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BIOLOGY OF *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE) REARED ON THIRD INSTAR LARVAE OF *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The biology of *Diachasmimorpha longicaudata* (Ashmead) was studied on third instar larvae of *Bactrocera dorsalis* (Hendel) to optimize its mass rearing in the laboratory. The mean parasitoid emergence rate ranged from 55–92% during its first 13 days of life. The number of females was higher than that of males. The mean development time from egg to adult emergence was 20.14± 0.02 days in females and 17.72± 0.02 days in males. Fed females lived longer than fed males. Food-deprived females and males lived 72 hr after emergence. The lifespan of males and females was 16.42± 0.53 and 15.86± 0.35 days, respectively, in pairs exposed to *B. dorsalis* larvae.

Key words: *Bactrocera dorsalis*. fruit flies, mango, *Diachasmimorpha longicaudata,* parasitism, sex ratio, mass rearing, biological control, host plants, emergence rate, survival rate, life span, male, female, longevity, egg laying

In Burkina Faso, the mango sector is threatened by tephritid fruit fly pests causing important losses. Significant losses linked to fruit fly attacks are observed in orchards and processing or packaging units. These pests are polyphagous and attack other cultivated fruit crops, such as guava and citrus fruit species (Ouédraogo, 2011; Zida et al., 2020; Nébié et al., 2021). They are also known as quarantine insects in Europe, and any mango shipment containing infested fruits is intercepted and destroyed. According to Nébié et al. (2023), 163.228 tons of mangoes were intercepted in Europe in 2017, causing an economic loss estimated at $USD 178,067. Several control methods include orchard sanitation, mass trapping through parapheromones and spot spray with food attractants have been implemented and popularized among producers to combat fruit flies (Zida et al. 2023). However, biological control agents such as parasitoids that naturally help reduce fruit fly populations in mango orchards are often overlooked. Over eight species of native parasitoids have been inventoried in Burkina Faso (Zida, 2019). These include Alysiinae, *Bracon* sp., *Diachasmimorpha* sp., *Ealata clava* Quinlan, *Fopius caudatus* Szépligeti, Microgastrinae, *Psyttalia concolor* Szépligeti and *Tetrastichus giffardianus* Silvestrii. The parasitoids that emerge from mango are *F. caudatus* and *P. concolor*, and their combined parasitism rate is very low on *Bactrocera dorsalis* (Hendel).

For this purpose, *Diachasmimorpha longicaudata* (Ashmead) was imported from the International Center of Insect Physiology and Ecology (ICIPE) to implement classical biological control. It parasitizes larvae of many fruit flies, including *Ceratitis cosyra* and *B. dorsalis* (Mohamed et al., 2008; Ndlela et al., 2020). Once released and established, *D. longicaudata* populations become self-perpetuating and persist in agrosystems for many generations (Ndlela et al., 2020). The same authors reported that in Kenya, *D. longicaudata* showed promising results in controlling *B. dorsalis* and can successfully parasitize and complete its life cycle in the indigenous fruit fly *C. cosyra*. One of the challenges for the successful establishment of *D. longicaudata*

in West Africa is the availability of high quantities of healthy parasitoids for release. The accomplishment of this mission requires previous laboratory studies to evaluate the parasitoid biology, parasitism capacity, parasitoid emergence, and parasitoid biological cycle in our laboratory facilities. This knowledge is very important in planning parasitoid mass rearing in the laboratory. This study was carried out to determine the development duration of the parasitoid from egg to adult emergence, assess the emergence rate of parasitoids and the sex ratio as a function of the age of the pairs, and assess the longevity and survival rate of male and female parasitoids reared on third instar larvae of *B. dorsalis*.

