

Factors influencing the burrowing behaviour of adult *Leucopholis lepidophora* Blanchard (Coleoptera: Scarabaeidae) under laboratory conditions

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Summary

The present study investigates the burrowing behaviour of the arecanut white grub, *Leucopholis lepidophora* was conducted in the laboratory. Mated adult female beetles were observed to burrow deeper than male and virgin female beetles. Male, virgin female, and mated female burrows were significantly deeper in wet soil compared to dry soil. Sandy loam soil recorded a lower percentage of beetles on the soil surface at 210 minutes when compared to 2 minutes. But in case of gravel soil, a higher percentage of beetles remained on the surface at 210 minutes compared to at 2 minutes, where no beetle was burrowed into to soil. The various soil characteristics, including soil texture and moisture, play a major role in the burrowing behaviour of adult beetles.

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Introduction

Arecanut (*Areca catechu* L.) has been cultivated as a commercial crop in the Asian continent. Arecanut is susceptible to a wide range of 102 insect and non-insect pests at various stages of its growth, either in the soil or on the plant, throughout the year. These pests are a major constraint to its production (Nair & Daniel 1982). Among the insect pests, white grubs are the most serious threat to arecanut in the Malnad and coastal belt of Karnataka (Veeresh et al. 1982). Three species of *Leucopholis* are pests of arecanut during their larval stage. The key species in this genus include *Leucopholis coneophora*, *L. lepidophora*, and *L. burmeisteri* (Muthusamy et al. 2017; Mahadeva Swamy et al. 2019). Kalleshwaraswamy et al. (2015) reported that *L. lepidophora* caused 27.86–36.97 per cent damage to arecanut, resulting in a yield reduction of 39.79–41.60 per cent in various districts of the hilly regions of Karnataka. Continuous feeding by white grubs on the roots causes yellowing of the leaves, a reduction in crown size, stem tapering, decreased inflorescence production, premature nut fall, lower nut yield, and overall loss of vigor, all of which significantly impact the productivity of arecanut palms (Nair & Daniel 1982; Kumar 1999; Padmanabhan & Daniel 2003). Palms that are severely damaged by grubs can be easily uprooted with a slight pull, as the grubs destroy the entire root system (Nair & Daniel 1982; Kumar 1999; Adarsha et al. 2018; Haldhar & Maheshwari 2021).

The white grub *L. lepidophora* is a soil-dwelling insect which spends most of its life cycle in the soil, and adult beetles spend a short period above ground for mating

(Adarsha et al. 2022). Adult beetles emerge from the soil for mating between 6:30 to 7:30 p.m., during June to October (Kumar 1999; Channakeshava 2006; Kalleshwaraswamy et al. 2016; Adarsha et al. 2019; Haldhar et al. 2023a). Several males were approaching the female for mating with buzzing sounds. After copulation, the males returned to the soil, while the females remained on the surface for a few minutes before flying down to the soil to lay their eggs. Most unmated males also returned to the soil shortly after the evening's active flight period (Adarsha et al. 2023). After mating, there is a noticeable change in the burrowing behaviour of the mated female. However, studies on the burrowing behaviour of *L. lepidophora* were not attempted in India. Therefore, in-depth ecological studies have been carried out to investigate the burrowing behaviour of adult beetles.

Materials and Methods

Laboratory studies were carried out at the Department of Entomology, College of Agriculture, Shivamogga. Soil was collected from the white grub-infested arecanut garden and placed in a plastic container (30 cm height x 18.50 cm diameter) (Plate 1). Male and female beetles were collected from the field during evening hours from 18:30 to 20:30. These beetles were placed individually in a plastic container and transferred to the laboratory for further studies. Virgin, mated females and males were placed individually in each container during evening hours of the day, and then the top of the plastic container was closed by using wire mesh to prevent of beetles. Observations were recorded with respect to depth of burrowing and head direction (downward, upward, and horizontal direction) of each beetle on every morning (10:00 to 11:00 hours) by slowly removing soil from the top. The data were then statistically analyzed using t-test.

