



LIFE STAGES OF COWPEA WEEVIL *Callosobruchus maculatus* (F)

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ABSTRACT

The cowpea weevil *Callosobruchus maculatus* (F) was reared on a cowpea to study its life cycle. The study revealed that egg, larval and pupal stages were completed in 4-5, 15-19 and 4-5 days, respectively. Similarly, the duration of the adult male and female was 10-12 and 10-14 days, respectively, with the total developmental period being 38 to 40 days. The male and female measured 2.30 ± 0.15 and 3.23 ± 0.19 mm long, and 1.25 ± 0.05 and 1.47 ± 0.11 mm broad, respectively.

Key words: *Callosobruchus maculatus*, lifecycle, development, duration, morphometry, legume, grain pest, damage, reproduction

The cowpea weevil *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae) is a major pest of economically important leguminous grains (Okonkwo and Okoye, 1996; Mulatu and Gebremedhin, 2000; Raja et al., 2000; Park et al., 2003; Sarwar, 2012; Beck and Blumer, 2014; Tahmasebi et al., 2022). They infest grain in the field and often cause severe damage in storage (Prevett, 1961; Ashamo et al., 2022). It particularly prefers to attack the stored grains of the leguminous genus *Vigna* (Cope and Fox, 2003), especially cowpea *Vigna unguiculata*, causing significant losses in grain weight, germination ability, and nutritional quality (Caswell, 1981; Babu et al., 2021; Hamzavi et al., 2022). In severe infestation by *C. maculatus*, seeds become completely hollow and are unmarketable (Khalil and Ali, 1999). Due to its feeding damage secondary pests or fungi may enter the grain, which lowers its nutritional quality (Hagstrum et al., 2012). Due to the short lifecycle, high reproductive capacity, rapid development and continual generations of *C. maculatus*, all stored grains can be lost in a few months (Turaki, 2012). In India, *C. maculatus* hibernates in the larval stage during winter and breeds freely from March to November. It causes maximum damage when all its developmental stages exist simultaneously from February to August (Tapondjou et al., 2002). For effective control analyzing the life stages is important. Detailed information on the life history of *C. maculatus*, including adult, egg laying, egg, grub hatching and boring, the day-wise series of grub development, pupa, adult emergence, adult longevity in grains of *V. unguiculata*, and measurements of life stages is still wanting. Its biology has been studied by Devi and Devi (2014) at Imphal, India, and Rahman et al. (2022) at Rajshahi, Bangladesh. The present study analysed its lifecycle from Kolhapur, Maharashtra.

MATERIALS AND METHODS

A study was conducted under laboratory conditions in the Department of Zoology, Shivaji University, Kolhapur from March 2022 to May 2022 ($28.05 \pm 2^\circ\text{C}$, $71.07 \pm 3\%$ RH) (Devi and Devi, 2014). A sampling of *C. maculatus* was done from a farmers' house, in Panhala, district Kolhapur along with cowpea-stored seeds in November 2021. The culture was maintained on cowpeas in a 1 kg capacity plastic rearing jar. The egg-laying female was placed in a separate 4x 3.5 cm sized container containing a single layer of cowpea. The culture was maintained for a limited period; freshly emerged adults were kept separate in another container. An equal sex ratio of 1-2 day old virgin adults was released for egg laying in a new plastic container for 24 hr. After that, on the 2nd day, the male and female were transferred into a container containing fresh seeds. Like this, adults were transferred for 10 days to check the development of beetle stages. Another set was also prepared for analyzing damage in which five containers (4x 3.5cm) are filled with a single layer of the measured quantity of cowpeas. Contech electronic weighing balance for weighing the grains to study damage. In every container, five virgin adults (2 males and 3 females) were released and kept for 45 days without being disturbed. Grains were again weighed after the emergence of adults. A blade was used to make an initial cut adjacent to the egg. Blunt and sharp forceps were used to remove the seed coat. Entomological pin no. 4 was used to remove frass, and grain particles, and to find the grub. The dissected portion was examined under a stereozoom microscope (Lynx LM-52- 3621). To confirm the stage of development, the observations were made every day and recorded with temperature

and relative humidity. Photography was done under an Olympus CX31RTSF microscope attached with a camera. The photographs were stacked in Helicon Focus 7 software and edited in Photoshop CS3. IMAGE J software was used for the measurement of the length and breadth of every stage. The experiment was carried out in five replications with standard mean deviation, and the data were statistically analyzed in Microsoft Excel software.

RESULTS AND DISCUSSION

Being a holometabolous insect, cowpea weevil *C. maculatus* consists of four life stages viz. egg, larva (grub), pupa and adult. A total of four larval instars were observed in the larval stage. Egg: After three to four hours of adult emergence mating takes place for 3 to 6 minutes, after which the female tightly grips the seed and lays the egg singly on the seed coat anywhere. The time duration between mating and egg laying is five to six minutes. Mating was observed with the female multiple times. The female secretes a glue-like substance during egg laying, which adheres the egg to the seed coat. A female laid eggs for up to 5 to 6 days. After mating, she lays maximum eggs i.e. 35 to 40, on 1st day, and then gradually decreased to 7 to 9 eggs. A single egg was laid by a female on a single seed for their oviposition markers (Wijeratne and Smith, 1998). However, when there is a scarcity of the required number of fresh seeds or a limited number of seeds, the female can lay multiple eggs on the single seed. The fecundity of a female is 120-132 eggs. The freshly laid eggs are transparent and shiny, later becoming yellowish white. The incubation period of the egg ranged from 4-5 days. The eggs measured 0.55 ± 0.01 mm long and 0.33 ± 0.01 mm in breadth.

