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Research Article

Studies on life history traits of the oophagous parasitoid, *Gryon fulviventre* Crawford (Hymenoptera: Scelionidae), a potential biocontrol agent of the cowpea pod-sucking bug, *Clavigralla tomentosicollis* Stäl. (Hemiptera: Coreidae) in Burkina Faso

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ABSTRACT: The pod-sucking bug, *Clavigralla tomentosicollis*, is a key pest of cowpea, an important food legume in West Africa. *Gryon fulviventre*, an egg parasitoid of this pest is a potential biological control agent and proved that inoculative or inundative releases in cowpea fields boost its natural parasitism rates. The current study aimed to determine some biological parameters of *G. fulviventre* on *C. tomentosicollis* eggs under laboratory conditions, and the effects of host age and host density on the parasitic abilities of the parasitoid. On average, *G. fulviventre* development time from egg to adult emergence was 13.14 ± 0.41 days. Female fecundity averaged 27.5 ± 11.21 eggs over their lifetime, with most eggs laid during the first two days of life. The average sex ratio was 73.82% female. The 24-h-old eggs of *C. tomentosicollis* are preferred by *G. fulviventre* females for oviposition. The intrinsic rate of population increase (r_m) was 0.206 ± 0.01 and the population doubling time was 3.37 ± 0.19 days. The functional response of *G. fulviventre* females was dependent on host density. Thus, *G. fulviventre* exhibits some characteristics that may make it a good biological control agent. The results will contribute to the mass production of this beneficial agent, an essential step before implementing the parasitoid release strategies.

KEYWORDS: Biological control, cowpea pod-sucking bug, egg parasitoid, functional response, mass production

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INTRODUCTION

Cowpea, *Vigna unguiculata* L. Walp., is a food legume crop that can contribute to food security and poverty alleviation in West Africa (Manda *et al*. 2019). Its production and consumption are increasing in the region, raising hopes for the sustainability of a key source of plant-based protein for millions of people (Omoigui *et al*., 2018). The annual demand for cowpeas increased by 3.87% in Burkina Faso and 3.60% in Nigeria (Sanginga & Bergvinson, 2015). In Burkina Faso, the production areas increased by 85% over the past 5 years, but this significant growth in cultivated areas is not proportional to the increase in production, which is only 20% (DGESS, 2020). The gap is due to low yields despite the use of improved high-yielding varieties (Ishikawa *et al*., 2013). The low productivity of cowpeas is also due to several constraints, mainly the impressive range of insect pests (Singh *et al.,* 1992).

Among the whole range of pests associated with cowpeas, pod-sucking bugs are particularly feared because of their attacks during the fruiting period. *C. tomentosicollis* Stäl is the main pod-sucking bug of cowpeas and pigeonpeas (Baoua *et al*., 2021; Bello *et al*., 2018). The females of *C. tomentosicollis* are the first to invade the cowpea field at the beginning of the pod setting (Dabiré, 2001). Peak hatching of eggs deposited by the first wave of colonizing females generally coincides with the pod-filling stage. Then, the neonate larvae feed on the tender and juicy pods. The pod maturity stage is favourable for the rapid development of *C. tomentosicollis* (Dabiré *et al.,* 2005) and the production of 2-3 generations at the end of the cowpea growing season (Abdourahamane-Harouna *et al*., 2018). The main consequences of *C. tomentosicollis* attacks are the fall of young pods which do not fill properly with seeds and eventually wilt. Furthermore, following the bug attacks, the most developed pods are malformed and the seeds are

destroyed (Singh & Jackai, 1985; Suh *et al.,*1986), thus reducing the seed germinative capacity. The yield losses due to the pod-sucking bug are estimated to be 60% (Jackai *et al.,* 1989).

Several control methods including cultural methods, varietal resistance and the use of plant extracts, although promising against the pest, have so far had their limits. The effective control of *C. tomentosicollis* is still based on the use of synthetic insecticides that are very harmful to the environment (Gomgnimbou *et al.,* 2009) and to human health (Nicolopoulou-Stamati *et al.,* 2016; Sarwar, 2015). In addition, the repeated and uncontrolled use of these chemicals can eliminate the pest's natural enemies and develop resistant strains within the pest population (Jan *et al.,* 2015). It has been shown in Burkina Faso that the increase in the area used for cowpea production is correlated with the increase in the amounts of synthetic insecticides used for plant protection (DGESS, 2020).

