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**Artículo Original**

# Functional responses of *Novius punicus* (Gordon) and *Novius cardinalis* (Mulsant) (Coleoptera: Coccinellidae) to *Crypticerya multicicatrices* Kondo and Unruh (Hemiptera: Monophlebidae)

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# Respuestas funcionales de *Novius punicus* (Gordon) y *Novius cardinalis* (Mulsant) (Coleoptera: Coccinellidae) a *Crypticerya multicicatrices* Kondo and Unruh (Hemiptera: Monophlebidae)

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**ABSTRACT:** The functional responses of *Novius punicus* (Gordon) and *N. cardinalis* (Mulsant) (Coleoptera: Coccinellidae) were investigated using eggs and first-instar nymphs of *Crypticerya multicicatrices* Kondo and Unruh (Hemiptera: Monophlebidae), respectively, as prey. Ten-day-old laboratory-reared adult individuals of each coccinellid species were placed individually in Petri dishes, and each one was offered a different prey density for a 24-hour period. For *N. cardinalis*, prey densities of 10, 20, 50, 100, and 150 first-instar nymphs of *C. multicicatrices* were evaluated. With *N. punicus*, densities of 10, 25, 50, 100, and 200 eggs of the fluted scale were evaluated. Using the proportion of prey consumed, a logistic regression was performed, which allowed the identification of the type of functional response described by the data. The random predator equation was then used to describe the functional response, using the R software v. 3.4.1. For the two species of coccinellids, a type II functional response was found, which describes a decrease in the proportion of prey consumed as the density of the prey increases. The estimated parameters of the random predator equation, attack rate (α) and handling time (in hours) ($T_h$) were as follows: *N. punicus*: $\alpha = 0.12$, $T_h = 0.64$; *N. cardinalis*: $\alpha = 0.05$; $T_h = 0.34$. These results represent an advance in the evaluation of the potential of these predators to be used in a pest management program for *C. multicicatrices*.

**Key words:** coccinellids, Colombian fluted scale, natural enemy, predator, attack rate.

**RESUMEN:** El objetivo de este trabajo fue determinar la respuesta funcional de *Novius punicus* (Gordon) y *N. cardinalis* (Mulsant) (Coleoptera: Coccinellidae) utilizando, como presa, huevos y ninfas del primer estadio de *Crypticerya multicicatrices* Kondo and Unruh (Hemiptera: Monophlebidae), respectivamente. Se individualizaron, en cajas Petri, especímenes adultos de 10 días de edad de cada especie de coccinélido, criados en laboratorio, y a cada uno se le ofreció por un periodo de 24 horas una densidad de presa diferente. Para *N. cardinalis*, se evaluaron las densidades de presa de 10, 20, 50, 100 y 150 ninfas del primer estadio de *C. multicicatrices*. Con *N. punicus* se evaluaron densidades de 10, 25, 50, 100 y 200 huevos de esta cochinilla acanalada. Con la proporción de presas consumidas se realizó una regresión logística, que permitió identificar la forma de la respuesta funcional que describen los datos. Se utilizó la ecuación de depredador aleatorio para la descripción de la respuesta funcional, mediante el uso del software R v. 3.4.1. Para las dos especies de coccinélidos se encontró una respuesta funcional tipo II, la cual describe una disminución en la proporción de presas consumidas a medida que la densidad de la presa incrementa. Los parámetros estimados de la ecuación del depredador aleatorio, tasa de ataque (α) y tiempo de manipulación (en horas) ($T_h$) fueron los siguientes: *N. punicus*: $\alpha = 0.12$, $T_h= 0.64$; *N. cardinalis*: $\alpha = 0.05$; $T_h = 0.34$. Estos resultados representan un avance en la evaluación del potencial que tienen estos depredadores para usarse en un programa de manejo de plagas para *C. multicicatrices*.

