Research Article

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Foraging behavior of different developmental stages of *Hippodamia variegata* (Coleoptera: Coccinellidae) on *Hyalopterus amygdali* (Hemiptera: Aphididae)

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Abstract: Biological control represents an effective approach for managing pest populations in horticultural crops, presenting an alternative to the reliance on pesticides. To ensure the successful integration of predators into biological control programs, it is imperative to conduct thorough assessments of how these predators respond to changes in prey density in laboratory settings. In this study, we investigated the functional response of various developmental stages of Hippodamia variegata (Coleoptera: Coccinellidae) when exposed to varying densities of Hyalopterus amygdali (Hemiptera: Aphididae) at 26°C, 65 ± 5% RH, and 16L: 8D h photoperiod. Each treatment was replicated 15 times and conducted on almond leaves placed within Petri dishes. Our findings revealed that all larval stages, as well as adult males and females of the predator, exhibited a type II functional response when presented with different prey densities. We quantified searching efficiency (0.0219, 0.0173, 0.0163, 0.0141, 0.0198, and 0.0128 h⁻¹) and handling times (5.7650, 2.6023, 0.7303, 0.3166, 0.7352, and 0.2920 h) for the first through fourth instar larvae, male and female adults, using Holling's disc equation. Notably, handling times for males were significantly longer than those for females or fourth instar larvae. Nevertheless, no statistically significant differences were observed in the attack rate between fourth instar larvae and either adult females or males. The maximum attack rate (T/Th) for the first to fourth instar larvae, male, and female H. variegata was estimated as 4.163053, 9.222611, 10.52909, 100.2925, 32.64418, and 82.19178, respectively. Consequently, our results indicate that the fourth instar larvae and adult females of H. variegata are recommended for utilization in biological control programs aimed at managing H. amygdali populations.

Keywords: Biological control, attack rate, Handling time, Hyalopterus amygdali, Hippodamia variegata

important pests (Favret, 2017). Almond leaf-curl aphid *Hyalopterus amygdali* (Geoffroy) is one of

the key insect pests that attacks almond, sometime

apricot and pear (Rechitean et al., 2021). The

almond trees in Iran are greatly affected by this

Introduction

Aphids (Hemiptera: Aphidoidea) are phytophagous insects which comprise more than 5,000 species, including numerous economically

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pest, which primarily feeds on the undersides of leaves. As a result, it stunts shoot growth, causes leaf deformations, and produces excessive honeydew (Ghorbali et al., 2008). Different species of predators, especially from the family Coccinellidae, including Hippodamia variegata (Goeze), Coccinella septempunctata (L.), Adalia bipunctata (L.), Oenopia conglobate (L.) and Scymnus syriacus Marseul were reported as the major natural enemies of H. amygdali. (Ghorbali et al., 2008). Among them, the variegated ladybird (VL) beetle H. variegata is a polyphagous and widespread species worldwide (Kontodimas and Stathas, 2005). This predator has been cited as a ubiquitous predator of aphids on various host plants in Ukraine, Bulgaria, Italy, India, and Turkmenistan (Orr and Obrycki, 1990; El-Hag and Zaitoon, 1996; Obrycki and Kring, 1998; Kontodimas and Stathas, 2005). H. variegata is widely distributed in Iran and has been the subject of many studies (e.g., Farhadi et al., 2010; Madadi et al., 2011; Davoodi and Sahragard, 2013). The functional response is the basic mechanism that reveals different aspects of prey-predator behavior, explains their coevolutionary interactions, and can determine the efficiency of a predator in regulating prey populations (Holling, 1965; Martinou et al., 2010; Houck and Strauss, 1985). According to Solomon (1949) and Holling (1959), the number of prey consumed per predator as a function of different prey densities is known as the functional response. Functional responses are generally classified into three types: type I assumes a linear increase in intake rate with food density, type II is characterized by a decelerating intake rate, and type III has a sigmoid increase. Despite some records of type III Functional responses (Seyfollahi et al., 2019; Zarghami et al., 2021), coccinellid predators are more commonly reported to have type II Functional responses (Atlihan and Guldal, 2009; Farhadi et al., 2010; Papanikolaou et al., 2011; Bayoumy and Awadalla, 2018). Type II functional response is characterized by unstablepredator-prey dynamics since, at lower densities, there is a risk of prey extinction, as predators can consume most available prey (Van Lenteren, 1975). In contrast, predators may exhibit reduced prey consumption at higher densities, resulting in limited regulation of prey populations. These unstable dynamic results in the correlation of predators demonstrating type II functional response to inundative biological control programs aimed at directly reducing pest populations (Van Lenteren, 2012). Various abiotic factors, such as prey type and size (Kalinoski, and DeLong, 2016), prey distribution (Feng et al., 2018), temperature (Madbouni, 2017), spatial complexity (Alexander, 2012), and presence of alternative prey (Murdoch, 1969) can affect the functional response type and its parameters: attack rate (a) and handling time (h). Although some studies evaluated the interactions of H. variegata with various aphid species, no information is available on the foraging behavior of this species on H. amygdali. Thus, the purpose of this study was to investigate the effect of different densities of H. amygdali on the number caught and consumed by various developmental stages of H. variegata. The results may be helpful to assess the potential efficiency of different life stages of this predator for controlling the abundance of aphids on almond trees as a part of an IPM program.

