

Life table of *Chrysoperla externa* (Neuroptera: Chrysopidae) reared on *Melanaphis sacchari* (Hemiptera: Aphididae)

Tabla de vida de *Chrysoperla externa* (Neuroptera: Chrysopidae) alimentado con *Melanaphis sacchari* (Hemiptera: Aphididae)

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Abstract: *Chrysoperla externa* is a widely distributed and highly adaptable predatory insect. In order to evaluate its capacity as a natural enemy of the sugarcane aphid, *Melanaphis sacchari*, its life table when reared on *M. sacchari* was inferred. The biological cycle lasted an average of 29.5 ± 6.2 d from egg to adult. The embryonic and larval period duration was 5.1 ± 0.3 and 13.8 ± 4.5 d, respectively. The pupa stage was completed in 10.6 ± 1.4 d, whereas adults lived 63.5 ± 26.7 d after eclosion. The mortality rate showed the highest values in the egg stage ($qx = 0.17$). The greatest survival rate appeared in the third instar (93.7 %). The period prior to reproduction was 6.5 ± 3.1 d. The average fecundity was 228.3 ± 139.1 eggs. The life table parameters were $R_0 = 113.2$, $T = 28.9$, $rm = 0.163$ and $\lambda = 1.17$. According to the results, it is possible to infer that *C. externa* is capable of accomplishing its life cycle feeding only on *M. sacchari*.

Keywords: Biological control, life table parameters, predator, sugar cane, phytophagous, *Melanaphis sacchari*, *Chrysoperla externa*.

Resumen: *Chrysoperla externa* es un insecto depredador ampliamente distribuido y altamente adaptable a diferentes ambientes. Para evaluar su capacidad como enemigo natural de *Melanaphis sacchari* se realizaron estudios de su ciclo biológico y tabla de vida utilizando a esta plaga como alimento. El ciclo biológico duró en promedio $29,5 \pm 6,2$ d de huevo a adulto. El periodo embrionario y la duración del estado larval fueron de $5,1 \pm 0,3$ y $13,9 \pm 4,5$ d, respectivamente. La pupa completó su ciclo en $10,6 \pm 1,4$ d y los adultos vivieron $63,5 \pm 26,7$ d. La tasa de mortalidad registró los valores más altos en el estado de huevo ($qx = 0,17$). La mayor viabilidad se presentó en la L3 (93,7 %). El periodo de preoviposición fue $6,5 \pm 3,1$ d y el promedio de huevos ovipositados por hembra fue de $228,3 \pm 139,1$. Las características reproductivas $R_0 = 113,2$, $T = 28,9$, $rm = 0,163$ y $\lambda = 1,17$. De acuerdo con los resultados, es posible inferir que *C. externa* es capaz de completar su ciclo de vida alimentándose sólo de *M. sacchari*.

Palabras clave: Control biológico, parámetros tabla de vida, depredador, caña de azúcar, fitófago, *Melanaphis sacchari*, *Chrysoperla externa*.

Introduction

Lacewings of the genus *Chrysoperla* (Neuroptera: Chrysopidae) are some of the most important and frequently used natural enemies in biological control of agricultural pests (Chang *et al.* 2000; Miller *et al.* 2004). *Chrysoperla externa* (Hagen, 1861) is a predator with a high degree of adaptability to different climates; it allows this species to have a wide geographical distribution (Gitirana *et al.* 2001). Its wide prey range, including soft-bodied insects such as aphids, whiteflies, and thrips, allows for its use in many biological control programs (Guarín 2003). The predatory potential of the Chrysopidae family increases with their size during the larval stage (Velázquez-Grisales 2004; Loera *et al.* 2001). Adults feed on nectar, pollen or honey produced by insects (Loera *et al.* 2001). They are easy to breed in laboratories (Cardoso and Lazzari 2003).

In order to work with a natural enemy, either a predator or a parasitoid, in a biological control program, it is important to know about its biology. This information is basic and necessary in order to implement it in the integrated pest management programs (Salamanca Bastidas *et al.* 2010).

Therefore, considering the potential of *C. externa* for integrated pest management programs, this study was performed in order to learn about the biological aspects of *C. externa* fed on *Melanaphis sacchari* (Zehntner, 1897) (Hemiptera: Aphididae), an invasive pest considered one of the aphid species of greatest economic importance on the world (ElSayed 2013; Peña-Martínez *et al.* 2015) and recently introduced to Mexico (SENASICA 2014).

Materials and methods

All experiments were performed in the Entomophagous Insect laboratory of the Centro Nacional de Referencia de Control Biológico (IE-CNRCB) (18°55'3452"N 103°53'01.95"W) under conditions of 25 ± 2 °C, 60-70 % R. H. and 14:10 h L:D cycle.