MATERIALS AND METHODS

The study was conducted from May to December 2020 at the entomology laboratory of the Farako-bâ research station (11º06′ N; 04º20′ W). During the study the mean temperature and relative humidity varied between 24.55 °C (July) and 28.7 °C (May) and 51.55% (May) and 71.86% (August), respectively. Mass rearing device was set up to rear *B. dorsalis* larvae following the procedures described by Nébié et al. (2023). The parasitoid *D. longicaudata* was reared on third instar larvae of *B. dorsalis* following the procedures described by Mohamed et al. (2003) and Mohamed et al. (2008). Parasitoid fecundity, emergence rate, sex-ratio and development time of male and female parasitoids were assessed by forming 80 pairs of *D. longicaudata* distributed in four rearing cages, i.e., 20 pairs emerged on the same day at the same time per cage. After 24 hr, an oviposition unit containing 100 third instar larvae of *B. dorsalis* was placed in each cage. After 12 hrs of exposure, the oviposition units were removed from the cages and replaced by others until all female parasitoids died. The larvae were extracted from each oviposition unit and then transferred to plastic pots containing sand and labelled with the following information: cage number, date, and exposure time. The pupae thus formed were collected after 48 hours and placed in labelled Petri dishes with the same previous information. Observations were performed daily from 7 a.m. to 11 p.m. at a frequency of 4 hours to collect parasitoids that emerged, recording the date of emergence and sex until almost all pupae hatched. The lifespan of male and female of *D. longicaudata* was determined under seven scenarios: (1) pairs fed with larval exposure for oviposition; (2) pairs fed without exposure of larvae; (3) pairs deprived of food and larvae; (4) females deprived of food; (5) fed females; (6) males deprived of food and (7) fed males. The number of dead parasitoids (male and female) were recorded every 12 hours at 7 a.m. and 7 p.m. The formula described by Harbi et al. (2016) and Kadio et al. (2016) were used to calculate the emergence rate, sex ratio, lifespan and development time of male and female parasitoids. Statistical analyses were performed using R software version 3.6.1. In these analyses, the Wilcoxon test was applied at the 5% probability level to compare the means when there were two treatments. The Kruskal-Wallis test was used for multiple comparisons.

RESULTS AND DISCUSSION

During the experiment, the mean time between egg-laying and adult emergence was 20.14 ± 0.02 days in females and 17.72± 0.02 days in males. This difference ($W = 7420320$, $p < 0.0001$) in emergence day between females and males could be explained by the protandry of the species, which is an asset because it improves reproductive efficiency. On larvae of *C. capitata*, the male of *D. longicaudata* emerge approximately two days before female parasitoids (Harbi et al., 2016). According to Paladino et al. (2010), preimaginal development of *D. longicaudata* on third instar larvae of *C. capitata* takes about 16 days at 25*º* C, 85% RH, and with an 18:6 L:D photoperiod. During the rearing, exposure times 7 a.m. and 7 p.m. did not significantly influence ($W = 2122$, $p = 0.4095$) parasitism rate that was 42.87± 3.62% and 47.76± 3.73%, respectively. The first emergence of parasitoid adults was observed from pupae of third instar larvae of *B. dorsalis* parasitized by one-day-old female parasitoids (Fig. 1). During the first 13 days, parasitoid emergence rates varied from 54.95% (13th day) to 91.67% (4th day). Exposures of *B. dorsalis* third instar larvae made after this period resulted in emergence rates of 10.56% in 15-day-old pairs to 24.41% in 14-day-old pairs. The decrease in the emergence rate is linked to the considerable age-related decrease in the number of mature oocytes in females. It can also vary according to parasitoid species and

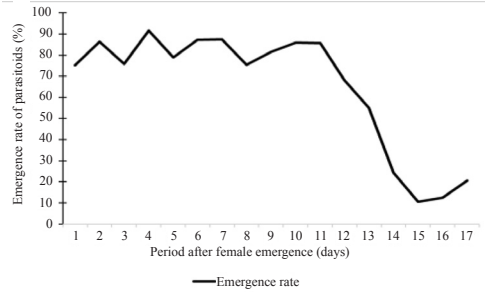

Fig. 1. Emergence rate of parasitoids reared on third instar larvae of *B. dorsalis* related to the age of *D. longicaudata* females

rearing conditions. Indeed, when second instar larvae of *B. dorsalis* are offered to *D. longicaudata* seven to nine days old, the parasitism rate can reach 64.33% after six hours of exposure in similar rearing conditions (25–27ºC; 60–70% RH; L12: D12) (Ndlela et al., 2020).

