THE FUNCTIONAL RESPONSE, WHIT DIFFERENT FLOWERS FOODS SUPPLY, OF *HIPPODAMIA VARIEGATA* (Goeze) ADULTS (COLEOPTERA: COCCINELLIDAE) IN LABORATORY

[a]Bertolaccini Isabel*., [b]Etelvina Núñez-Pérez, [c]María C. Curis, [d]Emilio. J. Tizado

[a] *Facultad de Ciencias Agrarias (Universidad Nacional del Litoral). Kreder 2805 (3080) Esperanza. Santa Fe (Argentina).*

[b] *Facultad de Ciencias Biológicas y Ambientales. Campus de Ponferrada (Univ. de León)., Ponferrada. León (España).*

[c] *Facultad de Ciencias Agrarias (Universidad Nacional del Litoral). Kreder 2805 (3080) Esperanza. Santa Fe (Argentina).*

[d] *F Facultad de Ciencias Biológicas y Ambientales. Campus de Ponferrada (Univ. de León). Ponferrada. León (España).*

A B S T R A C T

Functional response (FR) of the ladybeetle adults, *Hippodamia variegata* (Goeze) (Coleoptera, Coccinellidae) to varying densities (5, 10, 20, 40, 60 and 80) of third instar nymphs of *Acyrthosiphon pisum* (Harris) in the aphid range 1-40 was assessed in a growth chamber (23±2 ºC, 41±10% relative humidity, and photoperiod 16L:8D), reared on *Phaseolus vulgaris* (L.). The effect on the FR of the following factors was studied: (i) different sexes (male and female), (ii) different levels of aphids satiety (24 and 48 hours), (iii) different flowers foods supply (Brassicaceae, Apiaceae and Asteraceae) and (iv) different experimental arenas (petri dishes and alfalfa plants). The logistic approach showed a type II functional response. The males consume less aphids than females (a mean reduction of 53.2% in petri dish, and 71.1% in alfalfa plant). The attack coefficients (*a*) and the handing times (*Th*) were, generally, majors in the count realized to 24 hrs., than to 48 hrs. and in petri dishes, compared with the values realized on alfalfa plants and, in both cases, when the flowers supplied was *Brassica nigra*. The presence of floral resource plants from *B. nigra* and, with less impact, *Daucus carota* enhances the consumption of *H. variegata* and, females are most effective as predators than males.

Keywords: Functional responses, Coccinellidae, supplementary food, arena, prey density, hungry time.

INTRODUCTION

The functional responses (FR), is the change in attack response of a predator species when increasing prey population, is a main component of the stability of predator-prey systems (Hassell, 1978). Since Holling (1959) found the attack rates of natural enemy increase with increasing the prey density, several functional response`s types has been described: a rectilinear rise to a maximum (Type I), a constantly decreasing rate towards a maximum (Type II), a sigmoid increase (Type III), and a dome-shape (Type IV). However, some authors found that insect predators exhibit mainly a Type II FR (Asante, 1995; Pervez & Omkar, 2005; Davoodi Dehkordi & Sahragard, 2013), and only a few

* Corresponding Author:
Email: isabelb@fca.unl.edu.ar

group of insects seem exhibit a Type III FR (Almeida Sarmento *et al.*, 2007). The study of the FR is a key component of population dynamic models in biological control because it can determinate if a predator is able to regulate the density of its prey (Fernández-Arhex & Corley, 2003; Pervez & Omkar, 2005; Rossi *et al.*, 2006). Two parameters characterizing the predator FR Type II: the attack rate and the handling time. The number of prey eaten by a natural enemy is limited only by the time the predator takes to subdue, consume and digest the prey. However, the prey density is not the only factor in the natural enemy FR (Arditi & Ginzburg, 1989). Also the plant (Treacy *et al.*, 1987), the density and the size of prey population (Reis *et al.*, 2003), the interference or competition between prey species, the heterogeneous patch distribution of predators (Cosner *et al.*, 1999), the presence of alternative preys (Nielsen *et al.*, 2002), can

affect the natural enemies FR. In this hand, the age and sex of the predator is especially important because they can prefer different size or types of prey (Murdoch & Sih, 1978; Da Silva *et al.*, 2004).

Coccinellids are an important group of predatory insects but the number and distribution of the natural populations of many ladybirds are declining because of habitat destruction. They are can also feed on nectar, fungal spores, prey products such as honeydew, and pollen (Lundgren *et al.*, 2005). These supplementary foods are important to enhance their growth and development when prey is scarce, and to obtain critical nutrients for overwintering, dispersal and egg production (Hodek & Honek, 1996; Lundgren *et al.*, 2005).

Moreover, their biology exhibit a close synchrony with their prey species i.e. usually reproduces when the prey species are increasing in numbers. *Hippodamia variegata* (Goeze) is a worldwide beneficial coccinellids species, and is an important predator of *Acyrthosiphon pisum* (Harris) in legume crops in the province of León and potentially interesting to use in biological control of alfalfa aphids pest. With this antecedent and due to the numerous factors that affect the FR, the aims of the present work are evaluate the particular effects of the different flowers food supply, the sex and the number of aphid on the aphid eaten as a function of initial density using different experimental arenas. The main hypothesis was that the different experimental conditions affect the functional response of *H. variegata*.

MATERIAL AND METHODS

The FR studies of *H. variegata* were conducted in laboratory (Campus de Vegazana, Libertad Av., 25, University of León, León, Spain). Adults were collected from alfalfa fields and ladybird beetle were provided with *A. pisum* reared on *Phaseolus vulgaris* (L.). Experiments were run at 23±2 ºC, 41±10% relative humidity, and photoperiod 16L:8D. The effect on the FR was studied with the following factors: (i) different sexes (male and female), (ii) different levels of aphids satiety (24 and 48 hours), (iii) different flowers foods supply (Brassicaceae, Apiaceae and Asteraceae) and, (iv) different experimental arena (petri dish and alfalfa plants).

