

Life Cycle and Fertility Life Table of *Zelus vespiformis* (Hemiptera: Reduviidae)

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Abstract: *Zelus vespiformis* Hart (Hemiptera: Reduviidae) is a native predator which has been recorded frequently preying on some insects pest of coffee plantations in Colombia. In order to know its biological parameters, its life cycle and fertility life table were evaluated under laboratory conditions at Cenicafé, using *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae). To obtain the duration and survival of immature stages, 716 eggs were followed and 23 adults pairs (♂: ♀) were established to construct the life table. The mean duration from egg to adult was 112.65±0.76 days; the egg incubation period was 23.22±0.21 days; and nymphs had 11.75% survival to the adult stage. The longevity of adults was 25.86±2.94 and 30.39±2.26 days for females and males respectively. The mean number of eggs per female was 105.55±10.75. The parameters of the fertility life table were net reproductive rate $R_0=5.19$ (2.54–10.28); generation time $T=106.75$ (101.7–114.0) days, intrinsic growth rate $r_m=0.015$ (0.009–0.022), and finite growth rate $\lambda=1.015$ (1.009–1.023). This information forms a biological basis for initiating studies that pretend to determine the potential of this insect as predator.

Keywords: Predator, Biology, Assassin bug, Demography

1. Introduction

Studies carried out by Cenicafé with the coffee pest ‘Chamusquina’ coffee bug *Monalonion velezangeli* Carvalho & Costa (Hemiptera: Miridae) in Colombia, have resulted in field observations of some natural enemies of nymphs and adults of this species, including insects of the family Reduviidae (Hemiptera) [1]. Reduviid predators, commonly called predatory bugs or assassin bugs, belong to the subfamily Harpactorinae. Harpactorinae are diurnal insects that inhabit various weeds, shrubs and crops [2]. They feed on other insects from different orders; therefore, they are considered generalist predators [3]. Among the Harpactorinae reported to date as predators of *M. velezangeli* are *Zelus vespiformis* Hart and the genera *Arilus* Hahn, *Repipta* Stål [1], and *Castolus* Stål [4]. The reduviid *Z. vespiformis* is a Neotropical species distributed from Central America to northern South America; it is reported in Colombia, Costa Rica, Ecuador, El Salvador, Panama, Trinidad and Tobago, and Venezuela [5]. Its depredatory capacity on *M. velezangeli* was tested in confined field conditions by Laiton et al. 2018 (personal

communication), who found a consumption of 2.8±0.67 *M. velezangeli* individuals in 5 hours.

Although prior to this study, the biological attributes of *Z. vespiformis* was not known, progress has been made in knowledge of the biology of related species, such as *Zelus renardii* Kolenati [6], *Zelus tetracanthus* Stål (as *Z. socius*) [7], *Zelus exsanguis* Stål [8] and *Zelus longipes* Linnaeus [9].

Knowing of the biology of predators, especially factors such as the critical stages of development, birth rates, mortality and generation times, is important to identify the parameters that have the greatest influence on the population structure, which allows the development of an efficient rearing system [10] in order to evaluate its potential as a biological-control organism. The life table provides information on the development, survival, and reproduction of an individual cohort, as well as other data on population parameters [11]. Fertility tables are useful to study the population dynamics of arthropods, since the growth potential of a population is estimated [12]. Grundy et al. [13] developed a successful rearing method for *Pristhesancus plagipennis* Walker (Hemiptera: Reduviidae), a predator of

Creontiades spp. (Hemiptera: Miridae) in Queensland, Australia. Likewise, Tomson et al. [14] developed a rearing method under controlled conditions for *Rhynocoris fuscipes* Fabricius (Hemiptera: Reduviidae), a predator of *Aphis gossypii* Glover (Hemiptera: Aphididae), *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae), and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae).

Because of the lack of knowledge of the biological characteristics of *Z. vespiformis*, which may be exercising some control of *M. velezangeli* in the field, we studied the biological and reproductive parameters of *Z. vespiformis* fed on an alternative diet under controlled laboratory conditions; with the aim of supporting future studies that intend to test the predator's potential as biological controller.

