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Functional Responses of *Hippodamia variegata* (Goeze) and *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) on *Aphis fabae* Scopoli and *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae)

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ABSTRACT

In this study, the functional responses of aphid predators *Hippodamia variegata* (Goeze) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) were tested. In these experiments, the amount of prey consumed by the predator insects at different aphid densities and the effect of this on the reduction of aphid populations were examined. The larvae (L1, L2, L3, and L4) and adults of the predators were starved for 24 hours. Afterward, the second and third instar larvae of the aphid species *Aphis fabae* and *Acythosiphon pisum* were offered as prey in specific numbers (5, 10, 20, 40, 80, and 160). After providing the prey, the predators were allowed to feed for another 24-hour stage, and the amounts of prey consumed were recorded. The results showed that the attack rates (a) of the predator insects increased as their developmental stage progressed, while the handling time (Th) decreased. Based on the data, it was concluded that both predator species, especially in the later stages of their developmental stages, had high prey consumption on *A. fabae* and *A. pisum* and could be effective in the control of these pests.

Hippodamia variegata (Goeze) ve *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae)'nın *Aphis fabae* Scopoli and *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) Üzerindeki İşlevsel Tepkisi

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ÖZET

Bu çalışmada, yaprak bitleri avcıları *Hippodamia variegata* (Goeze) ve *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) türlerinin fonksiyonel yanıtları test edilmiştir. Bu denemelerde, predatör böceklerin, farklı yaprak biti yoğunluklarında tükettikleri av miktarı ve bunun yaprak biti popülasyonunun azaltılmasına etkisi incelenmiştir. Predatörlerin larvaları (L1, L2, L3 ve L4) ve erginleri 24 saat boyunca aç bırakılmıştır. Ardından yaprak bitleri *Aphis fabae* Scopoli ve *Acyrthosiphon pisum* Harris türlerinin 2. ve 3. dönem larvaları belirli sayılarda (5, 10, 20, 40, 80 ve 160) av olarak verilmiştir. Av verildikten sonra, 24 saatlik bir süre daha beslenmeleri için beklenmiştir ve tüketilen av miktarları kaydedilmiştir. Bu çalışmalardan, avcı böceklerin avlarına karşı saldırı oranlarının (a) gelişme dönemi ilerledikçe arttığı ve avı işleme sürelerinin (Th) azaldığı tespit edilmiştir. Elde edilen verilere göre, her iki avcı böceğin, özellikle gelişme dönemlerinin son aşamalarında, *A. fabae* ve *A. pisum* üzerinde yüksek av tüketimine sahip oldukları ve bu zararlılarla mücadelede etkili olabilecekleri sonucuna varılmıştır.

1. Introduction

In agricultural production, chemical control is largely preferred for combating diseases and pests. However, pests develop various resistances to new chemicals that emerge each year, which leads to increased chemical usage. Due to the negative effects of chemicals used in agricultural production on the environment and human health, researchers and producers around the world are focusing on alternative control methods (Lacey et al., 2001). In recent years, efforts have been made to explore these alternative methods to prevent these adverse effects. One of these methods, "Biological Control," is characterized by sustainability. Additionally, the methods applied do not cause environmental pollution and do not harm humans and wildlife (DeBach, 1969; Uygun et al., 1987).

Aphids, which are widely distributed worldwide, are among the herbivores that cause damage to a large number of plants (Nelson & Rosenheim, 2006). These pests directly damage plants by sucking their sap (Blackman & Eastop, 2000) and indirectly harm them by secreting toxic substances and carrying viral diseases (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı & Toros, 1994). *Aphis fabae* (Scopoli)

(Hemiptera: Aphididae) causes damage to many vegetables, beans, peas, sunflowers, and tomatoes, in addition to over 200 wild plants (Völkl & Stechmann, 1998; Barnea et al., 2005; Fericean et al., 2012). *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) is known to be a pest of wild weeds, as well as causing damage to beans, lentils, clover, vetch, and some legumes (Stary, 1970; Ali & Habtewold, 1994).