Experimental design: The adults emerged in laboratory were used in the trials and were fed with aphid nymph I and II of *A. pisum*. The main variable analyzed was the number of aphids eaten at 24 and 48 hours (day 1 and day 2) with different initial number (levels) of aphids (aphid factor): 1, 2, 3, 10, 20, and 40, each with 30 replicates. The number of aphids consumed was calculated subtracting the number of aphids remaining at an inspection from the number offered at the previous day. For each level of aphids, the flower supply effects (plant factor) was studied using three plant species *Brassica nigra* L. (Brassicaceae), *Daucus carota* L. (Apiaceae) and *Sonchus oleraceous* (L.) Koch in Röhling (Asteraceae) and a control treatment, without flowers. The flowers were examined to eliminate small insects or eggs, previously to use in each trial. We assumed that the coccinellids fed also pollen and nectar of the flowers. Also, for each level of aphids, the effect of two different experimental places (arena factor) was studied: (i) petri dish of 9 cm diameter and 2 cm height with a filter paper on the bottom, and (ii) plastic vial of 7 cm diameter with one alfalfa plant of 2-4 leaves developed, seeded on substrate (to avoid escapes, the aerial part was covered with muslin and sealed with dry sand at the base).

For each trial, the protocol was the following one: in the beginning of the first day one level of aphids to study, a single flower (Brassicaceae and Asteraceae) or inflorescence (Apiaceae), and one adult coccinellids (male or female) (sex factor) were introduced in the arena. After 24 hrs., the remaining number of live aphids was counted and was added more aphids to complete the initial level of study, and the flower was removed and replaced by newly cut one. After 48 hrs. of the beginning of the trial, the remaining number of live aphids was newly counted and the trial was finished. Then, we also study the effect of satiety comparing the aphid consumed after 24 and 48 hours (day factor).

Data analysis: To distinguish between the three forms of host dependence in the functional response, a first-order linear model with binomial distribution was used to fit a logistic regression of the proportion of prey eaten $p = Ne=No$ to aphid density No (Trexler *et al.*, 1988):

$$\ln[p/(1-p)] = \beta o + \beta 1 No$$

Where: β_0 and β_1 are the parameters to estimate by the logistic model. A Type I is characterized by no dependence of β_1, a Type II by negative dependence of β_1, and a Type III by positive dependence of β_1. The data was restricted to aphid range 1-40 to avoid the upper limit of the FR to aphid density (Mills & Lacan, 2004; Pervez & Omkar, 2005). To study the effect of satiety, flower, sex and level of aphid on the probability of aphid

eaten we analyzed for the following models of multiple logistic regressions.

$$\ln[p/(1 − p)] = βo + β1No + β2[F2] + ⋯$$

The predator FR Type II, which allows for prey depletion over the course of the experiment, is the random predator equation:

$$Ne = No[1 − \exp(a.\,Th.\,Ne − a.\,T)]$$

Where: N_e= the number of prey eaten; N_o= the number of prey present at start of trial; T= the total time of the trial; Th= the handling time per prey item; a= is the attack constant. For the estimation of the parameters, the main methods used in literature are nonlinear regression (Williams & Juliano 1996; Juliano, 2001).

A logistic approach is useful because Type II and III FR are difficult to discriminate. Thus, the logistic model applied was: Lijkl= logit [Pijkl]= β0 + β1 [FS]i + β3 [S]j + β4 [T]k + ε ijkl

Where: Lijkl= the logit model, [Pijkl]= probability for the response category ("aphids consumed frequency/ aphids offered"); $β0$= intercept; $β1$, $β2$, $β3$ =estimate parameter for the model; [FS]i: effect of flower supplements, in dummy variable (without flower: FS1=0, FS2=0, FS3=0; *Sonchus oleraceus*: FS1=1, FS2=0, FS3=0; *Brassica nigra*: FS1=0, FS2=2, FS3=0; *Daucus carota*: FS1=0, FS2=0, FS3=1); [S]j: effect of sex, in dummy variable (female: S=0; male S=1); [T]k: effect of control period, in dummy variable (24 h: T=0; 48 h: T=1); and ε ijkl = residual error.

Where: P is the probability that an aphid is eaten by the coccinellid (*Ne/No*), FS is the effect of flower supplement, S is the effect of adult sex, T is the effect of time (day), and e is the residual error.

The statistical analysis for logistic regression was estimated using R (The R Development Core Team, 2005) and the parameters of the FR was fitted Stat Graphic Logistic regression model.

RESULTS AND DISCUSSION

Analysis of the proportion of aphid eaten: The general logistic model applied to the proportion of aphid eaten and the initial aphids results in a negative dependence (β1= -0.03; P<0.001) between the both variables. However, the analysis with all other factors in the saturated logistic model results in independence with aphid and not significant three-way or upper interactions. Moreover, a more simplified model with only interactions of second-order give the same result (β1= 0.007; P=0.148). Therefore, initial inspection of the

data suggests that the response of *H. variegata* can be explained by other factors different of the initial density of aphids, i.e. there is not a FR of *H. variegata* in the aphid range 1-40 analyzed in laboratory.

Table 1. shows the analysis of deviance for the logistic-model with all second-order interactions. The analysis of deviance table shows a very important percentage on the deviance residual of the model explained by arena (45.9% of deviance) and a strong interaction between arena and aphid (14.6% of deviance). Also, the arena is the main factors that affect the probability of to be eaten (Figure. 1). Without considerer other experimental factors, was consumed 84.8% of prey in the petri dish and only 52.0% of prey in the alfalfa plant. A simple inspection of the response of *H. variegata* by arena and plant (Figure. 1) shows clear differences that respond to a different FR of *H. variegata* in both arenas.

