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Functional Response of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) to the Black Pea Aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae)

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Abstract

Solano Y, Delgado N, Morales J, Vásquez C. 2016. Functional Response of *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) to the Black Pea Aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae). Entomotropica 31(38): 311-318.

In this study, we determined feeding response, handling time (T_h) and search rate (*a*) of the ladybeetle *Cycloneda sanguinea* (L.), with respect to the black pea aphid, *Aphis craccivora* Koch. The experiments were conducted using larvae and adult males and females reared under laboratory conditions. Various mathematical models were applied to the feeding response data using regression analysis. The T_h and *a* values, were estimated by a nonlinear regression analysis. At the highest density tested, the fourth larval instar (L4) consumed daily an average of 132.2 aphids/cage, compared with the first (L1), second (L2) and the third (L3) larval instars, which consumed 10.6, 35.2 and 72.8 aphids/cage, respectively, while adult females consumed 233.8 aphids/cage and adult males consumed 209 aphids/cage. The functional response curve to L1 adjusted better to the exponential model, while L2, L3, L4, male and female adults adjusted better to linear or type II Holling's model. The values of T_h and *a,* were higher in L1 and L3, with respect to other larval instars, while in the adult stage, females presented lower T_h and greater *a,* compared with males. We conclude that L4 and adult females are the most voracious stages of *C. sanguinea* feeding on *A. craccivora*.

Additional key words: Biological control, handling time, predator, prey, search rate.

Resumen

Solano Y, Delgado N, Morales J, Vásquez C. 2016. Respuesta functional de *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) al áfido del frijol, *Aphis craccivora* Koch (Hemiptera: Aphididae). Entomotropica 31(38): 311-318.

En este estudio, se determinó la respuesta de alimentación, el tiempo de manipulación (T_h) y la tasa de búsqueda (*a*) de *Cycloneda sanguinea* (L.), sobre *Aphis craccivora* Koch. Los experimentos fueron conducidos usando larvas y adultos machos y hembras, criados bajo condiciones de laboratorio. Varios modelos matemáticos fueron aplicados a los datos de respuesta de alimentación usando análisis de regresión. Los valores de T_h y *a*, fueron estimados por análisis de regresión no lineal. El cuarto instar larval (L4) consumió diariamente un promedio de 132,2 áfidos/cápsula comparados con el primer (L1), segundo (L2) y tercer (L3) instar larval los cuales consumieron 10,6; 35,2 y 72,8; respectivamente, a las densidades más altas, mientras que las hembras adultas consumieron 233,8 áfidos/cápsula y los machos adultos 209 áfidos/cápsula . La curva de respuesta funcional de L1 se ajustó mejor al modelo exponencial, mientras que L2, L3, L4 y adultos machos y hembras se ajustaron mejor al modelo lineal y tipo II de Holling. Los valores de T_h y ***a,*** fueron más altos en L1 y L3, que en los otros instares larvales, mientras que en el estado adulto, las hembras mostraron un T_h más bajo y una *a* más alta, comparado con los machos. Estos resultados revelan que L4 y las hembras adultas son los estados más voraces de *C. sanguinea*.

Palabras clave adicionales: Control biológico, depredador, presa, tasa de búsqueda, tiempo de manipulación.

Introduction

One of the methods used to assess the impact of predators on prey population growth is the functional response, where the number of prey consumed by a predator is defined as a function of prey density. This has been represented by the Holling disc equation, which describes the relationship between search rate (a), handling time (T_h) and consumption rate for any predator (Holling 1959).

Three types of curves related to different feeding strategies have been proposed for showing the functional response of predators. Type I curve rises linearly to a saturation point beyond which no further increase in feeding rate can occur. Type II curve is curvilinear. As prey density increases, the number of prey eaten per predator also increases, but at a decreasing rate as the maximum value is approached. Type III is a sigmoid curve, and represents organisms such as birds and mammals that are capable of some type of learning behavior (Holling 1959). However, this type of curve has also been reported for coccinellids such as *Stethorus gilvifrons* Mulsant, *Exochomus flavipes* Thunberg and *Cryptolaemus montrouzieri* Mulsant feeding on *Tetranychus turkestani* Ugarov and Nikolski, *Phenacoccus manihoti* Matile-Ferrore and *Maconellicoccus hirsutus* (Green), respectively (Sohrabi and Shishehbor 2007, Essien et al. 2014, Torres and Marcano 2015).

