Research Journal of Animal, Veterinary and Fishery Sciences _____ ISSN 2320 – 6535
Vol. **2(6),** 1-5, June **(2014)** *Res. J. Animal, Veterinary and Fishery Sci.*

Studies on the Life History and Ovi positional Preference of *Callosobruchus maculatus* Reared on different Pulses

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Available online at: www.isca.in, www.isca.me
Received 11th June 2014, revised 16th June 2014, accepted 23rd June 2014

Abstract

The pulse beetle, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), is a worldwide insect pest that infests pulses in the fields and seeds in storage. An investigation has been conducted on the life history and ovipositional preference of Callosobruchus maculatus reared on five different pulses. The oviposition period had not varied significantly between the pulses. The adults exhibited a marked preference for smooth, well-filled seeds for oviposition.The order of preference for Callosobruchus maculatus for oviposition were: Vigna unguiculata (cowpea) > Vigna radiata (green gram) > Vigna mungo (black gram) > Dolichos biflorus (horse gram) > Pisum sativum (green peas). The pulses selected for the investigation were initially infested by the insect, but the degree of infestation varied among the pulses. This is due to the varied responses of the insect to different host seeds for oviposition and their appropriateness for the successful completion of the life cycle.

Keywords: *Callosobruchus maculatus,* life history, biological parameters, ovipositional preference, fecundity.

Introduction

The pulse beetle, *Callosobruchus maculatus* (Fabricus) (Coleoptera: Bruchidae) is one of the cosmopolitan pests affecting cowpea seeds, *Vigna unguiculata* .The beetle causes widespread infestation in the field condition, but most of the damage is done during storage[1]. The cowpea seeds are seriously affected by the beetle infestation and the insect multiplies very fast in storage, giving rise to a new generation every month causing weight losses of up to 60%. However, post harvest losses are a serious problem and in India, as much as 20-50% of grain is lost because of infestations from the pest[2].

The insect pests infesting stored products are included in the family Bruchidae which is found in the tropical and sub tropical countries of the world[3]. Out of five known species of Bruchidae, three are commonly met in stored products being their most important pests namely *Callosobruchus maculatus, Callosobruchus chinensis* and *Callosobruchus analis*[4]. The pulse beetle adults are small reddish brown coloured insects (2-3mm long), with the typical rounded appearance compared to the other members of the bruchid family. They are not true weevils and they lack heads prolonged into a long snout[5]. Their wing covers are distinct with black and grey colours possessing two black spots near the middle. The head is hypognathous and provided with a pair of segmented antennae, which are serrate or pectinate. Antennae are comparatively longer in males than in females. The mouthparts are biting type, which are best used during the larval stages[6]. The pro-thorax is freely movable. The thorax bears three pairs of legs with femora often swollen. There are two pairs of wings; the first pair is modified into elytra covering only a part of the abdomen. The hind pair of wings is membranous and longer than forewings and protected by the elytra. The abdomen is exposed at the posterior end and is with at least five free sternites.

The damage caused by this beetle is constrained to consumption quality only. It is not possible to consume the cowpea grains infested with bruchid holes[7]. Even though the cowpea seeds are infested with beetle, germination of cowpea is not affected, and even seeds that are full of holes will germinate very well during the next season. Damaged grains are filled with minute holes and lifeless beetles may be found inside the grains. The white eggs of the beetle are attached firmly to the outer surface of cowpea grains and are clearly visible as minute white dots on the grain. Severe damage and significant weight loss in stored seeds is caused by larvae, which grow within the grain, consuming the seed.

The adult *Callosobruchus maculatus* may be found feeding the nectar of flowers in early spring and at the end of the rainy season they starts to inhabit the cowpea culture where they continue to increase their population in very large numbers[8]. Eggs laid by females emerge in 5-20 days. The eggs are oval and translucent at the time they are laid. The incubation period varies with various species and also with the varying temperature. The first instar larva bores its way into the cowpea seeds where the rest of life cycle is completed.

The larva undergoes hypermetamorphosis. The first instar larva is caraboid with well-developed toothed thoracic plates to help entry into the seed on which it is laid. After the first moult, the larva becomes cruciform blind grub. The larva moults five times and before it enters into the pupal stage it cuts a round window on the testa of the seed coat[9]. Later, the adult emerges through the window leaving a neat round hole. The total development period is approximately 30 days.

