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Biology and morphometrics of the most predominant root grub species, *Holotrichia reynaudi* in groundnut crop in Andhra Pradesh

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Abstract
Holotrichia reynaudi was the predominant species of root grub collected from the groundnut growing areas of the Rayalaseema region of Andhra Pradesh. Studies on the biology of root grub, *H. reynaudi* in groundnut was carried out under laboratory conditions from June to December in 2014 and 2015 in the Department of Entomology, Agricultural Research Station, Utukur, Kadapa. The studies revealed that each female laid 18.68 ± 3.77 eggs with 76.95 ± 10.55 per cent hatchability. Incubation period was 11.16 ± 0.43 days. The duration of I, II and III instars were 15.40 ± 0.52, 17.36 ± 0.36 and 33.84 ± 0.55 days, respectively. Total grub period was 66.62 ± 0.48 days. Pupation occurred in earthen cell and pupal period was observed as 17.06 ± 0.46 days. Male and female adult duration were observed as 18.61 ± 1.28 and 21.38 ± 1.05 days, respectively. The total life cycle from egg to adult lasts for 114.81 ± 1.16 days under laboratory conditions. The average mating period was recorded as 48 minutes and the average sex ratio of male to female was 1:1.30.

Keywords: Andhra Pradesh, Biology, Groundnut, *Holotrichia reynaudi*, Morphometrics, Root grub

Introduction
Groundnut (*Arachis hypogaea* L.) belongs to the Fabaceae family and is a principal oilseed crop of India. It is cultivated in 4.68 million hectares with the production of 6.56 million tons and productivity of 1400kgha^{-1}was recorded in 2014-15 (www.agricoop.nic.in, 2016) [1]. In Andhra Pradesh, groundnut is cultivated in 8.74 lakh hectares of area with 4.93 lakh tons of production with a productivity of 564 kg ha^{-1}(www.apdes.ap.gov.in, 2014-15) [2]. Of this groundnut is cultivated in an area of 8.32 lakh hectares in Rayalaseema alone. The soil dwelling insects, which are the root feeders in groundnut ecosystem result in huge yield losses than foliage feeders. Soil insects are difficult to manage because farmers usually do not know about their occurrence until plants die (or) damage is noticed (or) until the crop is harvested. One of the most important soil pests affecting groundnut is root grub. *Holotrichia reynaudi* and was the predominant species of groundnut growing areas of the Rayalaseema region of Andhra Pradesh (Sunil Kumar *et al*.,) [3]. Root grub cause damage by feeding on the roots and underground parts of the plant from one to several inches below the soil surface. In endemic areas of root grub the damage to groundnut ranges from 20-100%. The presence of one grub/m^2 may cause 80-100% mortality (Yadava and Sharma, 1995) [4], it causes damage upto 39.40% (Umeh *et al*., 1999) [5], 12-60% (Pokhrel, 2004) [6]. Biology of the root grub, *H. reynaudi* should be thoroughly known for development of management practices. A very little attention has been paid for studying biology and morphometrics of *H. reynaudi* in the Southern Andhra Pradesh. Hence, studies on biology and morphometrics of *H. reynaudi* were carried out at Agricultural Research Station, Utukur, Kadapa, Andhra Pradesh.

Material and Methods
Studies on biology and morphometrics of root grub, *H. reynaudi* was carried out during June to December, 2014 and 2015 under laboratory conditions at mean temperature of 25± 2 ºC and relative humidity of 75 ± 2 per cent in the Department of Entomology, Agricultural Research Station, Utukur, Kadapa. The adult beetles of *H. reynaudi* collected from the light trap of ARS, Utukur were brought to the laboratory in the plastic containers with perforated lids containing moist soil. Pneumatic rearing troughs of 35 cm diameter and 15 cm depth was filled

with moist sand upto 10cm soil and was placed in aluminium rearing cages of 75cm × 45cm × 45cm, small twigs of neem were provided as food and fresh twigs were replaced every day. Ten pairs of morphologically identified male and female beetles were released into the 10 cages for mating and observed for oviposition. Starting time of mating and ending time of mating for each pair was recorded separately to know the mating duration. After mating, every day the soil in the trough was examined for eggs laid in the soil of particular cage. Immediately after egg laying, eggs were transferred to petridishes.