The determination of the functional response at each stage of development of a predator is a valuable parameter to visualize which of these stages will be more efficient in controlling the prey (Seko and Miura 2008). Several previous studies suggest that fourth instar larvae frequently exhibit a lower T_h than that adults, which indicates that this predatory stage of coccinellid may be considered most efficient as reported for *Adalia tetraspilota* (Hope) on *A. craccivora* (Shah and Khan 2013) and *Anegleis cardoni* (Weise) on *Aphis gossypii* (Omkar and Kumar 2013).

Extensive research has been conducted to study the functional response of *Cycloneda sanguinea* (L.). Morales and Burandt (1985) reported a type II curve for larvae, adult males and females fed with *Toxoptera citricida* (Kirkaldy). Santa-Cecilia et al. (2001) also found a type II response in larval instars of *C. sanguinea* at different densities of the aphid *Schizaphis graminum* (Rondani), except for the second instar. Işikber (2005) compared the functional response of *C. sanguinea* and *Scymnus levaillanti* Mulsant on cotton aphid *Aphis gossypii* Glover, and indicated that consumption of both predators showed a type II functional response.

The black pea aphid *Aphis craccivora* (Koch) has been recorded as a pest of economically important crops such as potatoes, peppers and beans, in which it is responsible for the transmission of viruses (Ofuya 1997, Carrera and Cermeli 2001, Leite and Beicher 2007). Vandenberg (2002) and Araujo-Siqueira and Almeida (2006) observed larval and adult stages of *C. sanguinea* feeding on this aphid. The purpose of this study was to determine the functional response of the larval instars, adult males and females of *C. sanguinea* feeding on *A. craccivora*. This information could be useful for predicting the importance and usefulness of this predator in a legume pest management system.

Materials and Methods

Rearing of *C. sanguinea*

Adult ladybeetles were collected on bean plants, *Vigna unguiculata* (L.) at the Universidad Centroccidental Lisandro Alvarado (UCLA) Agricultural Experimental Station, Tarabana, Lara State, Venezuela. The ladybeetles were brought to the laboratory and sexed following the method of Gordon (1985).

Ten males and females were placed in a glass jar (3.785 L), which was previously prepared with the bottom covered with filter paper moistened with distilled water, and the tap covered with an organdy cloth to allow ventilation. Aphids were obtained from infested bean plants growing in the field and taken to the laboratory to infest other bean plants in order to ensure a permanent supply of prey. Both predator and prey were placed in a rearing room at 25 ± 2 °C, 72 ± 10 % RH and 12:12 (L:D) photoperiod.

Daily, filter paper in the glass jar was moistened, new aphids were added on bean leaves and jars were checked for ladybeetle oviposition. Leaves containing eggs were isolated in Petri dishes (90 mm of diameter x 15 mm of height), and covered with an organdy cloth lid. These were observed daily until hatching. F1 individuals, larvae, male and female virgins were used as test organisms in the feeding trials.

Feeding trials

The experiment was conducted using the first instar larvae (L1) selected at random from the Petri dish cultures. Feeding tests for L1 consisted of 5 treatments, corresponding to densities of 10, 15, 20, 25 and 30 aphids per cage. The aphids used were nymphs between the second and fourth instars of development. L2 larvae developed from L1 were evaluated on 30, 40, 50, 60 and 70 aphids/cage. Similarly, L3 and L4 obtained during the experiments were evaluated at the densities 70, 80, 90, 100 and 110, and 110, 120, 130, 140 and 150 aphids/cage, respectively. Five replications were conducted for each treatment. The cages used consisted of petri dishes prepared as previously described, where the predator larvae and the respective number of aphids were placed together. The number of unconsumed aphids for L1, L3 and L4 were recorded each 24 hours, while L2 was checked at 12h, since ecdysis occurred within 24 h. New leaves and the respective density of aphids were placed in the cages. Development through the larval stage was determined by the presence of the exuvia left by each instar.

Adult feeding trials were conducted using those males and virgin females obtained from larvae used in the experiment described above. Both, males and females 24 hours old were randomly selected and separately kept in a Petri dish (same size as above) provided with filter paper moistened with distilled water, but without food for a period of 24 hours. One male or female virgin ladybeetle was separately placed in cages containing 50, 75, 100, 125, 150, 175, 200, 250 or 300 aphids for a total of 9 treatments. The experiment was replicated five times. Daily, new aphids on leaves were introduced to maintain the respective density of aphids in the cages as originally stated. Petri dishes were checked daily for 5 days and the number of unconsumed aphids was recorded.