Diachasmimorpha longicaudata offspring were mainly dominated by females (52.24–77.77%), except on the 14th day (17.94%) and 16th day (40.96%) of the oviposition period (Fig. 2). Consequently, the average number of offspring/day/pair was dominated by females (0.08 to 5.85 females emerged/day/pair vs. 0.16 to 2.7 males emerged/day/pair). According to Viscarret et al. (2005), sex ratios in Opiinae can be affected by different variables, such as larval stage, age, size of the host, time of exposure and age of the female parasitoid. On third instar of *C. capitata* under laboratory conditions, the progeny produced by *D. longicaudata* were mainly males during the first three days of the oviposition period, with a predominance of females observed thereafter (Harbi et al., 2016). Cruz *et al.* (2018) observed a significant interaction between the number of females in the cage and larval density in relation to the sex ratio.

Table 1 shows the mean lifespan of fed and unfed parasitoids (males and females) separately maintained in rearing cages. Fed females and males lived on average 69.24± 1.60 days and 40.08± 1.95 days, respectively. This long lifespan in favor of fed virgin female parasitoids was also reported by Meirelles et al. (2013) by using third instar larvae of *Anastrepha fraterculus* and *C. capitata* as host. Among the fed female parasitoids, the first mortalities (1.3%) were observed 37.8 days after their emergence. Approximately 50% of these parasitoids died 68.5 days after emergence. The last mortalities were observed 95 days after emergence. With regard to fed male parasitoids, the first mortalities (6.3%) were noted 15 days after their emergence. The death rate was 50% 36.5 days after the emergence of these parasitoids. All male parasitoids were dead within 73.5 days of emergence (Fig. 3). Females and males deprived of food (honey) and water lived on average 1.87 ± 0.06 days and 1.53 ± 0.05 days, respectively. No significant difference was observed. Among the unfed female parasitoids, 10% of adults died 0.83 days after emergence. The death rate reached 66.2% after 1.83 days. No unfed female parasitoids survived beyond 2.83 days after emergence. Among the unfed male parasitoids, 23.8% of adults died within 0.87 days of emergence. The death rate was 60% after 1.37 days. The last mortalities were observed 2.37 days after emergence (Fig. 3).

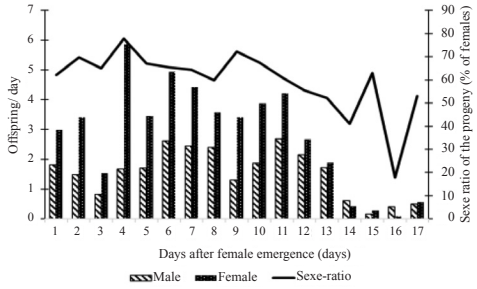

Fig. 2. Daily sex ratio of the progeny of *D. longicaudata* reared on third instar larvae of *B. dorsalis*

Table 1. Lifespan (days) of males and females (fed or unfed) of *D. longicaudata* separately maintained in rearing cages

Parasitoids	Lifespan (Mean± SD)
Fed males (n=80)	40.08± 1.95[b]
Unfed males (n=80)	1.53± 0.05[c]
Fed females (n=80)	69.24± 1.60[a]
Unfed females (n=80)	1.87± 0.06[c]
p-value	<0.0001***
χ^2	259.02

Values followed by the same letter not significantly different Kruskal– Wallis multiple comparison test (p=0.05). ***Very highly significant

Fed females and males of pairs exposed to *B. dorsalis*

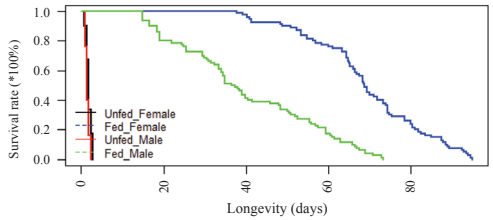

Fig. 3. Survivorship curves of fed females (n=80), fed males (n=80), unfed females (n=80) and unfed males (n=80) separately maintained in rearing cages

Table 2. Lifespan (days) of fed males and females of *D. longicaudata* pairs with and without oviposition on third instar larvae of *B. dorsalis*

Pairs	Sexe	Lifespan (Mean± SD)
Pairs fed without oviposition (n=80)	Male	42.05± 1.51[b]
	Female	54.68± 1.73[a]
Pairs fed with oviposition (n=80)	Male	16.61± 0.3[4]c
	Female	16.01± 0.2[1]c
p-value		<0.0001***
χ^2		230.8