The eggs collected from the rearing cages were transferred to petridishes (9cm diameter) filled with fine sand kept moist by filter papers. Egg period was recorded from the day of egg laying to emergence of neonate grub from the egg. The length and width of twenty eggs immediately after egg laying and before hatching were measured with the help of ocular and stage micrometer.

The freshly hatched grubs from eggs were transferred immediately into petridishes (9 cm diameter) containing a mixture of 1:1 sand and organic manure. Care was taken to maintain moisture in the petridish containing soil mixture. The petridishes were examined daily to see if the grubs had moulted. The second instars were then transferred to small pneumatic troughs of 10cm diameter filled with moist soil and organic matter 1:1 ratio and covered with muslin cloth. Pearl millet seeds were sown in these troughs to provide root material for the grubs as a feed. In each trough five second instar grubs were released and observed in alternate days for moulting.

After moulting, 3rd instar grubs were transferred to large pneumatic troughs of 35 cm diameter and 15cm depth, 75 percent of the trough was filled with sand and organic matter mixture and pre-sown pearl millet. Two grubs were released into each trough to avoid overcrowding. Pearl millet seeds were sown at every 3-5 days interval to ensure uninterrupted supply of root material to the voracious 3rd instar larvae. The 3rd instar grubs were left in the same jars for pupation. Care was taken to see that the jars were kept moist at all the time.

The morphometrics of first two instar grubs *viz.*, length, width of the body and width of the head capsule were recorded with the help of ocular and stage micrometers. Similar observations were made for third instar and were measured with the help of a graph sheet and scale. For each observation, twenty specimens were considered and duration of all the three instars were recorded for all the 20 specimens. Mean and standard deviation were calculated for all the parameters.

Pupal period *i.e.*, from the day of pupation to the day of adult beetle emergence was recorded on a sample of 20 pupae. The length and width of 20 pupae were measured with the help of graph sheet and scale.

As soon as the pupae turned to adults, they were transferred to rearing cages containing fresh neem twigs in the cages as a source of food material. Adult longevity in laboratory conditions were recorded for 20 specimens. The body length, width were measured with the help of graph sheet and scale. All the morphological characters and male genitalia were studied to describe the species.

To determine the sex ratio, pupae of known number were maintained in the rearing cages with moist sand. As soon as the adults emerged in each cage (sample) the beetles were categorized into sexes and male genitalia extraction was done based on the need. The sex ratio between male and female was taken as an average of all the cages which have been calculated separately.

Results and Discussion

Pooled data of biology and morphometrics of root grub *H. reynaudi* in groundnut carried out during *kharif*, 2014 and 2015

Studies on the biology and morhphometrics of root grub, *H. reynaudi* were carried out from June to December, 2014 and from June to December, 2015 under laboratory conditions with a mean temperature of 25 ± 2 ºC and relative humidity of 75 ± 2 per cent.

Pooled data of two years i.e., *kharif*, 2014 and 2015 on duration, morphometrics of different life stages of root grub, mating time, fecundity, per cent hatchability and sex ratio are presented under different headings.

I. Egg

Freshly laid egg was pearly white in colour, oval and measured on an average of 2.99 ± 0.08 mm in length and 1.71 ± 0.03 mm in width. Egg became spherical before hatching and measured about 2.89 ± 0.05 mm in width and 3.73 ± 0.07 mm in length, respectively (Table 1). The pooled mean of incubation period of root grub, *H. reynaudi* in groundnut was 11.16 ± 0.43days, the minimum and maximum incubation period observed was 10.25 and 11.75 days, respectively under laboratory conditions (Table 2).

