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ORIGINAL RESEARCH ARTICLE

Hyperpredation of local adults ladybirds on the eggs of *Cryptolaemus montrouzieri* a potential predator of carmine cactus cochineal *Dactylopius opuntiae* in Morocco

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Abstract

The mealybug destroyer *Cryptolaemus montrouzieri* has been introduced in many countries for biological control of mealybug species, recently the ladybeetle was tested on the biocontrol efficiency of *Dactylopius opuntiae* in many countries such as Brazil, Israel, and Morocco. Also, a total of thirteen species belonging to three orders and three families of local natural enemies associated to *D. opuntiae* were collected from Zemamra locality, and Gharbia locality in the Sidi Bennour region (120 km north-west of Marrakech), Morocco, and identified. A laboratory examination of *C. montrouzieri* eggs predation by eleven Morocco local generalist predators adults *Hyperaspis campestris*, *Scymnus interruptus*. *Scymnus loewii*, *Nephus redtenbacheri*, *Scymnus latemaculatus*, *Scymnus guttulatus*, *Exochomus nigripennis*, *Coccinella septempunctata*, *Hippodamia convergens, Chilocorus bipustulatus*, and *Chilocorus politus* showed that *C. septempunctata* had the highest predation on *C. montrouzieri* eggs (51 to 99% after 24 h) when provided with, or without, an alternative food supply (*D. opuntiae* first and second instars nymphs and *Aphis fabae* larvae). The addition of an alternative food source reduced egg predation by local polyphagous ladybeetles adults tested. These findings should be considered during biocontrol program on *D. opuntiae* and mass-rearing production.

Keywords Intraguild predation · Coccinellidae · *Cryptolaemus montrouzieri* · *Dactylopius opuntiae*

Introduction

Natural enemies associated with *Dactylopius opuntiae* and other Dactylopiidae comprise mainly predators (Vanegas-Rico et al., 2010; Bouharroud et al. 2018; Bouharroud et al. 2019; El Aalaoui et al. 2019a) including coleoptera (coccinellidae), dipetra, and lipedoptera. The predator, *Cryptolaemus montrouzieri* is native to Australia and commonly known as 'mealybug destroyer' since both adults and larvae prey on pests completely (Clausen 1978). Because of its efficiency, this predator has been introduced in many countries for biological control of mealybug species (Solangi et al. 2012). The predator was introduced into Brazil for biological control of *D. opuntiae*, and primarily cochineal attacking cassava and citrus (Sanches and Carvalho 2010). In Israel, 100,000 naturalist *C. montrouzieri* were successfully released in cactus crop infested by *D. opuntiae* in the north of the country (Protasov et al. 2017). Recently, the mealybug destroyer was tested in Morocco and results showed that inoculative releases of fourth instar larvae and adults could be used as part of an integrated management strategy to control *D. opuntiae* (Bouharroud et al. 2018; El Aalaoui et al.

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2019b). Several factors could affect the establishment, and predation potential of *C. montrouzieri* as a *D. opuntiae* predator and may include the density of the prey, the density and diversity of other non-prey species (Kratina et al. 2007), other predators (Kratina et al. 2009), pesticides (Martinou and Stavrinides 2015; El Aalaoui et al. 2019c), intraguild predation (Martinou et al. 2010), mutual interference (Chong and Oetting, 2006), prey nature, temperature (Van Lenteren et al. 2006), and other ecological factors.

The generalist predators ladybeetles can be important as scale pest predators (Fox et al. 2004) or potentially harmful in a variety of ways (Koch et al. 2003). The potentially harmful way is intraguild predation upon beneficial organisms (Cottrell 2005). Intraguild predation is defined as the interaction between predators that share the same resource, independently of their mode of nutrition, ecology, and taxonomic position (Lucas 2005). This has a direct implication for the fitness of the species implicated, intraguild predator (The aggressor), intraguild prey (victim), extraguild prey (common resource), and for the structure and dynamics of populations (Polis and Holt 1992). Many studies showed that the intraguild predation among coccinellids in natural situations when aphid prey become scarce (Musser and Shelton 2003). Also, other research has traditionally studied intraguild predation between predators in the field (Chacón et al. 2008) and laboratory conditions (Labbé et al. 2006). Lucas et al. (1998) reported that specialist predators are more likely to become intraguild prey when involved in intraguild predation interactions.

