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Adult emergence pattern and utilization of females as attractants for trapping males of white grubs, *Leucopholis lepidophora* (Coleoptera: Scarabaeidae), infesting areca nut in India

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ABSTRACT

White grub, *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae: Melolonthinae), is a major insect pest infesting areca nut in India. Larvae feed on roots (hence also called root grubs), causing reduced number of roots, loss of anchorage, stem tapering, reduced number of fronds, yellowing of leaves, yield reduction and death of the plants. The biology and ecology aspect of this scarabaeid are scanty. Hence, the aim of this study was to understand the ecological and behavioral aspects to develop IPM program. The adult emergence pattern of *L. lepidophora* was observed daily during the rainy season. The emergence period was defined between June and October during 2013 and 2014. Peak emergence of adult beetles occurred between 1900 and 2000 h. When raining occurred between 1800 and 2100 h, no emergence of adult beetles was observed. The sex ratio of *L. lepidophora* varied over time, but the overall sex ratio was female biased in both the study years (female:male; 1:1.18 and 1:1.46 in 2013 and 2014, respectively). Males were found to emerge first in the season resulting in protandry. Innovative idea emerged during our field experiments in 2013–2014 was the use of female beetles for attracting male beetles. Female adults were collected and placed individually in traps made of small pouches of nylon net and were tied to the areca palm at a height of 5 ft. Trap containing a female beetle attracted male individuals for about 8–10 days. The same technique was employed for the large-scale collection of beetles during 2014. A total of 1843 males were collected in an areca grower's field using female-baited traps during 2014. Male baited traps did not attract female beetles, indicating existence of only female produced semiochemical that attracts males. Our findings provide new insights for incorporating adult collection using female-baited traps in IPM against areca nut white grubs.

Introduction

White grubs or root grubs (Coleoptera: Scarabaeidae: Melolonthinae) are important pests of palms, mainly areca nut and coconut in Western Ghat region of India (Veeresh et al., 1982). Three important species that infests roots of these crops are *Leucopholis lepidophora* Blanchard, *Leucopholis burmeisteri* Brenske and *Leucopholis coneophora* Burmeister (Veeresh et al., 1982). Among these three, *L. lepidophora* is considered as a major species distributed throughout the high rainfall regions of India (Veeresh, 1983). Feeding by these root grubs damage the roots resulting in symptoms of yellowing of leaves, stem tapering and nut fall, ultimately leading to reduced vigor and yield (Nair and Daniel, 1982). Grubs show a particular preference for feeding on tender roots. The roots are fed on from the tip or cut across at various points. When population densities increase, the entire base of the bole is denuded (Kumar, 1997). Affected palms loose anchorage and topple when disturbed (Nair and Daniel, 1982). A few grubs (6–8) are enough to kill trees (Kumar et al., 2011). Kalleshwara swamy et al. (2015) reported 27.86–36.97% damage of this species with a yield reduction of 39.79–41.60% in different districts of hilly regions of Karnataka. Their cryptic life cycle and a much-to-be-understood biology limit the range of management options. Several strategies have been advocated (Veeresh et al., 1982; Kumar, 1997). These include larval collection by digging the soil, soil application of biocontrol agents and insecticides and adult collection during their emergence (Veeresh et al., 1982; Kumar et al., 2011). Insecticidal application *viz*., chlorpyriphos, imidacloprid and phorate to the soil targeting grubs is a widely followed method that provides considerable control (Prakash et al., 2011). However, their use on white grub management must be reconsidered in light of the fact that, the Western Ghat region is one of the biodiversity hot spots. Keeping in

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view the effect of chemicals on soil biodiversity, there is a need to identify eco-friendly techniques, which provides satisfactory control of white grubs. Hence, there is a need to understand the biology and ecology aspect of these insects.