**Palabras clave:** coccinélidos, cochinilla acanalada de Colombia, enemigo natural, depredador, tasa de ataque.

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## INTRODUCTION

The functional response of a predator describes the number of prey that an individual consumes as the density of the prey increases (1). It is an important concept in the field of ecology and evolution, also useful in the understanding of population dynamics at different trophic levels (2). The predator will consume a greater number of prey as density increases, and the maximum number of prey it can consume is determined by satiety (maximum consumption capacity) or by the maximum time available to manipulate the prey (2). The functional response is considered a criterion that allows the evaluation of the potential of a predator to be used as a biological control agent within a pest management program (3, 4, 5).

There are three types of functional responses, the type I response describes a linear increase in the number of prey consumed until a maximum consumption rate is reached, where the number of consumed prey remains constant despite the increase in prey density. In the type II functional response, the rate of consumption by a predator rises at a decelerating intake rate as prey density increases, until reaching an asymptote (hyperbolic curve) at which the rate of consumption remains constant regardless of increases in prey density. The type III response is described as a sigmoid curve, at low densities the number of consumed prey is reduced, but as the number of prey increases the rate of consumption also increases, until the limit is reached (4, 6, 7).

The attack rate, handling time and exposure time of the prey to the predator are the factors that determine the functional response of a predator (8, 9). Handling time is a term that involves different aspects of the predator's behavior, since it refers to the time it takes for the predator to capture, subjugate, kill, and digest the prey (10, 11). Handling time and attack rate are inversely proportional, high handling time leads to low attack rates, since the predator will spend a longer time on each prey reducing the number of attacks per unit of time. On the other hand, high attack rates are the consequence of short handling time (7).

*Novius punicus* (Gordon) and *Novius cardinalis* (Mulsant) (Coleoptera: Coccinellidae) are predators of the Colombian fluted scale *Crypticerya multicicatrices* Kondo and Unruh (Hemiptera: Monophlebidae: Iceryini), which was reported as an invasive insect in the archipelago of San Andrés, Providencia and Santa Catalina in 2010 (12, 13). After a search for natural enemies in the place of origin of this pest (continental region of Colombia), these two coccinellid species were considered as potential control agents for this fluted scale (13). The species *N. punicus* is native to the New World (14, 15) and preys on scale insects of the tribe Iceryini such as *Icerya purchasi* Maskell and *Crypticerya montserratensis* (Riley and Howard) (16). *Novius punicus* is an important natural enemy of *C. multicicatrices* (17), demonstrating a great predatory capacity on this pest. The immature and adult stages of this coccinellid feed preferably on the eggs of the fluted scale; however, it can also feed on nymphs (13). It is common to find larvae of this predator inside the ovisacs of adult fluted scales, where they hold their eggs. This species is the main one responsible for the reduction in the populations of *C. multicicatrices* on the islands of San Andrés, Providencia and Santa Catalina in 2013 (13). On the other hand, *Novius cardinalis*, until recently known as *Rodolia cardinalis*, is a species native to Australia (14, 15) and preys mainly on species of the family Monophlebidae. *Novius cardinalis* has been used in successful biological control programs for *I. purchasi* (13, 17). It was introduced to Colombia in 1948 to control *I. purchasi* in urban areas (12) and it has been reported preying on eggs and nymphs of *C. multicicatrices* (13). Both species of coccinellid predators play an important role in reducing the population levels of *C. multicicatrices* in urban areas of Colombia where it occurs. For this reason, the objective of this study was to determine the functional response of *N. punicus* and *N. cardinalis* on eggs and first-instar nymphs of *C. multicicatrices*, respectively.