Insect sources. *C. externa* and *M. sacchari* were collected in sorghum fields in the village of Tecuanillo in the municipality of Tecomán, Colima, México (18°51'30.37"N 103°52'52.03"W); 15 masl). Prior to the biological study, chrysopids were identified based on morphological characteristics (Brooks 1994). Aphids were identified using molecular analysis through the extraction of genomic DNA using the DNeasy® Blood and Tissue (QIAGEN® 2006). Specimens were kept at the Colección de Insectos Entomófagos del Centro Nacional de Referencia de Control Biológico, Tecomán Colima, México.

A colony of *C. externa*, feeding on *M. sacchari* was established in the department of IE-CNRCB. The second generation was used to carry out the subsequent experiments.

The aphids were reared on *Sorghum vulgare* L. plants in 5 L pots and placed in the CNRCB greenhouses.

Development and survival of *Chrysoperla externa*. The study started with a cohort of 135 *C. externa* eggs under 24 h old. They were placed in groups of 10 in 5 cm diameter Petri dishes and covered them with damp filter paper in order to prevent drying and mortality. The incubation period from egg laying to the emergence of larva 1 was recorded.

In order to monitor the larval stage, the recently emerged larvae were transferred individually to Petri dishes with a daily average of 50 *M. sacchari* nymphs as food. As a feeding substrate for the aphids, at the bottom of the dish, it was placed 5 cm diameter of a *S. vulgare* leaf. Every 24 h the density of the nymphs was adjusted, and the leaf of sorgho was changed to a fresh one. The duration of each larval stage was registered according to the size of the larva and the presence of exuviae.

The beginning of the pupa stage was established when the cocoon was observed, and it culminated in the emergence of the adult.

Once the adults emerge, their sex was determined, and they were transferred with entomological tweezers individually to vessels 350 mL. They were monitored until death. In order to facilitate ventilation and keep the adults from escaping, a 4 cm diameter opening covered with organza cloth was made. On the bottom side of the cup, a 1.5 cm diameter perforation was

made and covered with cotton and moistened daily to provide water to the adults. Inside the cup, a 2 x 10 cm impregnated paper piece with an artificial diet composed of honey, beer yeast, pollen, ascorbic acid and spirulina (Palomares-Pérez *et al.* 2017).

The duration of the biological cycle is presented as mean and standard deviation using the Excel program (Ott 1993). To compare longevity between females and males, a Student's t-test was conducted using the statistical program SAS 9.2 (SAS INSTITUTE 2008).

Reproduction of *Chrysoperla externa*. 50 pupae were selected and placed them in an 18 cm diameter, 7 cm tall dish. It was monitored 24 h periods, once the adults emerged; they were collected with entomological tweezers and using a stereoscopic microscope to determine sex, according to size and apical form of abdomen. Thirteen both females and males were selected and placed in pairs in 350 mL styrofoam cups until his death. When a male died, another replaced it. Observations were made every 24 h to register the start of egg laying, the number of eggs laid per day and mortality of females.

Life table analysis. The life table was studied through groups of individuals (cohorts) born in the same space of time, from birth until death of the last adult (Rabinovich 1980; Begon *et al.* 1999). A cohort of 163 eggs was analyzed with the methodology described in the biological cycle, specifying for each age interval the following parameters: X = age (days), N_x = total number of individuals observed at the beginning of each stage or state, l_x = proportion of survivors at the beginning of the age (N_x/N_0), d_x = number of deaths between ages l_x y $l_x + 1$, q_x = death rate (d_x/l_x), K = death force ($K = \log_{10}N_x - \log_{10}N_{x+1}$) and e_x = life expectancy (T_x/l_x).

Results and discussion

Development and survival. The biological cycle of *C. externa* presented an average duration of 29.5 ± 6.2 d from the egg until adult emergence, with a range of 18 to 45 d. Biological cycle may vary by feed type and temperature; Auaud *et al.* (2001) and Giffoni *et al.* (2007) who have observed the developmental time of *C. externa* tend to be faster at 26 °C fed *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) (26.1 ± 0.10 d), which is attributed to their generalist feeding behavior.

The eggs hatched on average at 5.1 ± 0.3 d, data fits with the range of 5 to 6 d reported by Ribeiro (1988) when using different prey. Similarly, Fonseca *et al.* (2015), using *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) as food, reported an embryonic period of 3 to 15.1 d. According to the above, it is concluded that depending on the prey and the temperature, the eggs of *C. externa* presents different periods of incubation. The temperature and the nutritional quality of prey are decisive factors in the biological performance of predator insects, altering for instance the developmental time of pre-imaginal stages and reproductive performances of adults (e.g. fecundity, fertility) (Michaud 2005; Cabral *et al.* 2006; Jalali *et al.* 2010).

