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Biological studies and life table of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) on *Aphis craccivora* Koch (Hemiptera: Aphididae)

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Abstract

Solano Y, Delgado N, Morales J, Vásquez C. 2016. Biological studies and life table of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) on *Aphis craccivora* Koch (Hemiptera: Aphididae). Entomotropica 31(34): 267-275.

The aim of the work was to estimate the biological and population parameters of *Cycloneda sanguinea* (L.) on *Aphis craccivora* Koch under laboratory conditions. The experiments were carried out in an acclimatized room at 25 ± 2 ˚C, 72 ± 10 % RH and photoperiod 12:12 (L:D). The development time of egg, larval, prepupal and pupal stages were 2 ± 0; 6.2 ± 0.5; 0.6 ± 0.2 and 3.1 ± 0.2 days, respectively. The sex ratio was 1: 0.76 and longevity of virgin adults was 83.8 ± 11.4 and 83.1 ± days for females and males, respectively. The fecund females lived during 75.7 ± 16.9 days and had a fecundity of 1 828 ± 385 eggs/female with pre-oviposition period of 6.0 ± 1.1 days. The survival curve was type I with mortality concentrated in old adults, and the fecundity curve showed two oviposition peaks on days 30 and 35. In addition, the net reproductive rate was 718.78 female egg/female, in a generation time of 35 days with intrinsic rate of natural increase of 0.180 and a finite rate of increase of 1.197. Biological data and life table results indicate that *C. sanguinea* can be a valuable natural enemy of *A. craccivora* due to its fast development and high reproductive capacity.

Additional key words: Biological control, life cycle, population growth, predator, prey.

Resumen

Solano Y, Delgado N, Morales J, Vásquez C. 2016. Estudios biológicos y tabla de vida de *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) sobre *Aphis craccivora* Koch (Hemiptera: Aphididae). Entomotropica 31(34): 267-275.

El objetivo de este trabajo fue estimar parámetros biológicos y poblacionales de *Cycloneda sanguinea* (L.) sobre *Aphis craccivora* Koch bajo condiciones de laboratorio. Los experimentos fueron conducidos en cuartos aclimatizados a 25 ± 2 ˚C, 72 ± 10 % RH y fotoperiodo 12:12 (L:D). El tiempo de desarrollo del huevo, larva, prepupa y pupa fue 2 ± 0; 6,2 ± 0,5; 0,6 ± 0,2 and 3,1 ± 0,2 días, respectivamente. La proporción sexual fue 1: 0,76 y la longevidad para adultos vírgenes fue 83,8 ± 11,4 días y 83,1 ± 9,8 días para hembras y machos, respectivamente. Las hembras fecundas vivieron durante 75,7 ± 16,9 días en los cuales tuvieron una fecundidad de 1 828 ± 385 huevos/hembra con un periodo de pre-oviposición de 6,0 ± 1,1 días. La curva de sobrevivencia fue tipo I, con mortalidad concentrada en adultos viejos y la curva de fecundidad mostró picos de oviposición en los días 30 y 35. Adicionalmente, la tasa neta reproductiva fue de 718,78 huevos hembra/hembra en un tiempo generacional de 35 días, con una tasa intrínseca de crecimiento natural de 0,180 y una tasa finita de crecimiento de 1,197. Los datos biológicos y resultados de la tabla de vida indican que *C. sanguinea* puede ser un enemigo natural valioso para el control de *A. craccivora*, debido a su rápido desarrollo y alta capacidad reproductiva.

Palabras clave adicionales: Ciclo de vida, control biológico, crecimiento poblacional, depredador, presa.

Introduction

The black cowpea aphid, *Aphis craccivora* Koch is considered one of the most injurious pests of legumes crops in Africa, Asia and America (Pettersson et al. 1998). Both nymphs and adults of *A. craccivora* produce a direct damage by sucking plant sap causing reduction in plant growth and affecting fruits and seeds production, as well as an indirect damage as vector of many viral diseases such as CpAMV and BICpMV on beans, and FBNYV and BLRV on broad beans (Cermeli 1970). This aphid is established in Venezuela since 1966 and is considered an important pest on species such as *Arachis hipogea* L., *Cajanus cajan* (L.), *Gliricida sepium* (Jacq.), *Phaseolus vulgaris* L. and *Vigna unguiculata* (L.) (Ordosgoitty 1972, Carrera and Cermeli 2001, Leite and Beicher 2007).