Data analysis

Cycloneda sanguinea larval and adult feeding response was analyzed using the statistical software CoStat for Windows version 4.21. Mean value consumption for larval and adult stages was analyzed by Tukey's test for determining differences one another. The mathematical models of Holling, linear, exponential and polynomial were fitted to the feeding response data using regression analysis. The F value and determination coefficient (r^2) were used as criteria to evaluate each model.

The Holling model is defined as follows: $N_e = (a)(T)(N_o)/[1+ (a)(T_h)(N_o)]$, Where N_e is the number of consumed prey, N_o is the initial prey number, T is total time, which was 24 hours, a is the search rate, and T_h is the handling time per prey.

Searching rate (a) and handling time (T_h) in each larval instar and adult males and females, were estimated by no linear regression analysis.

Results and Discussion

Aphid consumption rate by larvae (L1, L2, L3 and L4) and adult males and females of *C. sanguinea* increased as prey density increased (Table 1 and Table 2, Figures 1, 2, 3 and 4). The functional response curve to L1 adjusted better

Table 1. Average number of prey eaten/day by larvae instars of *C. sanguinea* at different prey densities.

Larval instar	Prey density	Eaten prey/day*
L1	10	6.6 ± 0.9 b
	15	8.4 ± 2.4 ab
	20	9.8 ± 2.6 ab
	25	9.2 ± 0.8 ab
	30	10.6 ± 1.1 a
L2	30	18.8 ± 3.3 b
	40	21.4 ± 5.4 ab
	50	23.0 ± 3.5 ab
	60	35.2 ± 10.7 a
	70	35.2 ± 7.3 a
L3	70	54.0 ± 8.9 b
	80	58.8 ± 10.2 ab
	90	65.2 ± 4.9 ab
	100	70.2 ± 19.0 ab
	110	72.8 ± 5.1 a
L4	110	98.4 ± 7.4 c
	120	103.6 ± 9.2 bc
	130	117.3 ± 6.7 ab
	140	130.2 ± 6.4 a
	150	132.2 ± 5.6 a

*Based on 5 observations. Means within a instar followed by different letters are significantly different (P < 0.01; according to Tukey`s test).

Table 2. Average number of eaten prey/day by *C. sanguinea* females and males at different prey densities.

Prey density	Eaten prey/day*	
	Female	Male
50	48.4 ± 0.7 hA	47.3 ± 1.6 gA
75	69.8 ±2.4 gA	67.5 ± 2.9 fA
100	90.2 ± 7.0 fA	88.1 ± 4.3 eA
125	107.3 ± 6.0 eA	92.3 ± 9.3 deB
150	125.6 ± 9.5 dA	106.9 ± 11.4 cdB
175	148.2 ± 7.9 cA	120.3 ± 7.1 cB
200	192.7 ± 1.9 bA	168.8 ± 5.8 bB
250	225.1 ± 8.2 aA	198.1 ± 7.8 aB
300	233.8 ± 5.1 aA	209.0 ± 6.6 aB

*Based on 5 observations. Means within the column followed by same letter and means within the row followed by the same capital letter are not significantly different (P < 0.01; According to Tukey's test).

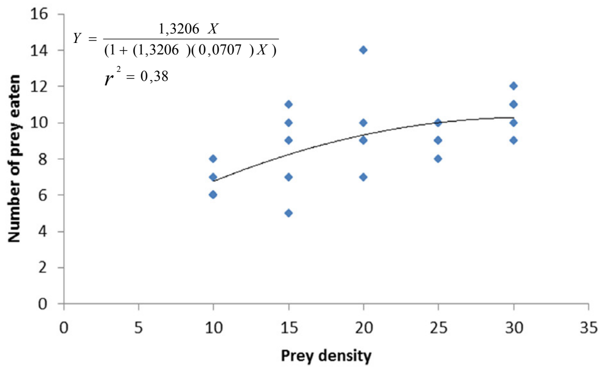

Figure 1. Functional response of the first larval instar of *C. sanguinea* to different *A. craccivora* densities (N= 5).

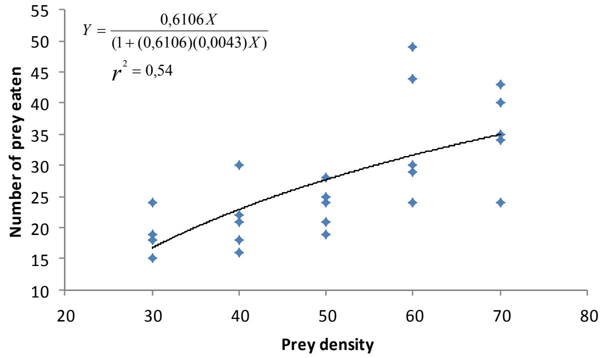

Figure 2. Functional response of the second larval instar of *C. sanguinea* to different *A. craccivora* densities (N= 5).

