

Functional responses of larval instars of *Cryptolaemus Montrouzieri* Mulsant (Coleoptera: Coccinellidae) to the Cochineal Scale, *Dactylopius Opuntiae* (Cockerell) (Hemiptera: Dactylopiidae)

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Abstract

*Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is native to Australia and commonly known as ‘cochineal destroyer’. This predator has been introduced in many countries worldwide for biological control of many scale pest species including the cochineal of cactus pear, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae). The functional responses of larval instars of *C. montrouzieri* on *D. opuntiae* (Cockerell) adult females were evaluated under laboratory conditions at 26°C and 12:12 (L:D) h. All larval instars of the predator were first starved for 12 h, then placed individually in Petri dishes (14.5 cm in diameter) with different densities (5, 10, 15, 20, 25) of *D. opuntiae* females for 24 h. The logistic regression for larval instars of the predator had a negative and significant linear parameter (P1) indicating a type II functional response. Attack rates (0.010, 0.028, 0.042, and 0.052) and handling times (11.945, 6.834, 4.878, and 3.971 hours) for first to fourth instar larvae, respectively, which were estimated using the Holling’s disc equation. This study provides a better understanding of the functional response of *C. montrouzieri* larval instars to *D. opuntiae*, which may be useful for effective use of *C. montrouzieri* in the management of the cactus pear cochineal infestations.

**Key words:** *Cryptolaemus montrouzieri*, *Dactylopius opuntiae*, Coccinellidae, predator-prey interaction, functional response

## Introduction

The interactions between a predator and prey system (the number of preys attacked per predator as a function of prey density) is known as functional response (Solomon, 1949; Holling, 1959) and it has been proved as a good indicator of the suitability of a predator as a biological control agent (Wiedenmann & O’Neil, 1991, Fernández-Arhex & Corley, 2004). Although there are several types of functional response (Van Alphen & Jervis, 1996), most ecological interest is in Holling’s type II and III functional responses, in which predators cause negative (type II) or positive (type III) density-dependent mortality of their prey. In addition, the type II response is the most dominant type of functional response in nature and is also common for coccinellids (Oaten and Murdoch, 1975; Collins et al., 1981; Seo & DeAngelis, 2011). In the past, different models have been implicated to explain predator-prey interactions. The pioneer and simplest of those was the Lotka–Volterra model (Lotka, 1925; Volterra, 1926) in the form of differential equations, explaining the dynamics of a pure resource consumer system. Today, the Holling’s disc equation model is the most frequently used model to describe the functional response due to its simplicity. The method most commonly used to estimate the handling time and attack coefficient parameters is the Holling’s disc equation modified by reciprocal linear transformation and least square regression (Omkar, 2004). The attack coefficient describes the steepness of increase in predation with increasing prey density and handling time is useful for estimating the satiation threshold (Pervez & Omkar, 2005). In recent years, the introduced cochineal *Dactylopius opuntiae* (Cockerell) have become an increasing threat to the cultivation of cactus pear crops in Morocco. Since 2016, *D. opuntiae* has been causing severe damage to cactus pear plantations in several regions of the country (El Aalaoui et al., 2019). Arthropod natural enemies associated with *D. opuntiae* and other *Dactylopiidae* include only predators (Mann, 1969; Zimmermann et al., 1979; Vanegas-Rico et al., 2010). The cochineal destroyer *Cryptolaemus montrouzieri* Mulsant (*Coleoptera: Coccinellidae*) has been introduced in many countries worldwide for biological control of scale pests (Solangi et al., 2012). This predator is a very efficient natural enemy of cochineals, and both adults and larvae of these beetles consume the cochineal completely (Bartlett, 1978). The successful control of cochineal species by this predator was reported in many control programs worldwide (Bartlett, 1978; Protasov et al., 2017). The lady beetle was introduced into Brazil for biological control of *D. opuntiae*, and primarily cochineal attacking cassava and citrus (Sanchez & Carvalho, 2010). In Israel, 100,000 *C. montrouzieri* were released in cactus crop infested with *D. opuntiae* in the north of the country (Protasov et al., 2017). The ladybeetle was also introduced into Morocco to control *D. opuntiae* (El Aalaoui et al., 2019).

However, there are no published studies on the functional response of larval instars of this predator to *D. opuntiae*, which could contribute to determining its regulatory capacity.