Mated female laid eggs singly in the moist loose soil at a depth of 5 to 10 cm, and egg laying was mostly done during early hours of day time. Egg laying continued upto 8 to 10 days leaving 3 to 5 days gap between two egg laying days. The pooled mean of fecundity was recorded as 18.68 ± 3.77 with a minimum and maximum number of 6 and 22.5 eggs, respectively under laboratory conditions. Average per cent of egg hatching was 76.95 ± 10.55% with a maximum and minimum of 55.50 and 92.50%, respectively (Table 2).

Incubation period and morphometrics are in close agreement with the findings of Anitha (1997) [7] who reported that duration of egg of *H. reynaudi* in groundnut was 11.5 days on an average and freshly laid egg measured about 3 mm in length and 1.78 mm in breadth and egg before hatching measured about 2.96 mm in diameter and 3.67 mm in length.

The fecundity and per cent hatching of root grub, *H. reynaudi* is first time reported in this paper.

II. Grub stage

A. First instar grub

The newly hatched neonate grub was creamy white in colour, head is light brown in colour, which later on becomes dark brown in few hours and grubs became active in about 3.5 to 5 hours. First instar grub feed on the organic matter supplemented in the petridish. First instar grub measured on an average of 14.86 ± 0.07 mm in length, 3.54 ± 0.05 mm in width and 1.98 ± 0.07 mm width of the head capsule (Table 1). The present results are comparable with the findings of Anitha (1997) [7], who reported that the first instar grub average length was 14.90 mm and width was 3.50 mm.

The pooled average duration of first instar grub under laboratory conditions was observed as 15.40 ± 0.52 days with a minimum and maximum of 14.0 and 16.0 days respectively (Table 2). The present results are in close agreement with the findings of Anitha (1997) [7] who reported that the average duration of first instar grub was 15 to 16 days.

B. Second instar grub

The second instar grub was dirty white in colour, the abdominal segments became more swollen and darker than first instar grub. The average length, width of grub and width of the head capsule of second instar grub was 21.62 ± 0.24 mm, 5.5 ± 0.12 mm and 3.04 ± 0.10 mm (Table 1). The reports of Anitha (1997) [7] confirm the present observations of second instar grub and are 21.7 mm in length and 5.5 mm in width and head capsule width being 3.1mm.

Pooled mean duration of second instar grub under laboratory conditions was 17.36 ± 0.36 days with a range of 16.5 to 18.0 days (Table 2). This observation is in conformity with those made by Anitha (1997) [7] who recorded an average second instar grub period as 17.5 days with the minimum and maximum being 15 and 20 days, respectively.

C. Third instar grub

The third instar grub was dirty white in colour, active root feeder, with strong and powerful mandibles. The thoracic segments are distinct, the fore legs shorter, the hind legs longer and the middle pair in between. The pooled average length, width and width of the head capsule was 40.65 ± 0.43mm, 8.21 ± 0.21 mm and 4.91 ± 0.22 mm respectively (Table 1). The present results are in close agreement with the findings of Anitha (1997) [7] who reported that average length, width of gruband width of its head capsule was 40.60 mm, 8.50 mm and 5.0 mm respectively in the third instar grub of *H. reynaudi* in groundnut.

The pooled mean of third instar grub duration was ranged between 33.25 and 34.75 days with an average duration of 33.84 ± 0.55 days (Table 2). These observations are in accordance with those of Anitha (1997) [7] who reported the average duration of third instar grub was 34 days with a minimum and maximum of 33 and 35 days respectively.

D. Total grub period

The pooled mean of total grub period from first instar to third instar grub was ranging from 66.0 to 67.38 days with an average duration of 66.62 ± 0.48 days (Table 2). These observations of present study are in close conformity with the observations recorded by Anitha (1997) [7] who reported the average total grub period as 67 days for *H. reynaudi* in groundnut under laboratory conditions.

IV. Pupal stage

The fully grown third instar grub burrows deep into the soil to the bottom of the jar and stops feeding. It prepared a small earthen cell in which they lay in a semi-circular fashion and pupated within earthen cell. The pupa was exarate and it measured about 21.29 ± 0.26 mm in length and 8.51 ± 0.21 mm in width (Table 1). The pupal period lasted for 16.50 to 17.75 days with an average of 17.06 ± 0.46 days under laboratory conditions (Table 2). Pupae did not survive when the earthern cell was damaged.