2. Materials and Methods

The study was conducted in the Entomology Department of Cenicafé, in the municipality of Manizales, Caldas, Colombia, in a rearing room under controlled conditions of temperature $25\pm 2^{\circ}\text{C}$, relative humidity $80\%\pm 10\%$, and photoperiod 12:12 [D:N]. The insects to found the rearing colony were obtained from coffee farms in the municipality of La Plata ($2^{\circ}20'44.7''\text{N} - 075^{\circ}52'21.5''\text{W}$), Huila, Colombia.

2.1. Rearing Colony

Considering that prior attempts to rear *M. velezangeli* in the laboratory have been unsuccessful, an alternative method using a diet with larvae of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) developed by Cenicafé [15] was adapted and used for rearing. A diet using *G. mellonella* has been successful in similar studies with other species of Reduviidae [16–18].

The *Z. vespiformis* nymphs collected were taken to the laboratory and maintained in rearing units in a climate-controlled room at $25\pm 2^{\circ}\text{C}$, $80\pm 10\%$ RH and a photoperiod of 12:12 h [L:D], until adult emergence. The rearing unit was composed of an acrylic box ($21 \times 11 \times 8$ cm) with two ventilation holes. The box contained a cotton wad moistened with a 5% honey solution as an alternate source of food, and three fresh coffee leaves. Each insect was fed 10 *G. mellonella* larvae three times per week. Emerging adults were separated by sex based on external characteristics of the last abdominal segment. The adults were placed in a ratio of 2: 2 (♂:♀) in acrylic boxes as described above, in order to provide an ample supply of males for mating. Each egg mass laid was placed in a new acrylic box with paper towels moistened three times per week, to maintain sufficient humidity for the nymphs. After emergence, the paper toweling was removed and three fresh coffee leaves, a cotton wad moistened with a 5% honey solution, and *G. mellonella* larvae were placed in the box.

2.2. Life Cycle of *Zelus vespiformis*

2.2.1. Immature Phase

To carry out the biological cycle of *Z. vespiformis*, 716 eggs were used, from 10 egg masses of different females

from the rearing colony (F2). The egg masses were placed in individual transparent acrylic boxes 21 cm long by 11 cm wide by 8 cm high, with two ventilation holes (experimental unit). The insects were observed daily from the egg to adult stage, to determine: duration of the incubation period, viability of the eggs, survival of nymphs, and sex ratio (♂: ♀). To determine the survival of each nymphal stage, newly hatched individuals of each egg mass were reared to adult stage together in a single rearing container. Insects were maintained in groups until the adult emergency because they showed higher mortality when they were kept in a separate container than placed together. To facilitate the counts of changes in the stage of each insect and identify the time of molt, each individual was marked with a fluorescent pigment (DayGlo® Color Corp, Cleveland, Ohio, USA) on the dorsal part of the abdomen, using a #0 brush. The moment of the molt was recognized when the exuvium fluorescing under black light and because the nymphal stage was unmarked. Each new nymph was again marked with a different-colored pigment to continue the study.

2.2.2. Adult Phase: Reproduction and Longevity

With the emerged adults, 23 couples of similar age were placed in a ratio of 1: 1 (♂: ♀) in acrylic boxes (each pair considered an experimental unit). The pre-oviposition period, gross fecundity (M_x), and longevity in days were estimated for each experimental unit. The evaluation concluded when the adults died.

2.3. Statistical Analysis

A descriptive analysis was carried out for each variable evaluated: measures of central tendency using weighted arithmetic means of the individuals obtained from each egg mass, and measures of variation (standard error SE), by means of the statistical program SAS version 9.4 [19]. The survival of immature stages was analyzed using a Kaplan-Meier survival curve, applying the PROC LIFETEST procedure of SAS version 9.4 [19].