The aim of this paper was to investigate the type of functional response of *C. montrouzieri* larval instars and its parameters (the attack rate and handling time) and to provide insights into the suitability of this predator as a biological control agent against *D. opuntiae*.

## Material and Methods

*Opuntia ficus-indica* (L.) Mill., cladodes were used for rearing *D. opuntiae*. A *D. opuntiae* colony was established from individuals collected from Kemis Zemamra locality (32°37'48" N, 8°42'0" W,) in the Sidi Bennour province, Morocco. A modified version of the 'cut cladode technique' of Aldama-Aguilera & Llanderal Cazares (2003) was used to increase the number of insects.

A *Cryptolaemus montrouzieri* colony originated from adults imported by the laboratory of entomology at INRA, Morocco (National Institute of Agricultural Research). The adults were placed in entomological cages (80-80-80cm) comprised of a wooden frame covered with a mesh fabric to allow ventilation. Access to water was provided via a cotton wick inserted into a 25 ml glass vial filled with water. Cladodes infested with *D. opuntiae* were introduced weekly into the cages to provide food and substrates for *C. montrouzieri* oviposition. All of the assays with *C. montrouzieri* larval instars were conducted at 26°C and 12:12 (L: D) h.

Each larval instar of *C. montrouzieri* was placed individually in Petri dishes (14.5 cm in diameter) and starved for 12h in order to standardize their hunger. Five densities (5, 10, 15, 20, and 25) of *D. opuntiae* (25 days old) females were taken from the colony established in the laboratory and added to each Petri dish. Ten randomly distributed replicates were used for each combination of predator and prey density. After 24 h, the number of killed females or with evidence of consumption by the predator was recorded. All experiments were repeated three times.

The logistic regression analysis of prey consumed ( $Ne/N_0$ ) against the number of preys offered ( $N_0$ ) was used to determine the shape of the functional response (Trexler et al., 1988). The polynomial function that describes the relationship between  $Ne/N_0$  and  $N_0$  is as follows:

$$Ne/N_0 = (exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)) / (1 + exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)),$$

Where  $Ne$  is the number of cochineals eaten;  $N_0$  is the initial cochineal density;  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the constant, linear, quadratic, and cubic parameters, respectively, related to the slope of the curve.

The data indicated a type II functional response and were fitted to the Holling's disc equation (Holling, 1959)  $Ne = aTN_0/(1+a N_0 Th)$  —where  $Ne$  is the number of preys consumed,  $a$  is the attack rate or the instantaneous rate of discovery,  $N_0$  is the initial number of prey offered or prey's density,  $T$  is the time when the prey and predator are mutually exposed or the experiment duration (24 h), and  $Th$  is the handling time associated with each prey consumed. Once the type of functional response was determined, both the disc equation (Holling, 1959) and the random attack equation (Royama, 1971; Rogers, 1972) were used to estimate handling time ( $Th$ ) and instantaneous rate of discovery or attack rate ( $a$ ). Significant differences between the parameters of the functional response model for all larval instars were tested with the superposition of 95% confidence intervals criterion. Mean values of  $Th$  were used to calculate maximum attack rates  $T/Th$  (Hassell, 2000), which is the maximum number of preys that can be attacked by a predator during the time interval considered. The predator searching efficiency ( $E$ ) was calculated by using the equation  $E = Ne/N_0$ . The searching time was determined using the equation  $Ts = T - ThNe$  (Juliano & Williams, 1987, Elliott, 2003).

The significance of the difference in the number of consumed preys at different densities, searching efficiency and searching time was determined using analysis of variance (ANOVA); the Tukey's LSD test was used for multiple comparisons among significant treatment effects where they occurred (IBM SPSS statistics 21).

## Results and Discussion

The linear coefficient  $P_1$  is significantly negative for all larval instars ( $P < 0.0001$ ) indicating that the proportion of prey consumed by all larval instars declined monotonically with increasing prey density, typical of a type II response (Juliano, 2001). The estimated parameters from the logistic regression analysis of the proportion of *D. opuntiae* females consumed by *C. montrouzieri* larval instars are presented in Table 1.

Table 1. Estimates of the parameters  $P_0, P_1, P_2,$  and  $P_3$  of the proportion of *D. opuntiae* adult females consumed by *C. montrouzieri* larvae.