The analysis by arena, in a logistic model more simplified, without significant differences with the saturated model (Table 2) moreover aphid is the main factor to explain the model. This mean that the percentage of prey eaten slows down when aphid increase as correspond to a FR Type II. However, the simplified logistic model for arena.alfalfa shows aphid with a zero coefficient (β1= 0.004; P= 0.465). This result suggests an independence of the response with aphid, i.e. no FR of *H. variegata* in alfalfa plant on the aphid range of the study.

When analyzing what happened in alfalfa plants (habitat more complex), the number of aphids consumed was lower (Figure 3-C y D). Males consume reached 15 aphids per day at 24 hrs. in the presence of flowers of *B. nigra*, and its next lower consumption was close to 4 aphids per day, at 48 hrs. into the study (greater satiety) and supplied when the flowers were of *B. nigra*. Females consumed aphid`s daily over 20 to 24 hrs. (more hunger), also when the flowers were supplied Brassicaceae.

Differences in the experimental arena give rise to one significant difference in the probability of capture, in petri dish was 12.09 higher times to the carried out in the alfalfa, and the level of satiety was reached before. The spatial complexity of the arena and their interaction with number of aphids seen to be a key factor in the FR of *H. variegata*, because predators could consume more aggregated prey than dispersed prey and increased consumption rate may result in an increase in the rate that energy moves through the food chain (Pitt & Ritchie,

2002; Hodek, 2003). Thus, in arenas with lower spatial complexity, was reduced the contact coccinellids/aphids and result in a no significate FR at this low density of aphids. This result suggests that is needed a minimum density of aphid to increase the FR (Anietie *et al.*, 2014). Thus, the satiety of aphid at beginning of the trials changes the activity and the proportion of aphid eaten. The predator FR is a key factor regulating population

dynamics of predator-prey systems. Numerous mechanistic and phenomenological models have used to describe FR (Wang & Tsai, 2001). Juliano (2001) stated that the random predator model is the appropriate model to use for such analysis, rather than the typical Holling Disk equation (Holling, 1959), because preys depleted during the experiment.

Table 1. Analysis of deviance table of the logistic model with all second order interactions (Null deviance= 3983.3), of adults of *Hippodamia variegata* fed on *Acyrthosiphon pisum.*

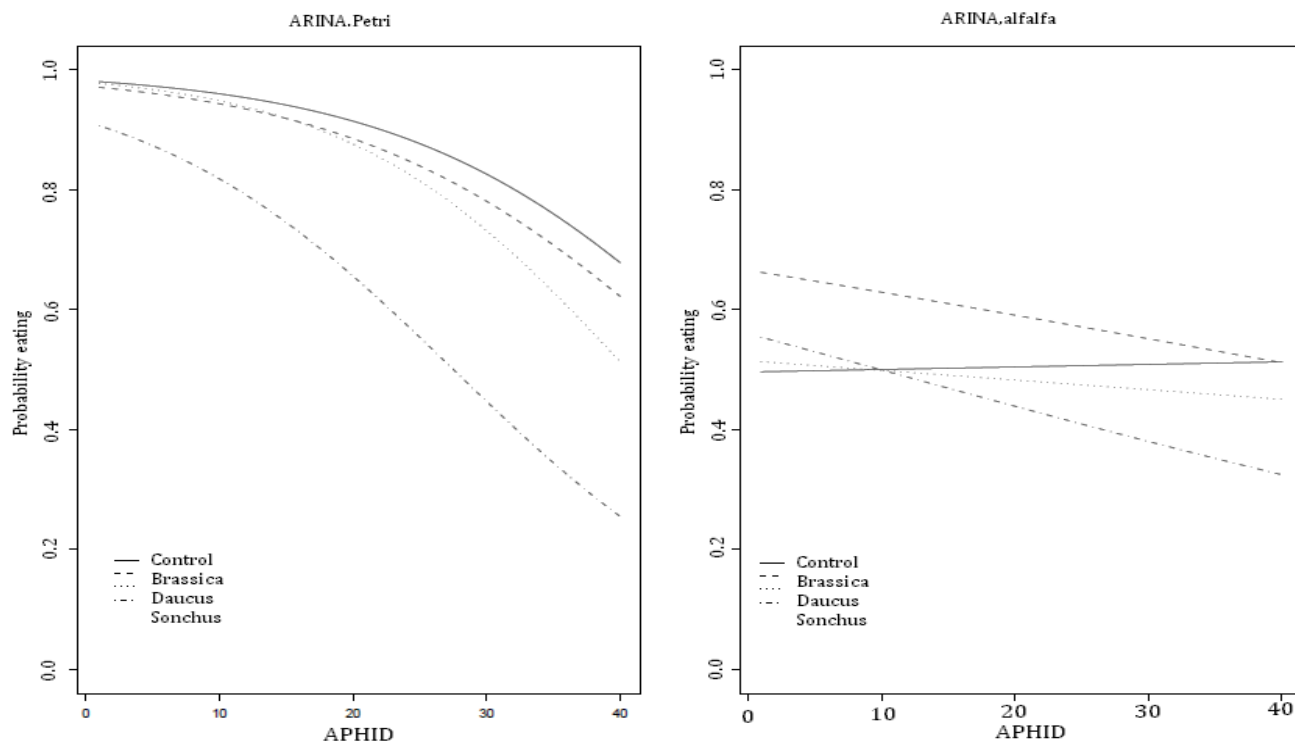|	df	Deviance Residual	P(>lChi l)
ARENA	1	898.7	0.001
APHID	1	315.9	0.001
DAY	1	152.5	0.001
PLANT	3	105.8	0.001
SEX	1	52.7	0.001
ARENA.APHID	1	287.2	0.001
ARENA.PLANT	3	103.3	0.001
ARENA.SEX	1	6.9	0.009
PLANT.SEX	3	3.3	0.3
PLANT.DAY	3	15.1	0.002
APHID.PLANT	3	15.7	0.001
APHID.SEX	1	1.5	0.2
APHID.DAY	1	6.4	0.001

Figure 1. The response of *Hippodamia variegata*, fed on *Acyrthosiphon pisum*, whit different flowers foods supply (Brassicaceae, Apiaceae and Asteraceae) and different experimental arena (petri dish and alfalfa plants).