Leucopholis lepidophora has a 2-year life cycle with the majority of time spent as a grub. Eggs of *L. lepidophora* are laid at 5 cm depth in the soil, and they hatch in 12–15 days. Larvae undergo three instars. The larval duration of the first, second and third instar grubs range from 30 to 40 days, from 35 to 60 days and from 400 to 550 days, respectively, with a pupal stage of 15 days (Kumar, 1997). Pupation occurs in the soil at a depth of >60 cm and adults emerge at night after a couple of rains in June-July and up to November (Kumar, 1997). Adult longevity is 20–45 days (Adsule and Patil, 1994; Naik et al., 2009). Unlike other scarabaeids such as *Holotrichia serrata*, which feeds on various host plants like neem, ber and acasia, the adults of *L. lepidophora* do not feed on any particular host plant (Kumar, 1997). Prathibha et al. (2013) studied the adult emergence pattern of another coconut and areca nut pest, *Leucopholis coneophora* Burmeister. This species has a restricted distribution confined to the coastal areas of Karnataka and Kerala states and has annual life cycle. They suggested mechanical capture and destruction of *L. coneophora* between 1835 h and 1915 h for 2 weeks commencing from the first day of monsoon as an IPM tool. However, there is no published information on the emergence pattern of adult *L. lepidophora* covering a complete emergence season, which is crucial for their collection and destruction. The detailed pattern of adult emergence may have important implications toward designing management strategies. Similarly, no information is available on the chemical ecology of this key pest species of palms. Hence, the objective of this study was to determine the adult emergence pattern of root grubs infesting areca nut and also to elucidate response of males to female-baited traps.

Material and methods

Experimental site

Field studies were conducted in two locations, *viz*., Harakere (Shivamogga district 13°53′N; 75° 33′E, 585 msl) and Gulukoppa (Hosanagara taluk, Shivamogga district; 13°52′N; 75° 12′E, 692 msl). Areca nut gardens were 15 years old with an incidence of white grub infestation about 40% and 75%, respectively.

Preliminary study to record the timing of adult emergence using nylon net field cages

A preliminary study was conducted in two locations to record the timing of adult emergence. Two nylon net cages (20 mesh/cm^2) were erected which were 3 m (L) × 3 m (B) × 2 m (H) (Plate 1) in two different locations for nine consecutive days to determine the timing of adult emergence. Adult emergence was observed throughout the day and timing of emergence and light intensity was noted down.

Plate 1. Nylon net field cage used for studying the adult emergence pattern of *L. lepidophora*.

Sampling for emerged adult *L. lepidophora* to record the adult emergence pattern

Two nylon net cages as described above (Plate 1) were placed in between four areca nut palms (plant spacing 3 m × 3 m) in a growers field in Harakere (Shivamogga taluk). The cages were set in the field on the day following the first day of the South West monsoon. The beetles that emerged from the soil and settled inside the nylon net cages were collected, counted and sexed. As per the results of preliminary experiment, observations on adult emergence were recorded daily from 1800 to 2100 h after the beginning of the monsoon (fourth week of June during 2013 and first week of June 2014) and sampling was terminated when no beetles had emerged for a period of 15 days (October of 2013 and 2014). Field visits were also made during hours of dusk whenever off-season rain was received, to know whether beetles emerged during these days. Beetles were sexed based on the character of hind tibial spur (female, spoon shaped; male, spine like; Prathibha et al., 2013). Data were subjected to simple correlation coefficient

Plate 2. Female-baited trap used for attracting *L. lepidophora* male beetles.