The stored product insects are a major problem prevalent throughout the world because they reduce the quantity and quality of stored grains[10]. In countries where recent storage technologies have not been introduced, insect damage in stored grains and pulses may account to 40%[11]. The use of synthetic insecticides to control pest infestation in grains cause harmful health hazards to warm-blooded animals and a threat of environmental disruption to the ecosystem[12]. The insect pests affecting stored product pests can be effectively controlled only by analysing the life cycle and host seed preference of the insect for oviposition. In the view of the above fact, an attempt has been made to evaluate the influence of different pulses on the life history and ovipositional preference of *Callosobruchus maculatus.*

Material and Methods

Five major pulses commonly grown in Tamil Nadu, namely green gram (*Vigna radiata*), Black gram (*Vigna mungo*), Cowpea (*Vigna unguiculata*), green peas *(Pisum sativum)* and Horse gram (*Dolichos biflorus*) were selected to study the ovipositonal preference of *Callosobruchus maculatus*. All insect cultures were grown in the laboratory at a temperature of 30 ± 2^0C, 12:12 L: D and with 70% RH during the experimental study. All the experiments were conducted in the growth chamber under similar conditions.

Laboratory Maintenance of the Experimental Insect: The pulse beetle, *Callosobruchus maculatus* (Fabricus) was used in the present experiment. A small population of beetles was reared under laboratory conditions on cowpea seeds (*Vigna unguiculata*) inside a growth chamber at *30* ± 2^0C, 12:12 L: D and with 70% RH. The growth chambers were sealed and the beetles were allowed for mating and oviposition. Then parental insects were removed and cowpea seeds containing eggs was transferred to fresh cowpea seeds in the breeding jars that were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles.

Bioassays: Ovipositional preference by no-choice test: 100 g of pulses of the different host seeds were taken in glass jars of 5x3.5'' size. Five pairs of freshly emerged adult insects were introduced into each of the containers. The mouth of the bottles was covered with a thin voil cloth. Each treatment had four replications. The number of eggs laid was counted for five days at 24 hr interval in a random sample of 100 grains, which were replaced after counting.

Ovipositional preference by free-choice test: 100 g of different pulses under study were taken in petridishes with 4'' diameter and randomly allocated to different position on the table of 5''x 2.5''x 2'' with provision for handling the grains with provision for handling the grains without disturbing the insects. This set up enabled the insects to move freely inside the cage while preventing their escape and also afforded protection against the lizards. Ten pairs of less than one-day-old insects were released into the cage and allowed to oviposit on the pulses of their choice. The number of eggs laid by the insect was counted for five days at an interval of 24 hrs in a random sample of 100 grains.

Development-Hatching: A pair of newly hatched adults was confined to specimen bottles of 4''x 2''size each containing about 200 grains of the various pulses taken for study. Ten eggs laid on the same day were separated from each type of pulse to small specimen tubes with the mouth covered with the thin cloth. There were four replications. The eggs were tested from the day for hatching and the time taken by the eggs to hatch was calculated and recorded. The percentage of hatching was worked out by taking another set of 100 grains each with an egg. This was replicated four times.

Larval and Pupal Development: Ten grains from each commodity with a single egg were separated to specimen bottles from the culture of the previous experiment. Care was taken to choose only those eggs, which were laid on the same day. After calculating the time of hatching they were allowed to continue their development. The larva bores its way into the seed and develops inside the seed. Since the larva moults within the seed, the interval between the various larval instars and the pupal instars were not calculated separately. At the end of the larval period, the larva drills a minute circular hole near the seed coat till only a thin layer of seed coat is left intact. This was an indication of pupation. The emergence of the adult insect was taken at the final stage of the growth phase. The mean number of days taken by the insect to complete its life cycle from the egg to the adult stage on different pulses was calculated.

Adult emergence: 100 grains each with a single egg from each type of pulse tested were separated to specimen bottles and there were four replications. These eggs were observed from the 15th day for the emergence of adults. As the adults emerged, they were counted and removed, so also the grains from where the insects emerged. The grains, which did not show any emergence holes, were kept and watched till the 14th day and then percentage of adult emergence calculated.

Fecundity: The influence of different host seeds on the fecundity of females was tested by confining a pair of newly emerged adults in 5x 3.5'' specimen bottles containing 25 g of seeds of different pulses under study with four replications. The eggs laid were counted after the death of the adult insect.