Parameter	1 <sup>st</sup> instar larva	2 <sup>nd</sup> instar larva	3 <sup>rd</sup> instar larva	4 <sup>th</sup> instar larva
$P_0$	0.605	1.454	2.147	2.496
$P_1$	-0.005*	-0.011*	-0.016*	-0.019*
$P_2$	-0.007	-0.032	-0.049	-0.054
$P_3$	-0.019	-0.058	-0.082	-0.101

\*Significant at  $P < 0.05$

The number of preys consumed by 1<sup>st</sup> instar larva, 2<sup>nd</sup> instar larva, 3<sup>rd</sup> instar larva, 4<sup>th</sup> instar larva increased from the first to fourth instar (Fig. 1). Estimated attack rates increased as larval age increased while handling times decreased as larval age increased (Table 2). The predator searching efficiency ( $E = Ne/N_0$ ) of all the predator instars decreased significantly as the prey density increased from 5 to 25 reflecting a direct increase in the probability of finding prey at higher densities (Fig. 2). Moreover, a decrease in larval handling time in relation with larval age indicates an increase in the upper level of the response, which is determined by the maximum attack rate ( $T/Th$ ). Maximum attack rate values were 2.021, 3.524, 4.899, and 6.043 for the first, second, third, and fourth instars, respectively (Table 3).

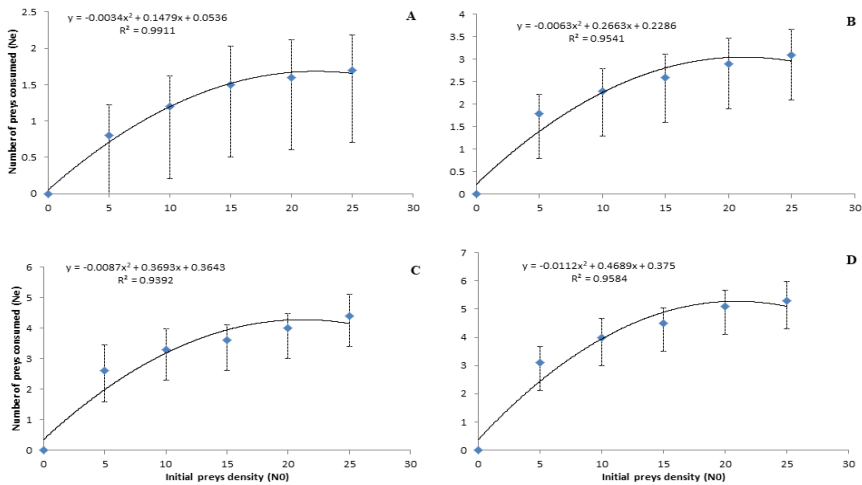


Fig. 1. Functional responses of *C. montrouzieri* larval instars to *D. opuntiae*. (A) first instar larva; (B) second instar larva; (C) third instar larva, and (D) fourth instar larva. Data fitted using the Holling's disc equation (Holling, 1959).

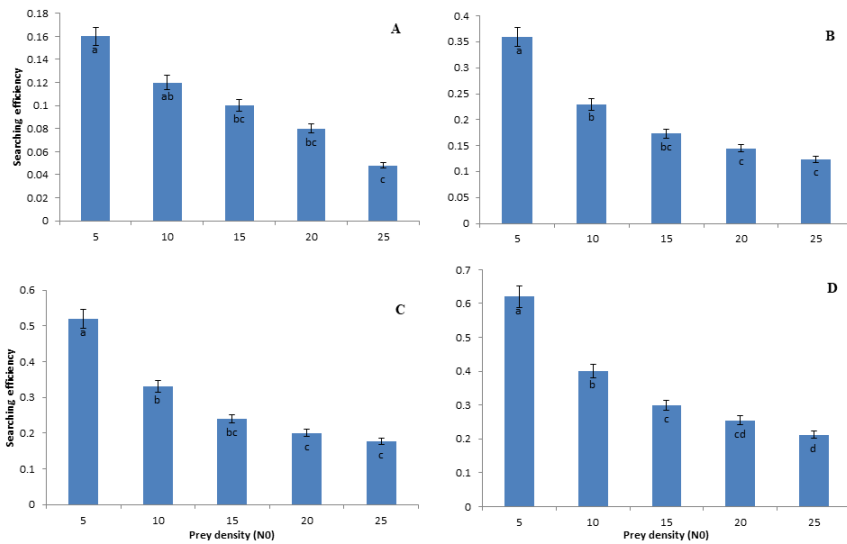


Fig. 2. Search efficiency of *C. montrouzieri* larval instars at different densities of *D. opuntiae* adult females. (A) first instar larva; (B) second instar larva; (C) third instar larva, and (D) fourth instar larva. Bars with different letters are significantly different according to the Tukey's LSD test ( $\alpha=0.05$ ).