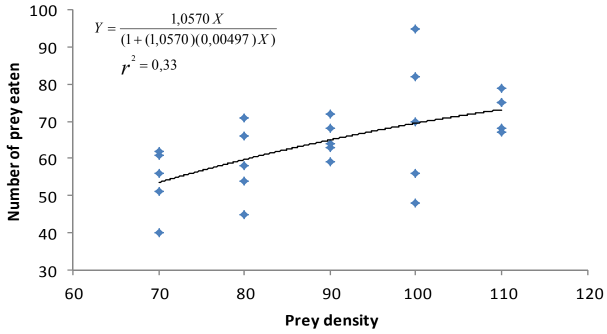

Figure 3. Functional response of the third larval instar of *C. sanguinea* to different *A. craccivora* densities (N= 5).

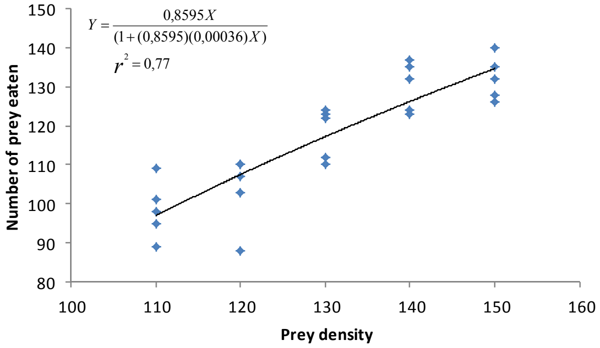

Figure 4. Functional response of the fourth larval instar of *C. sanguinea* to different *A. craccivora* densities (N= 5).

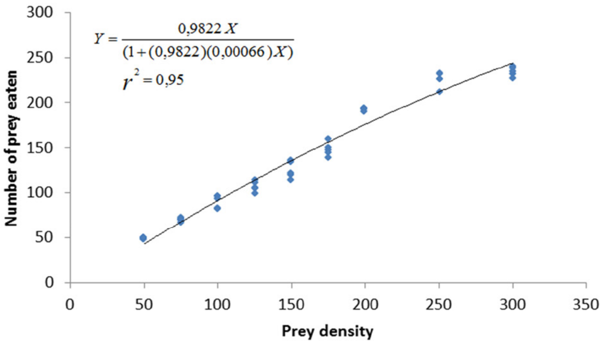

Figure 5. Functional response of *C. sanguinea* females to different *A. craccivora* densities (N= 5).

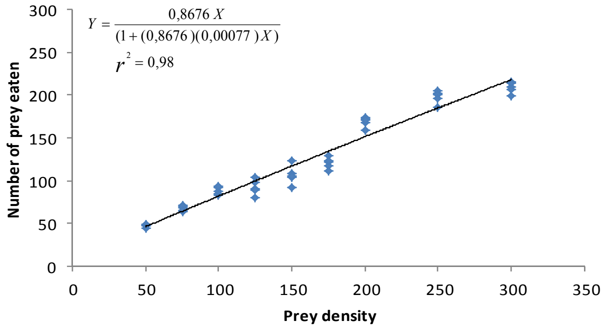

Figure 6. Functional response of *C. sanguinea* males to different *A. craccivora* densities (N= 5).

Table 3. Estimated parameters for mathematical models used to evaluate the functional response of different stages of *C. sanguinea* to *A. craccivora*.

Stage	Models											
	Lineal			Polynomial			Exponential			Holling		
	F	P	r^2	F	P	r^2	F	P	r^2	F	P	r^2
1st Instar	12.88	0.0015**	0.36	6.89	0.0048**	0.39	14.9	0.0008***	0.39	17.59	0.0003***	0.38
2nd Instar	24.48	0.0001***	0.52	11.88	0.0003***	0.52	5.52	0.0000***	0.57	27.99	0.0000***	0.54
3rd Instar	11.46	0.0025**	0.33	5.56	0.0111*	0.34	11.37	0.0026**	0.33	10.86	0.0032**	0.33
4th Instar	82.33	0.0000***	0.78	40.58	0.0000***	0.79	74.39	0.0000***	0.76	68.2	0.0000***	0.77
Female adult	1160.84	0.0000***	0.96	685.01	0.0000***	0.97	462.69	0.0000***	0.92	3550.56	0.0000***	0.95
Male adult	836.2	0.0000***	0.95	410.36	0.0000***	0.95	505.66	0.0000***	0.92	1186.86	0.0000***	0.98

* Significant at $P < 0.05$; ** Significant at $P < 0.01$; *** Significant at $P < 0.001$.