The day is another important factor to explain the response of *H. variegata*. The response shows a significantly increasing of aphid killed the day.B in both arena (2.3-4.0 times in arena.alfalfa; 3.4-6.2 in arena. petri). However, this response is different by sex and plant. The males consume less aphids than females (a mean reduction of 53.2% in arena.petri, and 71.1% in arena.alfalfa) but the main difference is the response of females in the day.B where there is not an important reduction of aphid killed with more than 80% of captures at aphid level 40 in plant.control (Figure. 2).
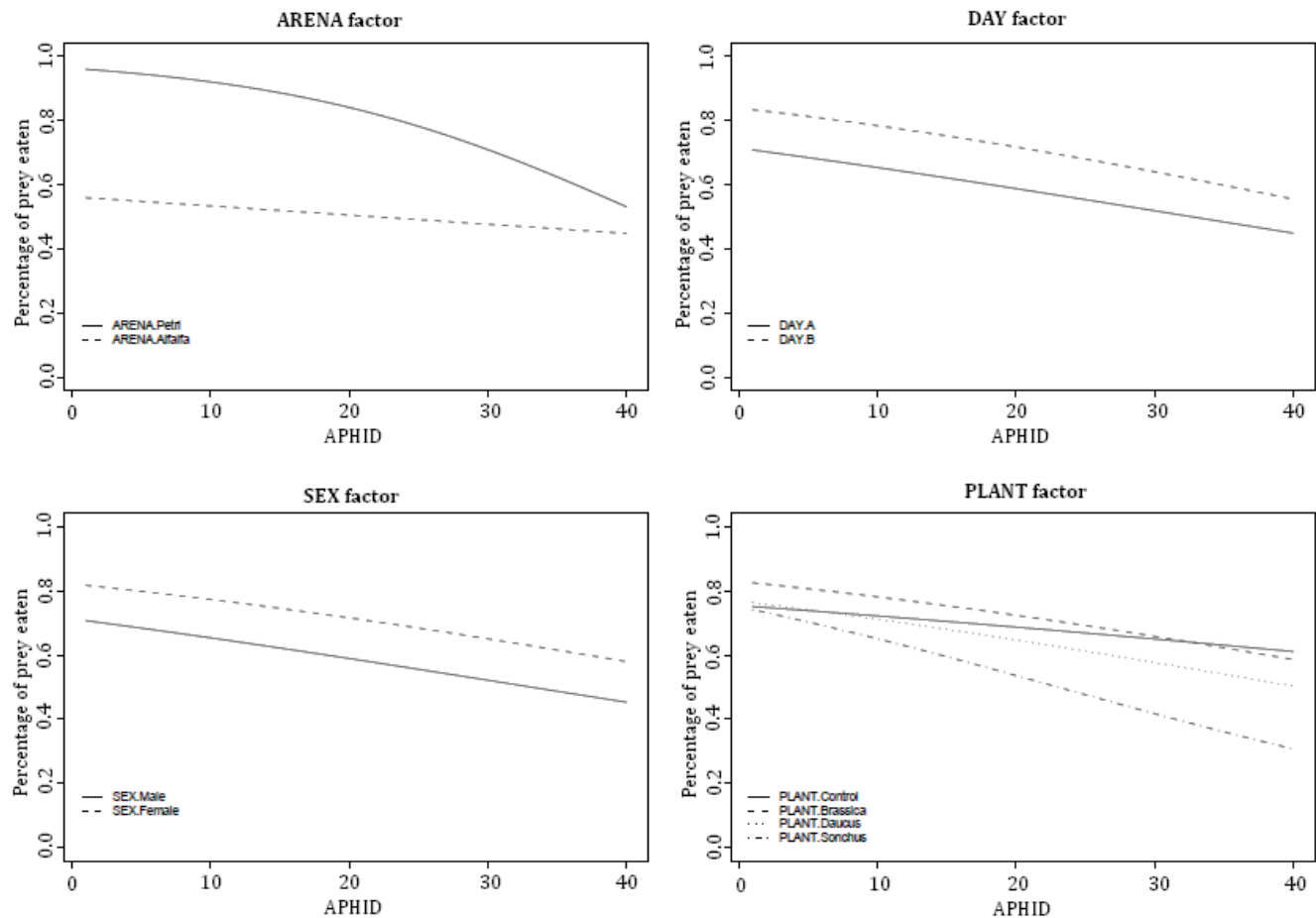

Figure 2. The response of *Hippodamia variegata*, fed on *Acyrthosiphon pisum*, and by the four factors considered: different experimental arena (petri dish and alfalfa plants), days (24 and 48 hrs.), sex (female and male) and different flowers foods supply (Brassicaceae, Apiaceae and Asteraceae).