Table 2. Parameters estimated by the Holling's disc equation.

Larval stage	Attack rate ( $h^{-1}$ ) ( <i>a</i> )		$R^2$	Handling time (h) ( <i>Th</i> )		$R^2$
	Mean $\pm$ S.E.	95% CI		Mean $\pm$ S.E.	95% CI	
1 <sup>st</sup> instar larva	0.010 $\pm$ 0.004	0.000-0.018	0.031	11.945 $\pm$ 4.006	6.324 $\pm$ 17.659	0.255
2 <sup>nd</sup> instar larva	0.028 $\pm$ 0.006	0.016-0.038	0.002	6.834 $\pm$ 1.595	4.697-11.375	0.009
3 <sup>rd</sup> instar larva	0.042 $\pm$ 0.008	0.025-0.066	0.000	4.878 $\pm$ 0.928	2.965-7.938	0.010
4 <sup>th</sup> instar larva	0.052 $\pm$ 0.007	0.034-0.067	0.014	3.971 $\pm$ 0.610	3.016-6.031	0.025

Table 3. Maximum attack rates ( $T/Th$ ) estimated for all four larval instars of *C. montrouzieri* using the Holling's "disc equation"

<i>C. montrouzieri</i> larval instars	1 <sup>st</sup> instar larva	2 <sup>nd</sup> instar larva	3 <sup>rd</sup> instar larva	4 <sup>th</sup> instar larva
Max. attack rate ( $T/Th$ )	2.021	3.524	4.899	6.043

The total searching time significantly decreased and the total handling time increased ( $p < 0.05$ ) at different prey densities for all *C. montrouzieri* larval instars tested (Fig. 3).

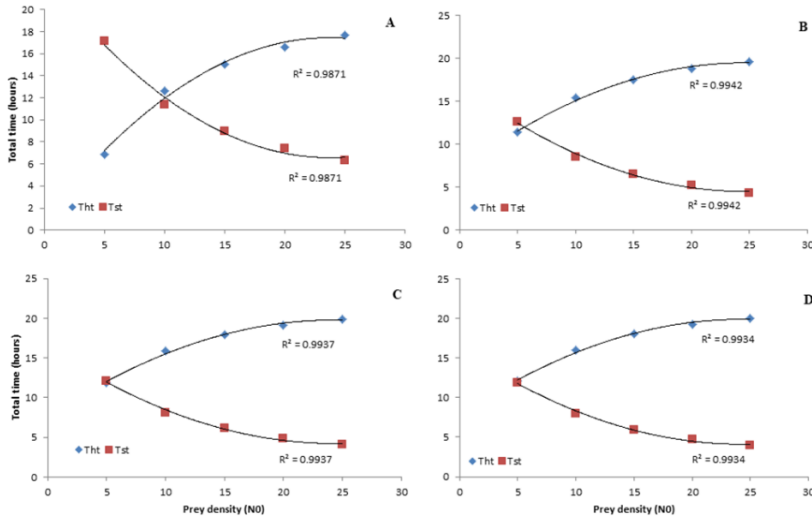


Fig. 3. Inverse relationship between the total handling time (Tht) and total searching time (Tst) of *C. montrouzieri* larval instars preying on *D. opuntiae* adult females, obtained by the estimated parameter Th and the equation  $Ts = T - ThNe$ .

The Holling's disc equation (Holling, 1959) indicated that all *C. montrouzieri* larval instars exhibited the functional response type II. Although various types of functional responses are described for coccinellids (Hodek, 1996), type II is the most common. Oaten and Murdoch (1975) reported that this type of response may destabilize predator-prey interactions and is theoretically less capable of suppressing prey populations compared to the type III functional response (Holling, 1965). The effect of the density of *D. opuntiae* females on each *C. montrouzieri* larval instar consumption may be ascribed to the simultaneous action of various phenomena, such as handling time, level of hunger of the predator and the nature of the experimental arena (O'Neil & Stimac, 1985, 1988). The attack rates were estimated to be 0.010, 0.028, 0.042, and 0.052 and handling times were estimated to be 11.945, 6.834, 4.878, and 3.971 h for first to fourth instar larvae, respectively. The higher Th for *C. montrouzieri* larval instars in our study compared with previous reports for other coccinellids attacking different scale pests (Hodek et al., 1984; Messina & Hanks, 1998; Lee & Kang, 2004; Isikber, 2005; Pervez, 2005; Cabral et al., 2009;

