

Functional Response and Predation Potential of *Hyperaspis Campestris* (Herbst 1783) (Coleoptera: Coccinellidae) on *Opuntiae* Cochineal *Dactylopius Opuntiae* (Hemiptera: Dactylopiidae) in Morocco

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Abstract:

Functional response of the lady beetle *Hyperaspis campestris* (Herbst 1783) to varying densities (1, 5, 10, 15, 20 and 25) of *Dactylopius opuntiae* (Cockerell) young females (20 days old) were determined under controlled conditions at $26\pm 2^\circ\text{C}$, $60\pm 10\%$ RH and 12:12 h L:D regime. The searching efficiency of *H. campestris* considerably decreased as prey density increased. The significant linear coefficient (P_1) obtained by logistic regression had a negative indicating functional response type II. Attack rates (0.151, 0.101, 0.097, 0.122, 0.124 and 0.135) and handling times (3.848, 5.171, 5.417, 4.245, 4.356 and 3.940) for 1 to 25 density, respectively, were recorded using Holling's disc equation. Most of this handling time was spent in removing the wax covering and protecting the young females of the mealybug. *H. campestris* feeds on young females of *D. opuntiae* and could therefore be an effective predator to regulate to low-density populations of *D. opuntiae*.

Keywords: Biological control, Functional response, *D. opuntiae*, *Hyperaspis campestris*, Cactus

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Introduction

Cactus is one of the most important economic crop worldwide (Kiesling 1999). It plays an critical role in the ecological system in semi-arid and arid areas, preventing desertification and preserving biodiversity, is also exploited as a vegetable source for human, and cladodes

as cattle feed (Kiesling 1999; Bouharroud *et al.* 2016). It is cultivated in Africa, Europe, Asia, America, Africa, Canada, Peru, Cuba, and other Caribbean islands (Casas & Barbera 2002; Bravo Hollis & Scheinvar 1995; Griffith 2004). In Morocco, cactus was first introduced in 1770 from south Africa and the cactus area

has considerably evolved over the last two decades from 50 000 ha in 1998 to more than 150 000 ha at present as a result of drought (Bouharroud *et al.* 2016). Mann (1969) reported that there are 167 Arthropod species associated to cactus (*Opuntia ficus indica*). Among these insects, *Dactylopius opuntiae* (Cockerell) is the most devastator pest of prickly pear cactus in Mexico (Githure *et al.* 1999; Portillo & Viguera 2006), Brazil (Silva *et al.* 2013), Israel and Spain (Spodek *et al.* 2014) and recently Morocco and others Mediterranean areas (Bouharroud *et al.* 2016; El Aalaoui *et al.*, 2019a; El Aalaoui *et al.*, 2019b). This sap-sucking insect was first detected and identified in Morocco in 2014 (Bouharroud *et al.* 2016) and caused severe damage to prickly pear in many regions of kingdom. *D. opuntiae* feed directly on cladodes, and severe infestations inferior or equal to 75 % of the cladode surface can result a death of the host plant (Mann 1969; Vanegas-Rico *et al.* 2015). This hemipteran females have 3 life stages – egg, nymph or crawlers (2 instars), and adult, lived permanently attached to their host cladodes, and produce wax cottony that protects their bodies against predators, and reduces the potency of pesticides, the female survival time varies from 90 to 128 days, also each female can laid more then 130 eggs, which hatch almost simultaneously into crawlers (Badii & Flores 2001). whereas males have 5 life stages egg, nymph, pre-pupa, pupa and adult, male is short lived without feeding and moves predominantly by walking (Badii & Flores 2001). No informations are available today on economical and environmental losses caused by *D. opuntiae*, in Morocco, where it is not native, around 400 ha of cactus damaged by this insect at Sidi Bennour in Doukkala region have been reported. The cochineal is today present almost in all Morocco regions. In Brazil the degas caused by this scale pest were estimated at \$25 Million Dollar (Lopes *et al.* 2009).

