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RESEARCH ARTICLE

LABORATORY REARING OF ROOT GRUBS

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ABSTRACT

The root grubs *Holotrichia serrata* Fabricius, *Holotrichia consanguinea* Blanchard, *Holotrichia reynaudi* Blanchard, *Leucopholis lepidophora* Burmeister and *Anomala dimidiata* Hope ,were reared on sliced carrots under laabortory conditions. Ambient temperature of 26±1^{0}C and 65% RH with food availability ad-libitum provided for successful rearing of the grubs. Biological attributes varied among the different species and the grub stage had three instars irrespective of the species. The average life cycle of *L.lepidophora* and *A.dimidiata* was completed in 291.8 and 301.9 days, respectively as compared to the *Holotrichia* species, where the duration ranged between 110.2 to 218.3 days. *H.consanguinea* had the highest average longevity of 38.6 days followed by *H.reynaudi* with 32.4 days. Fecundity of the root grubs ranged from 30-56 eggs/female. The benefit of laboratory rearing for taxonomic delineation of grub species and bioassay studies are discussed.

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INTRODUCTION

The family scarbaeidae is the second largest family with more than 30,000 species recorded worldwide (Mittal, 2000). About 2500 species are reported from India (Pathania *et al.,* 2015) and a majority of these are phytophagous (sub families Melonthinae, Rutelinae, Dynastinae and Cetoninae) (Mittal, 2000, Ali, 2001). The adult beetles and their grubs cause extensive damage to fruit crops, vegetables, ornamental plants, plantation crops pastures, turf and meadow grasses, lawns, golf courses and forest trees (Chandra and Gupta, 2011., Dadmal *et al*.,2013). Adults of the sub-family Melolonthinae and Rutelinae are pre-dominantly leaf feeders (Lawrence *et al.,* 2000., Dashad *et al.,* 2008) where as those of Cetoniinae feed on flowers and fruits, and are popularly referred to as flower beetles, prefer nectar, sap or juice of ripening fruits and vegetables. Members of Dynastinae usually attack stems or roots of plants. Grubs of Melolonthinae, Rutelinae and Dynastinae commonly referred to as whitegrubs are often soil dwelling and cause extensive damage to the roots of cereals, legumes, small fruit plants, shrubs and trees (Anitha *et al*., 2006., Bhat *et al*., 2005., Thakare and Zade, 2012). In India, the white grubs are pests of national importance (Mehta *et al*., 2010, Bhawane *et al*., 2012). An authentic classification of species is a pre-requisite for research in ecology and biodiversity. Lack of taxonomic understanding has been a major impediment to the study and management of scarabaeid beetles due to their phenotypic variation within a single species (Miller and Allsopp, 2000). Proper identification of the species and knowledge of their distribution, geographical variation, population dynamics, feeding and reproductive behavior are the first steps in developing environmentally compatible/sustainable integrated pest management strategies, which can be successfully implemented and optimised when the species have been identified Accurate identification of scarabaeid larvae is essential for understanding larval species biology (e.g. soil type and depth they occur in and at, oviposition preferences and host plants) and ecology (Miller *et al.,* 1999). Identification of scarabaeid species is a challenging task due to variable morphological differences among species and delineation among the immature forms, the grubs and adults. Studying the morphological characters requires laboratory rearing of the insects to enable availability of the various life stages of the pest (grubs and adults) for precise

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taxonomic identity. Rearing of the grubs in to adults convincingly establishes the species associated and their delineation. Rearing of root grubs is a challenging task due to their prolonged lifespan and extended grub periods (65-70 days)(total life cycle (96-102 days). Earlier, rearing methodologies under *insitu* field conditions and under laboratory,with soil and rooted saplings, were reported (Anitha *et al*., 2006.,Cock and Allard, 2013., Danuta Wand and Lidia, 2014., Theurkar *et al*., 2013). Therefore, an effective and simple technique for rearing of root grubs under laboratory conditions was attempted.

MATERIALS AND METHODS

Field survey and collection of scarabaeid beetles

The diversity of scarab beetles depends on the availability of food for larvae and adult, weather conditions and soil type. Collection of scarab beetles was made randomly by hand picking and light trapping. Grubs were collected from a soil depth of 0.25-0.5 nm in cultivated fields. The beetles were collected during May- June.

Collection of adult beetles using light traps

Light traps were used for four months (May – September) to collect the beetle populations. The light traps were placed in the centre of the fields at a height of about 3 metre above the ground and operated between 7:00 PM to 5:00 AM to attract the scarabaeid beetles which are positively heliotactic in nature. The light trap comprised of PVC plastic funnel of 25 cm in height, and 30 cm. diameter. The bottom diameter of the funnel was 5 cm. The rain shed cone for protecting the bulb was fixed at 17 cm above the funnel with the help of three white metal sheets. The diameter of the rain shed cone was 20 cm. The light source consisted of a 125-watt incandescent light bulb with copper wire choke. The light trap had three baffles (30 cm x 10 cm), placed at a uniform distance of 10 cm around the circumference of funnel. The baffles were fixed to emit light uniformly in all directions without any interference, when the beetles are attracted to light they collide with baffles and fall into the trap. A nylon bag was attached to the bottom of this funnel for collection of beetles. The collected beetles were preserved in a vial containing 70% alcohol and taken to the laboratory for morphological identification.