2.4. Fertility Life Table of *Zelus vespiformis*

An age-stage, two-sex fertility life table was constructed using the biological attributes of *Z. vespiformis*: duration of egg-adult development, survival of immature stages, preoviposition period, sex ratio, male and female life spans, and egg-laying capacity [20]. Data obtained for the number of females alive each day at each age (x) and the number of eggs oviposited each day were used to calculate the basic population parameters: gross fecundity (M_x), survival (l_x), fecundity (m_x), net fecundity function ($l_x \cdot m_x$), generation time (T), net reproductive rate (R_0), intrinsic growth rate (r_m), and finite increase rate (λ). Bootstrap technique was used to calculate the variance of the estimated parameters, according to Meyer et al. [21] using the statistical software R version 3.4.4. [22].

3. Results and Discussion

3.1. Life Cycle of *Zelus vespiformis*

3.1.1. Immature Phase

The egg-adult development period of *Z. vespiformis* was 112.65 ± 0.76 days (Table 1). The incubation period averaged 23.22 ± 0.21 days (Table 1). The duration of the egg phase was longer than reported for other species of *Zelus*: 16.87 ± 1.76 days for *Z. longipes* [9], 17.00 ± 0.28 days for *Sycanus galbanus* Distant (Hemiptera: Reduviidae) [18], and 9 ± 1.0 days for *Z. tetracanthus* [7]. The percentage of hatching was 88%. Several reports indicate that the species of subfamily Harpactorinae have 60% viability or higher, i.e., 75% for *Z. renardii* [6] and 91% for *Z. tetracanthus* [7]. Reported hatching percentages for other representatives of this subfamily are 64.3% for *Repipta flavicans* Stål (Hemiptera: Reduviidae) [23] and 71.29% for *Sphedanolestes variabilis* Distant (Hemiptera: Reduviidae) [24]. All nymphs hatched within 48 h; during this process, the opercular lid was released and was located on the side of the egg.

The duration of the nymphal phase was 56.95 ± 1.09 days, with five nymphal instars showing durations between 8.96 and 14.97 days (Table 1). This is a longer period than that reported for *Z. renardii*, which showed a mean nymphal phase of 33.18 ± 0.38 days, with durations between 4.75 and 10.36 days [6]. This difference may be due to factors such as food, temperature, and relative humidity, which may influence the duration and survival of nymphs and adults [25- 27]. Additionally, the grouped or individual conditions in which these insects breed can influence the development time and survival of the nymphs; accordingly, Sahayaraj [28] reported that a population of *Acanthaspis pedestris* Stål (Hemiptera: Reduviidae) fed in groups had a shorter nymphal period (61.13 days) compared to nymphs raised individually (70.47 days).

Table 1. Biological parameters of *Zelus vespiformis* fed on *Galleria mellonella* larvae under laboratory conditions. ($T 25 \pm 1^\circ\text{C}$, RH $80 \pm 3\%$ and photoperiod 12:12 [D: N]). ($n=10$ egg masses).

Biological parameters	mean \pm SE
Incubation period (days)	23.2 \pm 0.21
Duration of stage (days)	
Instar I	12.04 \pm 0.87
Instar II	10.93 \pm 0.31
Instar III	8.96 \pm 0.53
Instar IV	10.29 \pm 0.39
Instar V	14.97 \pm 0.49
Adult	104.52 \pm 14.64
Viability (fertility) (%)	88.22 \pm 2.96
Survival rate (I-V) (%)	11.75
Sex ratio (σ : ρ)	1:0.7

The survival of the nymphal phase was 11.75% (Figure 1). Nymphal mortality was highest in the first four stages (76%). Despite of nymphs were maintained in groups until the adult emergency to increase the individual survival, there was mortality during instars I and II as a consequence of cannibalism. Cannibalism is reported in different species of insects; for example, García-González *et al.* [29] reported in *Delphastus pusillus* LeConte (Coleoptera: Coccinellidae),

that when oviposition occurs in groups, the individuals that emerge first may consume their siblings. Cannibalism is a type of predation that arises from intra-specific competition and can play an important role in maintaining the population stability and age structure of some predators [30, 31]. Cannibalism can be considered a non-advantageous strategy, but in times of resource scarcity, the consumption of individuals of the same species can help to extend the life span [32]. This strategy has been found in other families of generalist predators, such as members of Chrysopidae (Neuroptera) [33] and Coccinellidae (Coleoptera) [34].