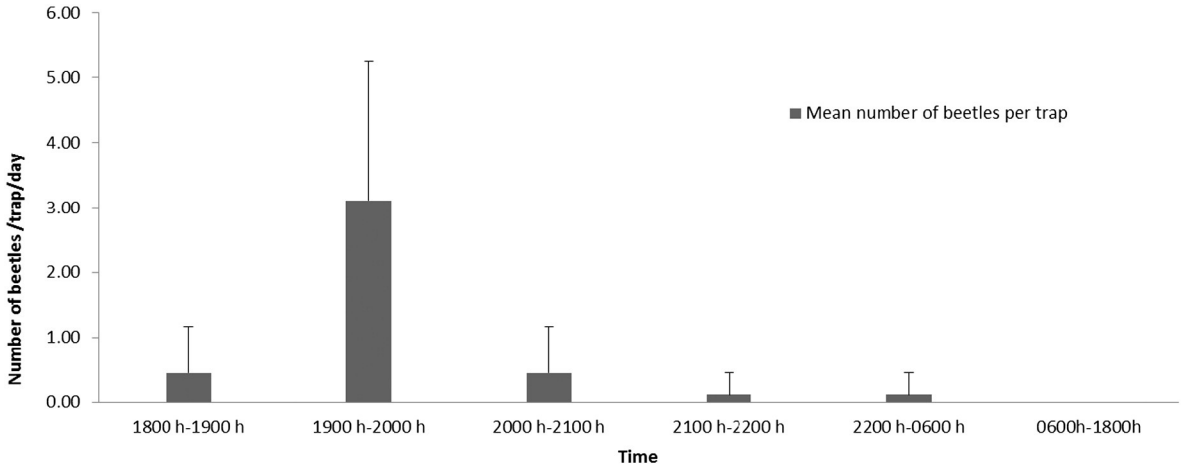

Fig. 1. Number of adults of *L. lepidophora* emerged per hour in a day for nine consecutive days inside two nylon net cages (each of 9 m^2 area).

analysis to determine the extent and nature of association between number of beetles trapped per day and different daily weather parameters. The weather parameters considered were daily maximum temperature (MT), minimum temperature (mT), relative humidity at 0730 h (RH 1) and 1330 h (RH 2) and rainfall (RF). Soil moisture (%) was taken when the adult emergence was began, i.e., during 1st week in June and at weekly intervals at depths of 60 cm; the beetles are generally found at this depth. Correlation was also performed between number of beetles emerged per trap per week and soil moisture (%) taken at weekly intervals using SPSS version 16.0 software. The significance of correlation coefficients were tested at $p = 0.05$ and $p = 0.1$ between calculated r value and table r value. Further, daily beetle collection data were converted to a weekly basis to calculate sex ratio and also to record the swarming trend or peak emergence of adult beetles.

Utilization of females for attracting males of *L. lepidophora*

During field studies, males were observed flying toward resting females and approaching them for mating. In order to further understand their behavior, trap was made with a small nylon pouch (15 cm length; 20 mesh/cm^2) in which a freshly emerged, field-collected adult female was placed as bait and tied to the areca palm at a height of 2 m (Plate 2). The number of responding males that settled on the nylon pouch was recorded daily until the attraction reached zero. Eight such female-baited traps were used in a garden of 0.4 ha area. Two traps with a male and two traps without beetles were also used. The experiment was conducted during adult emergence periods of 2013 and 2014 in an areca nut grower's field (Gulukoppa, Hosanagara taluk). The total number of adults attracted between 1800 and 2100 h to each trap was recorded. The settled males on the nylon pouch were collected at 2100 h (Plate 2). A light trap was also run at night on three different dates to record phototaxis behavior.

Mechanical collection of *L. lepidophora* males using female-baited traps

Based on the encouraging results obtained from the adult emergence periods in 2013 and 2014, an experiment was designed to collect

Table 1
Number of *Leucopholis lepidophora* adults collected from two nylon net field cages at weekly intervals and their sex ratio during adult emergence season of 2013 and 2014.

Weekly intervals	*L. lepidophora*							
	2013				2014			
	Male	Female	Total	Sex ratio (male:female)	Male	Female	Total	Sex ratio (male:female)
May-IV	0	0	0	0	0	0	0	0
June-I	0	0	0	0	4	3	7	0
June-II	0	0	0	0	5	5	10	0
June-III	0	0	0	0	6	6	12	0
June-IV	8	6	14	1:0.75	5	4	9	1:0.80
July-I	6	3	9	1:0.50	3	4	7	1: 0.80
July-II	9	4	13	1:0.44	3	11	14	1:2.75
July-III	2	4	6	1:2.00	1	1	2	1:1.00
July-IV	6	5	11	1:0.83	3	8	11	1:2.66
August-I	5	11	16	1:2.20	3	4	7	1:1.33
August-II	8	8	16	1:1.00	3	4	7	1:1.33
August-III	3	5	8	1:1.66	3	5	8	1:1.66
August-IV	6	10	16	1:1.66	1	3	4	1:3.00
Sept-I	2	6	8	1:3.00	3	4	7	1:1.33
Sept-II	3	5	8	1:1.66	0	1	1	0:1.00
Sept-III	1	3	4	1:3.00	−0	−0	−0	–
Sept-IV	1	1	2	1:1.00	1	2	3	1:2.00
Oct-I	−0	−0	0	–	5	7	12	1:1.40
Oct-II	−0	−0	0	–	3	4	7	1:1.33
Oct-III	−0	−0	−0	–	0	0	0	–
Total/overall	60	71	131	1:1.18	52	76	128	1:1.46

Table 2
Simple correlation values between number of *Leucopholis lepidophora* collected per nylon net (9 m^2 area) and daily weather parameters during 2013 (year 1) and 2014 (year 2) and mean weekly data of adults emerged and soil moisture (%).