If we analyze the daily consumption (Figure. 3), was higher in the petri dishes, than in alfalfa plants, and was higher in females than in males. In both cases the amount of consumed aphids was close to 40 individuals, and higher at 24 hrs. than 48 hrs. and in both cases when the flowers supplied was *B. nigra*. The relative rate of prey consumption by *H. variegata* was higher at lower densities, which indicates that this predator would be more effective at controlling the *A. pisum* population at lower densities. Therefore, for consideration of biocontrol of this aphid, the coccinellids have released early before the aphids reach high densities (Mandour *et*

al., 2006). The probability of contact with the prey at higher density would tend to increase per unit area, because the host plants and prey abundance significantly influenced number of prey attacked. Interaction between plant species and prey abundance indicated that the host plants influenced significantly the response of the predator (Carrell *et al.*, 1993). For that's results the behavior of *H. variegata* is an indication for the ability of this predator to high level control of *A. pisum*, and to similar conclusion arrived Davoodi Dehkordi & Sahragard (2013), to high control of *A. gossypii* infestations. The plant is a factor to influence the

FR, but it is complex to explain because their interactions with aphid and day. In arena.petri, the presence of S. oleraceus reduce the consume of aphids a mean of 27.5% mainly in high densities of aphids while in arena.alfalfa the presence of *B. nigra* increase the

aphid killed a mean of 2.7 times mainly in low densities of aphids. In general, D. carota has not differences with plant control and aphid consumed was only reducing in the second day in arena.alfalfa.
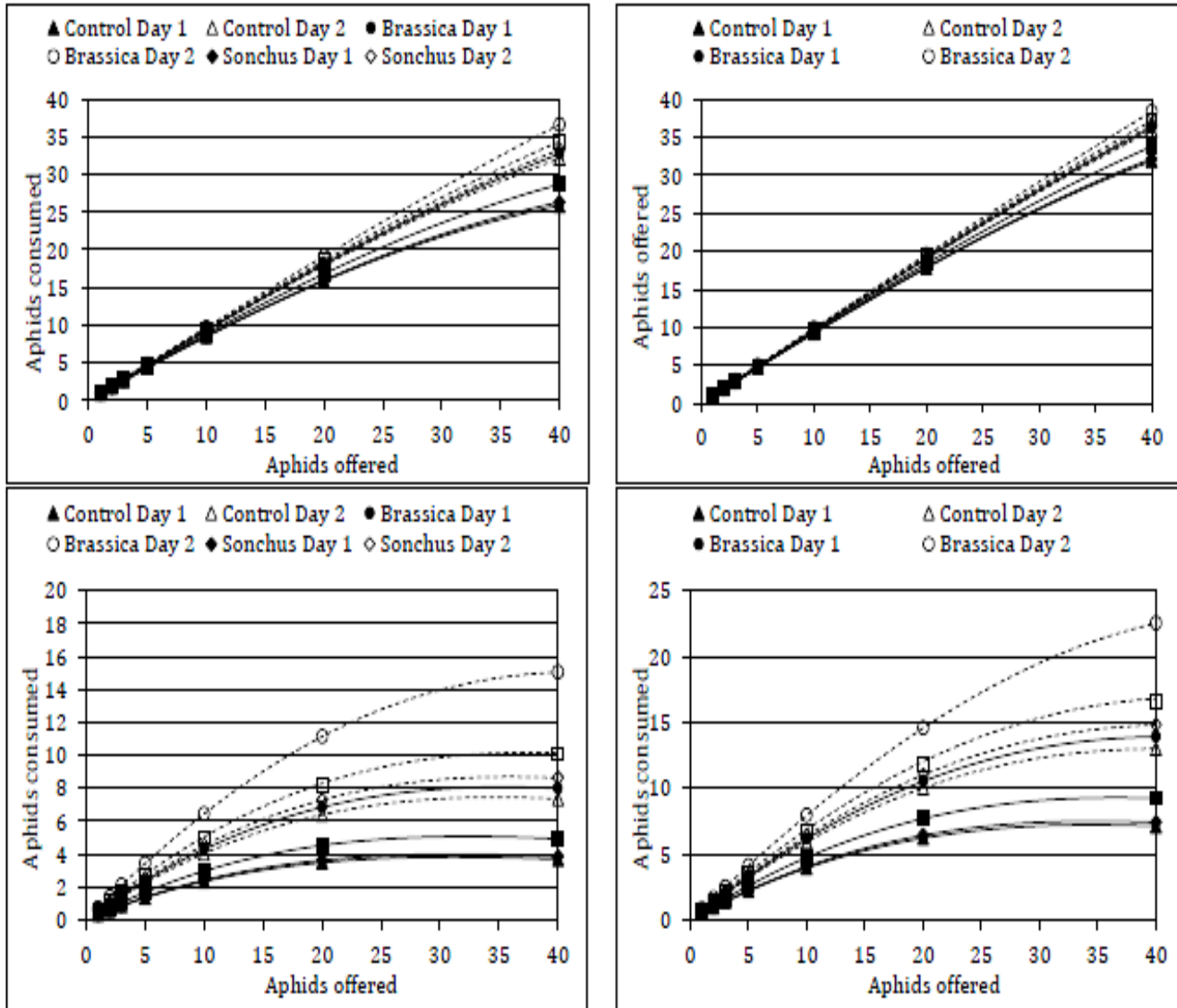

Figure 3. Functional response of adult of *Hippodamia variegata*, fed on *Acyrthosiphon pisum*, with *Brassica nigra*, *Daucus carota* and *Sonchus oleraceous* flowers, and control treatment. A) Males in petri dishes; B) Female in petri dish; C) Males in alfalfa plants; D) Female in alfalfa plants.

Effects on the parameters of the functional responses: The FR curve of adult females over that of the adult males indicates the possibility of larger body size than males; hence greater food requirement and higher nutritional demands, particularly for egg formation. Our results showed that consumption of *A. pisum* by *H. variegata* adults demonstrated a FR type II, consistent with the report by Davoodi Dehkordi &

Sahragard (2013) and Farhadi *et al.* (2010), and also whit another coccinellids investigated in the laboratory (Wang & Tsai, 2001; Mandour *et al.*, 2006, Anietie *et al.*, 2014). Percentage prey consumption and the handling time by adult male and female ladybeetles decreased significantly (P<0.001) with increase in prey density. Kumar *et al.* (1999) and Omkar & Pervez (2004), who studies *Propylea dissecta* (Mulsant) and *Coccinella*

septempunctata L., respectively, arrived to similar results. Farhadi *et al.* (2010) concluded that the voracity of female adults of *H. variegata* was grater that males, preying on *Aphis fabae* (Scolpoli). The killing efficiency of adult females is greater than males, who eat only 53.2% in arena.petri, and 71.1% in arena.alfalfa.