The Coccinellidae family, belonging to the order Coleoptera, is one of the most effective groups in biological control. It is known to encompass about 5200 species worldwide (Khan et al., 2007). *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), which is commonly found in the Palearctic region, is a polyphagous species. It primarily feeds on aphids, particularly those found on weeds, and it has also been observed feeding on species from the Aleyrodidae (Hem.) and Chaitophoridae (Hem.) families (Korchefsky, 1932; Klausnitzer, 1966; Horion, 1961; Elmalı & Toros, 1994; Aslan & Uygun, 2005; Elekçioğlu & Şenal, 2007). *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), an important predator of aphids, has also been reported to be effective against other soft-bodied insects that cause damage to plants (Ali & Rizvi, 2009).

To reliably predict the impact of predatory insects on their prey, it is crucial to understand their functional and numerical responses, which are significant in biological control studies (Davis et al., 1976; Trexler et al., 1988). The functional response is used in prey-predator models (Jeschke et al., 2002) and is one of the main components in the selection of agents in biological control (Lester & Harmsen, 2002). A predator's functional response demonstrates the rate at which the predator consumes prey at varying prey densities and its effectiveness in controlling harmful populations (Murdoch & Oaten, 1975). The numerical response is known as an indicator of the reproductive capacity of predators at changing prey densities. Predators with high numerical responses have been found to be able to keep increasing prey populations under control (Davis et al., 1976). Ecologically, changes in prey densities also affect predator densities (Solomon, 1949).

In order to protect against the adverse effects of chemicals used in the control of aphids, it is necessary to focus on control methods that do not negatively impact nature and human health. Predaceous coccinellids are associated with these arthropod pests as regulating populations naturally. Coccinellids provide effective control of these pests by devouring them (Kaçar & Koca, 2020). Therefore, determining the functional and numerical responses of *H. variegata* and *C. septempunctata*, which are effective against aphids *A. fabae* and *A. pisum* and are the main subjects of our study, is of great importance. This study analyzed the functional responses of *H. variegata* and *C. septempunctata*, the predators of aphids. In the functional response experiments, the amount of prey consumed by predators at varying prey densities and their effectiveness in reducing aphid populations have been determined.

2. Material and Methods

The main materials of this study are the broad bean plant (*Vicia faba* L.), two herbivores (*A. fabae* and *A. pisum*), and two predators (*H. variegata* and *C. septempunctata*). Plant production stages and the mass rearing of aphids and preadotors were conducted under controlled laboratory conditions, with the temperature maintained at 27±1°C, relative humidity (RH) at 65±5%, and 16:8 h light/dark (L/D) photostage.

2.1. Plant culture

The broad bean plants used in the experiments were grown in 200 ml plastic containers containing a 1:1:1 mixture of soil, peat, and perlite.

2.2. Rearing of aphids

The initial individuals of *A. fabae* and *A. pisum*, which would be produced collectively for the study, were obtained from laboratory mass production. Individuals collected from this production were transferred to laboratory-grown plants using a brush and placed into separate cages for use in the experiments. Clean plants were added to the cages covered with mesh until the number of aphids reached sufficient levels, and the rearing of pests continued stageically.

2.3. Rearing of predatory insects

The predatory insects used in the study (*H. variegata* and *C. septempunctata*) were collected from field conditions (from the clover gardens in Isparta province) and brought to the laboratory, where their identification was conducted. To avoid errors due to food differences in functional response experiments, the predatory insects were reared separately on different preys (*A. fabae* and *A. pisum*). Cages made of plexiglass, with sides and tops covered with tulle, were used for mass production. The individuals used in the experiments were also obtained from this mass production. The production of predatory insects was conducted entirely in climate chambers with a temperature of 27±1 °C, 65±5% relative humidity, and long-day lighting conditions (16:8).

2.4. Establishment of functional response trials

At this stage of the experiments, larvae (L1) were taken from the eggs of the predatory insects in the mass rearing containers as soon as they hatched and placed into separate Petri dishes. After this process, the larvae were starved for 24 hours, and the following day, a specific number of 2nd and 3rd instar aphids (*A. fabae* and *A. pisum*) were provided to each larva. Six densities of aphids (5, 10, 20, 40, 80, 160) were made available to the predators. After the food was provided, a waiting stage of 24 hours was observed, after which the amount of prey consumed by the larvae was counted and recorded. This process was carried out for all larval stages (L1, L2, L3, and L4) as well as for adult individuals (both female and male). These experiments conducted separately for each developmental stage and each predator (*H. variegata* and *C. septempunctata*) with 50 eggs. These tests were carried out in climatic chambers with a temperature of 27±1 °C, 65±5% relative humidity, and long-day lighting conditions (16:8). The functional response of the predatory insects was calculated using the formula applied by Holling (1959).