Weather parameter	*L. lepidophora*	
	2013 (year 1)	2014 (year 2)
Maximum temperature (°C)	−0.213**	−0.006
Minimum temperature (°C)	−0.19**	−0.14*
Relative humidity at 0730 h (%)	−0.082	0.18**
Relative humidity at 1330 h (%)	−0.313**	−0.216**
Rainfall (mm)	−0.171**	−0.165**
Soil moisture (%)[a]	0.68**	0.44**

Weather data: year 2013, $N = 116$ days, $r = 0.14$ at $p = 0.05$, $r = 0.17$ at $p = 0.1$; year 2014, $N = 142$ days; $r = 0.13$ at $p = 0.05$; $r = 0.16$ at $p = 0.1$.
* Significant at $p = 0.05$.
** Significant at $p = 0.1$.
[a] Data taken at weekly interval; $N = 18$ weeks; $r = 0.44$ at $p = 0.05$; $r = 0.37$ at $p = 0.1$.

beetles on a larger scale in order to include as a strategy in IPM. Eight female-baited traps (Plate 2) were used in an areca nut grower's field (Gulukoppa, Hosanagara taluk, Shivamogga district; 13°52′N; 75° 12′ E) of 4000 m^2 area, and females in the traps were replaced with a female freshly emerged from soil for every 10 days. The total number of males attracted between 1800 and 2100 h to these eight traps were counted daily during the trapping period between 10 June and August 2014.

Results

Timing of adult emergence in a day

Emergence began at ~1630 when illuminance fell below 65.5 ± 12.5 lx and extended till 2100 (2.5 ± 0.5 lx). An average of 3.11 ± 2.15 beetles per trap per day emerged between 1900 and 2000 h, which was followed by 1800–1900 h and 2000–2100 h (0.44 ± 0.73 beetles per trap per day). Only few beetles emerged between other night hours and no adults emerged during day time (Fig. 1). Hence, in order to study the adult emergence pattern, monitoring period was restricted to 1800–2100 h.

Adult emergence pattern of L. lepidophora and their relationship with weather parameters and soil moisture

In 2013, the number of *L. lepidophora* adults trapped in nylon net cages varied from 0 to 4 per cage per day. A total of 131 adults were collected during the trapping period from 30 June to 15 October 2013 (Table 1). Similarly, during 2014, a total of 128 beetles were recorded from 03 June to 22 October 2014 (Table 1). The trap catches of *L. lepidophora* were negatively correlated with maximum temperature [$r = -0.213$ (significant at $p = 0.1$) and $r = -0.006$ for 2013 (year 1) and 2014 (year 2), respectively]. A significant, negative correlation was also observed between minimum temperature [$r = -0.19$ (year 1) and −0.14 (year 2)], afternoon RH [$r = -0.313$ (year 1) & −0.216 (year 2)] and rainfall [$r = -0.171$ (year 1) & −0.165 (year 1)] (Table 2). The soil moisture (%) content taken at weekly interval varied from 25% to 37% and reaching field capacity (~40%) sometimes during June to October. Correlation between soil moisture (%) and average weekly trap catches indicated that there was strong positive correlation between *L. lepidophora* emergence and soil moisture content ($r = 0.68$ year 1 and 0.44 year 2)].

