

Biology of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) on different stored products and its host associated genetic variability

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Abstract

The biology of sawtoothed grain beetle *Oryzaephilus surinamensis* (L.) was assessed on seven different stored products; viz., neem seed kernel, groundnut kernel, groundnut cake, rice, wheat, dates and raisin. Significant differences were observed with the larval to pupal development and total developmental period from egg to adult emergence on different stored products compared to egg period. Larval and pupal development was found to be very faster on neem seed kernel than on all other stored products. The developmental period from egg to adult emergence was shortest on neem seed kernel and longest on raisins. Random Amplified Polymorphic DNA (RAPD) markers were used to check for host associated variation in larval populations that were cultured on neem seed kernel and groundnut kernel and identified the presence of host associated genetic variations between them.

Keywords: sawtoothed grain beetle, *Oryzaephilus surinamensis*, neem seed kernel, ground nut kernels, genetic variability.

1. Introduction

The sawtoothed grain beetle, *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae), is one of the key stored grain pest which occurs worldwide (Rossiter et al., 2001; Hashem et al., 2012). They are secondary feeders and infest and establish on whole grains with minor cracks or mechanical lesions (Prickett et al., 1990). It can feed on various stored product commodities such as cereals, millets, flours, oil seeds, confectionaries, dried meat and fruits, bran, nuts etc., (Barnes, 2002; Bowditch and Madden, 1997). In addition *O. surinamensis* thrives well on neem seed kernel which has insecticidal properties (Sarup and Srivastava, 1971).

Although the biology of *O. surinamensis* has been studied by many authors (Howe, 1956; Jacob and Fleming, 1989) on various hosts, but detailed studies on the biology of *O. surinamensis* on neem seed kernel, groundnut kernel and groundnut cake is lacking. Hence, studies on the biology of *O. surinamensis* in neem seed kernel and other stored products were carried out under laboratory conditions. In addition, only a few studies have been carried with respect to host associated genetic variability in *O. surinamensis*. In the present study molecular techniques were employed to characterize the host associated genetic variation present in *O. surinamensis* reared on hosts viz., neem seed kernel and groundnut kernel using Random Amplified Polymorphic DNA markers. RAPD markers have been commonly used to assess geographically and host

associated genetic variability (Stewart and Excoffier, 1996) and to determine the genetic structure of populations of various stored product pests (De Sousa et al., 1999).

Prevention of losses in stored products due to insect pests is of paramount importance. Hence the present investigations on biology of this insect on various stored commodities are informative and crucial for developing management practices. By characterizing host associated genetic variation in this cosmopolitan insect we attempted to obtain basic information with the potential to be used for future pest management strategies.

2. Material and Methods

Biology and host associated genetic variation of *O. surinamensis* was studied on various stored products viz., neem seed kernel, groundnut kernel, groundnut cake, rice, wheat, dates and raisin in the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, India.

2.1. Insect rearing

The adults of *O. surinamensis* were collected from infested stored grains and cultured under laboratory conditions in plastic containers and fed a diet of disinfested broken groundnut kernels and neem seed kernels for several generations.

2.2. Biology

Studies to determine the duration of different life stages of insect viz., egg, larva and pupa were carried out using glass vials of 3.60 x 2.0 cm size. A freshly laid egg was introduced using camel hair brush into the different vials containing one gram of broken neem seed kernels, broken groundnut kernels, groundnut cake, wheat and rice grains and cut pieces of dates and raisin. The mouth of the each vial was closed with a piece of muslin cloth and fastened with a rubber band. Twenty replications were maintained on each host. Daily observation were made to record the egg, larval and pupal periods.