Biological control of *C. tomentosicollis* is currently being considered to reduce pesticide use and limit their negative consequences. Hence, the oophagous parasitoid *G. fulviventre* is attracting much attention because it is the most abundant parasitoid on *C. tomentosicollis* eggs in West Africa (Asante *et al.,* 2000; Dabiré, 2001). The rate of natural egg parasitism is low at the cowpea pod setting stage but can reach 60 to 100% at harvest when damages to cowpea pods and seeds have unfortunately already occurred (Asante *et al.,* 2000; Dabiré, 2001). From this information, it appears that *G. fulviventre* is a good potential candidate for the sustainable management of *C. tomentosicollis*.

However, *G. fulviventre* is a poorly known parasitoid for which very little data is available on biology, ethology, and ecology. However, knowledge of the life trait parameters of a biological control agent determines its ability to control the pest population (van Lenteren *et al.,* 2003). In line with this assumption, we studied some aspects of the ethology of *G. fulviventre* exploring the factors associated with the host and its habitat location (Sanou *et al*., 2019). We then demonstrated that females of *G. fulviventre* use olfactory stimuli emitted by the pest to locate their hosts. These stimuli are thought to be an aggregation pheromone (Kpongbe *et al*., 2019). However, current knowledge of the biology of the parasitoid is still very insufficient to use it in a biological control program (Egwuatu & Taylor, 1977).

To assess the biological control potential of *G. fulviventre*, the current study aimed to determine its biodemographic parameters using *C. tomensicollis* eggs as hosts under laboratory conditions. The influence of host age on parasitism abilities and the parasitoid functional response were also determined.

MATERIALS AND METHODS

Insect origin, mass rearing and laboratory conditions

The strains of *C. tomentosicollis* and *G. fulviventre* used were collected from experimental plots located at the 'Insitut de l'Environnement et de Recherches Agricoles (INERA/Kamboinsé). *Clavigralla. tomentosicollis* was reared in 42 cm square cubic cages and fed with fresh cowpea pods according to the method described by Dabiré *et al.* (2005). The pods were renewed every two days, allowing the females to lay eggs. The eggs, collected daily, were then divided into two batches. The first batch was used for mass rearing of *C. tomentosicollis*. The second batch was subjected to *G. fulviventre* females for parasitism using 250 ml dishes. Parasitoids were fed with undiluted honey placed on cotton that was glued inside the boxes. Parasitized eggs were incubated and monitored until individuals of the new generation were obtained. These rearing procedures were repeated for each generation of bugs and parasitoids in order to maintain the strains and to have insects available for the experiments.

The rearing and bioassays were conducted in the 'Laboratoire Central d'Entomologie Agricole' of the 'Centre de Recherche Agricole et de Formation de Kamboinsé', near Ouagadougou in Burkina Faso. The research activities were carried out under uncontrolled laboratory conditions where the temperature varied from 25 to 34°C, and relative humidity from 30 to 70% with a photoperiod of 12h:12h LD.

Reproduction and life table parameters of *G. fulviventre*

At the beginning of the experiment, newly emerged parasitoids were placed in pairs (42 males and 42 females) inside 6 ml volume containers. Twenty (20) fresh eggs of *C. tomentosicollis* were introduced in each container and renewed daily until the females died. The longevity of male and female *G. fulviventre* was then determined. Eggs removed from the boxes, parasitized or not, were placed for incubation in emergency boxes on the benches. The number of parasitized host eggs was determined and used to estimate the number of eggs deposited by the female parasitoid during its lifetime. Parasitized eggs are easily recognized by their particular colouration. At the end of the embryonic and post-embryonic development, the first-generation parasitoids were collected daily, which allowed us to determine the duration of the development from egg to adult and to estimate the rate of parasitoid emergence. These different parameters were then used to calculate the life table parameters of *G. fulviventre* according to Birch's (1948) formula. These synthetic parameters are as follows :

- The net reproduction rate (R_0) corresponding to the number of females produced per female per generation:

$R_0 = \sum l_x m_x$

where, l_x is the survival proportion of individuals still alive at age x, m_x is the number of female offspring per female of age x

- The mean generation time: $TG = \sum xlxmx/\sum l_x m_x$
- The intrinsic rate of increase $R_m = \ln \sum l_x m_x/TG$
- The doubling time of the population T2 = (ln2)/Rm
- The finite rate of increase $\lambda = e^{Rm}$