Strategies to control *A. craccivora* have included insecticides due to its effectiveness in reducing population. However many of them cause resistance in aphids and damage to man and beneficial insects (Ali et al. 2013). In addition, varieties of resistant legumes also have been successfully used (De la Pava and Sepulveda-Cano 2015). Nevertheless this resistance could be broken by aphids (Souleymane et al. 2013). Therefore, natural enemies offer an ecological alternative because of their successfull application in the biological control of many injurious aphids. In consequence, different researches have been made in order to get more knowledge about parasitoid and predator species, which could be used in integrated pest management.

An essential step in this process is to determine biological parameters of the natural enemies feeding on the pest species. *Cycloneda sanguinea* (L.) is a common predator throughout the world used to control various aphid species such as *Aphis craccivora* Koch, *Macrosiphum euphorbiae* (Thomas), *Megoura viciae* Buckton, *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover (Işikber and Copland 2002, Boiça et al. 2004, Oliveira et al. 2005, Işikber 2008), and several studies have been undertaken to determine different biological aspects of *C. sanguinea* using aphids such as *A. gossypii*, *Toxoptera citricida* (Kirkaldy), *Dactynotus* sp., *Aphis citricola* Van der Goot, *Schizaphis graminum* (Rondani), *Cinara atlantica* (Wilson) and *Cinara pinivora* (Wilson) as prey (Hurtado 1997, Santa-Cecília et al. 2001, Cardoso and Lázzari 2003, Oliveira et al. 2004). There is thus an extensive literature on the subject. Nevertheless, information related to the population growth of this species remains scarce.

An understanding of the biology of *C. sanguinea* is vital in order to be able to analyse its life table. According to Duarte and Zenner (2009) the life table of a predator enables us to evaluate the role of a biological control agent in a pest-natural enemy system or to compare two populations of biological control agents, and is useful for the periodic evaluation of the health of a population raised under laboratory conditions.

This study was undertaken to determine the life cycle, life table, survival curves and fecundity of *C. sanguinea* when fed with *A. craccivora,* in order to get helpful information , which can be used in a biological control program against this pest.

Materials and Methods

Rearing

Capture and maintenance of *C. sanguinea*

Lady beetle adults were collected on bean plants (*Vigna unguiculata* L.) at the experimental agricultural field station of the Universidad Centroccidental Lisandro Alvarado, Tarabana, Lara state, Venezuela. The beetles were taken to the laboratory and sexed according to Gordon (1985) and fed on *A. craccivora* during one week, before beginning the experiments. A total of 10 male/female pairs were then immediately transferred to a 3.785 L glass container, one pair per container, previously prepared by covering

the bottom with filter paper moistened with water and the top with organdy cloth to ensure adequate ventilation. The glass jars containing the adults were maintained in an insect rearing room at 25 ± 2 ˚C, 72 ± 10 % RH and photoperiod 12:12 (L:D). Adults were daily fed with cowpea aphids infested bean leaves.

The filter paper on the bottom of the glass jars was moistened daily and fresh aphid-infested bean leaves added. The containers were checked twice daily for beetle egg masses. Bean leaves containing eggs were removed from the jars and placed in petri dishes (9 cm wide) covered with organdy cloth. These were observed daily until hatching. The F_1 individuals (larvae, recent emerged males and females) were used for determining the life cycle and life table of this predator. Larvae used in this study were kept in the same container during all experiment, so larval handling was reduced and damage to them or mortality was avoided.

Capture and maintenance of *A. craccivora*

A. craccivora was established in the laboratory on *V. unguiculata,* from individuals collected at the experimental agricultural field station of the Universidad Centroccidental Lisandro Alvarado, Tarabana, Lara state, Venezuela. The prey was kept on bean plants grown in a 350 cm^3 plastic plots, which were maintained in an acclimatized room with the same conditions described before. In the laboratory, bean plants were infested with adult aphids placed on leaves and stems, to ensure a permanent supply of prey. Daily, leaves with aphids were cut to feed predators in experiments.

Biological parameters of *C. sanguinea*

The life cycle of *C. sanguinea* was determined using the F_1 individuals raised in the laboratory as described above. Leaves containing eggs were placed in petri dishes, covered with organdie cloth, labelled with the oviposition date. The eggs were observed twice daily to record their incubation time. Once the larvae had emerged, 30 neonates were randomly selected, placed individually in petri dishes similar to those previously described and fed daily *ad libitum* with *A. craccivora*. The experiment was conducted using a completely randomized design whereby each larva was treated as a replicate. The development through the larval stage was assessed by observing the exuviae left in the container by each instar. Prepupal and pupal stages were observed until adults emerged. Since the second larval instar and the prepupa last less than 24 h, the experiment was checked twice daily. The number of days the predator spent in a particular development stage was also recorded and used to calculate average duration of development.