## MATERIALS AND METHODS

### Insect rearing

The individuals of *N. punicus* and *N. cardinalis* used in the experiments were taken from a rearing colony kept under laboratory conditions in Agrosavia, Caribia Research Center (temperature: 27.9 ± 2 °C, relative humidity: 65.3 ± 9.7 %). Individuals brought from the field were regularly added to the breeding stock to maintain genetic diversity within the population. Rectangular transparent plastic containers measuring 28 cm long x 18 cm wide and 13 cm high were used for maintaining the insects; the lid had a metal mesh (1 mm hole diameter) for ventilation. An average of 30 adult coccinellids were housed per container. Adults of *N. punicus* were fed with live adults of *C. multicicatrices* with fully developed ovisacs. On the other hand, the feeding of the adults of *N. cardinalis* consisted of first-instar nymphs of the fluted scale; in addition, the diets for both coccinellids were supplemented with pollen to stimulate oviposition. Individuals of *C. multicicatrices* used for feeding the coccinellids were collected in the field or brought from a breeding colony established in mesh house conditions; in both cases the host plant was mango (*Mangifera indica* L. variety “Azúcar”). Before offering the fluted scales as food, the specimens were checked to ensure that they were healthy (without fungi or affected by other natural enemies).

Every third day, 20 to 40 adult fluted scales were added to each *N. punicus* rearing plastic container (described above), and these were arranged in 9 cm diameter Petri dishes. Because the adults of the fluted scales also served as an oviposition substrate, the Petri dishes extracted from the rearing containers were preserved in order to recover larvae and pupae of *N. punicus* from the remains of the fluted scales, which were kept individually until the number of specimens needed for the experiments were obtained. In the case of *N. cardinalis*, plant structures infested with nymphs of *C. multicicatrices* were deposited in each rearing container every three days. When the plant structures were removed from the rearing containers, they were put under observation to recover coccinellid larvae, which were then kept individually in Petri dishes until the number of specimens necessary for the experiments was reached. The individuals of the two species of coccinellids that were not used in the trials were used to reinforce the breeding stock.

## Functional response experiment

The experiment was carried out under laboratory conditions at the Colombian Agricultural Research Corporation - Agrosavia, Caribia Research Center, (temperature: 27.9 ± 2.0 °C, relative humidity: 65.3 ± 9.7 %, photoperiod of 12:12 (h) Ligth to Dark), the abiotic variables were taken using a data logger (OM-EL-USB-2-LCD Omega®). In a completely randomized design, the coccinellid individuals were approximately 10 days old and were housed individually as larvae to ensure that they were age-compliant and that they had not mated before the trials. Although female coccinellids are commonly more voracious than males, we were not able to sex the adults by external morphological characters; therefore, the analyses were made for the adult stage regardless of sex related differences in prey consumption. This limitation should be taken into account when considering the results obtained.

The specimens chosen for the experiment were subjected to a 24-hour fast before the start of the experiment to standardize the hunger stimulus. The experimental units were composed of an adult coccinellid and different densities of the prey. For *N. punicus*, the prey population densities evaluated were 10, 25, 50, 100, and 200 eggs of *C. multicicatrices*. With *N. cardinalis*, densities of 10, 20, 50, 100, and 150 first-instar nymphs of *C. multicicatrices* were offered in the section of a mango leaf, where they were attached. In both cases, the coccinellids were kept individually in 9 cm diameter glass Petri dishes with dry filter paper at the bottom. The number of repetitions for each evaluated prey density was 10. The fluted scale prey was offered for a period of 24 hours, when the number of remaining prey was counted.

## Statistical analysis

For statistical data analysis the R v. 3.4.1 program (18) was used, and for the functional response analysis the (*frair*) package (19) was used. In this study, the methodology described by Paterson *et al.* (20) was used to identify the type of functional response described by our data, conducting a logistic regression based on the proportion of consumed prey against the number of offered prey (frair: frair_test). If the result of the logistic regression is a significantly negative first-order term, then the data fit a type II functional response (21).

Since the logistic regression indicated that the data for the two species of coccinellids fit a type II curve, the random predator equation (6) was used to describe the functional response. This equation defines a model where prey density decreases over time. The equation is as follows:

$$N_e = N_0\{1 - exp[a(T_h N_e - T]\}$$

Where $N_e$ is the number of eaten prey; $N_0$ is the number of initial prey; T is the total time (for this experiment it is 24 h), α is the attack rate; and $T_h$ is the handling time. The model was adjusted using maximum likelihood estimation (frair: frair_fit).