Influence of host age on the parasitism of *G. fulviventre*

In this experiment, 24, 48 and 72 h old *C. tomentosicollis* eggs were used. Twenty-five (25) *C. tomentosicollis* eggs from each egg age category were collected and placed in 250 ml dishes in the presence of ten 24-h-old female parasitoids. After 24 h of contact with the eggs, the parasitoids were removed and the eggs were incubated. For each egg age class, parasitized eggs were counted and the developmental time of the parasitoids was recorded. The parasitism rate and development time were then calculated for each egg age class. The experiment was repeated 16 times for each egg age class.

Functional response of *G. fulviventre*

Newly emerged *G. fulviventre* females were individually placed inside 6 ml cylindrical dishes in the presence of increasing density (5, 10, 15, and 20) of host eggs. For each egg's density, eleven replicates were performed. After 24 h of contact with the eggs, the females were removed from the dishes and the eggs were monitored to record the number of parasitized eggs and calculate the rate of parasitism in each case.

Data analysis

Biological data (fecundity, developmental period, emergence rate, sex ratio, life span) were presented as means (±sd). Life table data are presented as mean (±sd). Preference and functional response data were subjected to an analysis of variance (ANOVA PROC GLM) using SAS software version 9.1 (SAS, 2003). When ANOVAs were significant, means were compared by the Student–Newman– Keuls test at the 5% level.

RESULTS

Reproductive and developmental parameters

The total number of eggs laid by a female *G. fulviventre*, estimated from parasitized eggs, ranged from 13 to 63, with an average of 27.57 ± 11.21 eggs over the female lifetime (Table 1). Analysis of the parasitic activity of *G. fulviventre* showed that females were able to lay eggs and parasitize within 24 h following their emergence (Figure 1). The maximum number of eggs was thus deposited during the first 2 days of the females' life. However, oviposition declined rapidly afterwards, so by day 7, almost no more egg was recorded, although the females lived for up to 19.8 ± 7.29 days (Figure 1).

The development time from egg to adult emergence was 12 to 14 days with an average of 13.14 ± 0.41 days. The emergence rate was over 95%. The sex ratio (73.82 ± 9.3 %) was largely female-biased (Table 1). Under the experimental conditions, the intrinsic rate of increase of *G. fulviventre* on *C. tomentosicollis* eggs was 0.206 per day (Table 2). Thus, the parasitoid population multiplied 1.23 times per day (λ) with a doubling time (TD) of 3.37 days. The net reproductive rate (R_0) was 19.07 females for a generation time (TG) of 14.62 days.

Influence of host egg age on parasitism of *G. fulviventre*

Under ambient laboratory conditions, the parasitism of *G. fulviventre* was assessed on *C. tomentosicollis* eggs of different ages. The parasitism rate was significantly affected by host egg age ($F_{2;45} = 54.98$; $P = 0.001$). Parasitoid females preferred fresh eggs to older eggs for parasitisation. Thus, 24-h-old eggs were 99.2% parasitized, whereas 72-h-old eggs were not parasitized at all (Figure 2).

Functional response of *G. fulviventre*

The number of eggs parasitized by a *G. fulviventre* female increased with increasing numbers of *C. tomentosicollis* in a linear regression of equation ($y = 0.7964x + 0.275$; $R^2 = 0.9866$; $F= 52.12$; $P = 0.001$) (Figure 3). Therefore, the functional response was density-dependent, with the rate of parasitism increasing within the host densities tested.

DISCUSSION

We evaluated certain performance parameters of the oophagous parasitoid, *G. fulviventre* in the laboratory.