Longevity of *C. sanguinea* virgin adults was determined from those adults emerged during the life cycle experiments. Each specimen was sexed and placed individually in a 500 cm^3 container prepared as previously described. Each container was labeled to indicate sex, date of emergence and the replicate number. All individuals were daily fed with enough cowpea aphids until their natural death. Number of days each individual remained alive was noted and used to calculate mean longevity for both males and females.

The sex ratio of *C. sanguinea* also was assessed from those 30 F1 individuals used during the life cycle study. The adults emerged were sexed as previously described and expressed as a male: female ratio.

Life table for *C. sanguinea*

The life table for *C. sanguinea* was calculated following the methods described by Birch (1948) and Ravinobich (1980). Ten male/female pairs 48 hours old were raised under laboratory conditions. Each pair was maintained in a 3.785 L glass container prepared as described previously and covered with organdy cloth for 72 hours. Thereafter males were removed from the containers. Females were fed daily with

bean leaves infested with *A. craccivora*. Any *C. sanguinea* eggs found were removed and placed in petri dishes labeled with the oviposition date and identifying number of the female that laid them. The total number of eggs/female, eggs/female/day and female egg/female, together with longevity of the fecund female, were recorded until the natural death of the females. This data was used to calculate the following biological parameters:

Survival curve: obtained from the proportion of adult individuals alive at a specific age. Age-specific survival was calculated by the following equation: Survival = N_x / N_o

where x is the age in days of *C. sanguinea* females, N_x is the number of females alive at age x, N_o is the initial number of females.

Age-specific fecundity curve (m_x): obtained from the mean number of female egg mass laid by each female at a specific age.

Net reproductive rate (R_o)*:* indicates the number of female egg mass laid by each female in one generation, as calculated by the equation:

$$R_o = \Sigma(l_x)(m_x)$$

where x is the age in days of the *C. sanguinea* females, l_x is the number of females alive at age x, m_x is the number of daughters laid by each female at age x.

Generation time (T): represents time it takes a female, from the day the egg is laid, to reach reproductive age, as calculated by the equation:

$$T = (\Sigma x l_x m_x)/R_o$$

Intrinsic rate of natural increase (r_m) indicates the capacity of a population to multiply in one generation, such that:

$$r_m = (l_n R_o)/T$$

The finite rate of increase (λ) is the number of individuals added to the population per individual and unit of time, such that:

$$\lambda = e^{rm}$$

Statistical analyses

The data obtained for the life cycle were analysed using the statistical package STATISTIX for Windows® Version 7.0, using a descriptive statistic, while longevity data for virgin adults was analysed using the Student's t-test for homogeneous variances, which was applied for testing differences in mean longevity between sexes.

Results and Discussion

Life cycle of *C. sanguinea*

The mean development time of *C. sanguinea* (Table 1) from egg to adult, 11.8 days, was similar with previous reports as indicated by Figueira et al. (2003), Boiça et al. (2004) and Oliveira et al. (2004), who studied this predator feeding on *S. graminum* (12.4 days), *A. gossypii* (12.7 days) and *C. atlantica* (15.12 days), respectively, under similar conditions. However, mean larval development time found in this study, 6.2 days, was shorter than data obtained by those authors, who reported 7.5; 7.9 and 9.04 days, respectively. This could have occurred because larvae are the only feeding stage;, additionally, prey quality could cause such differences. Consequently, the high nutritional value of *A. craccivora*, an increase in the rate of consumption (possibly because this aphid is highly palatable) and/or the rate of its assimilation by the predator could be responsible for the results found.