Xue et al., 2009) may, in part, be due to the reduced accessibility to *D. opuntiae* as compared to the other prey species because *D. opuntiae* females produce a waxy covering that protect them from predators and insecticides. The Attack rates were increased for the first to fourth instar larvae due to the higher requirements for food and energy of the old larvae. The attack rate links functional response curve with increasing prey density. The results showed that the steepness did not differ among the four larval instars which indicates that the larvae have similar abilities to respond to increasing prey densities. Omkar and Pervez (2004) reported that satiation reduces the feeding performance of predators, which was not achieved at low prey densities as exhibited by high percentage of prey consumption. At high prey density, all larval instars continued to feed till satiation and did not completely devour the prey. This aspect of predator-prey association encourages the pragmatic utilization of ladybeetles, as they tend to increase prey mortality (Omkar & Pervez, 2004). The fourth instar was the most voracious stage followed by third, second, and first instars. High levels of energy are required by the fourth instar to complete development and attain a critical weight for pupation (Ferran & Larroque, 1977).

## Conclusion

Although *D. opuntiae* females are apterous and sessile, they produce a waxy covering that protects them from predators. Our results show that *C. montrouzieri* larval instars exhibit a Holling type II functional response to *D. opuntiae*, with the fourth larval instar being the most effective at detecting and consuming the cochineal adult females. The estimated attack rate increased, and handling time decreased as larval age increased. Prey handling time by all of the four larval instars tested was relatively higher at higher density of *D. opuntiae* (25 *D. opuntiae* adult females); most of this handling time due to the time needed for removing the waxy coating that protects *D. opuntiae* adult females. The search efficiency of the predatory larvae tested decreased with increasing prey density. In conclusion, there is always a relationship between predation and density of the prey; however, the density and diversity of other non-prey species (Kratina et al., 2007) and other predators (Kratina et al., 2009) can modify the functional response of the predators under field conditions. Therefore, field experiments are necessary for a good estimation of the predatory potential of *C. montrouzieri* and its effectiveness as a biological control agent against the *D. opuntiae*.



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Функционални одговор различитих узраста ларви *Cryptolaemus Montrouzieri* Mulsant (*Coleoptera: Coccinellidae*) на штитасте ваши *Dactylopius Opuntiae* (Cockerell) (*Hemiptera: Dactylopiidae*)

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Сажетак

Природно станиште инсекта *Cryptolaemus montrouzieri* Mulsant (*Coleoptera: Coccinellidae*) је Аустралија, а шире је познат је по називу 'истребљивач штитасте ваши'. Овај предатор је унесен у многе земље свијета ради биолошке контроле многих врста штитастих ваши укључујући штитасту ваш опунције (бодљикаве крушке), *Dactylopius opuntiae* (Cockerell) (*Hemiptera: Dactylopiidae*). У раду је извршена је евалуација функционалног одговора различитих узраста ларве *C. montrouzieri* на одрасле женке *D. opuntiae* у лабораторијским условима на 26°C и 12:12 (L:D) h. Сви ларвални узрасти предатора прво су били изложени условима без хране у трајању од 12 h, затим су појединачно смјештени у петријеве посуде (пречника 14.5 cm) са различитом густином (5, 10, 15, 20, 25) женки *D. opuntiae* у трајању од 24 сата. Логистичка регресија различитих узраста ларви предатора имала је негативан и значајан линеарни параметар (P1) који указује на тип II функционалног одговора. Ниво напада (0.010, 0.028, 0.042 и 0.052) и вријеме узроковања морталитета (11.945, 6.834, 4.878 и 3.971 сати) првог до четвртог стадијума ларви процијењени су Холинговом диск једначином. Овај рад пружа боље разумијевање функционалног одговора различитих узраста *C. montrouzieri* на *D. opuntiae*, које може бити корисно за успјешну употребу *C. montrouzieri* у контроли напада штитасте ваши бодљикаве крушке.

Кључне ријечи: *Cryptolaemus montrouzieri*, *Dactylopius opuntiae*, *Coccinellidae*, однос предатор – плијен, функционални одговор

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