Values followed by the same letter not significantly different Kruskal– Wallis multiple comparison test (p=0.05). ***Very highly significant

larvae lived 16.01± 0.21 days and 16.61 ± 0.34 days after emergence, respectively (Table 2). No significant difference was observed. After mating, females lay their eggs daily on exposed larvae; this gradually decreases their survival rate due to the energy used for copulation and oviposition. Among fed females and males of pairs without exposure to *B. dorsalis* larvae, the mean lifespan was 54.68± 1.73 days and 42.05± 1.51 days after emergence, respectively. Statistical analysis showed significant differences ($\chi^2 = 230.8$; df = 3; p value <0.0001). Appiah et al. (2013) reported that *D. longicaudata* females live significantly longer than males regardless of the rearing conditions. According to Ramadan et al. (1992), female parasitoids require additional time for reproductive organs to mature. Indeed, a virgin female without a host will live longer, and a female mated without a host has a doubled lifespan compared to an ovipositing mated female. Parasitoid mortality was faster in fed pairs with exposure to *B. dorsalis* larvae than in fed pairs without exposure to *B. dorsalis* larvae. In addition, females lived longer than males in fed pairs without larval exposure. The reverse situation was observed in the pairs exposed to the larvae. Statistical analysis showed that 55% of females from fed pairs with exposure to *B. dorsalis* larvae died 16.42 days after emergence, while approximately 50% of females from fed pairs without exposure to *B. dorsalis* larvae died 48.4 days after emergence. No females survived beyond 17.92 days in fed pairs with larval exposure. In fed pairs without exposure to larvae, the last mortalities of females were observed 93.4 days after emergence. Approximately 50% of males from fed pairs with larval exposure died within 15.92 days after emergence. The last mortalities of these males were observed 21.42 days after emergence. For the males of the fed pairs without exposure to the larvae, a survival rate of 50% was noted 41.9 days after emergence. Under these conditions, no males survived beyond 71.4 days (Fig. 4).

Short lifespan was observed in males and females of pairs deprived of food (honey), water and larvae for reproduction (1.96 ± 0.05 days vs 2.20± 0.05 days,

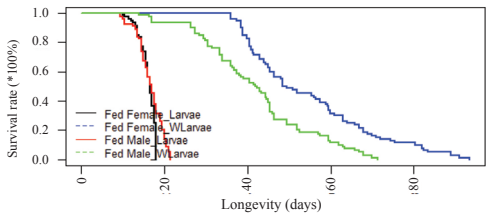

Fig. 4. Survivorship curves of fed males and females of *D. longicaudata* pairs without (n=80) and with (n=80) exposure to third instar larvae of *B. dorsalis*

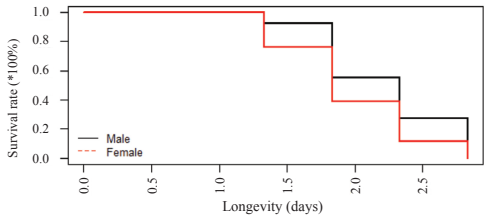

Fig. 5. Survivorship curves of males and females of *D. longicaudata* pairs deprived of food, water and *B. dorsalis* larvae for oviposition

respectively). A significant difference (W = 4058, p = 0.0022) was obtained. This short lifespan in unfed parasitoids is closely related to the lack of food substrate such as honey to feed the parasitoids. The presence of a food source (nectar, pollen or other sugary substances) is decisive or even essential for the survival of adult parasitoids (Lavandero et al., 2006; Araj et al., 2008). The first mortalities were observed after 1.33 days in both sexes, with mortality rates of 7.5% in females and 23.8% in males. In addition, 45% of females died within 1.83 days after emergence, while 61.2% of males died during the same period after emergence. No male or female parasitoid survived beyond 2.83 days after emergence (Fig. 5). These results will help optimize the mass rearing of *D. longicaudata* on third instar larvae of *B. dorsalis* in the laboratory and assist in responding to the demands for biological pest control of other countries in West Africa.

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AUTHOR CONTRIBUTION STATEMENT

KN, DAR and AK conceived and designed research. KN and AK conducted surveys and experiments. KN analyzed data and wrote the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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