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Plate 1. Plastic container used to observe the burrowing behaviour of *L. lepidophora* adults in soil

Effect of soil moisture on burrowing behaviour

Virgin males and females were placed separately in plastic containers containing dry soil (soil moisture 17%) or moist soil (soil moisture 25%) during late evening. Observations were recorded on the depth of burrowing and head direction (downward, upward, and horizontal direction) of each beetle on every morning (10:00 to 11:00 hours) by slowly removing soil from the top.

Effect of soil properties on burrowing behaviour of virgin males

Glass-framed boxes were constructed to study the effect of different types of soil properties (gravel soil and sandy loam soil) on the burrowing behavior of adult

beetles of *L. lepidophora*. Gravel and sandy loam soil were collected from a white grub-infested field. Two glass-framed boxes of 40 cm height × 2 cm width × 40 cm length, which were divided into eight blocks. Virgin males were introduced on the soil surface of each block of the box. Observations were recorded on a number of individuals remaining on the soil surface for every two minutes for the first 30 minutes and then for every 30 minutes for about 10 hours. The trial was repeated four times for each soil type with different individuals (Plate 2).

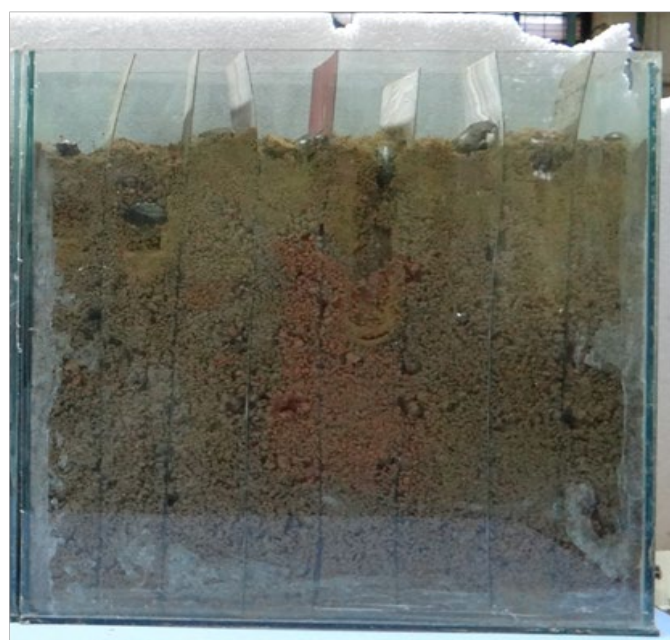


Plate 2. Glass-framed box used to observe the effect of soil properties on the burrowing behaviour of adult *L. lepidophora* A. Gravel soil B. Sandy loam soil

Results and Discussion

During 2015, male beetles burrowed to a depth of 8.11 ± 4.59 cm, ranging from 0.50 to 15.30 cm (mean $\pm$ SD, $N=30$). Average depth burrowed by virgin female was 11.20 ± 6.93 cm ($N=30$), which varied from 1 to 15.30 cm. However, mated females burrowed on an average of 19.34 ± 5.37 cm depth, which ranged from 6.90 to 25.6 cm. Here, mated females burrowed deeper than any of the above beetles. On the other hand, there was a significant difference among all three groups of beetles

(males versus virgin females, t test: $t=2.03$, $P<0.05$; males versus mated females, $t=7.30$, $P<0.05$; virgin females versus mated females, $t=3.98$, $P<0.05$). The head directions of burrowed adult beetles are presented in Table 1. Results indicated that 80 per cent of males, 56.66 per cent of virgin females, and 53.33 per cent of mated females directed their heads upward. However, 20 per cent of males, 43.33 per cent of virgin females, and 46.66 per cent of mated female beetles were found with the head facing downward.