Identification of the beetles

The scarab adults and grubs collected were identified up to the genus level at the Department of Entomology, University of Agricultural Sciences, Bangalore and the Division of Entomology, Indian Agricultural Research Institute, New Delhi, based on the keys and characters listed by (Veeresh, 1977, Mittal and Pajni, 1977. Khan and Ghai., 1982, Ahrens,2005, Sreedevi and Tyagi, 2013, Dittrich *et al.,* 2006). Adult beetles were identified based on the morphological characters such as body size, colouration, surface sculpture and male genitalia, while the grubs were differentiated based on the color, size of the cephalic capsule, number and form of dorsal sensorial maculae of the last antennomere, distribution, stridulatory structures in the maxilla and mandible , raster pattern arrangement of bristles and hairs on the underside of the abdomen, shape of anal slit (crescent, Y shaped, strongly Y shaped), shape and size of the respiratory plates, proportions of each pair of legs and tarsungulus size (Veeresh, 1977.,Chandel *et al.,* 2003, Dadmal *et al*., 2013., Dittrich *et al.,* 2009 and Ahrens *et al.,* 2011).

Rearing white grubs/root grubs

The field collected adult beetles were placed in large plastic containers containing organic matter and soil. The adult beetles were provided with neem leaves and allowed for mating and oviposition.

Plate 1 Rearing root grubs on sliced carrots

The eggs were collected by sieving with 5 mesh on paper trays, and then transferred to plastic crucibles containing moist soil. About 10 eggs were placed in each crucible and covered with another crucible of same size. After 4-5 days, the hatched grubs were transferred in to individual plastic containers filled with soil having adequate moisture and were provided with sliced carrots/potato, ad-libitum and changed every third day avoiding food and environmental stress during the rearing (Plate 1). Biological parameters such as, duration of egg, grub and pupa, adult longevity and fecundity were recorded. The pupae formed were again placed in plastic crucibles in a superficial pit made in the moist soil and covered with a similar crucible on top. Freshly emerged adults were transferred to large plastic containers containing organic matter and soil and the adult beetles were provided with neem leaves as food.

Rearing of root grubs

The technique of rearing as described was found congenial for the development of the root grubs when proper hygiene and soil moisture was maintained, with replenishment of food periodically. The ambient temperature of 26± 1^0C with 65% RH was optimum for the growth and development of the grubs. The results (Table 1) revealed that the root grubs *H. serrata, H. consanguinea, H. reynaudi, L. Lepidophora* and *A. dimidiata* could be effectively reared in plastic containers on sliced carrots placed in soil with adequate moisture, taking precaution to avoid stress due to non availability of food and moisture. The biological attributes varied with the pest species. The incubation period lasted for 12-15 days in *H. serrata, L.lepidophora.,* 7-13 days in *H. consanguinea* and 12-21 days in *A. dimidata*.

Table 1. Biology of root grubs reared on carrot slices under laboratory conditions *

Sl.No.	Root grub	Egg period	Grub period I	II	III	Total grub period	Pupal period	Egg to adult	Adult longevity	Fecundity (eggs/female)
1	*Holotrichia serrata*	12-15	24.5	36.2	126.8	187.5	15.8	218.3	23.8	56.0
2	*Holotrichia consanguinea*	7-13	17.5	34.6	52.2	104.3	14.6	131.9	38.6	49.0
3	*H.reynaudi*	10-12	16.2	18.4	35.5	70.1	18.1	100.2	32,4	30.0
4	*Leucopholis lepidophora*	12-15	76.6	43.6	141.3	261.5	15.3	291.8	24.3	34.0
5	*Anomala dimidiata*	12-21	16.5	40.1	208.2	264.8	16.1	301.9	34.5	38.0
CD at 0.05%			2.84	6.42	5.42		2.31	14.37	6.86	4.34
S.Em.			1.92	3.44	4.62		1.32	5.83	3.91	2.1

* Mean of 3 replications.

The rearing was carried out at 26± 1^0C, at 65% RH. The grub species reared were *Holotrichia serrata* Fabricius, *Holotrichia consanguinea* Blanchard, *Holotrichia reynaudi* Blanchard, *Leucopholis lepidophora* Burmeister and *Anomala dimidiata* Hope. Ten grubs of each species in three replicates were studied for their development on the diet. The observations were statistically scrutinised using ANOVA.