Sex ratio of adult beetles emerged from soil

Sixty males and 71 females were collected in 2013. For few weeks from the initiation of emergence, the sex ratio was male biased (Table 1). During 2013, in the first 3 weeks of trapping (June-IV week to July-II week), the sex ratio varied from 1:0.44 to 1:0.75 (male–female). However, from July-III week, the sex ratio was female biased except in a few weeks where a 1:1 ratio was recorded. The overall sex ratio was 1:1.18. During 2014, 52 males and 76 females were collected. During the initial emergence period (June-I week to July-I week), the sex ratio was 1:0.75 to 1:1.00. However, from July-I week, the sex ratio was female biased (up to 1:3.00) except in few weeks where in 1:1 ratio was recorded. The overall sex ratio was 1:1.46.

Utilization of females for attracting males of L. lepidophora

Female-baited traps attracted males, whereas male-baited traps did not attract any females. The frequency of attraction decreased as the days progressed during both years. In 2013, on the first day, on an average 6.12 ± 3.44 males, 2nd day—3.25 ± 1.67, 3rd day—3.12 ± 2.42, 4th day—1.37 ± 1.30, 5th day—1.75 ± 0.89, 6th day—1.25 ± 1.04, 7th day—1.0 ± 1.41, 8th day—1.37 ± 1.06, 9th day—1.12 ± 0.83 and 10th day—0.75 ± 0.71 were attracted on the female-baited trap (Fig. 3). In 2014, on an average 5.50 ± 2.14 males on 1st day, 2nd day—3.25 ± 1.49, 3rd day—2.62 ± 1.92, 4th day—3.00 ± 1.51, 5th day—1.50 ± 0.76, 6th day—2.00 ± 1.20, 7th day—0.75 ± 0.71, 8th day—1.00 ± 1.07, 9th day—1.25 ± 0.83 and 10th day—2.25 ± 0.71 were attracted

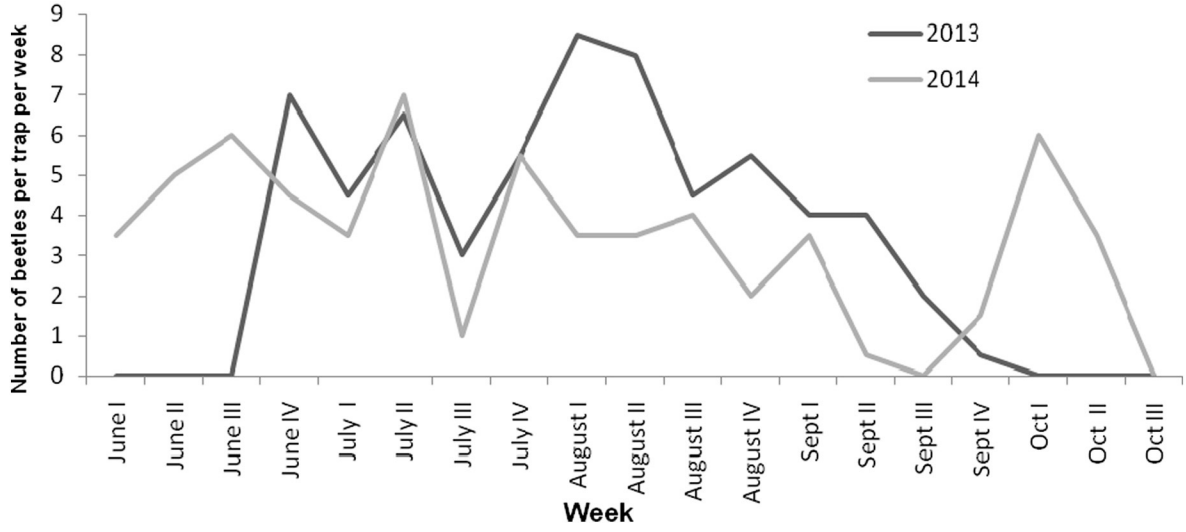

Fig. 2. Adult emergence of *L. lepidophora* beetles during 2013 and 2014 monitored using nylon net field cages of 9 m × 9 m in an areca nut garden.