Table 1. Burrowing behaviour of *L. lepidophora* adult beetles under laboratory condition during 2015

Particulars	Depth (cm)		Head direction (%)	
	Mean $\pm$ SD	Range	Downward	Upward
Males*	8.11 ± 4.59	0.5 to 15.30	20	80
Virgin females*	11.20 ± 6.93	1 to 15.30	43.33	56.66
Mated females#	19.34 ± 5.37	6.9 to 25.6	46.66	53.33

* $N=30$; # $N=15$

Particulars	t value	P value
Males vs Virgin females	2.03	*
Males vs mated females	7.30	*
Virgin females vs Mated females	3.90	*

* Significant at $P<0.05$

Similar behaviour was observed during 2016, where in mated females burrowed more deeply, 18.03 ± 8.34 cm, which differed significantly from other types of beetles, which ranged from 3.50 to 30.70 cm, $N=50$. This was followed by virgin females, which burrowed to 10.72 ± 5.70 cm depth with a range from 3 to 24.60 cm ($N=93$) and males with a depth of 10.5 ± 4.55 cm and range between 1 to 18.90 cm ($N=40$). However, mated females were observed to burrow deeper than male and virgin female beetles. Similarly, there was no statistical difference observed between males versus virgin females (t test: $t=0.21$ $P>0.05$). However, a significant

difference was noticed between males versus mated females ($t=5.12$, $P<0.05$) and virgin females versus mated females ($t=6.18$, $P<0.05$) (Table 2). Perusal of Table 2 indicated that variation in the head directions of adult beetles in soil. A higher number of male beetles (67.5 %), virgin females (79.57 %), and mated females (74 %) directed their heads upward. However, 30 per cent of males, 20.43 per cent of virgin females, and 26 per cent of mated female beetles were found with their heads facing downward. Only 2.50 per cent of males showed a horizontal position of the head.

Table 2. Burrowing behaviour of *L. lepidophora* adult beetles under laboratory conditions during 2016

Particulars	Depth (cm)		Head direction (%)		
	Mean $\pm$ SD	Range	Downward	Upward	Horizontal
Males*	10.5 ± 4.55	1 to 18.9	30	67.5	2.5
Virgin females**	10.72 ± 5.70	3 to 24.60	20.43	79.57	-
Mated females#	18.03 ± 8.34	3.5 to 30.7	26	74	-

* $N=40$; ** $N=93$; # $N=50$

Particulars	t value	P value
Males vs Virgin females	0.21	NS
Males vs mated females	5.12	*
Virgin females vs Mated females	6.18	*

NS=Non significant; *Significant at $P<0.05$

During the two-year study period, both males and virgin females were observed to stay close to the surface because they emerge again for mating. Mated females burrowed deeper than males and virgin females, as deep as >25 cm in the soil overnight. Most of the mated females did not reemerge from the soil. This indicated that they may move still deeper up to 90 cm (Kumar

1999). However, a few beetles may be searching for another oviposition site. In the case of *D. ishigakiensis* as reported by Harano et al. (2010), mated females burrowed deeper than males and virgin females. It is important to determine how deep they burrow to know the depth at which eggs are laid and the hatched larvae cause damage to the arecanut roots. Variation in head

direction was observed during burrowing. Most of the beetles were found facing heads upward. It may indicate completion of burrowing, and they were ready for emergence. Some of the beetles directed their heads downward and horizontally. Beetles with downward-facing heads indicated that they are still in the process of burrowing.

Effects of soil moisture on burrowing behavior of adult beetles

Results (Table 3) indicated that males burrowed deeper (10.54 ± 3.94 cm) in wet soil compared to dry soil (0.65 ± 0.88 cm). Male burrows significantly deeper into wet soil (t test: $t=10.95$, $P<0.05$, $N=20$), but in the case of dry soil, it did not go deeper than 0.65 cm. A similar trend was observed in both virgin and mated females. Virgin females burrowed to a depth of 11.55 ± 5.69 cm in

wet soil and 0.81 ± 0.88 cm in dry soil. Similarly, there was a significant difference among virgin females in wet soil versus dry soil ($t=8.34$, $P<0.05$, $N=20$). Mated females recorded 10.60 ± 5.10 cm depth in wet soil, which was deeper than in dry soil (0.78 ± 1.2 cm). On the other hand, mated females burrowed significantly deeper in wet soil compared to dry soil ($t=8.38$, $P<0.05$, $N=20$). In wet soil, 75 per cent of males, 85 per cent of virgin and mated female beetles were facing heads upward. However, the remaining per cent of adult beetles directed their heads downward. In dry soil, most of the males and virgin females directed their heads downward (25 and 35 %, respectively) except mated females (5 %). Whereas, males and virgin females (20 % each) and mated females (35 %) directed their heads upward.