The attack coefficients (*a*) and the handing times (*Th*) were, generally, majors in the count realized to 24 h, than to 48h (Table 2) (*a* varied from 0.015 to 0.024 at 24 hrs. and from 0.009 to 0.017 at 48 hrs. in alfalfa plant; from 0.042 to 0.052 al 24 hrs and from 0.21 to 0.027 in arena.petri. *Th* were smaller when the test was realized in petri dishes, compared with the values on arena.alfalfa (varied from 0.094 to 0.771 at 24 hrs. and 0.216 to 2.135 at 48 hrs., in alfalfa plant; from 0.281 to 1.789 at 24 hrs. and 0.014 to 1.902 at 48 hrs., in petri dishes), then both parameters depended on the studied habitat. However was not relation between these parameters when were analyzed the habitat and the treatments individually. Sakaki and Sahragard (2011) found that the kind of patch (open or closed) did not affect the type of FR for female *S. syriacus* to varying densities of *Aphis gosypii*. Glover.

The foods supply influenced significantly the number of prey attacked by *H. variegata*, in Control treatments, attacks coefficients was lower in petri dishes (Table 2) because the adults coccinellids only have the preys to find, however in alfalfa plants was lower with *B. nigra* flowers, perhaps because the pollen and nectar give them more energy to find the preys. In petri dishes whit *B. nigra* treatment the attacks coefficients (*a*) was lower in both sex (0.0052 and 0.042, at 24 hrs.), handing times was similar in all treatment. In alfalfa plants *a* and *Th* were dissimilar in all situations analyzed. Spellman *et al.* (2006) found that plants producing extrafloral nectar are being proposed to enhance biological control in apple orchards: because there was a significant reduction in *Aphis spiraecola* Patch, by *Harmonia axyridis* (Pallas) adults predation, in presence of extrafloral nectar glands of flowering *Fagopyrum esculentum* Moench, showing that alternative food resources potentially could interfere with rates of biological control.

Surrounding vegetation and plant patch shape seem to have a direct effect on the density of coccinellids by modifying their immigration and emigration patterns, but also seem to have an indirect effect by changing plant growth and its effect on herbivore recruitment (Grez & Prado, 2000). Reproductive diapause and migration are common features of aphidophagous coccinellids and both facilitate the sequential exploitation of different prey species. Consequently, dietary complementation could emerge as an important factor affecting the fitness of migrant coccinellids that change habitats as pre-reproductive adults. It might be worthwhile to test for similar effects in other beneficial insects that are predatory as both larvae and adults, as dietary complementation could have useful applications in augmentation biological control programs (Michaud & Jyoti, 2008).

Coccinellids preferentially colonizes weedy vegetation, which often offers an array of nectar resources in weed intercrops (Banks & Yasenak, 2003; Lundgren et al., 2005). Hodek & Honek (1996) founded that *Coleomegilla maculata* DeGeer females, consumed at field on average 10-fold more pollen than male, because require more and different nutrition to produce eggs. Plants can influence the performance of natural enemies of their arthropod herbivores in a variety of ways, because may provide them with shelter alternative foods. Whether the overall effect on the plant will be positive or not will thus depend on how pollen influences the predator: prey ratio near the moment of colonization of the plant by the herbivore, and the predator's numerical and aggregative response to herbivore density on the plant.

Under equilibrium conditions, pollen feeding promotes predator survival, development, or reproduction, and irrespective of how the pollen are distributed over the plant (Sabelis & van Rijn, 2005; van Maanen *et al.*, 2012). Conservation of naturally occurring omnivores is desirable because they may persist in the habitat at low prey densities, thus impeding any buildup of pest populations. The present study has provided us with a better understanding of the predator-prey relationship between *H. variegata* and *A. pisum*. Although our results suggest that *H. variegata* appears to be an efficient predator of *A. pisum* in León (Spain), especially with *B. nigra* and *D. carota* flowers in the area. The presence of high-quality plant resources, such as pollen and nectar, may be a good predictor of the abundance of omnivorous predators, and thus for potential suppression of pest populations (Sloggett & Majerus, 2000). The kind of weeds in a system can affect the performance of predators. We conclude from these

these findings that the presence of floral resource plants from *B. nigra* and, with less impact *D. carota* in landscapes enhances the consumption of *H. variegata* (Rebek *et al.*, 2005), and another practical conclusion of this study is that females are most effective as predators than males (Farhadi *et al.*, 2010). The conclusion of this work is that the presence of floral resource plants from *B. nigra* and, with less impact, *D. carota* enhances the consumption of *H. variegata* and, females are most effective as predators than males. This experiment took place at a small-scale, under controlled laboratory conditions and for a short period. In natural environments, more diversified and with greater distances, alternative food sources more varied and more architectural diversity, the interaction between predators and preys are, certainly, more complex.

Table 2. Attacks coefficients (*a*) and handing times (*Th*), in minutes, for *Hippodamia variegata* adults (male and female), fed on *Acyrthosiphon pisum*, in each treatment (Control, *Brassica nigra*, *Daucus carota* and *Sonchus oleraceous* flowers), and in petri dishes and alfalfa plants, at 24 and 48 hrs.