Na=TPaN/(1+aThN)

(Na: Number of preys consumed, T: The length of time the predator and prey are kept together, P: Number of predators, N: Prey density per unit area, a: Predator search rate, Th: Capture time of each prey).

2.5. Statistical analysis

At this stage, analysis of variance (ANOVA) was applied to determine the differences related to the data obtained from the functional response of predatory insects. If the difference between the means is statistically significant, the level of this significance was determined according to the TUKEY multiple comparison test. SPSS (Ver. 17) and Minitab (ver. 16) statistical applications were used in the analysis of the data.

3. Results and Discussion

3.1. *Hippodamia variegata* consumption of aphids

The results obtained from the functional response trials of *H. variegata* on *A. fabae* and *A. pisum* are given in Figure 1. It was observed that the late larvae of the predator insect consumed all the prey in 5 prey densities and almost all of the prey in following three prey densities (10, 20 and 40). It was determined that all the prey were consumed in the first two prey densities (5 and 10), and almost all in the next two prey densities (20 and 40). It was also observed that the number of aphids consumed by the first three development stages decreased when compared with the other stages. Where prey densities were high (80 and 160), it was determined that consumption decreased when compared with other prey densities. This was observed for all development stages. According to the obtained data, for both predators, at low prey density (5, 10, 20 and 40), the last instar larvae (L4) and adults (females and males) consumed more than 90% of the prey. In addition, the total predation rate decreased at 80 and 160 prey densities and this rate was between 50-15% in all developmental stages of predatory insects. In particular, it was observed that adult individuals of *H. variegata* consumed the entirety of the first two prey densities (5, 10); however, this rate decreased at other prey densities (particularly at 20, 40, 80 prey densities). The amount of food consumed in different prey densities according to different development stages in all of the trials and the statistical differences between them are given in Figure 1.

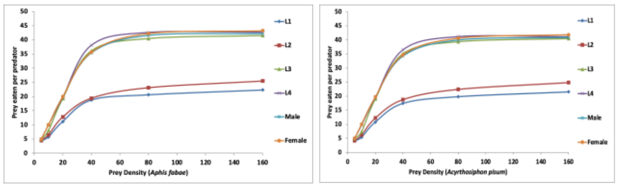

Figure 1. The quantity of aphids consumed by *Hippodamia variegata*
Şekil 1. Hippodamia variegata tarafından tüketilen yaprak biti miktarı

3.2. *Coccinella septempunctata* consumption of aphids

The results obtained from the functional response trials of *C. septempunctata* on *A. fabae* and *A. pisum* are given in Figure 2. It was observed that the last stage larva of the predator insect consumed the all of the prey in the 5 and 10 prey densities, and almost all of the prey in the 20 and 40 prey densities. When the results of the adults were examined, it was determined that all of the prey were consumed in the first three prey densities (5, 10 and 20), and almost all in the next prey density (40). It was observed that the number of aphids consumed in the first three development stages decreased when compared with other stages. In the trials where the prey densities were high (80 and 160), it was determined that consumption decreased when compared with the other prey densities. This was observed for all developmental stages. According to the data obtained for *C. septempunctata*, it was determined that the consumption amount of adults (males and females) and last stage larvae was approximately 100% in the first three prey densities (5, 10, 20) for both prey; however, it was determined that this rate decreased at other prey densities but was 90% and above. The amount of food consumed in different prey densities according to different development stages in all of the trials and the statistical differences between them are given in Figure 2.

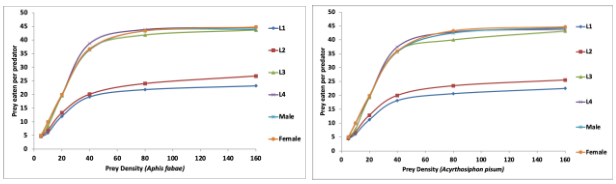

Figure 2. The quantity of aphids consumed by *Coccinella septempunctata*
Şekil 2. Coccinella septempunctata tarafından tüketilen yaprak biti miktarı

3.3. Hunting rates

The predation rates of predatory insects were calculated and are shown in Figure 3. It was observed that both *H. variegata* and *C. septempunctata* consumed the low-density foods to a large extent and after a certain level, the amount consumed by the predator insects decreased with increase in aphid density. According to the obtained data, for both predators, at low prey density (5, 10, 20 and 40), the last instar larvae (L4) and adults (females and males) consumed more than 90% of the prey. In addition, the total predation rate decreased at 80 and 160 prey densities and this rate was between 50-15% in all developmental stages of predatory insects.