Statistics: All data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan's Multiple Range Test (DMRT)

Results and Discussion

The biological parameters of *Callosobruchus maculatus* fed on different pulses was presented in table 1. The adults of *Callosobruchus maculatus* were found to mate between 50-60

minutes after their emergence. The mating lasted for 4–8 minutes. The oviposition commenced roughly 8-10 hours after mating.The eggs when first laid were transparent and the incubation period varied with various pulses. The mean incubation period on various pulses was found to range between 3 and 6 days. On green gram, cowpea and black gram, eggs hatched in 3 days while it took 4 days in horse gram and 4.5 days in green peas.

All the eggs laid were not found to hatch and there was significant difference in the percentage of hatching of eggs on different pulses tested. The hatching percentage was the highest (98%) in cowpea; followed by green gram (97.25%) and black gram (96%) Green peas and horse gram recorded 84.5 and 81.25%of hatching which was significantly low among all the pulses treated.

The larva about to hatch out was seen to exhibit wriggling movements inside the transparent eggshell. The larva feeds on the contents of the grain and moults 4 times inside the seed. The larva before pupation cuts a circular window on the testa of the seed indicating the beginning of the pupal period. The combined larval and pupal period significantly varied among the various pulses tested. In green gram, it took 18 days, cowpeas 19 days, horse gram 20 days and green peas 22 days, being the highest. There was no apparent difference in the time taken for the development of either sex.

The total number of days taken for the completion of life cycle (from the day of oviposition till adult emergence) varied significantly with different pulses. The minimum number of days taken by the insects that develop from green gram is 21 days followed by cowpea, 22 days. The total life cycle was completed in black gram, horse gram and green peas in 25, 24 and 25.5 days respectively. The longest duration to complete the life cycle was recorded to be in green peas.

The adults emerged out by drilling the window of the seed coat. There was significant difference in the percentage of adults emerged between different hosts. The highest percentage of adults (84.5%) emerged from green gram followed by cowpea (81.25%). In black gram, 63.5 % adults emerged from the seeds. The lowest percentage of adult emergence was recorded in horse gram (26.5%) followed by green peas (31.25%).

The fecundity tested in relation to different hosts exhibited significant variation. The number of eggs laid by a single female insect varied depending upon each host seeds. The highest number of eggs was recorded on cowpea (135.5), followed by green gram (121.0), horse gram (118.75) and black gram (108.5). The lowest number of eggs was recorded in green peas (45.5).

Table 2 deals the host preference of *Callosobruchus maculatus* on pulses under free choice test. The daily observations made on the number of eggs laid from the first to fifth day after infestation revealed that the insect laid more number of eggs in cowpea seeds when compared to other pulses. The least number of eggs were recorded in horse gram seeds. There was significant difference in the number of eggs laid by *Callosobruchus maculatus* on different pulses.

Table 3 explains the oviposition of *Callosobruchus maculatus* on five pulse grains under no-choice test. The results on the overall preference of the beetle for oviposition considering all the five days together revealed that the highest number of eggs (48.34) was laid in cowpea and black gram (44.76). Among the hosts, *Callosobruchus maculatus* laid significantly low number of eggs (19.26) on horse gram.

Table-1
Biological parameters of *Callosobruchus maculatus* fed on different pulses

Host seeds	Incubation Period (days)	Hatching (%)	Total Larval and pupal period (days)	Total life span (Days)	Adult emergence (%)	Fecundity (No of eggs)
Green gram (*Vigna radiata)*	3.0 ^{d}	97.25 ^{b}	18 ^{b}	21.0	84.5 ^{a}	121.0 ^{b}
Black gram (Vigna *mungo*)	3.0 ^{d}	96.50 ^{c}	23 ^{c}	25.0 ^{c}	63.5 ^{c}	108.5 ^{d}
Cowpea (*Vigna unguiculata)*	3.0 ^{d}	98.00 ^{c}	19 ^{e}	22.0 ^{e}	81.25 ^{b}	135.5 ^{a}
Horse gram *(Dolichos biflorus)*	4.0 ^{c}	81.25 ^{f}	20 ^{d}	24.0 ^{d}	26.5 ^{f}	118.75 ^{c}
Green peas (*Pisum sativum)*	4.5 ^{b}	84.50 ^{e}	22 ^{c}	25.5 ^{b}	31.25 ^{e}	45.5 ^{f}

Within a column means followed by a same letter is not significantly different 5% level of DMRT

Table-2
Influence of different pulses on the oviposition of *Callosobruchus maculatus* (free choice test)