Materials and Methods

Insect sources and identification

The initial populations of *H. variegata* and *H.* amygdali were collected from almond orchard in Agricultural and Natural Resources Research and Education Center, Shahre-Kord, Chahrmahal, and Bakhtiari province (southwestern Iran). Microscope slides from the aphid samples were prepared, and the identification process up to the species level was done using valid keys provided by Rezwani (2001, 2010) and Blackman and Eastop (2016). The predator was identified by the morphological characteristics including, body shape, colouration and size, and microscopic slides of male genitalia, according to Biranvand et al. (2021). The stock culture of the predator was maintained on fresh almond twigs infested with H. amygdali and kept in ventilated plastic jars (height 20 cm and diameter 15 cm). Several small folded papers were placed in each jar as an oviposition site. Cotton wool saturated with 20% honey water was used as a moisture source for ladybirds, as it can offer a more sustainable hydration option and provide additional nutritional value to them. Fresh almond twigs infested with *H. amygdali* were replaced in rearing jars regularly. Eggs were collected daily and transferred to Petri dishes (6 cm diameter). Hatched larvae were kept separately in Petri dishes to avoid cannibalism behavior. The aphids and predator stocks were kept in climate rooms at 26 ± 1 °C temperature, $65 \pm 5\%$ RH, and a photoperiod of 16: 8 (L: D) h.

Functional response experiment

The ladybirds were reared for two generations on different nymphal stages of the mealy peach aphid, H. amygdali, before starting the experiments. Adults used in the experiment were 5-d-old mated males and females. To standardize their hunger level. all developmental stages of H. variegata were starved for 12 h and placed individually in petri dishes (12 cm height \times 7 cm diameter) with various densities of third instar nymphs of H. amygdali on fresh almond leaves. The number of prey consumed significantly differs among the stages of ladybirds (Farhadi, 2010; Seyfollahi, 2019; Zarghami, 2021). Based on preliminary experiments on different stages, various densities of *H. amygdali* of third instar nymphs were used: for the first instar 2,4,6,8,10 and 15 aphids; for second instars 2, 4, 8, 12, 16, 24, and 32 aphids; for third instars 2, 4, 8, 16, 32 and 64 aphids; for last instars 8, 16, 32, 64, 80, and 100 aphids; for adult males 4, 8, 16, 32, 64, and 80 aphids and adult females 8, 16, 32, 64, 80, and 100 aphids. After 24 h the predators were removed, and the number of consumed aphids was recorded. Each experiment was replicated 15 times. The experiments were conducted under laboratory conditions at 26 ± 1 $^{\circ}C$, 65 ± 2% RH, and a 16L: 8D h.