Habitats	Sex	Treatment (flower)	24 hours			48 hours		
			n-1	*a* ± (SE)	*Th* ± (SE) (min)	n-1	*a* ± (SE)	*Th* ± (SE) (min)
Petri dishes	Male	Control	42	0.045 ± (0.002)	0.498 ± (0.015)	42	0.022 ± (0.0009)	0.522 ± (0.066)
		B. nigra	40	0.052 ± (0.005)	0.530 ± (0.08)	40	0.027 ± (0.002)	1.279 ± (0.148)
		D. carota	48	0.052 ± (0.002)	1.249 ± (0.58)	48	0.024 ± (0.002)	1.575 ± (0.157)
		S. oleraceus	47	0.044 ± (0.005)	1.789 ± (0.29)	47	0.022 ± (0.0009)	1.446 ± (0.131)
	Female	Control	66	0.048 ± (0.002)	0.416 ± (0.04)	66	0.022 ± (0.0002)	0.218 ± (0.012)
		B. nigra	68	0.042 ± (0.001)	0.281 ± (0.02)	68	0.021 ± (0.0003)	0.306 ± (0.027)
		D. carota	60	0.044 ±(0.001)	0.423 ± (0.03)	60	0.021 ± (0.0002)	0.014 ± (0.014)
		S. oleraceus	60	0.046 ± (0.004)	1.505 ± (0.15)	61	0.027 ± (0.002)	1.902 ± (0.141)
Alfalfa plant	Male	Control	51	0.015 ± (0.001)	0.182 ± (0.211)	51	0.013 ± (0.001)	0.216 ± (0.179)
		B. nigra	54	0.021 ± (0.003)	0.094 ± (0.345)	55	0.016 ± (0.002)	1.234 ± (0.277)
		D. carota	61	0.014 ± (0.001)	0.429 ± (0.21)	61	0.012 ± (0.001)	0.772 ± (0.333)
		S. oleraceus	49	0.016 ± (0.001)	0.153 ± (0.19)	49	0.015 ± (0.002)	2.135 ± (0.491)
	Female	Control	57	0.022 ± (0.002)	0.425 ± (0.16)	57	0.016 ± (0.0016)	1.236 ± (0.277)
		B. nigra	53	0.017 ± (0.001)	0.421 ± (0.14)	53	0.013 ± (0.0009)	0.288 ± (0.156)
		D. carota	47	0.024 ± (0.002)	0.296 ± (0.28)	47	0.009 ± (0.001)	1.951 ± (0.684)
		S. oleraceus	59	0.021 ± (0.003)	0.771 ± (0.54)	59	0.017 ± (0.001)	1.510 ± (0.362)

REFERENCES
Almeida, S. R., A. Pallini, M. Venzon, O. F. Fonseca de
Souza, A.J. Molina-Rugama and C. Lima de Oliveira.
2007. Functional response of the predator *Eriopis
connexa* (Coleoptera: Coccinellidae) to different
prey types. Brazilian Archives of Biology and
Technology. 24 (1): 13-16.

Anietie, R. E., J. O. Adebayo and M. E. Sunday. 2014.
Functional and numerical responses of *Exochomus
flavipes* Thunberg (Coleoptera: Coccinellidae), a local
predator of the cassava mealybug, *Phenacoccus
manihoti* Matile-Ferrore (Homoptera:
Pseudococcidae). Int. J. Entomol. Res. 02 (01): 01-05.

Arditi, R. and L. R. Ginzburg. 1989. Coupling in predator-
prey dynamics: ratio-dependence. Journal of
Theoretical Biology. 139: 311-326.

Asante, S. K. 1995. Functional responses of the european
earwig and two species of coccinellids to densities
of *Eriosoma lanigerum* (Hausmann) (Hemiptera:
Aphididae). Journal of the Australian
Entomological Society. 34: 105-109.

Banks, J. E. and C. L. Yasenak. 2003. Effects of plot
vegetation diversity and spatial scale on *Coccinella
septempunctata* movement in the absence of prey.
Entomologia Experimentalis et Applicata. 108:
197-204.

Carrell, J. E., M. H. McCairel, A. J. Slagle, J. P. Doom, J. Brill
and J. P. McCormick. 1993. Cantharidin production
in a Blister beetle. Experientia. 49: 171-174.

Cosner, C., D. L. DeAngelis, J. S. Ault and D. B. Olson. 1999.
Effects of spatial grouping on the functional
response of predators. Theoretical Population
Biology. 56: 65-75.

Da Silva, R. A., A. C. Busoli and N. R. Chagas Filho. 2004.
Aspectos biológicos de *Coccidophilus citricola*
Bretes, 1905 (Coleoptera: Coccinellidae). Ciencia
Rural. 34(3): 667-672.

Davoodi Dehkordi, S. and A. Sahragard. 2013. Functional
response of *Hippodamia variegata* (Coleoptera:
Coccinellidae) to different densities of *Aphis
gossypii* (Hemiptera: Aphididae) in an open patch
design. J. Agri. Sci. Tech. 15: 651-659.

Farhadi, R., H. Allahyari and S. A. Juliano. 2010.
Functional response of larval and adult stages of
Hippodamia variegata (Coleoptera: Coccinellidae)
to different densities of *Aphis fabae* (Hemiptera:
Aphididae). Environ Entomol. 39(5):1586-92.

Fernández-Arhex, C. and J. C. Corley. 2003. The
functional response of parasitoids and its
implications for biological control. Biocontrol
Science and Technology. 13(4): 403-413.

Grez, A. A. and E. Prado. 2000. Effect of plant patch shape
and surrounding vegetation on the dynamics of
predatory coccinellids and their prey *Brevicoryne
brassicae* (Hemiptera: Aphididae). Environmental
Entomology. 29(6): 1244-1250.

Hassell, M. P. 1978. The dynamics of arthropod predator-
prey systems. Princeton University Press,
Princeton, N.J. *In:* Wang, J. J. and J.H. Tsai. 2001.
Development and functional response of
Coelophora inaequalis (Coleoptera: Coccinellidae)
feeding on brown citrus aphid, *Toxoptera citricida*
(Homoptera: Aphididae). Agricultual and Forest
Entomology. 3: 65-69.