In Morocco, a total of thirteen species belonging to three orders and three families of natural enemies associated with *D. opuntiae* were collected from Zemamra locality, and Gharbia locality in the Sidi Bennour region (120 km northwest of Marrakech), Morocco and identified as: *Episyrphus balteatus* (De Geer) larvae (Diptera: Syrphidae), *Eupeodes corollae* (Fabricius) larvae (Diptera: Syrphidae), and *Hyperaspis campestris* (Herbst) adult (Coleoptera: Coccinellidae), *Scymnus interruptus* (Goeze) adult (Coleoptera: Coccinellidae), *Scymnus loewi* (Mulsant) adult (Coleoptera: Coccinellidae), *Nephus redtenbacheri* (Mulsant) adult (Coleoptera: Coccinellidae), *Scymnus latemaculatus* (Motschulsky) adult (Coleoptera: Coccinellidae), *Scymnus guttulatus* (LeConte) adult (Coleoptera: Coccinellidae), *Hippodamia convergens* (Guerin) adult (Coleoptera: Coccinellidae), *Exochomus nigripennis* (Erichson) adult (Coleoptera: Coccinellidae), *Chilocorus bipustulatus* (Linnaeus) adult (Coleoptera: Coccinellidae), *Chilocorus politus* (Mulsant) adult (Coleoptera: Coccinellidae), and *Emmelina Monodactyla* (Linnaeus) larvae (Lepidoptera: Pterophoridae). All these predators could feed on nymphs of mealybug and only *C. bipustulatus* and *Chilocorus politus* can be fed on both nymphs and adults stages of the prey (El Aalaoui et al. 2019a; Bouharroud et al. 2019). The *Coccinella septempunctata* larvae and adults were also observed to be associated to *D. opuntiae* in the field but they have not been observed feeding (El Aalaoui et al. 2019a). In the same case, other predators (*Exochomus* sp., *Chilocorus* sp., *Cryptolaemus* sp., *Hyperaspis* sp., and *Scymnus* sp) have been consistently observed associated with colonies of *D. opuntiae* in other countries where *D. opuntiae* is primarily pest such as Brazil and Mexico (Vanegas-Rico et al. 2010; Barbosa et al. 2014).

Egg predation is a key limiting factor that influences the interaction between community of predators population in the same ecosystem. The elucidation of this factor may play a role as to how generalist predators could impact beneficial coccinellids such as *C. montrouzieri* as a good introduced *D. opuntiae* predator in Morocco. Therefore, the objective of this study was to examine *C. montrouzieri* egg predation by eleven local coccinellid species that overlap spatially and temporally in *D. opuntiae* infected cactus. This was carried out in no-choice tests and for adults of each species when provided with, or without, an alternative food.

Materials and methods

Insect rearing

D. opuntiae nymphs used in this study originated from individuals collected from unsprayed cactus in Zemamra locality (32°37′48“ N, 8°42’0” W) in the Sidi Bennour region, Morocco. A modified version of the ‘cut cladode technique’ of Aldama-Aguilera and Llanderal-Cázares (2003), was used to increase numbers, and monitor the dynamic of insect stages. Each cladode was perforated at the basal end by a wooden stake, left to scar for 24 h under laboratory conditions, and then hanged vertically from metal grids. The gravid *D. opuntiae* females were isolated from infested cladodes and placed in an open waxed paper bag (8 cm^2). Each bag was then attached to the apex of each cladode; the other cladodes were placed horizontally beneath for nymphs which were not initially fixed on to the vertical cladodes. The infested cladodes were maintained under controlled conditions at 26 ± 2 °C, 60 ± 10% RH, and 12:12 h L:D (light:dark). *C. montrouzieri* colony was established from adults imported by the laboratory of entomology at INRA, Agadir-Morocco. Adults were placed in entomological cages (80–80-80 cm) comprised of a wooden frame covered with a mesh fabric to allow ventilation until they were used under laboratory conditions at 26 ± 2 °C and 60 ± 10% R.H. Access to water was provided via a cotton wick inserted into a 25 ml glass vial of water. To provide food and substrates for *C. montrouzieri* oviposition, cladodes infested with *D. opuntiae* were introduced weekly into the cages. *C. montrouzieri* larvae were transferred to another cage to complete their development and to avoid cannibalism. The polyphagous ladybeetle adults *H. campestris*, *S. interruptus*, *S. loewii*, *N. redtenbacheri*, *S. latemaculatus*, *S. guttulatus, H. convergens, E. nigripennis*, *C. septempunctata*, *C. bipustulatus*, and *C. politus* (Coleoptera: Coccinellidae) were collected from prickly pear plantations in Zemamra locality and Gharbia locality and maintained on cladodes infested with *D. opuntiae* nymphs in entomological cages (same cages and conditions described above). The identification of the specimens was done based on the following keys: Canepari et al. (1985), Stubbs and Falk (1996) (El Aalaoui et al. 2019a, Bouharroud et al. 2019). Also, all the ladybird adults were fed by a complementary diet (a mixture of water, honey, and brewer's yeast in a 20:40:40 proportion), which was replaced daily.