Statistical analysis

The two-stage approach of Juliano (2001) was used to analyze functional response data. First, the type (shape) of the functional response curve was determined using a cubic logistic regression of the proportion of prey consumed (N_a/N_0) as a function of initial prey provided (N_0) as follows:

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}{1 + \exp\left(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}$$
(1)

Where N_0 is the initial prey density, and P_0 (intercept), P_1 (linear), P_2 (quadratic), and P_3 (cubic) are coefficients estimated using the maximum likelihood method (PROC CATMOD, SAS 9.2, SAS Institute 2004). If the linear term does not differ from 0, it indicates a type I functional response that is density-independent. Suppose the linear parameter is significantly negative (P1 < 0). In that case, the proportion of prey consumed by the predator declines monotonically as the initial prey density increases, indicating a type II functional response. In contrast, a significant positive linear parameter $(P_1 > 0)$ with a negative quadratic parameter ($P_2 < 0$) demonstrates density-dependent mortality, i.e., a type III functional response (Juliano, 2001). The second step used the nonlinear least squares method (NLIN procedure; SAS Institute, 2004) to fit Rogers' type II random equation (Rogers, 1972) and estimate the predator handling time and attach rate. Because prey was depleted during the experiment, Rogers' model, which does not assume constant prey density, is appropriate for this experiment (Rogers, 1972; Juliano, 2001). Holling's disc equation, in contrast, is based on an assumption of unchanging prey density (Rogers, 1972) and is thus inappropriate for this experiment (Juliano, 2001). Therefore, the random attack equation (Rogers, 1972) was used to estimate the handling time (T_h) and attack rate (a) of the predator as follows:

$$N_{a} = N_{0} \left[1 - \exp(-\frac{aTP_{t}}{1 + aT_{h}N_{0}}) \right]$$
(2)

Where, N_a is the number of prey consumed, N_0 is the initial prey density, T is the time of exposure, a is the attack rate, and T_h is the handling time. The maximum predation rate (T/T_h) , which represents the maximum number of prey eaten by one predator during 24h (Hassell, 2000), has been calculated using the estimated T_h . Pairwise comparisons of parameters of the functional responses for different stages of predator were performed using the indicator variable method (Juliano, 2001) as follows:

$$Na = N_0 \{ 1 - exp \left[-(a + D_a (j)) (T - (T_h + D_{Th} (j)) N_a) \right] \}$$
(3)

Where *j* is an indicator variable that takes a value of zero or one. The parameters D_a and D_{Th} estimate the differences between the data sets being compared for the parameters attack rate and handling time values, respectively. In other words, the handling time for one stage is T_h , and that for another stage is $T_h + D_{Th}$. Testing for a significant difference in handling times between the two stages is accomplished by testing the hypothesis that D_{Th} includes 0 (Juliano, 2001).

Results

According to the logistic regression results, the positive or negative values of the linear parameter (P_1) indicated the types of functional response. In all life stages of a predator, the linear parameter of the polynomial regression of the proportion of aphids consumed (N_a/N_0) against initial prey density (N_0) was negative (Table 1). Furthermore, in all experiments, the number of prey consumed by H. variegata increased by increasing the initial prey densities also suggested type II functional response for all different stages of predator (Fig. 1) and the proportion of prey consumed by each growth stage of predator declined with increasing prey density (Fig. 2). Our result showed the functional responses of H. variegata fed on H. aymydali for the 24-h period were accommodated to Rogers random equation (equation 2).

Table 1 Maximum likelihood estimates of proportion of *Hyalopterus amygdali* consumed by different stages of *Hippodamia variegata* against initial density of aphids offered.