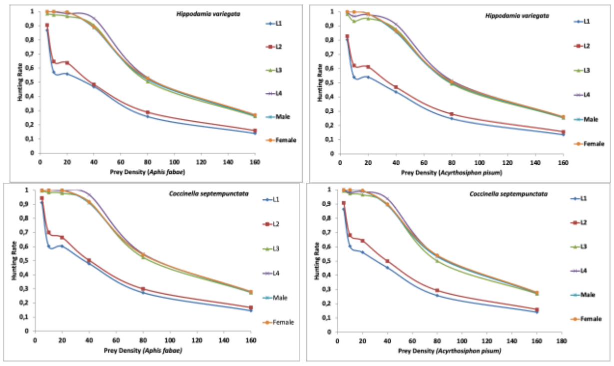

Figure 3. Hunting rates of *Hippodamia variegata* and *Coccinella septempunctata* on preys

Şekil 3. Hippodamia variegata ve Coccinella septempunctata'nın avlar üzerindeki avlanma oranları

3.4. Attack rates (a) and Handling times (Th) of the predators

The attack rate (a) and handling times (Th) of predatory insects for different foods were calculated according to Holling (1959). The highest attack rates were seen in the 4th larvae and adults of predatory insects. When comparing the two predators, it was observed that the values of *H. variegata* were higher than *C. septempunctata*. It was determined that the attack rate for both predatory insects was lower in the first three development stages when compared with the other stages. When handling times were examined, it was seen that the lowest values were in the last larval stage and the adult individuals. When comparing the two predators, it was observed that the values obtained in *C. septempunctata* were lower than the values obtained in *H. variegata*. It was determined that catching time for both predatory insects was high in the first three developmental stages and decreased in the latter stages, (Table 1).

In this study, the functional responses of *C. septempunctata* and *H. variegata* were determined separately on *A. fabae* and *A. pisum*. It was observed that the functional response type of both predators on their food was Holling (1959) Type-II. It was also noted that the amount of food consumed grew in proportion to the amount of food offered. As the larvae progressed through their stages and into adulthood, the food consumption continued to increase. In these studies, attack rate (a) and capture time (Th) values, which are functional response parameters, were also calculated. It was determined that the attack rates of the first instar larvae were low for both predators. It was found that this value increased with developmental stage. Although both predator species have a long capture period in their early stages, it has been estimated that this period gets shorter as the developmental stage advances. It was also observed that predatory insects consume the low-density foods to a large extent but after a certain level (40 and 80 prey densities), consumption decreases with an increase in density.