Table 3. Effect of soil moisture on burrowing behaviour of arecanut white grub adult beetles

Particulars	Wet soil			Dry soil				
	Depth (cm)	Head direction (%)		Depth (cm)	Head direction (%)			
	Mean±SD	Downward	Upward	Mean±SD	Downward	Upward	t value	P value
Males	10.54±3.94	25	75	0.65±0.88	25	20	10.95	*
Virgin females	11.55±5.69	15	85	0.81±0.88	35	20	8.34	*
Mated females	10.60±5.10	15	85	0.78±1.2	5	35	8.38	*

$N=20$; * Significant at $P<0.05$

In case of *D. ishigakiensis*, virgin and mated males, as well as virgin and mated female beetles of *D. ishigakiensis* burrowed deeper in moist soil than dry soil (Harano et al. 2010). The present study indicated that soil moisture also influences the depth to which beetles reach. In dry soil, males, virgin and mated females stay within 0.81 cm of the soil surface. Beetles burrowed deeper in wet soil compared to dry soil, but virgin and mated female beetles stayed relatively shallow. It clearly indicates that soil moisture also plays the main role in the burrowing behavior of the adult beetles.

Effect of soil properties on burrowing behaviour of males

Results indicated that sandy loam soil recorded less percentage of beetles (31.25 %) on the soil surface after

210 minutes, when compared to after 2 minutes (84.38 %). But in the case of gravel soil, more percentage of beetles (59.37 %) remained on the surface even after 210 minutes (Table 4).

In case *D. ishigakiensis*, soil properties and topography influence the burrowing behaviour and the depths that the beetles reach (Harano et al. 2010; Haldhar et al. 2023b). The present study showed that burrowing behavior is greatly influenced by soil properties. It may be due to sandy loam soil facilitating easy movement for beetles to push their body downwards. Gravel soil, which contains mineral stones/coarse particles which obstructs the movement of beetles compared to sandy loam soil.

Table 4. Effect of soil properties on burrowing behaviour of arecanut white grub adult male beetles[#]

Particulars	Per cent*														
	2 min	4 min	6 min	8 min	10 min	12 min	14 min	16 min	18 min	20 min	22 min	24 min	26 min	28 min	30 min
Sandy loam soil	84.38	78.13	68.75	68.75	68.75	68.75	53.13	50.00	50.00	43.75	43.75	43.75	40.63	40.63	40.63
Gravel soil	100.00	96.88	84.38	81.25	75.00	75.00	75.00	75.00	75.00	71.88	71.88	71.88	71.88	71.88	71.88
	30 min	60 min	90 min	120 min	150 min	180 min	210 min								
Sandy loam soil	40.62	37.50	34.37	31.25	31.25	31.25	31.25								
Gravel soil	71.87	65.62	65.62	62.50	59.37	59.37	59.37								

[#] $N=32$; *Males beetles remaining of male beetles on the soil surface

The current study demonstrates that *L. lepidophora* adult beetles exhibit distinct burrowing preferences based on soil texture and moisture. Mated female beetles burrow deeper than virgin males and females, with both mated and virgin females showing a preference for deeper burrows in wet soil compared to dry soil. Furthermore, sandy loam soils are preferred over gravel soils for burrowing, likely due to the favorable texture, moisture retention, and aeration properties of sandy loam. These findings contribute to a better understanding of the factors influencing beetle burrowing behaviour and habitat selection.

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Declaration of interests

The authors do not have any conflict of interest, and the research was conducted in the absence of any commercial relationship with anyone.

Data Sharing

All relevant data are within the manuscript and are available from the first author.

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