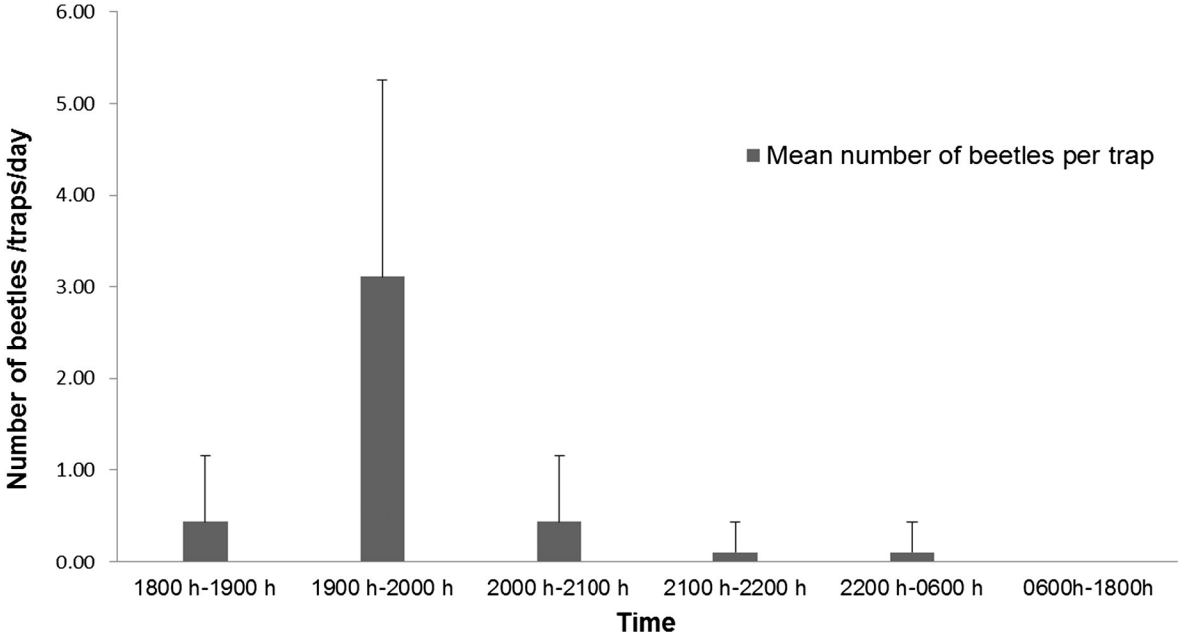

Fig. 3. Number of *L. lepidophora* male beetles attracted to eight female-baited traps in an areca nut garden during August 2013.

(Fig. 4). Attraction stopped almost from 11th day onward (Fig. 4), but the female lived up to 45 days. Only males were attracted to traps. When the males were kept in trap, no beetles were attracted and also traps with no beetle inside did not attract any beetles.

Mechanical collection of L. lepidophora using female-baited traps

A maximum of 75 beetles were attracted to female-baited traps in a single night. A total of 1845 beetles were collected during the emergence season (Fig. 5). There was no clear trend across days, but greater numbers of beetles were collected during July but emergence was progressively reduced in August.

Discussion

Adults of *L. lepidophora* emerged between 1800 h and 2100 h with a maximum emergence between 1900 and 2000 h (IST). Emergence starts roughly 30 min after sundown (1930 h) and continues for 2.5 h. The highest number of trapped beetles was observed in the first 2 h of flight activity. Perusal of Fig. 2 indicated that the trend of emergence was not same during both the years of study. There was no particular peak period of emergence (Fig. 2). This observed variation might be due to variation in climatic variables, especially rainfall and soil moisture. Padmanaban and Daniel (2003) showed that the maximum number of *L. burmeisteri* adult beetles emerged within 3–4 days during evening hours at 1845 and 1930 h. Prathibha et al. (2013) reported that