The larval period presented durations of 13.8 ± 4.5 d, superior to the data from Lima (2004), who reported a period of 10.8 d fed on *Brevicoryne brassicae* (L., 1758) (Hemiptera: Aphididae). This difference can be linked to food quality in

which a shorter life cycle is the result of good nutrition and faster adaptation by the predator (Soffiantini Lira and De Luna Batista 2006).

The pupa state was completed in 10.6 ± 1.4 d. In the study by Giffoni *et al.* (2007), average duration of 7.5 d was found when they were fed on *Aphis craccivora* (Koch, 1854) (Hemiptera: Aphididae).

Adult longevity was 63.5 ± 26.7 d (Table 1). These results are within the average life range of these predators reported by Soffiantini Lira and De Luna Batista (2006).

Out of a total of 86 adults, 50 were females and 36 males; this represents a 1.4:1 sex ratio. The developmental time was slightly longer for females, although the difference was not statistically significant ($F = 1.42$; $Pr = 0.2785$) (Table 1). Duration of the larval stage with respect to sex did not show a significant difference ($F = 1.09$; $Pr = 0.6153$) (Table 1).

The survival of the different biological stages indicates full development of *C. externa* fed on *M. sacchari*, the opposite is reported by Giffoni *et al.* (2007) and Salamanca Bastidas *et al.* (2010); they mention that the biological cycle of *C. externa* is incomplete, and it only reaches the pupal stage when they feed on *Aphis nerii* (Boyer, 1841) (Hemiptera: Aphididae) and immature states of *Neohydatothrips signifer* (Priesner, 1932) (Thysanoptera: Thripidae). Giffoni *et al.* (2007) also refer a wide variation in the biological cycle of *C. externa* feeding on different species of aphids; when it consumes *A. craccivora* and *R. maidis* its biological cycle from egg to adult is 20.7 and 36.8 d, respectively. It is important to remember that insect development, in general, is also affected by laboratory conditions, mainly temperature, relative humidity and exposure to light (McEwen *et al.* 2001).

Without a doubt, the results of this study show that *C. externa* is capable of completing its biological cycle by feeding only on *M. sacchari* nymphs. This is important to consider if one seeks to introduce and establish this predator in an area where it is not found naturally.

Reproduction of *Chrysoperla externa*. The pre-oviposition period to egg laying ranged from 2 to 11 d with an average of 6.5 ± 3.1 d, time similar to the reported by Saini and Salto (1999), but longer to the reported by Elkarmi *et al.* (1987) for other species of the same genus, *Chrysoperla rufilabris* (Burmeister, 1839) and *Chrysoperla carnea* (Stephens, 1836) they presented a period of 3 d. In nature, a lengthy period prior to egg laying is a disadvantage for the survival of the species, since adults who have not reproduced yet are exposed longer to their natural enemies (Canard 1981).

The average number of eggs laid per female was 228.3 ± 139.1 , with a range from 39 to 542. The daily number of eggs laid was 2.8 ± 0.2 . The longest-lived female lived for 105 d, while the shortest-lived lasted 12 d.

The fertility curve indicates cyclical behavior with a descending trend and maximum production peaks at 15, 45 and 53 d. Subsequently, egg laying decreases constantly until the death of the female (Fig. 1).

In population terms, the fecundity was $mx = 168.9$. The net reproduction rate R_0 registered a value of 113.2, meaning that population will increase approximately 113 % in generation time (T) of 28.9 d. The intrinsic growth rate (rm) was 0.163, this means the population would multiply 16.3 % from one unit of time to the next. Finally, the finite reproductive rate (λ) of 1.17 indicates that for each chrysopid at any given time,

Table 1. Biological cycle (days) of *Chrysoperla externa* fed on *Melanaphis sacchari* at 25 ± 2 °C, 60-70 % RH, 14 light h.

State and/or Stage	n ¹	Total			Females (n = 50)		Males (n = 36)	
		Mean ± S.D. ²	Range	Mean ± S.D. ²	Range	Mean ± S.D. ²	Range	
Egg	135	5.1 ± 0.3	5-6	5.1 ± 0.2	5-6	5.1 ± 0.2	5-6	
Larva 1	135	4.3 ± 1.4	1-9	4.5 ± 1.2	4-9	4.3 ± 1.0	3-8	
Larva 2	111	3.2 ± 1.1	1-7	3.3 ± 1.1	1-7	3.1 ± 0.9	3-4	
Larva 3	104	6.3 ± 2.0	1-11	6.2 ± 1.9	3-10	6.4 ± 2.0	3-10	
Pupa	100	10.6 ± 1.4	10-12	10.7 ± 0.9	9-13	10.5 ± 1.0	8-12	
Adult	86	63.5 ± 26.7	6-104	65.5 ± 28.6	6-104	60.8 ± 24.0	9-97	

¹Number of individuals = n. ²Standard deviation = S.D.