V. Adult stage

Adults were emerged from the pupae of root grub during the October and November months of 2014 and 2015 under laboratory conditions.

A. Male adult

The freshly emerged adults were brick red in colour and dark brown in four to five weeks. Males are slightly smaller than the females in size and hind tibial spur was pointed. The pooled mean length, width of adult male and width of the head capsule were 20.0 ± 0.47 mm, 10.17 ± 0.3 mm and 3.23 ± 0.19 mm respectively (Table 1). The present findings are in accordance with those of Jini Jacob (2010) [8] who reported that the *H. reynaudi* adult male morphometrics as 19.59 ± 0.49 in length, 10.05 ± 0.32 in width and 3.21 ± 0.29 mm width of the head capsule.

The pooled average duration of adult male was 18.61 ± 1.28 days with minimum and maximum duration was 16.5 and 20.0 days, respectively under laboratory conditions (Table 2). The results of present studies are in close agreement with the findings of Anitha (1997) [7] who reported that average duration of male adult was 18.75 days with a minimum and maximum of 16 to 20 days under laboratory conditions.

B. Female adult

Elytrae of freshly emerged beetles were brick red in colour and became dark brown in a month. Female adult was slightly bigger than male adult in size and hind tibial spur was broad. It measured about an average of 21.51 ± 0.48 mm in length, 11.05 ± 0.25 mm in width and 3.14 ± 0.15 width of the head capsule (Table 1). The present results are in conformity with that of Jini Jacob (2010) [8] who reported that the average length of female adult of *H. reynaudi* was 21.93 ± 0.35 mm, width was 10.35 ± 0.51 mm and width of the head capsule was 3.19 ±0.17

Female adult of *H. reynaudi* lasts for 18.75 to 22.5 days with a pooled mean of 21.38 ± 1.05 days (Table 2). The present findings are in accordance with Anitha (1997) [7] who reported the average duration of female adult was 21.25 days with a range of 17 to 22 days under laboratory conditions.

VI. Total life cycle

The pooled total life cycle from egg to adult lasts for 112.00 to 116.88 days with an average of 114.81 ± 1.16 days (Table 2).The present observations are in conformity with that of Anitha (1997) [7] who reported that total life cycle lasts for 114.96 days with minimum and maximum of 111 to 118 days for completing one life cycle from egg to adult in *H. reynaudi* in groundnut.

VII. Mating period

Morphologically identified male and female beetles were released into the cages for mating, female attracted male adult possibly by pheromonal action. Mating took place by inserting adeagus into the female genital chamber through female gonopore and spermatophores were released. During copulation initially male holds female just behind the head latter on male hangs freely down ward in upside down position. The pooled data of mating period revealed that mating started at 18.48 hours and disrupted at 20.08 hours with a pooled average mating period of 0.48 hours with a range of 0.43 to 0.52 hours (Table 2). Mating period of *H. reynaudi* was not reported by any researchers. The results of present investigation are in close agreement with that of Bhawane *et al.* (2011) [9] who reported the mating behavior of *H. karschi* and its mating period as 0.40 hrs to 0.60 hrs which was found to be nearer to that of *H. reynaudi.* However the sex ratio may vary among species and depends on the environment, habitate etc., and further research has to be carried out.

VIII. Sex ratio

The pooled data of sex ratio of two years revealed that among

the 152 pupae observed, 66 male and 86 female adults of *H. reynaudi* emerged with an average sex ratio of 1:1.30 (Table 4) which indicates that more females are emerged than males. The present results of sex ratio are in slight deviation with the Anitha *et al*. (2006) [10] who reported that the sex ratio of male and female *H. reynaudi* in groundnut was 1:1. The slight deviation in the sex ratio might be due to environmental effect or due to region or habitat changes.