Similarly low survival rates to the nymphal phase of *Z. vespiformis* were reported by Barrera *et al.* [6] for *Z. renardii*, with values of 23.7%; and by Swadener and Yonke [7] for *Z. tetracanthus*, with 23.6%, which indicates that members of the *Zelus* characteristically show survival rates of less than 25% during this phase. The survival curve obtained for this species (Figure 1) resembles the type III survival curve of Rabinovich [35], which is characterized by higher natural mortality in the immature stages of the population, and lower mortality in the adult stage, characteristic of predators.

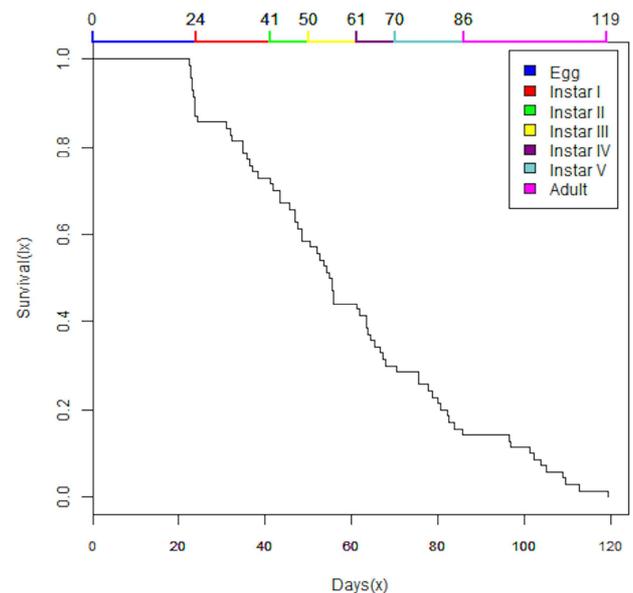


Figure 1. Kaplan-Meier survival analysis of *Zelus vespiformis* in laboratory conditions.

The sex ratio obtained was 1.0: 0.7 (σ : ρ) (Table 1), consistent with those reported in other species of the subfamily Harpactorinae, such as *R. flavicans* [23], *S. variabilis* [24] and *Z. renardii* [6], for which a sex ratio of 1:1 (σ : ρ) was reported.

3.1.2. Adult Phase: Reproduction and Longevity.

Of the 23 established pairs, 20 females produced at least one egg mass. The pre-oviposition period of *Z. vespiformis* was within the ranges of *Z. longipes* (from 15 to 18 days) [9] (Table 2) and *Z. renardii* (from 11 to 20 days) [6]. During the post-oviposition period, the females did not guard the egg masses as some other members of Reduviidae do, as described by Odhiambo [36]. On average, each female oviposited 1.55

egg masses, varying between one and four masses per female, which were oviposited in 25.86 days (Table 2).

Zelus vespiformis showed a fecundity of 68.10 ± 2.00 eggs / mass / female (Table 2). Of these egg masses, 74% contained between 55 and 78 eggs, and only 16% had more than 80 eggs per mass (Figure 2). The frequency distribution of the number of eggs per mass showed positive asymmetry ($G1=0.35$) with a mode of 63 eggs per oviposition (Figure 2). This asymmetry indicates that, in terms of fertility, the population is better represented by the mode than by the mean of the data. This accords with Matesco et al. [37], who stated that some biological parameters are better estimated by measures of central tendency other than the mean, an argument based on the study of *Chinavia longicorialis* Breddin (Hemiptera: Pentatomidae).