The difference between longevity of *C. sanguinea* adults was not significant according to the Student T-test (Table 1). Mean longevity of *C. sanguinea* virgin adults was greater in this study than that reported by Santos and Pinto (1981) for the same species, but fed on *Toxoptera citricida* (Kirkaldy) (63 days). Nevertheless, other studies have reported even higher longevities for this species, for example Cardoso and Lazzari (2003): 167.1 days on *Cinara* spp., and Oliveira et al. (2004): 125.70 days on *C. atlantica*. In addition, Torres (2008) registered longevities

Table 1. Development time in days of *C. sanguinea* fed with *A. craccivora* under laboratory conditions. N = 30

Stages	Development time (Days)	
	Mean ± SE	Range
Egg	2.0 ± 0.0	0
1st instar	1.9 ± 0.2	1.5 – 2.0
2nd instar	0.6 ± 0.2	0.5 – 1.0
3rd instar	1.6 ± 0.2	1.5 – 2.0
4th instar	2.1 ± 0.3	2.0 – 3.0
Larval period	6.2 ± 0,5	5.5 – 7.0
Prepupa	0.6 ± 0.2	0.5 – 1.0
Pupa	3.1 ± 0.2	3.0 – 3.5
Egg – Adult	11.8 ± 0.5	11.0 – 13.0
Female	83.8 ± 11.4 a	73 – 100
Male	83.1 ± 9.8 a	74 – 100

Means with same letter are not significantly different. Student T-test for homogeneous variances P = 0.89.

of 258.33 and 364.50 days for *Cryptolaemus montrouzieri* Mulsant virgin males and females, respectively, fed with *Maconellicoccus hirsutus* Green. The sex ratio of *C. sanguinea* males to females produced in the laboratory was 1: 0.76. This differs from that registered by Oliveira et al. (2004) for *Hippodamia convergens* Guérin-Méneville (1:1), *C. sanguinea* (1:1.17) and *Eriopis connexa* (Germar) (1:1.17).

Life table of *C. sanguinea*

The survival curve (lx) for female *C. sanguinea* was highest during the first 40 days, but then started to decline progressively until reaching zero on day 90 (Figure 1). The pattern of survival of *C. sanguinea* females fits a type I curve (Deevey 1947) in which individuals die at an advanced age. Previous studies have reported similar survival patterns for other coccinelid species such as *Stethorus pauperculus* (Weise), *S. siphonulus* Kapur, *C. transversalis*, *Eriopis connexa connexa* (Germar), *Coccinella septempunctata*, *Propylea quatuordecimpunctata* L. (Rattanatip et al. 2008, Ali and Rizvi 2009, Duarte and Zenner 2009, Ali and Rizvi 2010, Duarte et al. 2013, Papanikolaou et al. 2014).

Mean longevity for *C. sanguinea* fecund females was 75.7 ± 16.9 days, with a pre-oviposition period of 6.0 ± 1.1 days. Omkar and Bind (2004) reported similar values for *C. sexmaculata*, which showed longevity of 77.7 days and pre-oviposition period of 5.5 days on *A. craccivora*, while Omkar and James (2004) observed longevity of 79.50 days and pre-oviposition period of 10.30 days for *C. transversalis* fed with this same aphid species. According to Lewontin (1965), species with a short pre-oviposition period are generally successful colonisers. Therefore, the short pre-oviposition period exhibited by *C. sanguinea* proves to be an important feature in biological control as it reflects a capacity for rapid invasion by this predator of aphid-infested crops.

The fecundity curve (m_x) for *C. sanguinea* showed two oviposition peaks on days 30 and 35 with an average of 27.43 ± 5 eggs/day, and the total number of eggs laid reached a mean of 1 828 ± 385 eggs/female (Figure 1). Omkar et al. (2005) observed a mean daily oviposition rate of 29.42 for *C. sexmaculata*, while Omkar et al. (2009) reported for *Anegleis cardoni* (Weise) 21.15 eggs/day, both fed on *A. craccivora*. With regard to the total number of eggs laid,

