virgin females of *Dasylepida ishigakiensis*, but few males were captured by mated females [19]. In general, virgin females exhibit a strong attraction to males of western corn root worm [20]. Females secret sex pheromone which attracts male beetles for 8 to 10 days [13]. This indicated that only virgin females secrete sex pheromone and they can be utilized for the large scale collection of male beetles. This information has got implication in the selection of virgin females for isolation and synthesis of sex pheromone in future studies.

Table 1: Number of mature eggs in common and lateral oviducts of mated females of *L. lepidophora* dissected at different intervals

	Number of mature eggs in oviducts*					Mean ±SD	Status of females
Age (in days)	1	2	3	4	5		
5	30	24	32	26	5	23.4±10.76	Gravid
10	0	0	19	32	0	10.2±14.70	
15	1	24	0	31	30	17.2±15.48	Laying
20	0	0	0	0	0	0	
25	0	0	10	0	0	2±4.47	
30	0	0	0	0	0	0	Spent

*Five beetle were dissected at each time period

Table 2: Number of eggs laid by mated females of *L. lepidophora* at different intervals

	Egg production/female					Mean ±SD	Status of females
Age (in days)	1	2	3	4	5		
5	0	0	0	0	0	0	Gravid
10	17	16	16	0	15	12.8±7.19	
15	5	0	2	0	0	1.4±2.19	Laying
20	4	13	18	14	25	14.8±7.66	
25	9	6	0	31	30	15.2±14.34	
30	0	0	0	19	9	5.6±8.44	Spent

Table 3: Reproductive status of *L. lepidophora* hand collected female beetles in areca garden Gulukoppa, Hosanagara taluk, during 2015

Weeks	Reproductive status								
	Virgin females		Gravid females		Laying females		Spent females		
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Total
Jun I	6	66.67	3	33.33	0	0.00	0	0.00	9
Jun II	7	70.00	3	30.00	0	0.00	0	0.00	10
Jun III	9	75.00	1	8.33	2	16.67	0	0.00	12
Jun IV	3	37.50	4	50.00	0	0.00	1	12.50	8
July I	8	80.00	1	10.00	0	0.00	1	10.00	10
July II	6	40.00	4	26.67	2	13.33	3	20.00	15
July III	3	30.00	2	20.00	4	40.00	1	10.00	10
July Iv	10	62.50	3	18.75	0	0.00	0	0.00	16
Aug I	7	58.33	2	16.67	0	0.00	3	25.00	12
Aug II	6	60.00	1	10.00	0	0.00	3	30.00	10
Aug III	4	40.00	1	10.00	1	10.00	4	40.00	10
Aug IV	1	7.69	3	23.08	2	15.38	6	46.15	13
Sep I	0	0.00	2	13.33	1	6.67	12	80.00	15
Mean	70	46.67	30	20.00	12	8.00	34	22.67	150

Table 4: Reproductive status of *L. lepidophora* hand collected female beetles in areca garden Gulukoppa, Hosanagara taluk, during 2016

Weeks	Reproductive status								
	Virgin females		Gravid females		Laying females		Spent females		
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Total
Jun III	4	100.00	0	0.00	0	0.00	0	0.00	4
Jun IV	7	100.00	0	0.00	0	0.00	0	0.00	7
July I	9	90.00	0	0.00	0	0.00	1	10.00	10
July II	6	60.00	2	3.33	0	0.00	2	20.00	10
July III	8	80.00	2	2.50	0	0.00	0	0.00	10
July IV	5	35.71	7	19.60	1	7.14	1	7.14	14
Aug I	13	92.86	0	0.00	0	0.00	1	7.14	14
Aug II	8	53.33	1	1.88	1	6.67	5	33.33	15
Aug III	4	28.57	2	7.00	2	14.29	6	42.86	14
Aug IV	9	50.00	2	4.00	2	11.11	5	27.78	18
Sep I	0	0.00	0	0.00	2	13.33	13	86.67	15
Mean	73	55.73	16	12.21	8	6.11	34	25.95	131

Table 5: Number of male *L. lepidophora* attracted by traps baited with different staged females in the field

Particulars	Mean number of males attracted/female/week (Mean±SD)
Virgin females	22.8±13.85
Mated females	0
Laying females	0

*N=25

Plate 1: Dissected Virgin female abdomen

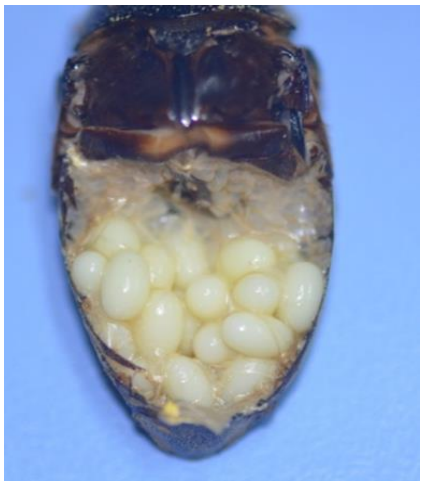

Plate 2: Dissected gravid female abdomen

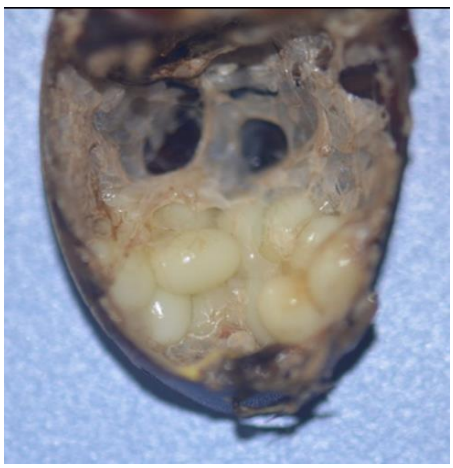

Plate 3: Dissected laying female abdomen

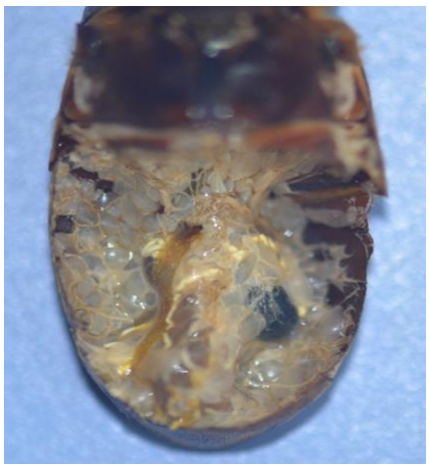

Plate 4: Dissected spent female abdomen

Conclusion

The study of the reproductive status of areca nut white grub, *L. lepidophora* helps in nature of female emerged during a different period of emergence season. Based on the groups made from laboratory study, the reproductive status of field-collected female beetle was assessed. Our results revealed that most of the newly emerged females were virgin; they are reproductively active with matured oocytes and release a large

quantity of sex pheromone. These virgin females were used as a bait to attract male beetles in large numbers for management of areca nut white grub. So pheromone trapping could be an essential method in integrated pest management strategy for management of areca nut white grub. White grub, *L lepidophora* is challenging to manage because it remains in the soil for the whole period of its life cycle except for a short period adult beetles emerged for mating. Because of this distinctive life cycle, conventional methods of pest control, i.e. application of insecticides may not give effective control. These conventional insecticides cause negative effect on other soil arthropods and earthworms [21]. The exploitation of synthetic sex pheromone has the potential for monitoring and mass trapping in the field. Hopefully, this study also will help to develop new management strategies for effective control of areca nut white grub.

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