# RESULTS

The results of the logistic regression show that the two species of coccinellids have a type II functional response. For both cases, the estimated value was negative and statistically significant (Table 1), which indicates that the curve of the proportion of eaten prey fits a function with a decreasing rate, characteristic of a type II functional response.

The functional response adjusted by the random predator equation for *N. punicus* and *N. cardinalis* are shown in Figures 1a and 1b, respectively. In the case of *N. punicus*, it can be observed that the relationship between the number of consumed prey and their density followed the typical curve of the type II functional

**Table 1.** Estimated first-order parameters of the logistic regression adjusted to the proportion of prey consumed and the density of prey (*Crypticerya multicicatrices*) offered. / *Parámetros de primer orden estimados de la regresión logística ajustada a la proporción de presas consumidas y la densidad de presas (Crypticerya multicicatrices) ofrecidas.*

| Predator | Estimated | S.E. | Z-value | Pr (> \|z\|) |
|---|---|---|---|---|
| *N. punicus* | -0.0117 | 0.0006 | -21.295 | < 2.2 e-16 *** |
| *N. cardinalis* | -0.0100 | 0.0008 | -12.553 | < 2.2 e-16 *** |

*** Statistical significance at P < 0.001; Standard Error: S.E.

response, clearly reaching an asymptote that shows the maximum rate of consumption of eggs of the fluted scale by this predator in a 24-hour period. On the other hand, the graph that describes the functional response of *N. cardinalis* feeding on first-instar nymphs of *C. multicicatrices* does not clearly show the predation limit in 24 hours, so it can be assumed that this coccinellid reaches its maximum rate of consumption at densities higher than those tested in this study.

The calculated parameters of the random predator equation are shown in Table 2. The attack rate of *N. punicus* on eggs of *C. multicicatrices* is higher than that observed in *N. cardinalis* preying on nymphs of Colombian fluted scale. On the other hand, the calculated handling time for *N. cardinalis* is 0.34 hours, and for *N. punicus* is 0.64 hours.

## DISCUSSION

Some authors have reported that there may be factors inherent to the experiment that make it difficult to differentiate between the curves that are characteristic of a type II and type III functional response, for example, not offering different numbers of prey at low densities, or not establishing an evaluation time that is in accord with the biological characteristics of the insect (6, 22). Logistic regression is an effective way to distinguish between type II and type III functional responses (21). Carrying out a comparison using the proportion of eaten prey at different densities allows greater sensitivity in the differentiation of the curves that represent type II and type III functional responses.

Different equations can be used to describe the functional response of a predator, each of them is used to estimate different values for the attack rate and handling time. Rogers (22) established that the random predator equation predicts the number of attacks that a predator can carry out in a given period of time, in a system where the exploitation of prey is allowed. This equation is suitable for systems where the density of the prey decreases as the predator consumes them (21); that is, experiments where the prey are not replaced to maintain a constant density throughout the evaluation. It is common to find the type II functional response in predators of the family Coccinellidae [e.g. Sakaki and Sahragard (10)]; this type of functional response is characterized by a decreasing proportion of eaten prey as prey density increases (10); in addition, the maximum rate of consumption is determined by the handling time of the prey (5, 6).