RESULTS AND DISCUSSION

Collection and identification of beetles

The collection of the beetles was restricted to the phytophagous group, belonging to the subfamilies Melolonthinae and Rutelinae. The populations were collected from various trees and crop plants (arecanut, coconut, groundnut, mulberry, millets, neem, soybean, sugarcane and vegetables). The collected adult beetles were identified based on the morphological characters up to the genus level. The beetles were distinguished based on antennae, mandibles, maxillae, size, colouration of surface, male genitalia and size of cephalic capsule. Identity of the grubs was based on the shape of the anal slit, raster pattern, arrangement of bristles and hairs (palida), spiracles and legs. The grubs of melolonthinae had a Y shaped anal slit with varying raster pattern (inverted V, lemon shaped and circular fashion) (Plate 2). In the rutelinids the anal slit is transverse (crescent shaped) with a triangular raster (Plate 3). The adult rutelinidae were prominently differentiated based on the tibial claws with two setae.

Irrespective of the species, the grub stage had three instars and the third instar in all the species studied had a prolonged duration (average 35.5 -208.2 days) than the first and second instars (Table 1, Plate 4). The average life cycle of *L.lepidophora* and *A.dimidiata* was completed in 291.8 and 301.9 days, respectively as compared to the *Holotrichia* species, where the duration of life cycle ranged from 110.2 to 218.3 days. *H.consanguinea* had the highest average longevity of 38.6 days followed by *H.reynaudi* with 32.4 days. Fecundity of the root grubs ranged from 30-56 eggs/female. The biological attributes observed in the different species of root grubs in the present studies under laboratory conditions are broadly in agreement with the reports of several workers (Gupta and Avasthy, 1956., Majumdar and Teotia.1965.,Garg and Verma, 1993., Lingappa and Girradi, 1995., Chandel *et. al*., 2003., 2013., Mathur *et al.,* 2010.,Mehta *et al*., 2010) who had studied the biology of the species of root grubs on different crops. Earlier, rearing of the root grubs was reported under *in-situ* field and *in-vitro* conditions. Pardo-Locarno *et al.,* (2005) reported rearing of root grubs on chopped carrots in disposable cups. Development on artificial diets of potato, groundnut and tree yam was reported (Yubak Dhoj *et al.,* 2008). Rearing of the grubs in earthen crucibles (AICRP on soil arthropods, Report 2014), oil drums filled with soil and stools of sugarcane planted and covered with mosquito nets (Cock and Allard, 2013)., reared on roots of two year old saplings of forest trees *Quercus petraea, Q. robur, Fagus sylvatica, Betula pendula, Larix decidua, Alnus glutinosa and Pinus sylvestris* (Danuta and Lidia, 2014), kept in pots filled with soil.

Plate 2. Anal slit and raster in Melolonthinae

Plate 3. Anal slit and raster pattern Melolonthinae in rutelinae

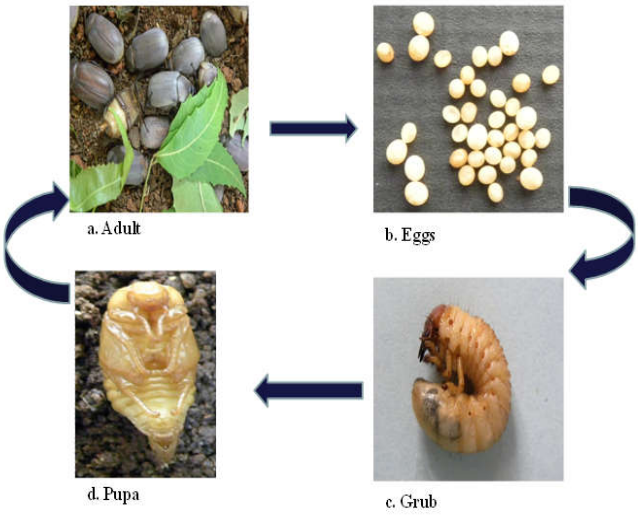

Plate 4. Life cycle of *Holotrichia serrata*

Rearing in net house providing rooted Pearl millet crop in a relay fashion as food for development (Anitha *et al.,* 2006). The methodologies adopted however were either unwieldy or protracted for critical observations on the morphology of the pest. Further, *in-situ* rearing under field conditions are subjected to the vagaries of environmental stress, leading to mortalities during the development and growth. Laboratory rearing therefore has an added advantage of optimised development that would enable availability of the species culture for taxonomic delineation of grub and adult stages, bioassays and breeding natural enemies to programme strategies for their management.

Conclusion

Root grubs *H.serrata. H.consanguinea, H.reynaudi, L.lepidophora* and *A.dimidiata* were successfully reared on sliced carrots placed in soil with adequate moisture at ambient temperature of 26$\pm$1^{0}C and 65% RH. All the species of the grubs studies had three instars and the life cycle was completed on an average of 100.2 to 301.9 days. The fecundity ranged from 30-56 eggs/female depending upon the species. Laboratory rearing provides for taxonomic species delineation and bioassay studies to strategise management programmes.

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