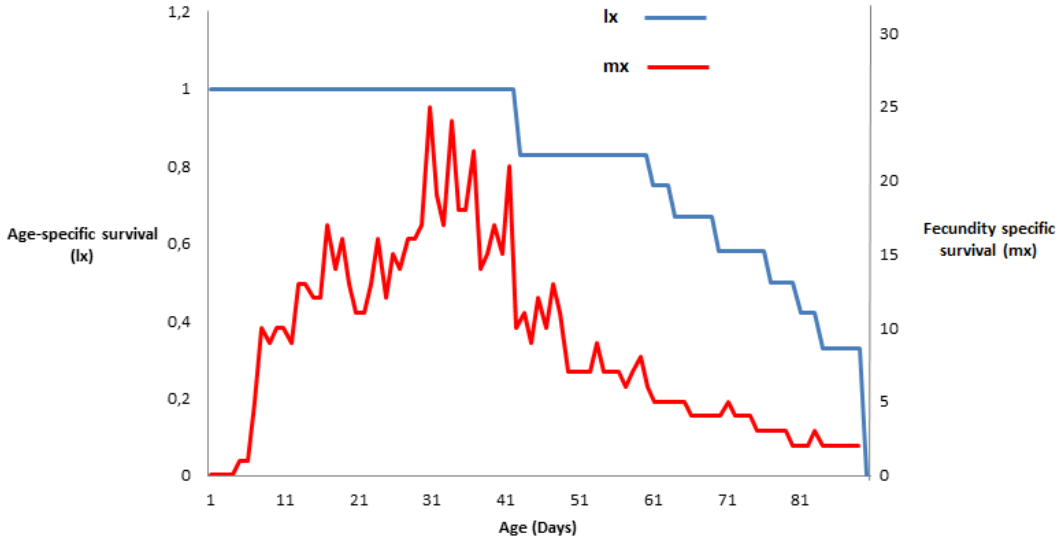

Figure 1. Age-specific survival (l_x) and age-specific fecundity (m_x) curves of *C. sanguinea* when fed on *A. craccivora*.

several other studies have registered high values for species fed with *A. craccivora*, such as *C. transversalis*: 1 155.9 eggs/female (Omkar and James 2004), *C. sexmaculata*: 1 096.9 eggs/female (Omkar and Bind 2004), *H. variegata* 1 075 eggs/female (Mandour et al. 2011).

The differences observed between results of this study and those obtained by other researchers confirm that factors such as the nutritional quality of the prey and rearing conditions can affect fecundity (Hodek and Honek 1996). Evans (2003) indicated that female coccinelids tend to lay large numbers of eggs when prey densities are high in order to guarantee that individuals will reach the end of the larval stage before the aphid colony collapses or emigrates. In this study, rearing conditions favored high rates of oviposition by *C. sanguinea* due to the high densities of aphids available to them in a confined space in which they were maintained. This enabled the females to direct all their energy towards the production of eggs, rather than searching for oviposition sites. The fecundity and longevity values registered for *C. sanguinea* thus reflect a high reproductive profile in egg production and reproductive period. In this sense Dixon (2000) suggested that a high fecundity could be associated with coccinelids that live longer.

The reproductive parameters for *C. sanguinea* are shown in Table 2. The net reproductive rate, generation time and finite rate of increase was low compared to that reported for *C. septempunctata* on *Aphis fabae* Scopoli which was Ro= 1 004.1 female egg/female, T = 58.6 days and λ = 1.13 (Kontodimas et al. 2008). Nevertheless, other studies have reported lower replacement rates than that observed in *C. sanguinea*, but with similar generation times and finite rates of increase, for example *Propylea quatuordecimpunctata* L. on *A. fabae* (R_o = 375.1; T = 35.7; λ = 1.18), *Harmonia axyridis* Pallas on *C. atlantica* (R_o = 632.7; T = 26.74; λ = 1.27) and *H. axyridis* on *Anagasta kuehniella* Zeller (R_o = 346.55; T = 39; λ = 1.159) (Kontodimas et al. 2008, Santos et al. 2014, Zazycki et al. 2015). The differences observed by different researchers can be attributed to biotic factors such as the predator species, the type of prey and the host plant, or abiotic factors such as prey and predator rearing conditions.

Table 2. Life table parameters for *C. sanguinea* fed on *A. craccivora* under laboratory conditions.

Parameters	Values
Net reproductive rate (Ro) (female egg/female)	718.79
Intrinsic rate of natural increase (r_m)	0.18
Generation time (T) (days)	35
Finite rate of increase (λ)	1.197

The intrinsic rate of natural increase (r_m) was close to that reported by Kontodimas and Stathas (2005) for *H. variegata* (0.178), Dehkordi et al. (2013) for *H. variegata* on *A. gossypii* (0.187) and Zazycki et al. (2015) for *H. axyridis* on *A. kuehniella* (0.148).

The biological parameters observed in this species are compatible with its mass-rearing, not only with respect to its short life cycle, but also considering its capacity for quick population growth under laboratory conditions, when compared to other ladybird predators. Regarding the duration of larval development, Santos et al. (2013) indicated that reduction of immature phase may represent a strategy to remain in that phase for a short time, particularly as immatures are vulnerable to predation. Thus, *C. sanguinea* larvae could be more effective than other predator species.

Hence, this kind of laboratory studies might help to better understand population dynamics under both laboratory and field conditions, and consequently to apply this knowledge in biological control tactics contained in integrated pest management on *A. craccivora* and other aphid species attacking legume crops.

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