The damage caused by this scale pest to fruit and cladodes in Mexico resulted higher production and economical costs (Badii & Flores 2001; Portillo & Viguera 2006). Also in Lebanon and Israel a pest risk analysis of the insect was carried out to protect natural areas covered by *O. ficus-indica* (Spodek *et al.* 2014). *D. opuntiae* is mostly controlled by organophosphate insecticides such as cypermethrin and chlorpyrifos which can affect human health, the environment, and limit international commercial exchange (Vanegas-Rico *et al.* 2016). Many alternative strategies such as the use of predators have been used around the world to reduce pesticides use for *D. opuntiae* control (Viguera *et al.* 2009; Borges *et al.* 2013; Vanegas-Rico *et al.* 2016). Natural enemies associated with *Dactylopius* species include much predators belonging to Lepidoptera, Coleoptera, Diptera and Neuroptera groups (Grissell 2004; El Aalaoui *et al.*, 2019b). Coccinellidae *Hyperaspis trifurcata* and *Chilocorus cacti* are the most predators of *D. opuntiae* in USA and Mexico (Vanegas-Rico *et al.* 2016). After detection of *D. opuntiae* in Morocco, surveys were conducted in different cactus production areas with a view to find biological control agents with the potential to be used as a predators against this scale pest. In July 2017, *Hyperaspis campestris* was observed on cactus crop actively feeding on cactus mealy bug (*D. opuntiae*) in Sidi Bennour, region (Bouharroud *et al.* 2019). A few studies showed the utilization of *H. campestris* as biological control agent. *Hyperaspis campestris* Herbst was able to reduce the population of *pulvinaria floccifera* (Westwood) within two years of release (Bogdanova 1956). Also *Hyperaspis* spp. eggs are generally deposited near their prey, on the bark or growth rings of twigs, but not inside the scales (El-Ali 1972). However, in Hubbard and Potter's (2005) study in Kentucky, *Hyperaspis* spp. emerged only from under

adult females. The body length measured 2 to 5 mm (Van Goethem 1975; Gunst 1978). The head is yellow with black on upper side hidden by pronotum (Bouharroud *et al.* 2019). The anterior pronotum border was yellow and the sides are partially yellow (Bouharroud *et al.* 2019). The red spot is typically placed at three-quarter part of elytron. The humeral spots is absent (Van Goethem 1975).

The functional response is defined by the number of preys devoured by predator in well determined time and is a good indicator of the efficiency of a biological control agent as a predator of prey (Fernández-Arhex & Corley 2004). Most coccinellids showed type II response (Gunog & Donald 2011). Many models have adopted in the past to explain the interaction between predator and prey (Omkar 2004). Holling disc equation was the most model used to describe this interaction (Holling 1959, Fenlon & Malcolm 2006). This method is frequently used to calculate handling time parameters, the attack rate and maximum attack coefficient of predators (Omkar 2004).

The aim of this study was to determine the type of functional response of *H. campestris* on *D. opuntiae*, to estimate its predation parameters (handling time and attack rate), and to have an idea about the suitability of this ladybeetle as a predator of *Opuntiae* scale in Morocco.

Material and Methods

Mealybug source

Opuntia ficus-indica cladodes not infested were used for *Dactylopius opuntiae* rearing. The *Opuntiae* scale (*D. opuntiae*) colony was established from specimens collected from zemamra locality. This area is located at the following coordinates: longitude W8° 22, latitude N 32° 21 and altitude 168m. The estate is in the semi-arid ecological zone. Rainfall varies between 112.6-607 mm/year respectively

in (1980-81) and (1996-97). The rainfall annual average of 30 years is 330 mm. Temperature varies in -1°C (Dec-Jan) and from 40 to 45 (July-August). For soil there are two zones: Zone 1: Vertisol with an angular structure over the first 15 cm and with high moisture content, deep up to 1.5 m, difficult to work in dry conditions. Zone 2: Light soil with a clay-sand-silty hydromorphic structure with an alkaline Ph (Khattabi *et al.* 2004). A version modified of the 'cut cladode technique' of Aldama-Aguilera and LlanderalCazares (2003) described by Vanegas-Rico *et al.* (2016) was used to follow the age and to increase numbers of scale insect. The attacked cladodes were then maintained under controlled conditions at 26±2 °C, 60±10 % RH and 12:12 h L:D regime and the colony was allowed to develop for use in experiments.