Host seeds	**No of eggs laid (days)**					***Mean***
	1	2	3	4	5	
Black Gram(*Vigna mungo*)	40.2[b]	63.5[b]	72.5c	79.2[b]	80.3[a]	67.14[b]
Cowpea(*Vigna unguiculata*)	38.5[c]	72.5[a]	79.8a	79.9[a]	80.0[a]	70.14[a]
Green gram (*Vigna radiata*	38.2[c]	56.0[d]	73.5[b]	74.0[c]	75.2[b]	63.38[c]
Horse gram *(Dolichos biflorus)*	6.5[e]	12.4[f]	16.5[f]	13.0[f]	15.2[e]	12.72[f]
Green peas (*Pisum sativum)*	31.2[d]	49.2[e]	55.2[e]	56.0[e]	53.5[d]	49.02[e]

Within a column means followed by a same letter is not significantly different 5% level of DMRT

Table-3
Influence of different pulses on the oviposition of *Callosobruchus maculates* (no-choice test)

Host seeds	**No of eggs laid (days)**					**Mean**
	1	2	3	4	5	
Green gram (*Vigna radiata)*	24.5[c]	31.5[d]	32.5[d]	37.0[d]	37.3[d]	32.56[d]
Black Gram(*Vigna mungo)*	34.5[b]	41.3[c]	44.0[c]	51.5[c]	52.5[c]	44.76[c]
Cowpea(*Vigna unguiculata)*	34.6[b]	43.0[b]	48.5[b]	58.3[b]	57.3[b]	48.34[b]
Horse gram *(Dolichos biflorus)*	14.5[e]	16.0[f]	21.5[f]	22.0[f]	22.3[f]	19.26[f]
Green peas (*Pisum sativum)*	15.0[d]	19.3[e]	24.8[e]	30.7[e]	31.3[e]	24.22[e]

Within a column means followed by a same letter is not significantly different 5% level of DMRT.

Cowpea is an important protein supplement in the diet of many people in tropical countries like India[13]. In the field, the cowpea is often attacked by many insect pests and diseases, whereas in seed storage, the main problem, apart from moulds and rodent damage is caused by only one insect species, the cowpea beetle, *Callosobruchus maculatus*. This bruchid lays its eggs on the ripe pods or seeds, the larvae feed on the contents of the seed and emerge from the seeds as adults. The life span of the beetle is about 3 weeks, but it differs depending on the temperature and the humidity of the seeds and their environment.

The present study has confirmed the following findings: The order of preference for oviposition and development of pulses were: 1. Seeds, which are favourable for oviposition and development – *Vigna unguiculata* (cowpea) > *Vigna radiata* (green gram) > *Vigna mungo* (black gram). This may be due to the smooth nature of the seed coat and also its smaller size, which was suitable for the insect to hold the seeds while depositing the eggs. Seeds, which are moderately preferred for oviposition and development – *Dolichos biflorus* (horse gram). *Dolichos biflorus* seeds are much flattened and its rough surface inhibited the egg laying of *Callosobruchus maculatus*. Seeds, which are less, preferred for oviposition and development – *Pisum sativum* (green peas).

Ouali-N'goran has conducted a similar research on the influence of dietary factors of five varieties of beans sold in Côte d'Ivoire on some biological parameters of *Callosobruchus maculatus.*Six biological parameters of *C. maculatus* were studied on the seeds of five varieties of beans: two varieties of *Phaseolus vulgaris* and three of *Vigna unguiculata* Walp.,according to their physical and chemical characters. The results revealed that white cowpea IT97K499-38 has more energy with the highest level of protein, fat, and sugar with a thin tegument[14]. The morphological characters of the seeds and their chemical content play an important role in the biology of the *Callosobruchus maculatus*. The white cowpea (*Vigna unguiculata* Walp, varieties IT97K499-38), very rich in protein and in sugar proved to be more favourable to the development of the beetles than other pulses.

Conclusion

The data presented in this study demonstrated that *Callosobruchus maculatus* always prefer cowpea seeds for oviposition than other pulses. From this study, it can be concluded that cowpea seeds are the most vulnerable legume seeds and the most suitable hosts for *C. maculatus.* These host seeds had the maximum number of eggs laid, shortest developmental period, highest susceptibility index and maximum weight loss. The current research paves the way to provide awareness to the farmers not to store cowpea, green gram and other seeds in the same place and/or at the same time

to avoid cross infestation because of their high susceptibility to *C. maculatus.*

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