Egg clusters

To obtain sufficient egg clusters used in the current experiments, 10 pairs of *C. montrouzieri* ladybirds were placed in ten separate Petri dishes (14.5 cm in diameter) containing different developmental stages of *D. opuntiae*. Egg clusters were collected daily and stored in the fridge at 10 °C until use. Eggs used in these studies were < 3-day-old. All experiments in this study were conducted under similar conditions 26 ± 2 °C, 60 ± 10% RH, and 12:12 h (L:D).

Adults feeding without food assays

Feeding assays were carried out using laboratory-reared generalist predators adults *H. campestris*, *S. interruptus*, *S. loewii*, *N. redtenbacheri*, *S. latemaculatus*, *S. guttulatus*, *E. nigripennis*, *C. septempunctata*, *H. convergens, C. bipustulatus*, and *C. politus* (El Aalaoui et al. 2019a). Adult beetles of each species were starved for 8 h, and fed with 40 eggs of *C. montrouzieri* eggs [$n = 30$ adults for each species and sex ratio (male: female) was 1:1] in separate Petri dishes (9.5 cm in diameter). Numbers of eggs consumed were determined using a binocular magnifier at 2, 4, 8, and 24 h (Cottrell 2005). The experimental design adopted was a completely randomized block, and each experiment was replicated thrice.

Adults feeding with food assays

The polyphagous ladybeetles adults were collected in the field and reared in the laboratory as previously described. Number of *C. montrouzieri* eggs consumed by each species of adult ladybeetle was counted when they were fed only by *C. montrouzieri* eggs, or *C. montrouzieri* eggs plus an alternative food source (*D. opuntiae* first and second instars nymphs, and *Aphis fabae* larvae). Adult beetles of each species were kept individually in Petri dishes (9.5 cm in diameter) containing *C. montrouzieri* eggs (40 eggs) on white filter paper (placed in center of the Petri dish) or *C. montrouzieri* eggs (20 eggs) plus the alternative food source provided ad libitum (*D. opuntiae* first and second instars nymphs, and *Aphis fabae* larvae) scattered throughout the dish. The experimental design adopted in this study was a completely randomized block for each species using 20 beetles per treatment, and each experiment was replicated thrice. Beetles were assayed for 24 h and then the percentage of eggs consumed by each adult was determined (Cottrell 2005).

Statistical analysis

The percentages of *C. montrouzieri* eggs consumed by polyphagous lady beetles adults when provided with, or without, an alternative food supply were subjected to analysis of variance using a Tukey's test ($p \leq 0.05$) using the software package SPSS ver. 18.0 (Carver and Nash 2011).

Results

Adults feeding without food

Percentages of *C. montrouzieri* eggs consumed by polyphagous lady beetles adults are listed in Table 1. The highest percentages were showed by *C. septempunctata* during 2 h ($F = 830.65$, $df = 10$, $P = 0.000$). The lowest percentages of *C. montrouzieri* eggs consumed were recorded during 2 h for the following 8 ladybeetles adults: *H. campestris, S. loewii, N. redtenbacheri, S. latemaculatus, S. guttulatus, E. nigripennis, C. bipustulatus,* and *C. politus*. During 4 h the highest percentage of *C. montrouzieri* eggs consumed was observed with *C. septempunctata* ($F = 769.14$, $df = 10$, $P = 0.000$), and the lowest percentage of *C. montrouzieri* eggs consumed was observed with *S. loewii, N. redtenbacheri, S. latemaculatus, S.guttulatus,* and *E. nigripennis.* During 8 h to 24 h the highest percentages of *C. montrouzieri* eggs consumed was observed with *C. septempunctata* (after 8 h $F = 1045.99$, $df = 10$, $P = 0.000$; after 24 h $F = 1714.66$, $df = 10$, $P = 0.000$). The lowest percentages of *C. montrouzieri* eggs consumed were recorded during 24 h for the following lady beetles adults: *H. campestris, S. loewii, N. redtenbacheri, S. latemaculatus, S. guttulatus, E. nigripennis, C. bipustulatus,* and *C. politus*. Among the treatments, *C. septempunctata* had the highest predation for *C. montrouzieri* eggs.