Predator source

During surveys, the adults of *Hyperaspis campestris* were collected from cactus plantations in Zemamra locality, identified (Bouharroud *et al.* 2019; El Aalaoui *et al.* 2019b), and maintained on cladodes infested with *D. opuntiae* until their use under controlled conditions (26±2°C and 60±10% relative humidity) at the experimental station of INRA (National Institute of Agronomic Research, Morocco) in Zemamra. Adults were kept in entomological cages (80-80-80cm) comprised of a wooden frame with a mesh fabric to allow ventilation. Access to water was ensured via a white cotton inserted into a 25 ml glass vial of water. To provide food the cladodes attacked with *D. opuntiae* nymphs were introduced into the cages (Vanegas-Rico *et al.* 2016). During rearing a mixture diet with honey, brewer's yeast and water in a ratio of 40%:40%:20% was offered. Dreyer *et al.* (1997) reported that honey increase survival time of *Hyperaspis notata* Mulsant.

Experimental procedure

Each adult of *H. campestris* was placed individually in Petri dishes (9.5 cm diameter) and starved for three days in order to standardize their hunger. A fixed number of scale pest adults young females (20 days) were collected and added to each Petri dish to obtain a prey density of 1, 5, 10, 15, 20 or 25. *D. opuntiae*, young females are acceptable prey for *H. campestris* due to their smaller size (personal observation). For a given predator, there is a size of acceptable prey (Sabelis 1992). After 24 h, the adults predators were eliminated and young females consumed were recorded (Flores *et al.* 2013). Completely randomized block design with ten replicates were used for each predator-prey density combination and all experiment was replicated three times. To preserve prey density for each predator, prey replacement was done during the period of the study (24 hours) (Rogers 1972). All experiments of this study were done under controlled conditions (26±2 °C, 60±10 % RH and 12:12 h L:D regime).

Functional response

The functional response type was recorded by logistic regression fitted to the proportion of adult females devoured Ne/N_0 against the number of adult females offered (N_0) using the XLSTAT (XLSTAT 2017). This kind of regression provides more admirable results than the linear regression of Ne against N_0 (Pervez 2005). The equation reported by Juliano (2001) describes the relationship between Ne/N_0 and N_0 is as follows:

$$Ne/N_0 = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}$$

Where P_0 , P_1 , P_2 and P_3 indicated the intercept coefficient, linear coefficient, quadratic coefficient and cubic coefficient

respectively. If P_1 is negative, that represent functional response type II and if P_1 is positive that indicates prey density-dependent predation and thus represent a functional response type III.

Both, random attack equation (Rogers 1972) and Holling disc equation (1959)— $Ne = aTN_0/(1+a N_0 Th)$ — were adopted to calculate the functional response parameters (handling time (Th) and attack rate (a)): Ne represent the number of scale pest consumed by each ladybeetle, a represent the attack rate of predator, N_0 the initial number of scale pest offered, T is the experiment duration (24 hours) and Th the prey handling time estimated.

The ladybeetle searching efficiency (E) was recorded using the equation $E = Ne/N_0$ (Flores *et al.* 2013). The high number of scale pest females consumed (Nem) and prey searching time (Ts) were recorded using the equations: $Nem = \frac{T}{Th}$ (Elliot 2003) and $Ts = T - ThNe$ (Juliano & Williams 1987).

The significance difference in number of supping scale pest females consumed at different densities, in maximum attack rate, and in searching time was recorded using analysis of variance ANOVA (SPSS 2012). For searching efficiency, Fisher test was adopted for multi-comparisons between significant treatment effects where they appeared, using STATISTICA (ver. 6) software.

Results

The results showed a significant difference ($F = 20.686$, $df = 5$, $p < 0.05$) between the number of preys devoured by *H. campestris* at different prey densities. The maximum consumption was recorded at densities between 15 and 25 prey, and it was significantly higher ($p < 0.05$) than that recorded at the lowest densities (1, 5, 10) (Fig 1; Fig 2).