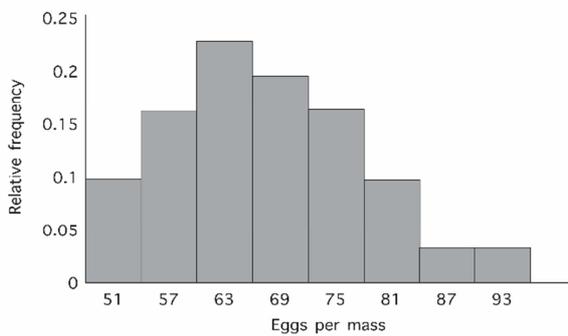


Figure 2. Relative frequency distribution of the number of eggs per *Zelus vespiformis* mass (n=31 masses).

Regarding the total fecundity, estimated as the mean of eggs placed per female (Table 2), several values have been reported for species of *Zelus*. For example, for *Z. renardii*, individuals fed on *Drosophila* spp. (Diptera: Drosophilidae) produced 95.25 ± 5.3 eggs / female [6]; *Z. longipes* fed on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) produced 134.10 ± 14.08 eggs / female [9]; and *S. galbanus* fed on *G. mellonella* produced 96.60 ± 5.65 eggs / female

[18]. Reported fecundity for *Z. exsanguis* is 406 eggs / female [8], and for *Z. tetracanthus* is 68 eggs / female [7].

The longevity of males and females differed (Table 2), and are lower than reported for other congeners, such as *Z. longipes* (σ 42.6 ± 4.6 and ♀ 44.6 ± 5.77 days) [9] and for the female of *Z. tetracanthus* (52.7 days) [7]. The differences in longevity of the adults may be due to genetic factors, influenced by the diet provided or the abiotic rearing conditions. The biocontrol effect of predators is based on the need for both immature and adult stages to consume more than one prey individual to support their vital functions [29]. Therefore, a constant supply of prey with good nutritional quality is crucial for the proper development of these predatory insects.

Table 2. Biological attributes of adults of *Zelus vespiformis*, under laboratory conditions (Temperature $25 \pm 1^\circ\text{C}$, RH of $80 \pm 3\%$ and photoperiod 12:12 [D: N]).

Parameters	mean \pm SE
Pre-oviposition period (days)	18.25 \pm 1.68
Number of masses per female	1.55 \pm 0.17
Number of eggs per mass	68.10 \pm 2.00
Number of eggs per female (Mx)	105.55 \pm 10.75
Male Longevity σ (days)	30.39 \pm 2.26
Female Longevity ♀ (days)	25.86 \pm 2.94

The relationship between fecundity and survival of the females of *Z. vespiformis* showed three oviposition peaks (Figure 3). The maximum rate of population growth, represented by the intersection of the specific survival (lx) and the gross fecundity (Mx) occurred between the second and fourth week after the females emerged. This is similar to that reported by Barrera et al. [6] for *Z. renardii*, which showed the highest oviposition rates between the second and fifth week after the females emerged. This may be due to the maturation of the eggs and the reproductive compatibility, or the ability to copulate immediately after the formation of the pairs [38]. According to Rabinovich [35], the adult phase of non-social insects is generally marked by a pre-oviposition period, followed by the reproductive phase where the effort reaches a maximum and then declined rapidly as the females age.