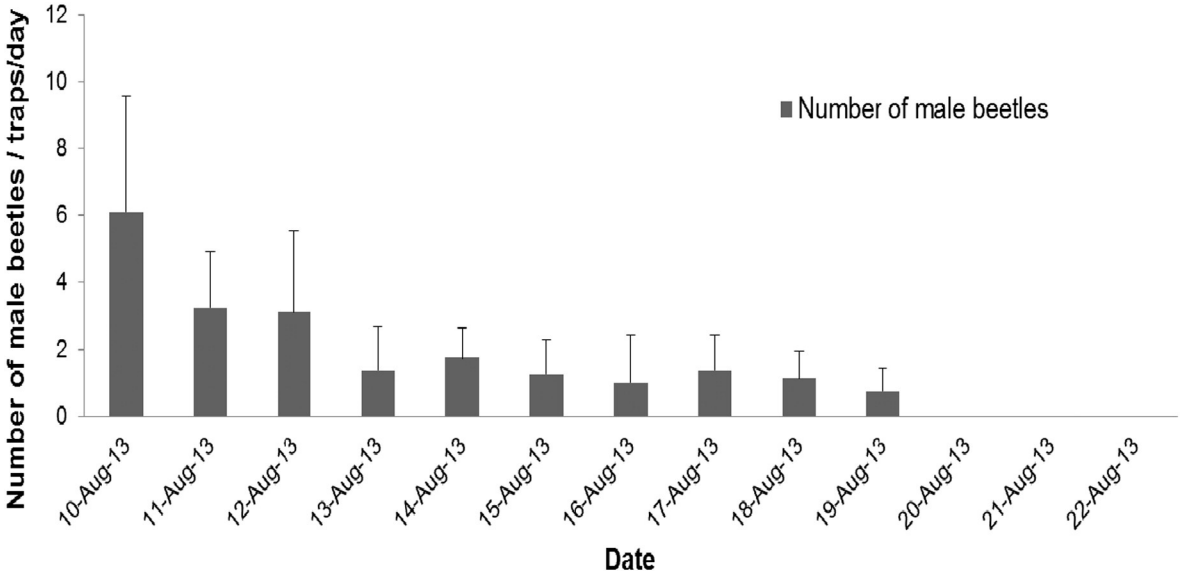

Fig. 4. Number of *L. lepidophora* male beetles attracted to eight female-baited traps in an areca nut garden during July 2014.

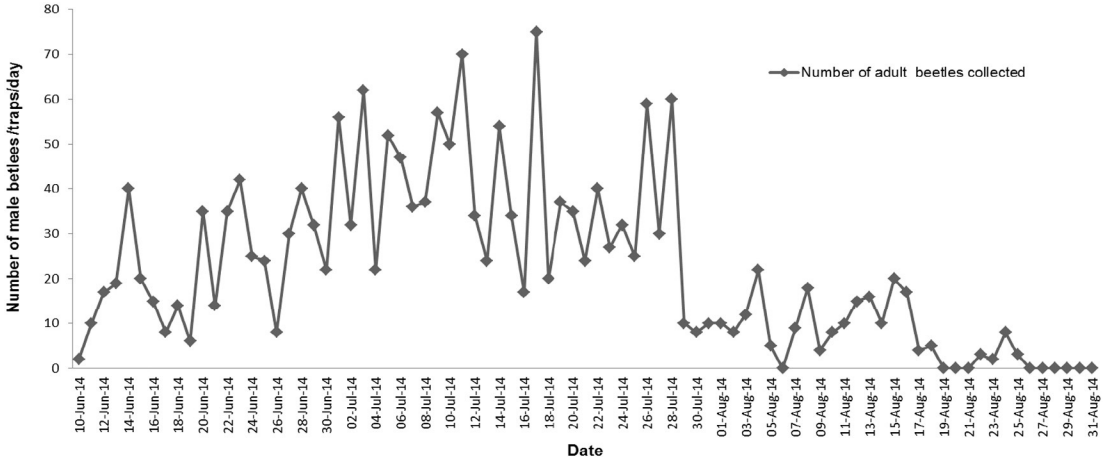

Fig. 5. Number of *L. lepidophora* male beetles attracted to eight female-baited traps during the adult emergence season of 2014 in an areca nut garden.

the emergence initiated when evening illuminance fell below 124.37 ± 75.5 lx (around 1835 h IST in June) and activities continued until 1.2 ± 0.4 lx (~1910 h). Light trap did not attract adults of *L. lepidophora*; hence, the use of light trap is not an option for their management.