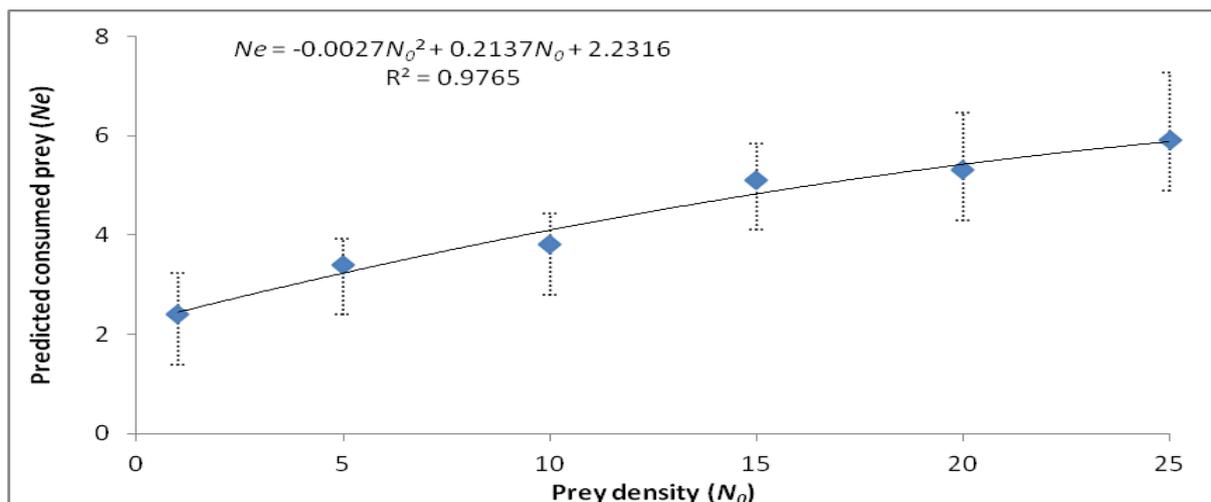


Fig 1 Numbers of *D. opuntiae* young females consumed by *H. campestris* at different prey densities ($T=24$ h).

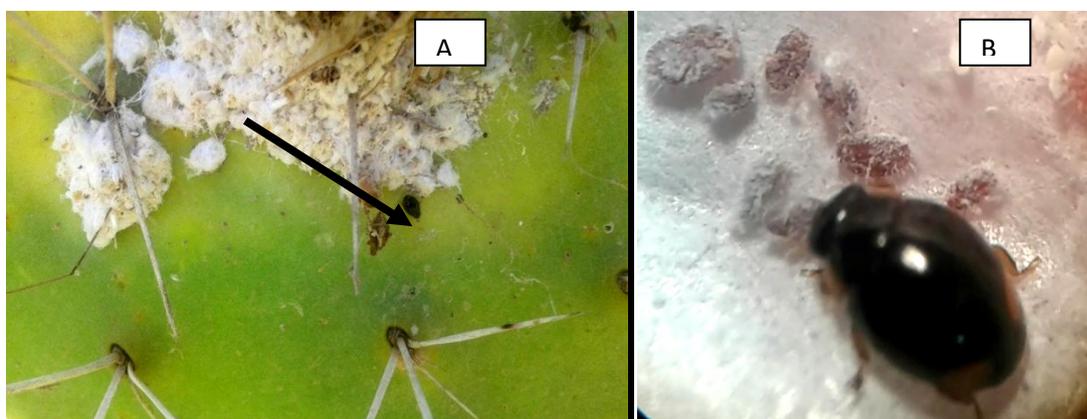


Fig 2. Adults of *Hyperaspis campestris* from prickly pear plantations infested with *D. opuntiae* in Morocco, **A.** *Hyperaspis campestris* feed on *D. opuntiae* young females, **B.**

The highest coefficient of determination ($R^2=0.9765$) indicated that cubic polynomial was the model that gave the best fit ($p<0.05$) to the observed data (Ne), with. Attack rates (0.151, 0.101, 0.097,

0.122, 0.124 and 0.135) and handling times (3.848, 5.171, 5.417, 4.245, 4.356 and 3.940) for 1 to 25 density, respectively, were determined using Holling's disc equation (Table1).

Table 1 Effect of density upon the instantaneous rate of discovery and the handling time.

Density N_0	Inst. rate of discovery (a)	Handling times (Th)
1	0.151±0.053	3.848±1.686
5	0.101±0.015	5.171±0.742
10	0.097±0.016	5.417±0.930
15	0.122±0.018	4.245±0.646
20	0.124±0.027	4.356±1.219
25	0.135±0.031	3.940±0.841

The predator searching efficiency (E) significantly decreased as prey density increased from 1 to 15 and does not significantly change from 15 to 25 (Fig 3).