Table 1. Reproduction parameters of *G. fulviventre* (Hymenoptera: Scelionidae) reared on *C. tomentosicollis* (Hemiptera: Coreidae) eggs at ambient conditions and adults fed with honey

Parameters	Mean value (±SD)
Fecundity (Number of eggs laid per female)	27.57 ± 11.21
Development time (days)	13.14 ± 0.41
Emergence rate (%)	96.77 ± 4.24
Sex ratio (% females) (n =1122)	73.82 ± 9.3
Females' longevity (days)	19.8± 7.29
Males' longevity (days)	19.0 ± 7.11

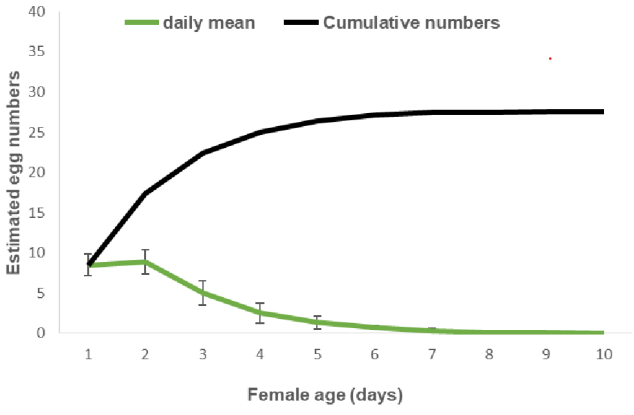


Figure 1. The dynamic of estimated egg numbers laid (Mean±SD) by a female *G. fulviventre* over her lifetime.

Table 2. Life table parameters of *G. fulviventre*

Parameters	Value
Intrinsic rate of increase (R_m)	0.206 ±0.011
Finite rate of increase ($_\lambda$)	1.23± 0.14
Generation time (TG) (days)	14.18± 0.10
Population doubling time (TD) (days)	3.37± 0.19
Net reproduction rate (R_0) females produced/ female/generation	19.07 ± 3.31

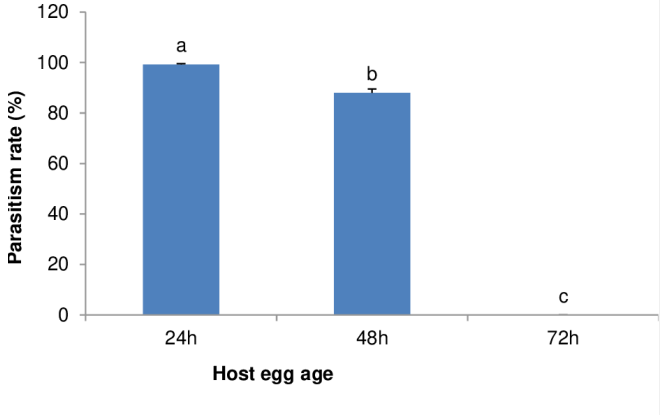


Figure 2. Mean parasitism rate (± SE) of *G. fulviventre* females on *C. tomentosicollis* eggs of different ages.

Females lay fewer eggs (27 eggs/female) than other species of the same genus such as *G. pennsylvanicum* (146 eggs; Peverieri *et al*., 2012), *G. gallardoi* (68 eggs; Canto-Silva *et al*., 2006), *G. gonikopalense* (59 eggs; Martel *et al*., 2019), and *G. philippinense* (82 eggs; Dasilao & Arakawa, 2005). The precise determination of fecundity requires a thorough observation of parasitized host eggs and the consideration of

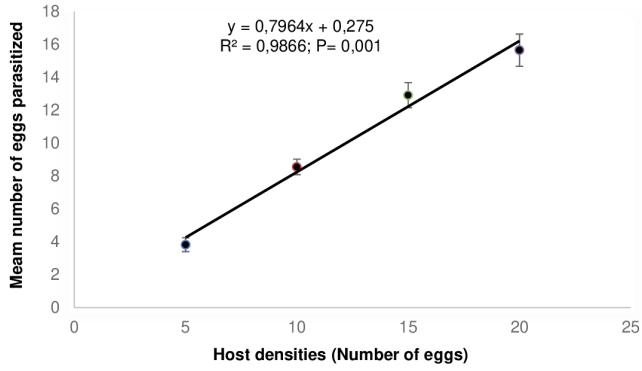


Figure 3. Host density dependent parasitisation of *G. fulviventre.*

possible cases of superparasitism, which could not be done in our experimental conditions. Furthermore, the availability and type of food and temperature are the main factors that impact parasitoid fecundity (Kaboré *et al.,* 2019; Pilkington & Hoddle, 2007).

Analysis of the parasite and oviposition strategy showed that females concentrated eggs during the first days of their life although they lived much longer. This result, which was also observed in other oophagous parasitoids (Dasilao & Arakawa, 2004; Canto-Silva *et al*., 2006; Romeis *et al*., 2000), could be useful in determining the parasitic efficiency of *G. fulviventre* for its practical use in biological control.