2.3. Genetic variation of *O. surinamensis* using RAPD markers

Host associated genetic variation was studied on *O. surinamensis* cultured on two hosts viz., neem seed and groundnut kernel. Total genomic DNA was extracted from the larvae of *O. surinamensis* cultured on respective hosts by the CTAB method according to Doyle and Doyle, 1987. The genomic DNA from *O. surinamensis* were subjected to Random Amplified Polymorphic DNA (RAPD) marker analysis using different OPERON random primers and polymerase chain reaction (PCR) was carried out using 25 µl mixtures containing 23 µl of cocktail mixture and 2.0 µl of DNA samples as the template. Genomic DNA 2.0 µl (25 ng), dNTPs (mixture of dATP, dCTP, dGTP and dTTP) 1.0 µl (200µM of each dNTP), assay buffer 2.5 µl (1X), RAPD primer 2.0 µl (10 µM), Taq polymerase 0.5 µl (1.5 units), magnesium chloride 1.0 µl (1.2 µM), sterile distilled water 16.0 µl, were added and PCR programme (thermocyclic conditions) was performed in a BIORAD DNA engine. The PCR was programmed for 5 min at 94°C for initial denaturation, following the initial denaturation 40 cycles of 1 min at 94°C for denaturation, 1.5 min at 40°C for annealing and 2 min at 72° C for extension. An additional cycle of 7 min at 72°C was also used for primer extension.

PCR products were separated by electrophoresis in 1.5% agarose gels using a 1x TBE (Tris base, Boric acid and EDTA-Ethylene diamine tetra acetic acid) buffer stained with ethidium bromide. The electrophoresis was performed for 3 hr at 75 V until good separation of RAPD bands occurred. The reproducible DNA bands in images were scored for the presence (1) and absence (0) for each sample and data matrices were formed for genetic variation analysis (RAPD marker analysis). Similarity matrix generated by using the NTYS - PC Software 2.02 version (Rohlf, 2000). The similarity coefficients were used for the cluster analysis and dendrogram were constructed by the unweighted paired group method with arithmetic average (UPGMA).

3. Results and Discussion

3.1. Biology

Results of the biology studies indicated that the different life stages of *O. surinamensis* varied significantly among the different stored products (Table 1). The duration of the egg stage on all the stored products *viz.*, neem seed kernel, groundnut kernel, groundnut cake, rice, wheat, dates and raisin was almost uniform ranged from 4.90 to 5.50 days. This finding gave a clear indication that the different types of food materials had no influence on the hatchability of eggs. Even though neem seed kernels have different insecticidal properties, there was no effect on the duration of the egg stage.

There was a significant difference in the mean duration of the larval period among the different stored products tested. The shortest larval developmental period (18.80 days) was recorded on larvae which fed on neem seed kernels. The anti-feedent and insecticidal properties in the neem seed kernel did not affect feeding of the larvae and their development. The successful development of *Oryzaephilus acuminatus* Halstead on neem seeds without any detrimental effect was reported by Sarup and Srivastava (1971) and Thomas and Woodruff (2001). The larval development period on dates was 22.05 days, which was similar to groundnut kernels (23.75 days) and groundnut cake (23.30 days). This can be attributed to the rich nutrient status of dates which contains protein, carbohydrates, minerals, amino acid and vitamins (El Sohaimy and Hafez, 2010). The larvae fed on raisins developed slowly and reached the pupal stage after 35.90 days, with a range of 27-41 days. Similarly, longer development times of larvae in raisin was also reported by Fraenkel and Blewett (1943) and Curtis and Clark (1974).

The pupal period ranged from 4.75 to 6.60 days on different food materials. As in the case of larval development, the pupal stage was also shortest when reared on neem seed kernel (4.75 days). In other stored products pupal stage ranged from five to six days, indicating these stored products had no significant impact on pupal development. Studies conducted by Curtis and Clark (1974) also indicated no significant variations of pupal stage on different stored products. The time period for completing the total developmental from egg to adult emergence greatly varied among different stored products. The developmental period from egg to adult emergence was shortest (28.45 days) in neem seed kernel and longest (47.50 days) in raisins. Next to the neem seed kernel the total development period was 32.70 days in dates. The time periods for total development on wheat (39.05 days) and rice (37.50 days) were similar. Komson (1967) and Curtis and Clark (1974) recorded a significant difference on total development period

O. surinamensis on different hosts. The significant variation of prolongation or shortening of total development period can be correlated with the larval development period.

Table 1 Duration of different life stages of *O. surinamensis* on different stored products.