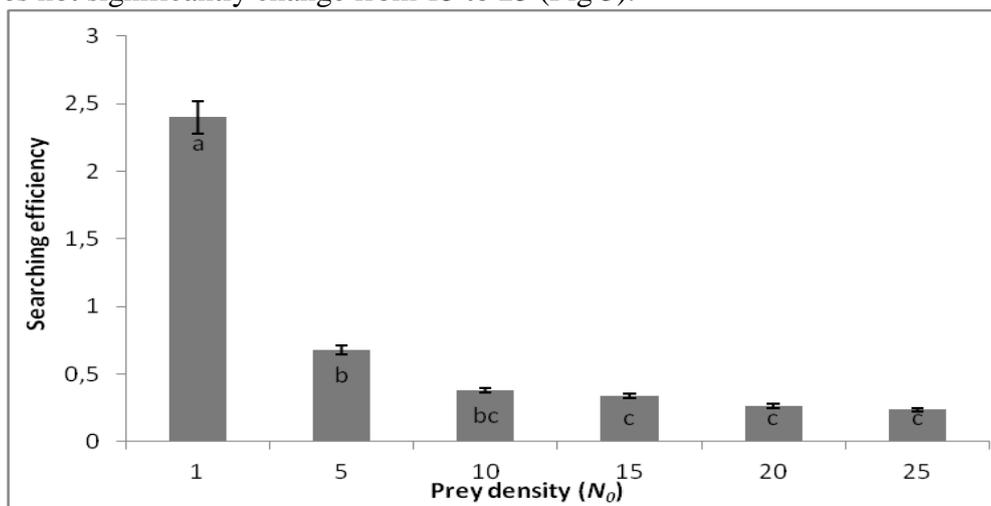


Fig 3 Searching efficiency of *H. campestris* at different *D. opuntiae* female densities. Bars with different letters are significantly different according to the Fisher test ($\alpha=0.05$).

In addition, a change in adult handling time indicates a change in the response level, which is determined by instantaneous rate of discovery (T/Th) or maximum attack rate. Maximum attack rate values were 3.961, 4.056, 4.260, 5.440, 5.701, and 6.214 for 1, 5, 10, 15, 20

and 25 densities, respectively (Table 2). It significantly increased as scale pest females density increased from 1 to 25 ($f=5.701$, $df=5$, $p<0.05$). The total searching time decreased significantly and the total handling time increased ($p<0.05$) at different scale pest densities (Fig 4).

Table 2 Maximum attack rates (T/Th) estimated for all six densities of *D. opuntiae*. Mean values of Th estimated by non-linear least squares regression using Holling's "disc equation".

Density N_0	1	5	10	15	20	25
Max. attack rate	3.961	4.056	4.260	5.44	5.70	6.21
(T/Th)	± 1.75	$\pm 1.03bc$	$\pm 1.14b$	$\pm 0.59a$	$\pm 1.40a$	± 1.43
	c		c	bc	b	a

Bars with different letters are significantly different according to the Tukey's test ($\alpha=0.05$).

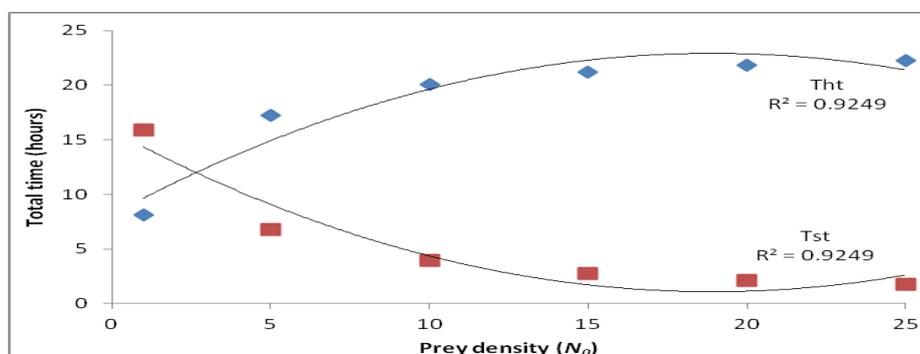


Fig 4 Inverse relationship between the total handling time (Tht) and total searching time (Tst) of *H. campestris* preying on *D. opuntiae* young females, obtained by the estimated parameter Th and the equation $T_s = T - ThNe$.