L. lepidophora adult emergence was restricted to the monsoon period. In this part of India, the South West monsoon starts during the third week of May to first week of June. In both the years, emergence began in June, after the receipt of >50 mm rainfall and soil moisture reached >30%. However, rainfall were found to have significant negative effect on the adult emergence ($r = -0.171$ (year 1) and -0.165 (year 2)). This could be directly due to the effect of rain on the flight activity which prevent beetle emergence from soil. Thus, rainfall appears to stimulate the adult emergence by increasing the soil moisture, but it inhibits flight activity as indicated by correlation coefficients (Table 2). No adults were found flying when rained between 1800 and 2100 h (personal observation). Similar observation was made by Prathibha et al. (2013) for the coconut root grub chafer, *L. coneophora.* Other scarabaeids like *Schizonycha* spp. initiate flight before the beginning of the rainy season as a result of irrigation, which, like rain, probably softens the soil and enhances both emergence of the mature beetles (Wilson, 1969; Robertson and Walker, 2001). Unseasonal rainfall and irrigation had no influence on the adult emergence of both species studied here. In general, factors affecting scarabaeid beetle flight activity are rainfall patterns, soil moisture, air and soil temperatures and height and spacing of light traps (Gaylor and Frankie, 1979; Potter, 1981; Guppy, 1982; Stone, 1986). In our experiment, the beetles did not respond to light traps, but soil moisture had significant positive correlation, indicating that soil moisture was the primary stimulant for beetle emergence.

During initial period of emergence, the sex ratio was male biased. This indicates that males emerge first and await female emergence and mate. However, the sex ratio was female biased after 3–4 weeks. This type of sex-biased emergence patterns are common in insects, resulting in protandry (males emerging before females) or more rarely protogyny (females emerging before males) (Fogerstrom and Wiklund, 1982). Protandry is, by far, the most commonly observed of these two patterns (Timmerman et al., 2000). The longevity of male and female beetles is more or less same in *L. lepidophora* (22.16 and 26.09 days, respectively) (Naik et al., 2009); protandry is an advantage rather than protogyny. The possible explanation for this type of early male emergence pattern and skewed sex ratio toward *Leucopholis* spp. females are as follows: (i) early emerging males will have access to more females, (ii) males are available throughout the emerging season for females and multiple mating would occur and (iii) as the emergence occurs only during rainy season and females need not to wait for long period for oviposition, protogyny is not an option. However, further studies with respect to reproductive biology of *L. lepidophora* may through a light on this sort of behavior. Such understanding on the behavioral ecology of areca nut white grubs may help us in designing further management strategies.

Only female-baited traps attracted males but male-baited traps did not attract any females. This may be due to presence of a female produced semiochemical in *L. lepidophora.* At the same time, no other species of *Leucopholis* adults found approaching to female-baited traps. The emerged beetles (both males and females) found resting on various plants including areca nut but were not found feeding or aggregated on any of the plants in the garden. This eliminates the possibility of existence of plant-mediated volatiles or visual cues in male attraction toward females. This strongly indicates the presence of female produced semiochemical in *L. lepidophora.* Evidence for the occurrence of female produced sex pheromones has been documented in Cetoniinae (Domek and Johnson, 1987), Melolonthinae (Yarden and Shani, 1994), Dynastinae (Potter, 1980) and Rutelinae (Leal et al., 1992), but so far sex pheromones have been unambiguously identified only for beetles in the most advanced rutelines and the primitive melolonthines. This needs to be elucidated in case of *L. lepidophora*. As the male-baited traps did not attract the females, the possibility of existence of sex pheromone cannot be eliminated. This is the first field evidence in which females were exploited for large-scale attraction of males of *L. lepidophora*. This can replace the earlier suggested practice of manual collection of beetles which are emerging from soil and resting/mating on the surface or on the trees at a reachable height. Wide spread beetle collection has also been suggested for the management of *Leucopholis irrotata* Chevrolat in the Philippines (Leopoldo, 1930). In India, Prakash et al. (2011) reported <3% adoption of manual collection of *L. lepidophora* beetles by the growers as an IPM strategy. The required labor and searching for beetles during night hours limits applicability of manual collection. Hence, till the female produced semiochemical is identified and isolated, capturing males using female-baited traps represents an important strategy for the management of white grubs.

From a practical perspective, the females of *L. lepidophora* could be used as a source of attraction for males in an IPM. Therefore, future research should aim to isolate, identify and synthesize the *L. lepidophora* pheromone, which could be incorporated into traps for monitoring and/or control of this pest.

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