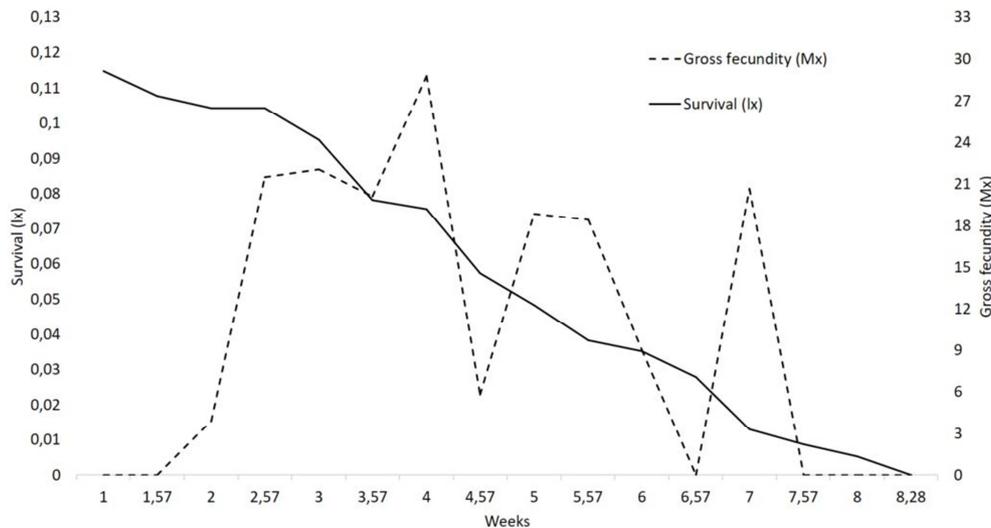


Figure 3. Relationship between gross fecundity (Mx) and survival (lx) of females of *Zelus vespiformis* (n=20 females).

3.2. Fertility Life Table of *Zelus vespiformis*

The generation time (T) of *Z. vespiformis* was 106.75 days, during which the species multiplied 5.19 times (R_0), being able to produce 3.4 generations per year in laboratory conditions (Table 3). Other members of Reduviidae show similar net reproductive rates; *Amphibolus venator* Klug (Hemiptera: Reduviidae) showed an $R_0=8.52$ when fed on *Tribolium confusum* J. DuVal (Coleoptera: Tenebrionidae) under laboratory conditions [39]. Predator species have low net reproductive rates, since they need to consume a certain amount of prey in order to produce a female [40], and the presence of large numbers of individuals can generate intense intraspecific competition, which can negatively affect population levels.

The population of *Z. vespiformis* showed an intrinsic growth rate (r_m) greater than 0 (Table 3), indicating that the population increases with each generation [41]. The potential for laboratory reproduction of *Z. vespiformis* is similar to that reported by Imamura *et al.* [39], who calculated an intrinsic growth rate for the reduviid *A. venator* of $r_m=0.019$ when fed on *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) in the laboratory; but differs from that reported for *Rhynocoris longifrons* Stål (Hemiptera: Reduviidae) ($r_m=0.09$) when fed on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in the laboratory [42].

The finite rate of increase (λ) indicates that the females of *Z. vespiformis* have an increase capacity of 1.5% for each generation (Table 3).

Table 3. Estimated parameters for the fertility life table of *Zelus vespiformis*. (Temperature $25\pm 1^\circ\text{C}$, RH $80\pm 3\%$, and photoperiod of 12:12 [D: N]). ($n=20$ females).

Parameter	Estimation (CI)
T	106.75 (101.7–114.0)
R_0	5.19 (2.54–10.28)
r_m	0.015 (0.009–0.022)
λ	1.015 (1.009–1.023)

T=generation time, R_0 =Net reproductive rate, r_m =Intrinsic growth rate, λ =Finite rate of increase. (95% Bootstrap Confidence Intervals).

4. Conclusions

Although the nutritional sources provided and the abiotic conditions used in this study may have influenced the development, survival, reproduction and longevity of *Z. vespiformis* individuals, as described by Eilers-Kirk and Fleischer [43], the results found for the different population parameters of *Z. vespiformis* are also affected by genetic factors. More research will be needed to determine the optimal biotic and nutritional conditions to allow the highest possible reproductive potential of this species in the laboratory. Additionally, these results can be used as a basis to test the biological parameters of *Z. vespiformis* fed on the pest *M. velezangeli* in future works, which pretend to know its viability as biological controller of the pest.

Author Contribution Statement

Pablo Benavides Machado planned, supervised and administratively executed the study; Pablo Benavides Machado and Marisol Giraldo Jaramillo designed the study; Laura Alexandra Laiton Jiménez collected insects in the field and reared the stock of *Z. vespiformis*; Laura Alexandra Laiton Jiménez executed the experimental work; Laura Alexandra Laiton Jiménez, Marisol Giraldo Jaramillo and Pablo Benavides Machado analyzed the data; and Laura Alexandra Laiton Jiménez, Marisol Giraldo Jaramillo, and Pablo Benavides Machado wrote the manuscript.

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