Life stage	Parameters	Estimate value	SE	χ2 value	P value
1 st instar	Intercept (P ₀)	3.3854	1.0484	10.43	0.0012
	Linear (P1)	-1.2930	0.4525	8.16	0.0043
	Quadratic (P2)	0.1239	0.0573	4.69	0.0304
	Cubic (P ₃)	-0.00398	0.00213	3.48	0.0621
2 nd instar	Intercept (P ₀)	1.1519	0.4068	8.02	0.0046
	Linear (P ₁)	-0.2117	0.0865	5.99	0.0144
	Quadratic (P2)	0.00685	0.00535	1.51	0.2185
	Cubic (P ₃)	-0.00008	0.000097	0.63	0.4289
3rd instar	Intercept (P ₀)	2.4941	0.4527	30.36	<.0001
	Linear (P1)	-0.1301	0.0627	4.30	0.0381
	Quadratic (P2)	0.00249	0.00227	1.21	0.2719
	Cubic (P ₃)	-0.00002	0.000022	0.79	0.3734
4 th instar	Intercept (P ₀)	3.6582	0.4996	53.62	<.0001
	Linear (P1)	-0.0740	0.0304	5.92	0.0149
	Quadratic (P2)	0.000640	0.000531	1.45	0.2282
	Cubic (P ₃)	-2.89E-6	2.776E-6	1.08	0.2982
Male adult	Intercept (P ₀)	2.6162	0.3306	62.63	<.0001
	Linear (P ₁)	-0.0990	0.0262	14.27	0.0002
	Quadratic (P2)	0.000926	0.000600	2.38	0.1227
	Cubic (P ₃)	-3.38E-6	4.137E-6	0.67	0.4142
Female adult	Intercept (P ₀)	3.8112	0.5239	52.92	<.0001
	Linear (P1)	-0.0757	0.0316	5.73	0.0167
	Quadratic (P ₂)	0.000614	0.000550	1.25	0.2637
	Cubic (P ₃)	-2.44E-6	2.866E-6	0.72	0.3955

Estimates were generated from logistic regression, PROC CATMOD Procedure of SAS.

SE: standard error, χ^2 : Chi-square, P: probability level.



Figure 1 The functional response curve from different growth stages of *Hippodamia variegata* on *Hyalopterus amygdali*.

Table (2) shows the attack rate (*a*) and handling time (T_h) estimated by the logistic regression models. The highest and lowest handling time were observed in first instar larvae (5.7777 ± 0.4253 h⁻¹) and adult females (0.2920 ± 0.00919 h⁻¹), respectively. Moreover, the theoretical maximum attack rate values (T/T_h) increased as the predator developed from one life stage to another (Table 2). The highest values of this parameter were observed in fourth instar larvae (100.2925) and adult females (82.19178), and the lowest was in the first instar larvae (4.153902), respectively.

Based on the asymptotic 95% confidence interval for D_a , no significant pairwise differences between the attack rate of fourth instar larvae and either adult females or adult males (Table 3), but significant differences between the handling times of adult males and either last instar larvae and adult female were observed. Handling times for males were significantly longer than those of females and fourth-instar larvae (Table 3).



Figure 2 Percentage of prey consumed by different growth stages of *Hippodamia variegata* on *Hyalopterus amygdali*.

Table 2 Parameters estimated by Rogers's random predator equation as well as R^2 and maximum predation rate (T/T_h) for developmental stages of *Hippodamia variegata* feeding on *Hyalopterus amygdali*.

Life stage	Functional response type	$a \pm SE$ (Lower–Upper)	$T_h \pm SE$ (Lower–Upper)	R ²	T/T_h
1 st instar	Π	$0.0219 \pm 0.00762 \ (0.00674 \text{-} 0.0370)$	$5.7650 \pm 0.4314 \ (4.9077 \hbox{-} 6.6223)$	0.2824	4.163053
2st instar	П	$0.0173 {\pm} 0.00477 (0.00788 {-} 0.0268)$	2.6023±0.1321 (2.3403-2.8644)	0.5265	9.222611
3st instar	Π	$0.0163 {\pm} 0.00375 (0.00883 {-} 0.0237)$	$0.7303 {\pm} 0.0308 (0.6691 {} 0.7916)$	0.7779	10.52909
4st instar	Π	$0.0141 {\pm} 0.00321 \; (0.00770 {\text -} 0.0205)$	$0.3166 {\pm} 0.00760 (0.3015 {-} 0.3317)$	0.8525	100.2925
Male adult	Π	$0.0198 {\pm} 0.00551 (0.00882 {\text -} 0.0307)$	$0.7352{\pm}0.0268(0.6820{\text{-}}0.7883)$	0.6979	32.64418
Female adult	II	$0.0128 {\pm}~ 0.00357~ (0.00575 {-} 0.0199)$	$0.2920{\pm}0.00919(0.2737{-}0.3102)$	0.8407	82.19178

a: Attack rate (h⁻¹), T_h : Handling time (h), T/T_h : Maximum attack rate.