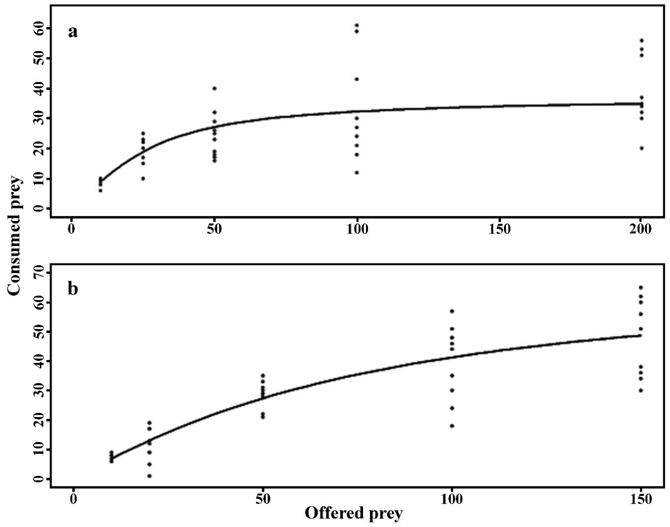

**Figure 1. a.** Functional response of *Novius punicus* on eggs of *Crypticerya multicicatrices*. **b.** Functional response of *N. cardinalis* on first-instar nymphs of *C. multicicatrices*. / ***a.** Respuesta funcional de Novius punicus sobre huevos de Crypticerya multicicatrices.* ***b.** Respuesta funcional de N. cardinalis sobre ninfas de primer estadio de C. multicicatrices* .

**Table 2.** Estimated parameters of the random predator equation, α ($h^{-1}$) is the attack rate and $T_h$ (h) the handling time, for *N. punicus* preying on eggs of *C. multicicatrices*, and for *N. cardinalis* preying on first-instar nymphs of *C. multicicatrices*. / *Parámetros estimados de la ecuación del depredador aleatorio a ($h^{-1}$) es la tasa de ataque y $T_h$ (h) es el tiempo de manipulación para N. punicus depredando huevos de C. multicicatrices, y para N. cardinalis depredando ninfas de primer estadio de C. multicicatrices* .

| Predator | Parameter | Estimate | S.E. | Z-value | Pr (> \|z\|) |
|---|---|---|---|---|---|
| *N. punicus* | α | 0.1191 | 0.0125 | 9.487 | < 2.2 e-16 *** |
|  | $T_h$ | 0.6423 | 0.0268 | 23.973 | < 2.2 e-16 *** |
| *N. cardinalis* | α | 0.0542 | 0.0041 | 13.312 | < 2.2 e-16 *** |
|  | $T_h$ | 0.3441 | 0.0225 | 15.263 | < 2.2 e-16 *** |

*** Statistical significance at $P < 0.001$; Standard Error: S.E.

The experiments reported here were carried out in a relatively small area so the prey density per unit area could be very high, increasing the encounter rate between predator and prey and reducing the search time; on the other hand, the sedentary habit and size of the prey does not represent a major challenge for their submission by the predators. Thus, the difference in handling times presented by these species may be mainly due to their ability to consume and digest prey. In addition, Munyaneza and Obrycki (5) mentioned that the resting time can be an important component of the total handling time that a predator has on a given prey.

The differences observed in the parameters (α) and ($T_h$) in the studied species reflect different feeding strategies. *Novius punicus* optimized the attack rate (α) by specializing in an immobile and confined prey (eggs in ovisac), but it showed a longer handling time ($T_h$); in contrast, *N. cardinalis* had a lower attack rate (α) for a mobile prey (first-instar nymphs), which compensated with a shorter handling time ($T_h$). This last characteristic is related to high rates of prey consumption.

FoRAGE (Functional Responses from Around the Globe in all Ecosystems) is a specialized database that includes more than 2000 entries of functional response experiments for all kinds of predators (23), among these, multiple coccinellid species. To put into context, the values of the parameters estimated in this experiment for *N. punicus* and *N. cardinalis*, the findings reported by other authors for functional response experiments with adults of various coccinellids species and various prey are summarized in Table 3.

The search efficiency values found in this study are similar to those reported by De Bortoli *et al.* (24) for adults of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (α = 0.01-0.05) preying on *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae). In contrast, the handling time values are much lower than those reported for *C. montrouzieri* ($T_h$ = 0.94-3.36 hours) in the above experiment (24), but much higher than those of other species with more mobile prey (Table 3).