Table 1: Pooled data of morphometrics of the life stages of root grub, *Holotrichia reynaudi* in groundnut carried out during *Kharif*, 2014 and 2015

Sl. No.	Stage of the insect	Minimum (mm)	Maximum(mm)	Mean (mm)	Standard deviation (±)
1	Egg				
	a) Length	2.81	3.12	2.99	0.08
	b) Width	1.67	1.78	1.71	0.03
2	Egg before hatching				
	a) Length	3.51	3.84	3.73	0.07
	b) Width	2.81	2.98	2.89	0.05
3	I instar lava				
	a) Length	14.73	14.97	14.86	0.07
	b) Width	3.43	3.61	3.54	0.05
	c)Width of the head capsule	1.93	2.12	1.98	0.07
4	II instar lava				
	a) Length	21.2	21.95	21.62	0.24
	b) Width	5.3	5.8	5.5	0.12
	c)Width of the head capsule	2.87	3.2	3.04	0.10
5	III instar lava				
	a) Length	39.7	41.3	40.65	0.43
	b) Width	7.75	8.55	8.21	0.21
	c) Width of the head capsule	4.55	5.25	4.91	0.22
6	Pupa				
	a) Length	20.95	21.7	21.29	0.26
	b) Width	8.1	8.85	8.51	0.21
7	Adult male				
	a) Length	19.3	20.95	20.0	0.47
	b) Width	9.6	10.75	10.17	0.30
	c) Width of the head capsule	3.0	3.6	3.23	0.19
8	Adult female				
	a) Length	20.6	22.3	21.51	0.48
	b) Width	10.7	11.55	11.05	0.25
	c) Width of the head capsule	2.85	3.35	3.14	0.15

Average of 20 individuals

Table 2: Pooled data of duration (days) of different developmental stages of root grub, *Holotrichia reynaudi* in groundnut of *Kharif*, 2014 and 2015.

Sl. No.	Stage of the insect	Minimum (days)	Maximum (days)	Mean (days)	Standard deviation (±)
1	Egg incubation period	10.25	11.75	11.16	0.43
2	Grub period				
	a. I instar	14.0	16.0	15.40	0.52
	b. II instar	16.5	18.0	17.36	0.36
	c. III instar	33.25	34.75	33.84	0.55
	Total grub period	66.0	67.38	66.62	0.48
3	Pupal period	16.5	17.75	17.06	0.46
4	Adult longevity				
	a. Male	16.50	20.0	18.61	1.28
	b. Female	18.75	22.50	21.38	1.05
	Average	19.13	21.25	19.99	0.70
5	Total life cycle	112.0	116.88	114.81	1.16
6	Fecundity (No.)	6	22.5	18.68	3.77
7	Percent hatching (%)	55.50	92.5	76.95	10.55