S.No.	Host	Egg period (Days)*	Larval period (Days)*	Pupal period (Days)*	Total developmental period (Days)* (Egg to adult emergence)
		Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
1	Neem seed kernel	4.90 \pm 0.64 ^a	18.80 \pm 1.91 ^a	4.75 \pm 0.85 ^a	28.45 \pm 1.76 ^a
2	Groundnut kernel	5.35 \pm 0.67 ^{abc}	23.75 \pm 1.65 ^c	5.35 \pm 0.67 ^b	34.45 \pm 1.73 ^b
3	Groundnut cake	5.45 \pm 0.76 ^{bc}	23.30 \pm 2.85 ^{bc}	5.35 \pm 0.81 ^b	34.10 \pm 3.23 ^b
4	Rice	5.45 \pm 0.76 ^{bc}	25.85 \pm 1.87 ^d	6.20 \pm 0.77 ^c	37.50 \pm 1.99 ^c
5	Wheat	5.50 \pm 0.89 ^c	27.35 \pm 1.93 ^d	6.20 \pm 0.77 ^c	39.05 \pm 2.09 ^c
6	Dates	5.50 \pm 0.89 ^c	22.05 \pm 2.56 ^b	5.15 \pm 0.93 ^{ab}	32.70 \pm 2.74 ^a
7	Raisin	5.00 \pm 0.65 ^{ab}	35.90 \pm 4.45 ^e	6.60 \pm 0.75 ^c	47.50 \pm 4.10 ^d

*Mean of 20 observations.

In a column, means followed by same letter (s) are not significantly different at (P = 0.05) by DMRT.

3.2. Genetic variation of *O. surinamensis* using RAPD markers

RAPD markers represent an efficient and inexpensive method to generate molecular data and have been used in various taxonomic groups and phylogenetic studies of insects (Jones et al., 2005). The DNA samples from *O. surinamensis* were evaluated for genetic diversity using RAPD markers and the size of the amplicons ranged from 150 to 1000 bp. The RAPD marker profile of two populations generated by different primers is shown in Figure 1 for illustration. The binary data from the polymorphic primers were used for computing similarity indices and the similarity index was 0.367 between two populations of *O. surinamensis* reared on neem seed kernel and groundnut kernel (Table 2). An UPGMA dendrogram based on the similarity coefficient was constructed for the two populations, the principle cluster A comprised of *O. surinamensis* populations from neem seed kernel and groundnut kernel host while cluster B had population which had more range than those of *Tribolium* (Fig. 2). The results of the present study indicated that host associated genetic variation existed between the two populations of *O. surinamensis*. The genetic variation among the populations collected from two hosts might be due to host characteristics. Host-associated genetic differentiation has already been documented in various insects (Subramanian and Mohankumar, 2006). Also, Lessard and Pronier (2008) detected intra-specific genomic differences of stored product insects at collected from various cosmopolitan geographical populations using RAPD primers.

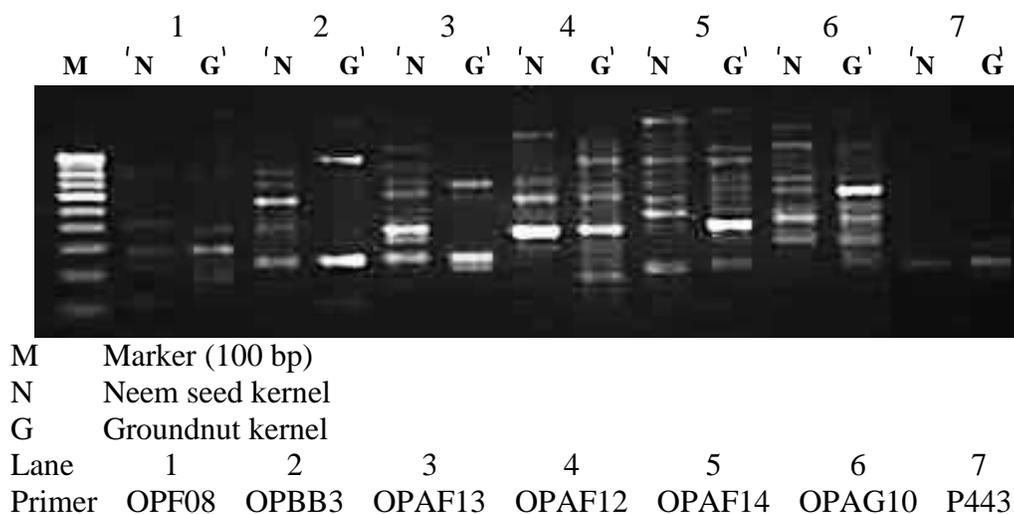


Figure 1 RAPD banding profile of two genotypes of *O. surinamensis*.