Discussion

A negative value of linear coefficient ($P_1 = -0.675$) obtained by logistic regressions of proportion of scale pest females consumed (N_e/N_0) against number of females initially provided (N_0) to adults of ladybeetle *H.campestris* over 24 hours, indicated that the functional response exhibited by this predator was type II (Holling 1959). Many types of functional response are described for ladybird species (Hodek 1996). Among these types, type II and III considered most important (Murdoch *et al.* 2003), because most natural enemies show these types. Oaten & Murdoch (1975) reported that response type II may destabilize biological control agent-prey interactions and is theoretically less able to remove prey populations compared to response type III. Hassell *et al.* (1977) reported that in the response type II, consumed prey intensity does not increase with prey density. In same case Hodek & Honek (1996) recorded that hungry predators completely devour the limited preys they encounter and then utilize subsequent prey with progressively reduced voracity. The decrease of the proportion of prey consumed with increasing of prey density, may be due to the effect of various phenomena, such as the size of experimental arena, handling time and degree of hunger of the ladybeetle (O'Neil & Stimac 1985, 1988). Prey handling time of the predator was relatively higher (ranging 3.848 ± 1.686 – 5.417 ± 0.930 hours) at different densities of *D. opuntiae* females (1 to 25 females). Because of *D. opuntiae* females cover themselves with white waxy cottony and remain attached to the cladode, *H.campestris* spent most of the T_h cleaning the waxy cottony to access the adult females bodies (Flores *et al.* 2013). This “cleaning” process uses time not required by other predatory insects that have direct access to their uncovered prey (Flores *et al.* 2010; Flores *et al.* 2013). The experimental data on handling time at

high *D. opuntiae* females density can be comparable with field conditions where the prey density is also high (Omkar 2004), due to heavy *D. opuntiae* infestation. Also Akhtaruzzaman & Ahmad (1998) recorded that handling time of each predator was higher at lower pest density. Variation in predator and prey size, predator voracity, predator satiation time, predator hunger levels, predator digestive ability, predator walking speed and accessibility to prey are the most factors that must be affected total handling time of each biological control agent (Pervez 2003, Omkar 2004). Although T_h and a were generally constant at all densities of prey and consumed prey is density dependent, in practice this is not usually true even in a functional response type II (Flores *et al.* 2013). The highest Maximum attack rates recorded at high prey density (25 females) may be explained by these suggestions: the engorgement of predator on the first prey they encounter then become less competent to exploit the prey, the predator consumes more than the minimum required for growth when prey is abundant (Honek 1996), or the combination of these factors.

Feeding on *D. opuntiae* young females and shorter life cycle of *H.campestris* in comparison with *D.opuntiae* could be beneficial for its use as a predator for the control of this scale pest. The searching efficiency of *H.campestris* at different *D.opuntiae* young female densities indicates that it may become less potent at high scale pest densities. Therefore, *H.campestris* would be an effective predator at the early stages of the invasion, this is the case today in many regions of Morocco. *H.campestris* adult consumes first and second instar crawlers of *D. opuntiae* but cannot consume gravid females (personal observation). A few studies showed the utilization of *H.campestris* in biological control.

Bogdanova (1956) reported that *H. campestris* Herbst was able to reduce the population of *pulvinaria floccifera* (Westwood). Scale populations were reduced below the economic injury level two years after release (Bogdanova 1956). *H. campestris* Herbst, a predator of scale insects on citrus, grapes and other subtropical crops, was release against *Pulvinaria floccifera* (Westwood) (Bogdanova 1956). *Hyperaspis* spp. were also able to reduce *Eulecanium cerasorum* (Cockerell) fecundity by 48% (Robayo 2015). Much previous research on biocontrol potential around the world reported that biological control agents predation could be modulated by other predators (Kratina *et al.* 2009), the density of the prey, the diversity and density of other non-prey species (Kratina *et al.* 2007). Abiotic factors can also modify the functional response of predators. So further studies under Morocco ecological system conditions need to be carried out to confirm the predation potential of *H. campestris* on *D. opuntiae* in Morocco.

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