Hodek, I. and A. Honek. 1996. Ecology of Coccinellidae.
Boston: Kluwer Academic Publishers. 464 pp. *In*:
Holling, C.S. 1959. The components of predation as
revealed by a study of small mammal predation of
the European pine sawfly. Canadian Entomology.
91: 293-320.

Hodek, M. S. 2003. The effect of prey species and
environmental complexity on the functional
responses of *Franklinothrips orizabensis*: a test of
the fractal foraging model. Ecological Entomology.
28: 309- 318.

Holling, C. S. 1959. Some characteristics of simple types
of predation and parasitism. Canadian
Entomology. 91: 385-398. *In*: Iperti, G. 1999.
Biodiversity of predaceous Coccinellidae in
relation to bioindication and economic
importance. Agriculture, Ecosysten and
Environment. 74: 323-342.

Juliano, S. A. 2001. Non-linear curve-fitting: predation
and functional response curves. Design and
Analysis of Ecological Experiment, 2nd. ed (Eds.
Scheiner, M. and J. Gurevitch). Chapman & Hall,
New York. pp. 293-374.

Kumar, A., N. Kumar, A. Siddiqui and C. P. M. Tripathi.
1999. Prey-predator relationship between *Lipaphis
erysimi* Kalt. (Hom., Aphididae) and *Coccinella
septempunctata* L. (Col., Coccinellidae). II. Effect of
host plants on the functional response of the
predator. J. Applied Entomology. 123: 591-601.

Lundgren, J. G., A. Huber and R. N. Wiedenmann. 2005. Quantification of consumption of corn pollen by the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Agricultua, Forest and Entomology. 7: 53-60.

Mandour, N. S., N. A-S. El-Basha and T-X. Liu. 2006. Functional response of the ladybird, *Cydonia vicina nilotica* to cowpea aphid, *Aphis craccivora* in the laboratory. Insect Science. 13: 49-54.

Michaud, J. P. and J. L. Jyoti. 2008. Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*. Entomol. Exp. Appl. 126: 40-45.

Mills, N. J. and I. Lacan. 2004. Ratio dependence in the functional response of insect parasitoids: evidence from *Trichogramma minutum* foraging for eggs in small host patches. Ecologycal Entomology. 29: 208-216.

Murdoch, W. W. and A. Sih. 1978. Age-dependent interference in a predatory insect. The Journal of Animal Ecology. 47(2): 581-592.

Nielsen, F. H., M. S. Hauge and S. Toft. 2002. The influence of mixed aphid diets on larval performance of *Coccinella septempunctata* (Col., Coccinellidae). Journal of Applied Entomology. 126: 194-197.

Omkar and A. Pervez. 2004. Functional and numerical responses of *Propylea dissecta* (Col., Coccinellidae). Journal of Applied Entomology. 128: 140-146.

Pervez, A. and Omkar. 2005. Functional responses of coccinellid predators: An illustration of a logistic approach. 6 pp. Journal of Insect Science, 5:5, Available online: insectscience.org/5.5.

Pitt, E. C. and M. E. Ritchie. 2002. Influence of prey distribution on the functional response of lizards. OIKOS. 96: 157-163.

Rebek, E. J., C. S. Sadof and L. M. Hanks. 2005. Manipulating the abundance of natural enemies in ornamental landscapes with floral resource plants. Biological Control. 33: 203-216.

Reis, P. R., E. O. Sousa, A. V. Teodoro and M. P. Neto. 2003. Effect of prey density on the functional and numerical responses of two species of predaceous mites (Acari: Phytoseiidae). Neotropical Entomology. 32(3): 461-467.

Rossi, M. N., C. Reigada and W. A. C. Godoy. 2006. The role of habitat heterogeneity for the functional response of the spider *Nesticodes rufipes* (Araneae: Theridiidae) to houseflies. Applied Entomology and Zoology. 41 (3): 419-427.

Sabelis, M. A. and P. C. J. van Rijn. 2005. When does alternative food promote biological pest control?. 2nd. International Symposium on Biological Control of Arthropods. September 12-16, 428-437. Davos, Switzerland.

Sakaki, S. and A. Sahragard. 2011. A new method to study the functional response of *Scymnus syriacus* (Coleoptera: Coccinellidae) to different densities of *Aphis gossypii*. Journal of Asia-Pacific Entomology. 14: 459-462.

Sloggett, J. J. and E. N. Majerus. 2000. Habitat preferences and diet in the predatory Coccinellidae (Coleoptera): an evolutionary perspective. Biological Journal of the Linnean Society. 70: 63-88.

Spellman, B., M. W. Brown and C. R. Mathews. 2006. Effect of floral and extrafloral resources on predation of *Aphis spiraecola* by *Harmonia axyridis* on apple. BioControl. 51: 715-724.

The R Development Core Team 2005: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Treacy, M. F., J. H. Benedict, J. D. López and R. K. Morrisom. 1987. Functional response of a predator (Neuroptera: Chrysopidae) of bollworm (Lepidoptera: Noctuidae) eggs on smooth leaf hirsute, and pilose cottons. Journal of Economic Entomology. 80: 376-379.

Trexler, J. C., C. E. McCulloch and J. Travis. 1988. How can the functional response best be determined?. Oecologia. 76: 206-214.

van Maanen, R., G. Broufas, M. F. Oveja, M. W. Sabelis and A. Janssen. 2012. Intraguild predation among plant pests: western flower thrips larvae feed on whitefly crawlers. BioControl. 57: 533-539.

Wang, J. J. and J. H. Tsai. 2001. Development and functional response of *Coelophora inaequalis* (Coleoptera: Coccinellidae) feeding on brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae). Agriculturl and Forest Entomology. 3: 65-69.

Williams, F. M. and S. A. Juliano. 1996. Functional responses revisited. Environmental Entomology. 25(3): 549-550.