Larval stage comprises four larval instars. 1st Instar develops within the egg and makes a circular hole in the seed coat with the help of chewing mouthparts and bores into the seed by feeding on grain endosperm and grows. Total grub duration varied from 15 to 19 days. The whole grub stage is completed within the grain. 1st Instar: Embryonic development is completed in 3 to 4 days and the 1st instar is formed within the egg before penetrating the seed coat. A pigmented head capsule is easily seen in the egg. They bore from the egg through the seed coat and entered into the grain endosperm. The transparent egg becomes opaque white due to the frass formed by the grub while boring the seed. Grub is small, white, slightly curved, and has a pigmented head. The body is broad behind the head and tapered at the end. About 6 to 7 days after oviposition, the grub hatched

from the egg and burrowed straight into the bean. Its length measures from 0.45 to 0.49 mm (0.47 ± 0.02 mm) while the breadth was from 0.20 to 0.25 mm (0.23 ± 0.03 mm). The first instar lasts for 6-7 days. The second instar grub burrowed and fed on the bean endosperm. This instar stage lasts for 3 to 4 days; segmentation on the body is seen in this instar, with body broad behind the head and has a humpback appearance at the end. It grows in size than the 1st instar by eating the endosperm of the seed; it was 1.06 to 1.45 mm (1.20 ± 0.16 mm) long and 0.5 to 0.69 mm (0.61 ± 0.09 mm) broad. The 3rd instar is yellowish-white, C-shaped, with a small blackish-brown head; most active and increases in size by feeding on the endosperm voraciously; in this instar leg buds and segmentation on the body are visible, with duration of 3 to 4 days; it measured 2.78 to 2.89 mm (2.84 ± 0.05 mm) long and 1.26 to 1.42 mm (1.33 ± 0.06 mm) broad. The 4th instar is also yellowish-white, C-shaped, with a small blackish-brown head, appeared to grow in size and devoured endosperm; with leg buds and segmentation ridges being prominent. It bores the seed into a position just underneath the seed coat before pupation, with duration ranging from 3 to 4 days; it measured 3.95 to 4.18 mm (4.10 ± 0.10 mm) long and 1.72 to 1.89 mm (1.80 ± 0.06 mm) broad.

Pupa revealed that the grub structure was broken down and the adult structure was formed; on the 1st day, rudiments of the wing, antennae, legs, and proboscis developed, while on the second day, the wing, antennae, legs and proboscis developed; sclerotization of the eye was started and cuticular hair development on the head, forewing, and legs appeared. The intersegmental region of the abdomen remained colourless and the forewings were pale green. On the 4th day, sclerotization was completed, and the forewings became dark brown with black patches. The pupal period ranged from 4 to 5 days, and pupa measured 3.97 ± 0.11 mm long and 1.64 ± 0.05 mm broad. The adult remained in the grain for several days after pupation by chewing and removing a circular piece of the seed coat. Adults emerging from seeds are well adapted to storage conditions, reproductively mature and require neither food nor water to reproduce (Cope and Fox, 2003). Whereas after 24-48 hours of emergence, adults become sexually mature (Beck and Blumer, 2014). The adult male was smaller and possessed a round shape than the female. A male is oval, whereas a female is elongated. The pygidium is covered by elytra in the male and exposed in the female due to eggs (Kafom et al., 2017). Both the male and female have long and erected 11 segmented slightly serrate antennae. Sexual dimorphism is observed according

to the number of antennal sensilla (Mbata et al., 1997). Pubescence is present all over the body. Females were blackish and larger as compared to males (Beck and Blumer, 2014) whereas male was chocolate or reddish brown, and had a whitish spot above the scutellum. Females have a prominent black spot in the middle of each elytron surrounded by white stripes and a white band at the middle of the pygidium. The female's abdomen is pointed and modified for egg laying, whereas the male's is blunt (Serum et al., 2022). The male was very active and many times followed the female for mating. The male and female periods ranged from 10-12 and 10-14 days, respectively. The length of the male and female was 2.15 to 2.48 mm and 2.88 to 3.38 mm (2.30 ± 0.15 , and 3.23 ± 0.19 mm), while the breadth was 1.17 to 1.31 mm and 1.29 to 1.58 mm (1.25 ± 0.05 , and 1.47 ± 0.11 mm).

Bruchids are the most notorious insect pests, causing 40% damage to pulses (Ofuya and Agele, 1989); in chickpeas, it is 50% during storage (Caswell, 1981); in cowpea, it is up to 60% (Allotey and Oyewo, 2004); in mung bean, it is up to 98.06% (Chauhan et al., 2022). Losses become about 100% if storage conditions are uncontrolled (Somta et al., 2007). It was observed that 59.34% of the damage was done by the grub of *C. maculatus* which corroborates with the observations of Allotey and Oyewo (2004). The nature of the damage observed now is in agreement with the observations of Furk and Hines (1993) i.e. grubs feed on the inner endosperm and leaves a hole, from which adult insects emerge. Rahman et al. (2022) studied the life history traits of *C. maculatus* reared in black gram, but these contrast with the present study. A slightly serrate type of antennae is present in *C. maculatus* but not the pectinate type. The elytral pattern differs from the details given by Kingsolver (2004). The present study reveals that the egg, larval and pupal duration are completed within fewer number days as compared to observations of Devi and Devi (2014). Morphometrics of life stages observed now are more or less similar to the observations made by Devi and Devi (2014) except first instar adult stages, which might be due to the green gram used as host.

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AUTHOR CONTRIBUTION STATEMENT

SMG involved in design of the research, identification of specimen and writing up the manuscript. DRS collected, identified, reared and gathered biological data of species under study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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