Treatments	Parameter	Estimate	Standard error	Approximate 95% CI	
				Lower	Upper
Female/Male	D_a	-0.0898	0.0779	-0.2436	0.0639
	D_{Th}	0.6041	0.0746	0.4569	0.7512
Female/fourth instar larvae	D_a	0.1819	0.1874	-0.1880	0.5518
	D_{Th}	-0.1043	0.0349	-0.1732	-0.0354
Male/ fourth instar larvae	D_a	-0.2717	0.1652	-0.5977	0.0543
	D_{Th}	0.7083	0.0812	0.5481	0.8686

Table 3 The parameters of the combined equation for comparing the attack rate and handling time of female, male and fourth instar larvae of *Hippodamia variegata*.

CI: Confidence intervals, Significant parameters are shown in the boldface. **Discussion** f

The success or failure of coccinellid biocontrol agents in inundative releases can be determined by the results of functional response studies (Obrycki and Kring, 1998). This study evaluated the functional responses of different larval stages, adult male and female, of H. variegata to different densities of H. amygdali. Based on the results, all growth stages of H. variegata exhibited type II functional response to different densities of H. amygdali, which means the declining proportion of prey eaten by increasing prey density. Although different types of functional response are demonstrated for coccinellids (Hodek and Honk, 1996), generally, type II is the most common among arthropod predators. This type has been reported for various species of coccinellids, such Coccinella undecimpunctata (L.) preying on Aphis fabae Scopoli and Aleyrodes proletella (L.) (Moura et al., 2006), third instar larvae and adults of Harmonia axyridis and *C*. septempunctata feeding on Aphis glycines (Xue et al., 2009), Aphidecta obliterata (L.) and A. bipunctata preying on Elatobium abietinum (Walker) (Timms et al., 2008), female adults of A. bipunctata preying on Myzus persicae (Jalali et al., 2010), adult female of H. variegata feeding on Aphis gossypii Glover (Davoodi et al., 2012), H. axyridis feeding on Aphis citricola (Feng et al., 2018). In contrast, coccinellid predators have occasionally exhibited type III functional responses. Zarghami et al. (2021) found adult female of Nephus arcuatus in some days of life showed type III functional responses. Seyfollahi et al. (2019) showed that the adult female stage of *Hyperaspis polita* only when feeding on the first instar of *Phenacoccus solenopsis* Tinsley nymphs exhibited type III functional response. Predators that show type II functional response can be used in inundative biological control programs that aim at direct pest suppression, while a high predator-prey ratio is needed for effective long-term pest control (Van Lenteren, 2012).

Attack rate (*a*) and handling time (T_h) were estimated to determine the magnitude of the functional responses. The number of function contacts between predator and prey is the attack rate and depends on the maximum distance at which a predator can begin attacking a prey, the quickness of movement exhibited by the predator and prey, and the amount of successful attacks (Begon *et al.*, 1996).

Our findings showed no significant differences in the estimated attack rates across the various growth stages of H. variegata. The attack rate determines how steeply the functional response curve rises with increasing prey density. Thus, according to the findings, the steepness did not change among different developmental stages of H. variegata, and the different growth stages had similar capacities to respond to higher prey densities. In contrast, the handling times decreased as the larval age of this predator increased. Our results indicated that the handling times of female and fourth instar larvae were shorter than other stages. In other words, the 1st instar larva of H. variegata spent more time and the last instar larvae and adult females spent less time to consume H. amygdali than other growth stages. Handling time (T_h) is an important and effective parameter for predation

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rate since it exhibits the total accumulative time spent in catching, subduing, killing, and digesting the prey (Veeravel and Baskaran, 1997). Therefore, being larger benefits 4th instar larvae and adult females in subduing, consuming, and digesting more prey. This was observed by Farhadi *et al.* (2010) in their study on *H. variegata* feeding on *A. fabae.* Additionally, Bayoumy (2011) found that the functional response of 4th instar larvae and adult females of *N. includens* to *A. gossypii* differed in handling time.

Pairwise comparisons revealed a significant difference between the handling times of female and fourth-instar larvae. Theoretical maximum attack rates per day (T/T_h) was highest for the last instar larvae of predator due to their greater needs for food and energy to grow and obtain the critical weight for pupation, the second highest maximum attack rate was for adult female due to nutrient requirement for egg production and oviposition (Hodek and Honek, 1996). Therefore, these two stages can be considered the most efficient predatory stages of *H. variegata*.