Table 2 Jaccard's Similarity Co-efficient showing relationship among two populations of *O. surinamensis*.

Population	Similarity Coefficient		
	<i>O. surinamensis</i> from neem seed kernel	<i>O. surinamensis</i> from groundnut kernel	<i>Tribolium castaneum</i>
<i>O. surinamensis</i> from neem seed kernel	1.000		
<i>O. surinamensis</i> from groundnut kernel	0.367	1.000	
<i>Tribolium castaneum</i>	0.000	0.000	1.000

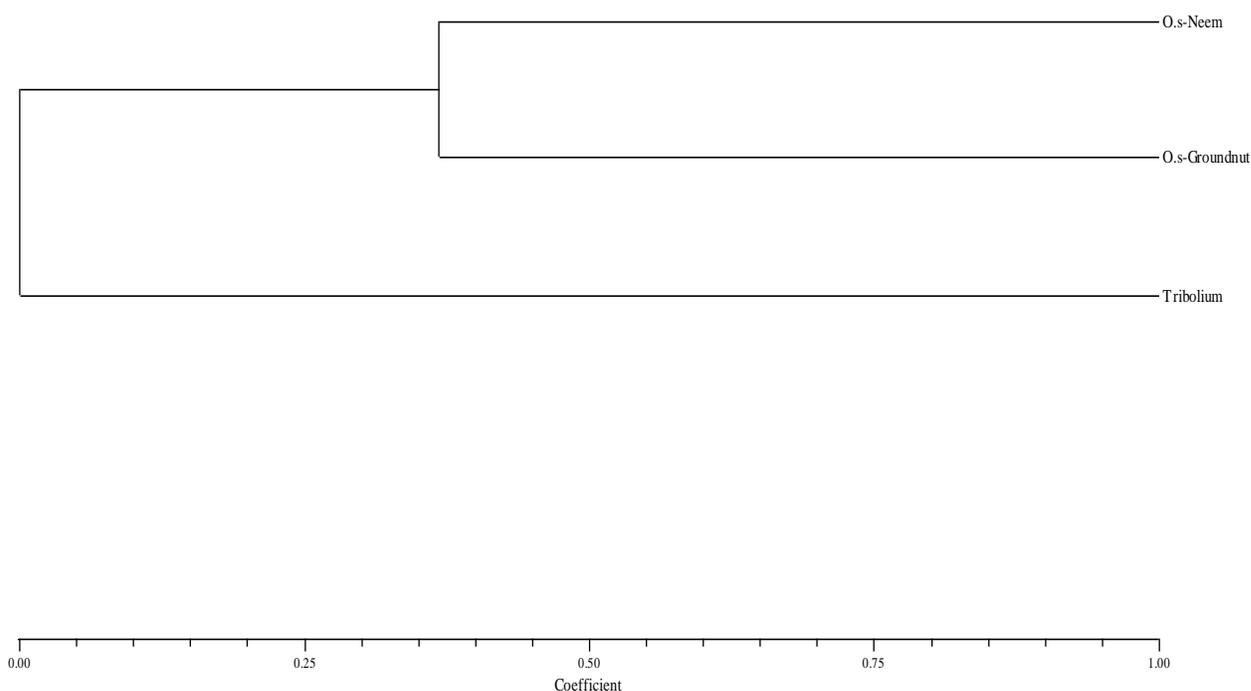


Figure 2 Dendrogram for two populations of *O. surinamensis* based on Jaccard's similarity coefficient.

4. Conclusions

The present study is a contribution to the biology of insects on different stored products and focuses on improving management practices for minimizing storage losses. The basic knowledge on host associated genetic variation can also be used for future pest management practices.

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