Adults feeding with food

One way ANOVA revealed significant differences in the percentages of *C. montrouzieri* eggs consumed by polyphagous ladybeetles adults when provided with, or without, an alternative food supply (*D. opuntiae* first and second instars nymphs, and *Aphis fabae* larvae). A

Table 1 Percentages (±SE) of *C. montrouzieri* eggs consumed by polyphagous lady beetles adults

Predator	Percentages (±SE) of *C. montrouzieri* eggs consumed at different intervals (h)			
	2	4	8	24
H. campestris	0.17 ± 0.63 c	6.75 ± 3.02 def	9.50 ± 3.91 c	12.33 ± 4.20 c
S. interruptus	8.50 ± 2.24 b	11.83 ± 3.00 c	17.67 ± 1.96 b	21.83 ± 1.30 b
S. loewii	0.08 ± 0.46 c	4.75 ± 1.78 g	9.33 ± 3.21 c	11.08 ± 5.03 c
N. redtenbacheri	0.25 ± 0.76 c	5.17 ± 1.85 fg	9.58 ± 3.09 c	11.33 ± 4.86 c
S. latemaculatus	0.42 ± 0.95 c	5.67 ± 1.85 defg	10.33 ± 2.43 c	12.83 ± 3.58 c
S. guttulatus	0.33 ± 0.86 c	5.42 ± 1.75 efg	9.92 ± 2.82 c	11.92 ± 4.24 c
E. nigripennis	0.50 ± 1.02 c	6.08 ± 1.93 defg	10.58 ± 2.52 c	13.17 ± 3.41 c
C. septempunctata	32.00 ± 3.04 a	44.58 ± 1.33 a	81.67 ± 7.29 a	99.58 ± 1.15 a
H. convergens	7.50 ± 4.05 b	14.42 ± 2.34 b	19.50 ± 3.11 b	23.67 ± 1.43 b
C. bipustulatus	0.42 ± 0.95 c	7.17 ± 2.69 de	10.25 ± 3.43 c	13.33 ± 3.17 c
C. politus	0.58 ± 1.08 c	7.50 ± 2.86 d	10.67 ± 3.21 c	13.92 ± 2.43 c

By column means with different letters are significantly different according to Tukey's test ($p \leq 0.05$).

significantly higher percentage of *C. montrouzieri* eggs were consumed by adult lady beetles in the absence of an alternative food source than in the presence of this alternative food (Table 2) ($F = 57.16$, $df = 1$, $P = 0.000$). Analysis of the main effect of alternative food availability on adult species eggs predation revealed that *C. septempunctata* consumed significantly more *C. montrouzieri* eggs in the presence of alternative food source ($F = 1010.06$, $df = 1$, $P = 0.000$) but no significant difference was observed for percentage consumed by other ladybeetles adults: *H. campestris, S. interruptus, S. loewii, N. redtenbacheri, S. latemaculatus, S. guttulatus, H. convergens*, *C. bipustulatus*, *C. politus*, and *E. nigripennis* consumed more.

Discussion

The introduction of a new coccinellid predator more specialist and efficient on scale pests may negatively be affected by native coccinellid species through resource competition and egg predation, despite coccinellid eggs are generally protected from predation by defensive alkaloids, pyrazines, and quinolenes (Cottrell 2005). Many studies reported that the alkaloids synthesized by coccinellids were found in all developmental stages (Agarwala and Yasuda 2001). This would explain why coccinellids eggs are attacked by a low number of predators compared to eggs of other insects (Cottrell and Yeargan 1998). The current study tested eleven local generalist predators in Morocco for their hyperpredation on *C. montrouzieri* eggs since this 'mealybug de-

Table 2 Direct comparison of percentages (±SE) of *C. montrouzieri* eggs consumed by polyphagous lady beetles adults when an alternative food source (*D. opuntiae* first and second instars nymphs) was or was not provided

Predator	Percentages (±SE) of *C. montrouzieri* eggs consumed after 24 h		
	With food	Without food	Significance
H. campestris	6.83 ± 1.60	11.92 ± 4.03	**
S. interruptus	6.00 ± 1.93	21.17 ± 1.70	**
S. loewii	5.50 ± 1.79	11.83 ± 4.55	**
N. redtenbacheri	6.08 ± 1.26	11.92 ± 4.29	**
S. latemaculatus	6.92 ± 1.42	13.08 ± 3.39	**
S. guttulatus	6.75 ± 1.49	11.50 ± 3.45	**
E. nigripennis	7.67 ± 1.60	13.33 ± 3.24	**
C. septempunctata	50.83 ± 5.31	99.17 ± 1.78	**
H. convergens	6.25 ± 2.15	24.08 ± 1.54	**
C. bipustulatus	6.17 ± 2.05	13.00 ± 3.18	**
C. politus	7.00 ± 1.90	13.50 ± 2.51	**