The development time of *G. fulviventre* from egg to adult of 13.14 days was significantly shorter than that of other *Gryon* parasitoids associated with several bug species (Dasilao & Arakawa, 2005; Martel *et al.,* 2019; Peverieri *et al.,* 2012). In addition, the estimated emergence rate was high, reflecting low mortality among parasitoids during their development.

The sex ratio was largely female-biased by previous observations in offspring analysis of *G. pennsylvanicum* (70.42%), *G. clavigrallae* (75%) and *G. gallardoi* (79%); (Canto-Silva *et al*., 2006; Peverieri *et al.,* 2012; Romeis *et al*., 2000). This result indicates that females are fertilized when they begin to lay eggs. Females of some parasitoids (Scelionidae) use strategies to regulate the sex of their offspring (Waage, 1982). According to Dasilao and Arakawa (2004), the first eggs laid are males to produce a minimum number of males needed to fertilize females. *G. fulviventre* would adopt the same behaviour since, in our study, males emerge before females. Thus, males were ready to mate with newly emerged females, which would reduce the pre-oviposition time as we observed.

The life table parameters of *G. fulviventre* are synthetic parameters that provide information on the growth and

reproductive capabilities of the parasitoid (Kidd & Jervis, 1996). The values obtained were quite high and indicated a good growth ability of *G. fulviventre*. The maximum intrinsic rate of natural growth (Rm) at 30°C for *C. tomentosicollis* was 0.152 (Dreyer & Baumgärtner, 1996). This value is lower than that obtained for *G. fulviventre* (0.206) under similar conditions. The parasitoid could therefore reproduce faster than its host, this is a characteristic of a good biological control agent (Kidd & Jervis, 1996).

The preference for fresh eggs seems to be common in oophagous parasitoids as we observed in *G. fulviventre*. This behaviour was observed with *Gryon gallardoi* (Brethes) (Hymenoptera: Scelionidae) a parasitoid of *Spartocera dentiventris* (Berg) (Hemiptera: Coreidae) eggs (Da Rocha *et al*., 2006), as well as with *Telenomus remus* (Hymenoptera: Scelionidae) on the eggs of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Peñaflor *et al.,* 2012). This phenomenon was also observed with *Cleruchoides noackae* Lin and Huber (Hymenoptera: Mymaridae), a parasitoid of *Thaumastocoris peregrinus* Carpintero and Dellape (Hemiptera: Thaumastocoridae) eggs (Barbosa *et al.,* 2018). This preference may be explained due to the nutritional quality of the eggs, which would depend on their stage of embryonic development (Vinson, 1998). Young eggs offer more nutrients favourable to the larval development of the parasitoid (Godin & Boivin, 2000; Sousa & Spence, 2001) while old eggs with advanced embryonic development are no longer suitable for parasitoid development (Strand, 1986). The latter hypothesis helps to understand our results where *G. fulviventre* females successfully parasitize only 1–2-day-old eggs while the incubation time of *C. tomentosicollis* eggs is 3-4 days (Abdourahamane-Harouna *et al*., 2018; Dabiré, 2001).

Functional response analysis showed that the number of parasitized eggs increases with the density of eggs submitted to the female. *G. fulviventre* females can increase their parasitization performance if they are in the presence of high numbers of *C. tomentosicollis* eggs. This is a good indicator of performance for a biological agent (Luna *et al*., 2007). The mechanisms responsible for the increase in parasitism are thought to be a decrease in host handling time and an increase in host search rate (Stiling, 1987; Walde & Murdoch, 1988). However, the functional response of a parasitoid is influenced by many factors, such as the densities tested, and the duration of the experiments (Islam *et al*., 2006; Tazerouni *et al*., 2017).

CONCLUSION

This study aimed to determine certain parameters of the biology of *G. fulviventre* in order to assess its potential for biological control of *C. tomentosicollis* in cowpea. This study showed that *G. fulviventre* normally develops on new eggs (24-48-h-old) of *C. tomentosicollis*, giving a high percentage of females. Its intrinsic growth rate is higher than that of its host and the functional response of females is density dependent. These results contribute to a better knowledge of the biology of *G. fulviventre* and can be used in the development of a biological control strategy against *C. tomentosicollis*, the main pod-sucking pest of cowpea in Burkina Faso.

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