Studies on predatory insects worldwide have shown that especially late-stage larvae of lady beetles (Coccinellidae) consume large amounts of food (Omkar & Pervez, 2004; Moura et al., 2006; Bayoumy, 2011; Lee & Kang, 2014). This is believed to be due to the high energy requirements for development and the weight needed for the pupal stage (Hodek & Honěk, 1996). Research on the hunting efficiency of predatory insects has reported that factors such as prey exploitation (Matter et al., 2011), prey type (Sarmento et al., 2007), prey age (Koch et al., 2003), cannibalism, collective hunting (Burgio et al., 2002), larval parasitism (Bayoumy, 2011; Bayoumy & Michaud, 2012), and temperature (Skirvin et al., 2007) influence this consumption. Kaydan & Yaşar (1999), studied the functional and numerical responses of the predatory insect *Scymnus apetzi* (Mulsant) (Coleoptera: Coccinellidae) on the aphid *Hyalopterus pruni* (Hemiptera: Aphididae) found on plum and peach trees, as well as the effects of starvation duration. They reported that the amount of food consumed by *S. apetzi* during its larval stages increased with the prey density in the environment. Additionally, they observed that both male and female adults consumed more food as the prey population increased, and found a linear relationship between prey density and food consumption. Atlıhan & Güldal (2008), examined the functional and numerical responses of *Scymnus subvillosus* (Goeze) (Coleoptera: Coccinellidae) to varying numbers (5, 10, 20, 40, 80) of *H. pruni* (Geoffroy) (Homoptera: Aphididae). Their data aligned with a Holling Type-II functional response. Khan (2010), assessed the functional response of different larval stages and adult males and females of *Harmonia eucharis* (Mulsant) on *Aphis pomi*, concluding that the species exhibited a Type-II functional response throughout its life cycle, with the highest search rate and shortest capture time observed in the fourth larval stage. This suggests that the fourth instar larvae of *H. eucharis* may be particularly effective in controlling *A. pomi*, though further field studies are needed to confirm this. Madadi et al. (2011), studied *H. variegata* (Goeze) (Coleoptera: Coccinellidae) using pea aphids and cotton aphids as prey. They found that prey consumption decreased as the pest populations increased, indicating a Type-II functional response. Dehkordi & Sahragard (2013), investigated the functional response of female *H. variegata* to various densities (5, 10, 20, 40, 60, and 80) of third-instar nymphs of *Aphis gossypii* (Hemiptera: Aphididae). They determined that the maximum theoretical predation (T/Th) for females was 121,475 and reported that the survival and vitality of *H. variegata* females at the end of the trials were influenced by prey density. Saleem et al. (2014), examined the predatory effect of *Menochilus sexmaculatus* (Fab) (Coleoptera: Coccinellidae) on *Macrosiphum rosae* (Linnaeus) (Hemiptera: Aphididae) (rose aphids). They found that the predatory effects of the 1st, 2nd, 3rd, and 4th instar larvae of *M. sexmaculatus* were 8.40±0.50, 13.60±0.81, 28.60±1.50, and 57.40±4.67 aphids, respectively. The predator effects of male and female adults were 802.40±2.56 and 916.60±1.69 aphids, respectively, with females being more consistent in their consumption than males. Zarghami et al. (2014), studied the functional response of *Nephus arcuatus* (Kapur)

(Coleoptera: Coccinellidae) to mealybug *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), and determined that the functional response followed Type-II. In their experiment, they used different densities of mealybugs (2, 4, 8, 15, 40, 65, 90, and 115) as prey and calculated the attack rate and capture time for females at 0.2819 h and 0.00811, respectively. The study also revealed that the predators preferred eggs and females of mealybugs, while the first nymph stage was the least preferred. El-Zahi (2017), explored the hunting potential and preference of *Chrysoperla carnea* (Stephens) and *Coccinella undecimpunctata* L. for cotton mealybug *Phenacoccus solenopsis* and *A. gossypii* under laboratory conditions. They found that the fourth instar larvae of *C. undecimpunctata* consumed 112.4±1.63 A. gossypii nymphs, 546.4±3.69 first-instar mealybug nymphs, 174.1±1.07 second-instar nymphs, and 40.2±1.22 third-instar nymphs. Both predators were suggested as promising biological control agents for *P. solenopsis*. Bayoumy and Awadalla (2018) studied the effect of different prey densities [*Myzus persicae* Sulzer and *Aphis craccivora* Koch (Hemiptera: Aphididae)] on the third larval stage of *C. carnea*, the fourth larval stage of *C. septempunctata*, and the fourth larval stage of *H. variegata*. Their results indicated that the functional response type remained Type-II, despite significant effects from prey type, predator type, and prey density on aphid consumption. Moradi et al. (2020), assessed the functional response of *Scymnus syriacus* (Coleoptera: Coccinellidae) on *Aphis spiraecola* and *A. gossypii* (Hemiptera: Aphididae) at varying prey densities (3, 5, 7, 10, 20, 30, 40, 60, and 80). They determined that the functional response was Type-II, with high attack rates and short capture times for both prey types in adult predators. Kumar et al. (2020) investigated the functional response of two predator species, *Delphastus catalinae* and *D. pallidus* (Coleoptera: Coccinellidae), to *Bemisia tabaci* eggs at different densities (20, 40, 60, 80, 100, and 120). Both species exhibited a Type-II functional response. The attack rates and capture times were also calculated for the predators, and the study supported previous findings that food consumption increased with prey density.