** significant by Tukey's test ($p \leq 0.05$); ns = no significant by Tukey's test ($p \leq 0.05$)

stroyer' was introduced in many countries among them Morocco for biological control of mealybug species. *C. septempunctata* had high predation for *C. montrouzieri* eggs when provided with, or without, an alternative food supply, and a higher percentage of *C. montrouzieri* eggs were consumed by adult ladybeetles in the lack of an alternative food source than in the presence of this source. In addition to the diversity of other non-prey species (Kratina et al. 2007), pesticides (Martinou and Stavrinides 2015; El Aalaoui et al. 2019b), mutual interference (Chong and Oetting, 2006), prey nature, temperature (Van Lenteren et al. 2006), and other ecological factors, intraguild predation can also reduce the efficiency of biological control (Noia et al. 2008). Intraguild predation can lead to changes in the biological and reproduction parameters of the protagonists (Polis et al. 1989), and limit the effectiveness of biological control (Noia et al. 2008). Other studies indicated that intraguild predation is an important cause of mortality of larvae in the field (Yasuda and Shinya 1997). Symondson (2002) reported that the use of generalist predators in biological control programs can be controversial because of their potential to prey on other biological control agents and non-target species. The predation and the competition for the intraguild prey are two components of intraguild predation, and the population dynamics of pests and natural enemies depends on the preference of the intraguild predator for the intraguild prey (Rosenheim 1998). Also, the size and the degree of feeding specificity of the protagonists and prey abundance are other important factors that determine the nature, symmetry, frequency, and outcome of intraguild predation (Lucas et al. 1998).

The presence or absence of intraguild prey influences the intensity of the interaction between intraguild predators (Felix and Soares, 2004). Intraguild predation between larvae of *Chrysoperla carnea* and *C. septempunctata* was greatly reduced when aphids were added (Sengonca and Frings 1985). Also, predation of the phytoseiid mite *Amblyseius cucumeris* by the predatory bug *Orius tristicolor* decreases with increasing densities of their common extraguild prey western flower thrips *Frankliniella occidentalis* (Gillespie and Quiring 1992).

The presence of extraguild prey decreases predation by intraguild predators (Yasuda et al. 2004). *Harmonia axyridis* intraguild predator is more aggressive than *Coccinella undecimpunctata* L., as in several coccinellid guilds (Yasuda et al. 2004). *H. axyridis* is invading aphidophagous ladybird guilds in North America, and in Japan, this intraguild predator was commonly co-occurred with *C. septempunctata* and *Propylea japonica* (Sato et al. 2003). Yasuda et al. (2001) reported that fourth-instar larvae of *H. axyridis* attack those of *C. septempunctata*. In our study, only *C. septempunctata* can feed on both *C. montrouzieri* eggs and first instar nymphs (personal observation). Sato et al. (2003) showed how *H. axyridis* larvae are well adapted to feed on other species and are well protected against predation. Also, survival and development of larvae of *C. septempunctata* worsen after consuming larvae (Yasuda et al. 2001) and eggs (Sato et al. 2008) of other ladybird species, which indicate that *C. septempunctata* is not well adapted for intraguild predation.

In this study, alternative prey was chosen based upon laboratory observations that indicated which available alternative prey would be acceptable to the coccinellids tested. *Aphis fabae* larvae were considered highly palatable to *C. septempunctata* that they do not observe feed on *D. opuntiae* nymphs, and *D. opuntiae* first and second instars nymphs and *A. fabae* larvae for other tested generalist predators; thus preventing *C. montrouzieri* eggs from being the preferential feeding choice for the predators tested. Also, ladybirds often resort to hyperpredation or intraguild predation when the number of their main prey decreases, and usually, this situation does not occur until the larval stage has achieved its development, which does not last long. This is rarely referred to as a food preference situation, for this reason, intraguild predation between larvae has not been taken in detail in this manuscript. A direct field evaluation on the impact of these local coccinellid species may have upon introduced beneficial ladybeetles such as *C. montrouzieri* is needed.

The laboratory results presented here suggest that the local polyphagous ladybeetles were able to impact negatively on beneficial introduced ladybeetle *C. montrouzieri* however an extrapolation of these results to the field will not be entirely accurate.

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Compliance with ethical standards

Conflict of interest Author A declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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