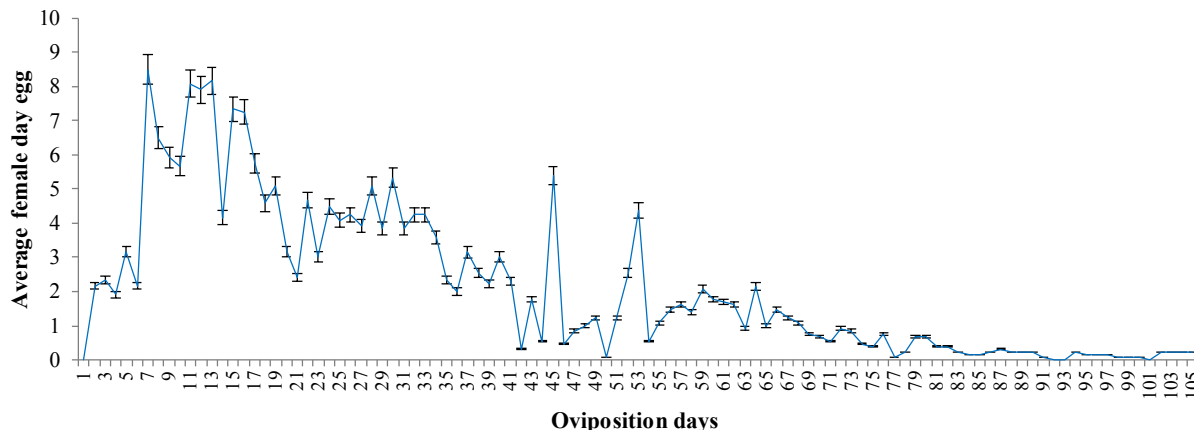


Figure 1. Eggs oviposited per day by females of *Chrysoperla externa* fed on *Melanaphis sacchari* at 25 ± 2 °C; 60- 70 % RH; 14 light h.

Table 2. Horizontal life table (days) of *Chrysoperla externa* fed on *Melanaphis sacchari* at 25 ± 2 °C, 60-70 % RH, 14 light h.

X	Nx	lx	dx	qx	ex	K
Egg	163	1.00	28	0.17	4.00	0.08
Larva 1	135	0.82	24	0.17	3.64	0.08
Larva 2	111	0.68	7	0.06	3.08	0.01
Larva 3	104	0.63	4	0.03	2.20	0.01
Pupa	100	0.61	14	0.14	1.86	0.07
Adult	86	0.52	86	1	1	0.00

X = biological state; Nx = number of individuals; lx = proportion of survivors; dx = number of deaths; qx = death rate; K = mortality force; ex = life expectancy.

there will be 1.17 individuals in the next time unit. Without a doubt, these results show the reproductive and colony establishment potential of *C. externa* is possible when it feeds on *M. sacchari*.

Life table analysis. The population parameters of cohort table showed that specific death rate (qx) maintains high values in the egg stage (0.17). This is reflected in mortality intensity (K) (0.08) (Table 2).

Both the egg stage and the first larval stages showed a higher mortality (qx) (Table 2). This data suggests a type III survival curve (Southwood 1978; Rabinovich 1980), which indicates a high mortality rate in the early stage of *C. externa*.

In the evaluated population, 86 individuals reached the adult stage (Table 2), it means that 63.7 % of insects handled complete their development, more than the reported by Salamanca Bastidas *et al.* (2010), who indicate that only 30 % of *C. externa* individuals complete their cycle when they feed on *R. maidis*.

The greatest viability was registered in larva 3 (96.2 %), this is important when considering that this stage is the most voracious and therefore the most predatory. Carvalho *et al.* (1998) and Figueira *et al.* (2000) report inferior viability in larva 3 when they are fed on eggs of *Alabama argillacea* (Hübner, 1823) (Lepidoptera: Noctuidae) (90 %) and nymphs of *Schizaphis graminum* (Rondani, 1852) (Hemiptera: Aphididae) (70 %).

Conclusions

The 63.7 % of insects completed their biological cycle; therefore, it is concluded that *M. sacchari* satisfies the necessary nutritional requirements to complete the four biological stages of *C. externa*. The net reproduction rate R_0 , the intrinsic growth rate (rm) and the finite reproduction rate (λ) show the reproductive potential of *C. externa* when is fed on *M. sacchari*.

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Author contribution

Martín Palomares-Pérez: Information, analysis and writing.

Teresa de Jesús Molina-Ruelas: Data collection.

Manuel Bravo-Núñez: Data Collection.

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