Consistent with these studies, our research also found that the predatory insects exhibit a Type-II functional response to both *A. fabae* and *A. pisum*, with consumption rates increasing as prey density in the environment rises. Moreover, the consumption of fourth-stage larvae and adults was higher than in other developmental stages. As evidenced by previous studies, functional response experiments conducted under laboratory conditions offer valuable preliminary insights into the prey consumption capacity of natural enemies. These trials are crucial for assessing the hunting efficiency of predatory insects and provide a basis for exploring their potential use in biological control applications. In this study, we examined the consumption rates of *H. variegata* and *C. septempunctata* at different growth stages on *A. fabae* and *A. pisum*, and observed that the predator insects used in this study were highly effective in consuming aphids.

4. Conclusions

The change in the attack response of a predator species with increasing prey population is a key component of the stability of predator-prey systems (Hassell, 1978). Since Holling (1959), discovered that the attack rates of natural enemies increase with higher prey density, several types of functional responses have been described: a rectilinear rise to a maximum (Type I), a continuously decreasing rate towards a maximum (Type II), a sigmoid increase (Type III), and a dome-shaped response (Type IV) (Figure 4).

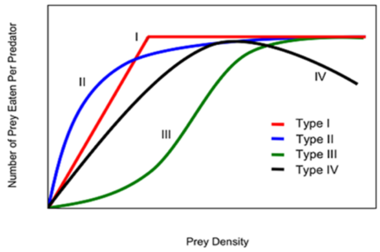

Figure 4. Types of Functional Response according to Megane (2013)
Şekil 4. İşlevsel Tepki Türleri Megane (2013)'a göre İşlevsel Tepki Türleri

According to the results, it was determined that both predatory insects showed Type-II functional response. In addition, it has been determined that both predators, especially the late larvae and adults, are very effective on aphids used as food. It was concluded that the late development stages of both *H. variegata* and *C. septempunctata* are effective on *A. fabae* and *A. pisum* and show potential as biocontrol agents in the management against these pests.

When all the data obtained were examined, it was observed that the pest-control potential of the predatory insects in the study was high. Accordingly, it is thought that mass production of these predatory insects can be made in the laboratory environment, especially in the areas where production is made under controlled conditions, and the populations of the pests (especially aphids) can be kept under the threshold of economic damage.

Table 1. Attack rates (a) and Handling times (Th) of *Hippodamia variegata* and *Coccinella septempunctata* on different prey densities (*Aphis fabae* and *Acyrthosiphon pisum*)
Tablo 1. Hippodamia variegata ve Coccinella septempunctata'nın Hippodamia variegata ve Coccinella septempunctata'nın farklı av yoğunlukları (Aphis fabae ve Acyrthosiphon pisum) üzerindeki saldırı oranları (a) ve Taşıma süreleri (Th)

Hippodamia variegata on *Aphis fabae*						
	1st Instar	2nd Instar	3rd Instar	4th Instar	Male	Female
Attack rate (a)	0.9752	1.0145	1.0102	1.0643	1.0695	1.0669
Handling time (Th)	0.950 h	0.799 h	0.324 h	0.216 h	0.233 h	0.223 h
Hippodamia variegata on *Acyrthosiphon pisum*						
	1st Instar	2nd Instar	3rd Instar	4th Instar	Male	Female
Attack rate (a)	0.8986	0.9227	1.0102	1.0615	1.0753	1.0724
Handling time (Th)	0.970 h	0.782 h	0.324 h	0.240 h	0.254 h	0.245 h
Coccinella septempunctata on *Aphis fabae*						
	1st Instar	2nd Instar	3rd Instar	4th Instar	Male	Female
Attack rate (a)	1.0267	1.0670	1.0102	1.0597	1.0635	1.0621
Handling time (Th)	0.905 h	0.758 h	0.324 h	0.192 h	0.211 h	0.209 h
Coccinella septempunctata on *Acyrthosiphon pisum*						
	1st Instar	2nd Instar	3rd Instar	4th Instar	Male	Female
Attack rate (a)	0,98319	1,02533	1,02176	1,06067	1,06496	1,06349
Handling time (Th)	0,936 h	0,7704 h	0,2928 h	0,216 h	0,2208 h	0,2136 h

5. Acknowledgements

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