The functional response parameters of the predators *N. punicus* and *N. cardinalis* reflect the result of the specialization of these coccinellids in consuming scale insect prey of the tribe Iceryni (13, 16), where the developmental stages on which they carry out the greatest predation (eggs and first-instar nymphs) are of low mobility or are contained in the ovisac of adult females. These characteristics make *N. punicus* a species with a high affinity to its main prey and less prone to attack other non-target species.

Some authors have pointed out that for functional response studies to be useful to evaluate the potential of a species as a biological control agent, it is necessary to carry out the experiments in such a way as to include the greatest number of conditions that the insect predator will encounter in the field (10, 26, 27). Messina and Hanks (27) showed that the characteristics of the plant where the prey is found can alter the functional response of the predator. These authors found that *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae), showed a different type of functional response (type II or type III) when feeding on the aphid *Diuraphis noxia* Mordvilko (Hemiptera: Aphididae) depending on the architecture of the leaf, especially because of the shelter that these structures provide to the prey (27).

## CONCLUSIONS

The coccinellids *N. punicus* and *N. cardinalis* are both important predators of eggs and first-instar nymphs of *C. multicicatrices*, respectively. Both coccinellids have a type II functional response, in which the proportion of prey consumed decreases as the prey density increases. The attack rate and handling time parameters observed for *N. punicus* and *N. cardinalis* reflect the specialization of both species in consuming prey from the tribe Iceryini, in particular, eggs and newly emerged first-instar nymphs of the adult female fluted scales. Taking into account the experimental conditions of the study, it can be said that the differences in the number of consumed prey observed between *N. punicus* and *N. cardinalis* are mainly due to their ability to consume the prey. The present experiment is a first step in evaluating the potential of these coccinellid species to be used in a management program for *C. multicicatrices*.

**Table 3.** Parameters of attack rate (α) and prey handling time ($T_h$) for adults of various species of coccinellids in functional response studies with various prey. / *Parámetros de tasa de ataque (a) y tiempo de manipulación ($T_h$) de la presa para adultos de varias especies de coccinélidos en estudios de respuesta funcional con diversas presas.*

| **Predator (Coleoptera: Coccinellidae)** | **Prey** | **α ($h^{-1}$)** | **$T_h$ (h)** | **T(°C)** | **Experimental unit** | **Information source** |
|---|---|---|---|---|---|---|
| ***Cryptolaemus montrouzieri* Mulsant** | *Planococcus citri* (Hemiptera: Pseudococcidae) | 0.03 | 1.47-7.61 | 25 | Petri dish 15 cm diameter | (24) |
| ***Cryptolaemus montrouzieri* Mulsant** | *Planococcus citri* (Hemiptera: Pseudococcidae) | 0.01-0.05 | 0.94-3.36 | 30 | Petri dish 15 cm diameter | (24) |
| ***Cheilomenes sexmaculata* (Fabricius)** | *Aphis craccivora, Myzus persicae* (Hemiptera: Aphididae) | 1.06 0.9 | 0.09 0.1 | 26-28 | Glass Beaker 8 x 11 cm | (9) |
| ***Propylea dissecta* (Mulsant)** | *A. craccivora*, *M. persicae* (Hemiptera: Aphididae) | 0.94 0.7 | 0.18 0.24 | 26-28 | Glass Beaker 8 x 11 cm | (9) |
| ***Coccinella transversalis* Fabricius** | *A. craccivora*, *M. persicae* (Hemiptera: Aphididae) | 1.05 0.94 | 0.09 0.14 | 26-28 | Glass Beaker 8 x 11 cm | (9) |
| ***Harmonia axyridis* (Pallas)** | *Myzus persicae* (Hemiptera: Aphididae) | 2.55 | 0.16 | 25 | Petri dish 9 cm diameter | (25) |

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