Table 4. Estimated parameters from the Holling's Equation for Functional Response of *Cycloneda sanguinea* at different prey densities.

Stage	Search rate (a)	Handling time (*Th* in days)	r^2
1st Instar	1.3206 ± 0.46798	0.07071 ± 0.02020	0.38
2nd Instar	0.6106 ± 0.01833	0.00432 ± 0.00089	0.54
3rd Instar	1.0570 ± 0.00643	0.00498 ± 0.00177	0.33
4th Instar	0.8595 ± 0.00404	0.00036 ± 0.00078	0.77
Female	0.9822 ± 0.02839	0.00066 ± 0.00093	0.95
Male	0.8676 ± 0.02926	0.00077 ± 0.00082	0.98

to the exponential model, while L2, L3 and L4 adjusted better to linear or type II Holling's model. L4 consumed daily the greatest number of prey compared to L1, L2 and L3. At the highest density evaluated, L4 consumed an average 132.2 aphids/cage, while L1, L2, and L3 consumed an average of 10.6, 35.2 and 72.8, respectively. As expected, morphological and physiological changes during larval development of the predator could account for differences observed in aphid consumption rate (Omkar and Pervez 2004).

Adult consumption rate was significantly higher in females than males at all densities tested. For example, at the highest prey density, females consumed an average of 233.8 aphid/cage, while males consumed an average of 209 aphids. Increasing voracity with increasing prey density showed by this predator is comparable to findings by other researchers (Agarwala et al. 2009, Dehkordi and Sahragard 2013, Mrosso et al. 2013, Barbosa et al. 2014).

Since lineal, Holling, polynomial and exponential models fitted data very close as verified by F and r^2 values (Table 3), we proposed the type II Holling's model to describe the feeding behavior of the ladybeetle (Figures 5 and 6).

Our results are consistent with those obtained by Morales and Burand (1985) for the first instar larvae of *C. sanguinea* on *T. citricida*, by Santa-Cecilia et al. (2001) for the larval instars of *C. sanguinea* preying *S. graminum* and Torres and Marcano (2015) for the first instar of *C. montrouzieri* on *M. hirsutus*. Other research has shown a Holling type II functional response for 4th instar larvae of different species of ladybeetles

(Timms et al. 2008, Cabral et al. 2009, Omkar and Kumar 2013).

Values of *a* and T_h were higher in L1 and L3, when compared to other larval instars (Table 4). This meant a more efficient search rate (*a*), but simultaneously represented the highest handling time (T_h) so those instar can find their prey quickly, but can take a long time to feed on it. L4 showed the lowest T_h of all larval instars, which was explained by its larger body size that allowed a faster sucking of the prey haemolymph required during its subsequent pupal stage (Hodek and Honek 1996, Omkar and Kumar 2013).

With regard to adult stages, females showed greater *a,* and lower T_h compared with males (Table 3). High aphid consumption shown by *C. sanguinea* adults can be explained by their ability to move and thereby increasing the probability of encountering prey, especially in a confined space such as Petri dishes, since the smaller cage leads to greater success in finding prey (Yasar and Özger 2005). Results reveal that L4 and adult stages of *C. sanguinea* are the most voracious due to the fact that they can consume a high amount of aphids with short handling time.

Searching rate and handling time values shown by L4 larvae, females and males could have a synergistic effect in regulating populations of *A. craccivora* in the field, even more so when these features are combined with the particular ability of larvae and adults to disperse. However, fourth instar larvae are considered more effective than adults in controlling prey population, due to the fact that larvae keep feeding on the prey at specific places. In this regard, Hodek and Honek (1996) indicated that fourth instar larvae have a very intense search activity on sites where prey is grouped in colonies, while adults are able to find prey in a more extensive way thus ensuring feeding and oviposition sites.

Higher voracity showed by larval and adult stages of *C. sanguinea* on *A. craccivora* demonstrates the biological potential of this predator as part of pest management system for several agricultural crops. However, more research is necessary for a better understanding of the effect of predator in field conditions and its impact on other prey.

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