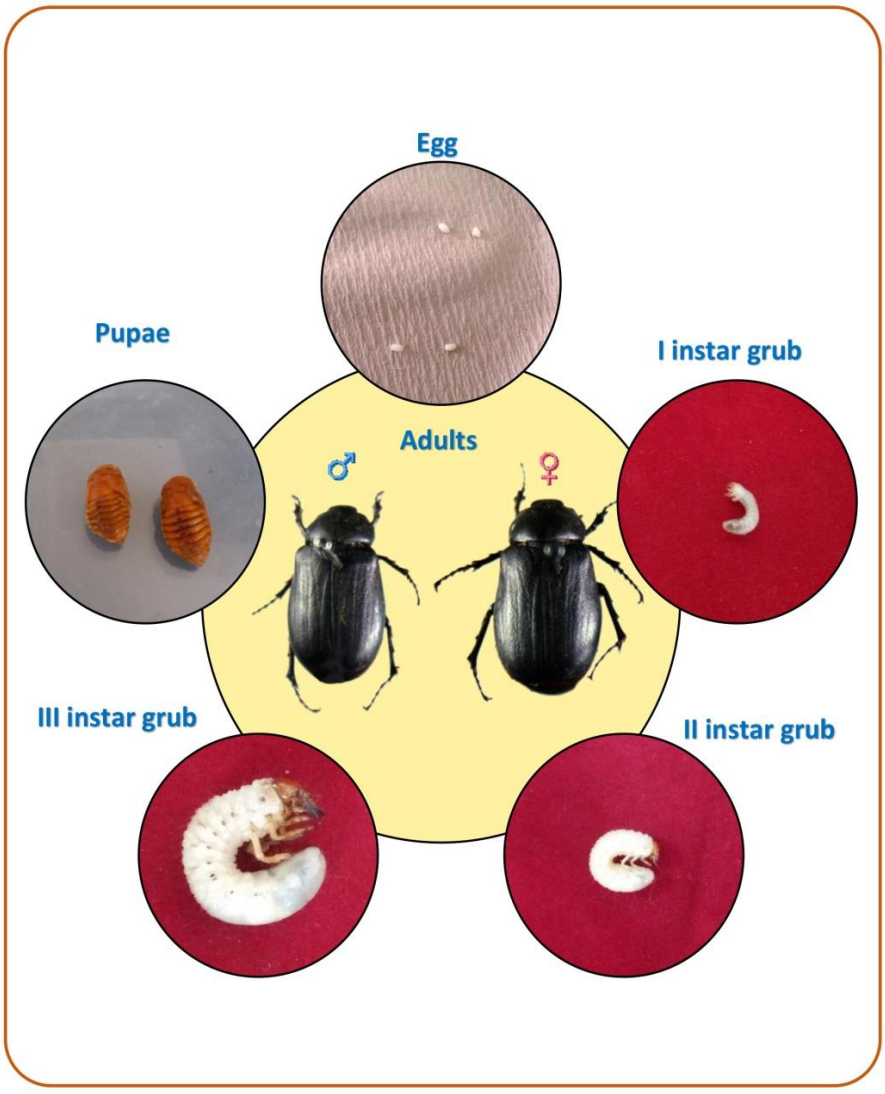
Average of 20 individuals

Table 3: Pooled data of duration of mating period of root grub *Holotrichia reynaudi* in *Kharif*, 2014 and 2015

Sl. No.	Starting time of mating (hr:min)	Completion time of mating (hr:min)	Duration of mating (hr:min)
1	19.10	20.02	0.52
2	19.11	19.58	0.47
3	19.20	20.08	0.48
4	19.22	20.08	0.46
5	19.05	19.54	0.49
6	19.03	19.50	0.47
7	19.08	19.51	0.43
8	19.09	20.01	0.52
9	18.48	19.38	0.50
10	19.04	19.48	0.44
Average			0.475

Table 4: Pooled data of sex ratio of the root grub, *Holotrichia reynaudi* observed in *Kharif*, 2014 and 2015

Sl. No.	Number of pupae observed	Male	Female	Sex ratio
1	15.5	6.5	9.0	1:1.38
2	16.0	6.5	9.5	1:1.46
3	21.0	9.5	11.5	1:1.21
4	12.5	6.0	6.5	1:1.08
5	14.0	5.5	8.5	1:1.55
6	18.0	9.0	9.0	1:1.00
7	14.5	6.0	8.5	1:1.42
8	13.0	5.5	7.5	1:1.36
9	11.0	4.5	6.5	1:1.44
10	16.5	7.0	9.5	1:1.36
Total	152.0	66	86	1:1.30



Life cycle of root grub, *Holotrichia reynaudi* in groundnut crop

Conclusion

Studies on the biology of the root grub, *H. reynaudi* revealed that each female laid 18.68 ± 3.77 eggs with 76.95 ± 10.55 percent of hatchability. Incubation period was 11.16 ± 0.43 days. The duration of I, II and III instars were 15.40 ± 0.52, 17.36 ± 0.36 and 33.84 ± 0.55 days respectively. Total grub period was 66.62 ± 0.48 days. Pupation occurred in earthen cell and pupal period was observed as 17.06 ± 0.46 days. Male and female adult duration were observed as 18.61 ± 1.28 and 21.38 ± 1.05 days respectively. The total life cycle from egg to adult lasts for 114.81 ± 1.16 days under laboratory conditions. The average mating period was recorded as 48 minutes and the average sex ratio of male to female was 1:1.30.

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