The results of the current study expand our comprehension of the functional response of one of the most important aphid predators of almond aphids and accommodate a better understanding of prey-predator interactions. We conclude that the fourth instar larvae and adult female stage of H. variegata were more efficient than the other stages and can also be used in the biological control of H. amygdali. Since fourth instar larvae remain longer in one patch of aphids, while adult females usually fly and disperse to find new patches, the last instar larvae can be very efficient in controlling the population of aphids in each patch (Hodek and Honek, 1996; Kindlmann and Dixon, 2001). According to Berry et al. (1998), functional response experiments conducted under laboratory conditions may not illustrate the field conditions because of climatic factors such as humidity and temperature changes. Therefore, more fieldbased studies, especially on numerical response, interference competition, foraging behavior, and long-term predation capacity, are needed to

consider the possibilities for using this predator in inoculative biocontrol programs of *Hyaopterus amygdali* in almond orchards.

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رفتار جستجوگری سنین مختلف رشدی کفشدوزک Hippodamia variegata (Hemiptera: Aphididae) روی شته آردی بادام (Coleoptera: Coccinellidae) Hyalopterus amygdali

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چکیدہ: کنترل بیولوژیک از روشھایموفقیتآمیز تنظیم جمعیت آفات مییاشد که امکان کاهش وابستگی به آفتکشها را فراهم میکند. ارزیابی واکنشهای رفتاری شکارگر نسبت به تغییر تراکم میزبان در تحقیقات آزمایشگاهی برای کاربرد موفقیتآمیز آن در برنامههای کنترل بیولوژیک ضروری است. در پژوهش حاضر واکنش تابعی سنین مختلف لاروی، حشرات کامل نر و مادہ کفشدوزک(Hippodamia variegata (Coleoptera: Coccinellidae بے تراکم های مختلف شته آردی بادام، Hyalopterus amygdali در شرایط آزمایشگاهی (دمای ۲۶ درجه سلسیوس، رطوبت نسبی ۵ ± ۶۵ درصد و دوره نوری ۱۴ ساعت روشنایی و ۸ ساعت تاریکی) مورد مطالعه قرار گرفت. آزمایش در ظروف پتری حاوی برگ بادام در ۱۵ تکرار انجام شد. نتايج نشان داد که واکنشتابعي تمامی مراحل رشدی مورد مطالعه شكارگر نسبتبهتراكمهاي مختلفشته ازنوعدوم میباشد. قدرت جستجوی (a) سنین اول، دوم، سوم، چهارم لاروی، کفشدوزک بالغ نر و ماده بهترتیب ۰/۰٬۰۱۷۳/۰۲۱۹، ۰/۰، ۰/۰۱۹۱ ، ۰/۰۱۹۸ و ۲۸۱۰/۰ بر ساعت و زمان دستیابی (T_h) برای مراحل رشدی فـوق بـهتـرتـیب ۵/۷۴۵۰، ۲/۴۰۰۳، ۷۳۰۰٪، ۳۱۹۴/۰، ۰/۷۳۵۲ و۲۹۲۰٬۰ ساعت برآورد گردید. مقایسه پارامترهای برآورد شده نشان داد که زمان دستیابی حشرات نر بهطور معنیداری بیشتر از زمان دستیابی حشرات ماده و لارو سن چهارم است ولی تفاوت معنیداری در قدرت جستجوی بین این مراحل رشدی مشاهده نشد. حداکثر نرخ حمله (T/T_h) برای لارو سن اول تا چهارم، بالغ نر و ماده بهترتیب ۴/۱۹۳۰۵۳، ۹/۲۲۲۹۱۱، ______ و ۲۰/۵۲۹۰۹، ۲۰۰/۲۹۲۵ و ۸۲/۱۹۱۷۸ پوره میزبان محاسبه شد. یافته های این پژوهش نشان میدهد که لارو سن چهارم و حشرات بالغ ماده این کفشدوزک میتواند گزینه مناسبی برای کنترل بیولوژیک شته H.amygdali در باغهای بادام باشد.

واژگان کلیدی: کنترل بیولوژیک، نرخ حمله، زمان دستیابی، Hippodamia variegata ،Hyalopterus amygdali