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Functional and numerical response of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) on *Macrosiphum rosae* (L.) (Hemiptera: Aphididae)

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Abstract
In this study, functional and numerical response tests, which are important components in the selection of biological control agent, were carried out. In functional response trials, the amount of food consumed, attack rate (a) and handling time (Th) were calculated for each developmental period, depending on the number of preys given after 24 hours. The obtained results were evaluated with the Holling. In numerical response experiments, the development of the predator insect was examined depending on the number of preys given in certain numbers (5, 10, 20, 40 and 80) and the data were recorded. This phase of the trials continued until the individuals died. At this stage of the trials, the reproductive response of the predator on the aphid and the prey use efficiency were calculated. All of the productions and trials were carried out in climate rooms with a temperature of 27±1 °C, 65±5% RH. Experiments were carried out with 50 replications for each growth period and prey densities. According to the results obtained in the functional response trials, it was determined that the development periods with the highest productivity were the fourth larval and adult stages. When the results obtained from the numerical response experiments were evaluated, it was observed that the reproductive response increased with the increase of the prey given to *H. variegata*. Considering all the data obtained as a result of the study, it was concluded that *H. variegata* is effective on *M. rosae* and can be used in the control of this pest.

Keywords
Biological control, Coccinellids, Reproductive response, Attack rate, Handling time

Introduction

Rosa genus (Rosales, Rosaceae), known as rose, is one of the important plant species in the world for humans. This genus is distributed in the Holarctic and contains more than 250 species. The members of this genus, which are found naturally in almost all parts of Turkey, are distributed in a wide area including Central and Western Asia, the Caucasia, Europe, Northwest Africa and North America (Cairns, 2003; Ekincialp et al., 2007; Özçelik, 2013). According to some studies, it is estimated that there are around 500 local genotypes depending on 70 species in Turkey (Özçelik, 2010; Özçelik and Özçelik Doğan, 2018). These plants are especially used by people for decorative purposes. In addition, rose oil obtained from its leaves is used in the perfumery industry (Seneta and Dolatowski, 2003; Jaskiewicz, 2000; 2006). Considering some studies conducted around the world, it has been reported that over a hundred aphid species are harmful on roses (Blackman and Eastop, 2006; Holman, 2009; Blackman and Eastop, 2021). Among these species, *Macrosiphum rosae* (Linnaeus, 1758) is one of the most important rose pests worldwide (Blackman and Eastop, 2021). *M. rosae* damages the young leaves, upper parts of the shoots and buds of plants. They feed by sucking the plant sap, and if their population is high, conditions such as downward bent stems, weak leaves and premature leaf fall occur in the plant. In addition, it creates sooty mold on the flowers and leaf surface due to a substance it secretes (Mehrparvar and Hatami, 2007). It has been reported that this pest mostly causes damage to 1 year and 2 years old rose shoots (Margina et al., 1999). Different strategies are being developed to keep aphid populations below the economic damage threshold (Holtzer et al., 1996). It has been observed that when chemical control is preferred against these pests, aphids develop resistance, negative effects on human and environmental health occur, and the populations of natural enemies decrease (Bielza, 2008; Saleem et al., 2008). Researchers and manufacturers focus on different alternative methods in order to prevent such negativities (Lacey et al., 2001). Coccinellidae is a family that is used in biological control studies and has 5200 species worldwide (Khan et al., 2007). A species belonging to this family, *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) is a polyphagous and is widely found in the Palearctic region. This species is effective on different pests, including aphids, in greenhouses and open fields due to its high nutritional capacity (Franzman, 2002; Aslan and Uygun, 2005; Elekçioğlu and Şenal, 2007).

Functional response, which is one of the important components in the selection of biological control agents, is used in models of the predator-prey relationship (Jeschke et al., 2002; Lester and Harmsen, 2002). The functional response of a predator insect indicates the rate of prey consumed by the predator at varying prey densities and its effectiveness in preventing pest populations (Murdoch and Oaten, 1975). The numerical response is known as an indicator of the reproductive abilities of the predator insect at varying prey concentrations. In addition, species with a high numerical response are known to be able to control growing prey populations (Davis et al., 1976). In this study, experiments were carried out on the functional and numerical response of *H. variegata* on *M. rosae*.

Materials and Methods

The main materials of this study are rose seedlings, rose aphid (*M. rosae*) and predator insect *H. variegata*.

Supply of rose seedlings

The rose seedlings were obtained commercially and transferred to 10 liter pots containing a 1:1:1 soil:peat:perlite mixture. The seedlings transferred to the pots were left in a climate room with 27±1 °C temperature and 65±5% RH and long daylight (16:8/L:D) conditions.

Production of *Macrosiphum rosae*

The first individuals of *M. rosae* were obtained from mass production in the laboratory. Individuals of *M. rosae* were transferred to plants grown in the laboratory with the help of a sable brush and were taken to separate climate rooms to be used in the experiments. New plants were transferred to the environment and the production of pests continued periodically until the number of aphids was sufficient. All aphid productions were carried out in climate rooms with 27±1 °C temperature and 65±5% RH and long daylight (16:8/ L:D).

Production of *Hippodamia variegata*

Hippodamia variegata individuals were collected from field conditions and brought to the laboratory and then diagnosed. In the experiments, predatory insects were fed on *M. rosae* for a while (one generation). In this way, food-borne trial errors were prevented. In mass production, cages (50x50x50 cm) made of plexiglass material covered with tulle were used. The individuals used in the experiments were also obtained from this mass production. Predator production was carried out in climate rooms with 27±1°C temperature, 65±5% RH and long daylight (16:8/ L:D).

Establishment of Trial

Functional Response Experiments

At this stage of the experiments, as soon as the larvae (L1) emerged from the eggs of the predatory insects in the mass production dishes, they were taken into separate petri dishes. After this process, the larvae were starved for 24 hours and the next day, a certain number of (5, 10, 20, 40 and 80) 2nd and 3rd instar *M. rosae* were given to each larva and adults. After the prey was given, it was waited for 24 hours, and then the amount of prey consumed by the larvae and adults were counted and recorded. All of these procedures were performed for all larval stages (L2, L3 and L4) and adult individuals (female and male). These trials started with 50 eggs separately for each development period (*H. variegata*). These experiments were carried out in a climate room with a temperature of 27±1°C and 65±5% RH with long daylight (16:8/L:D). The functional response of the predator insect was calculated by the formula used by Holling (1959).

Na=TPaN/(1+aThN)

(Na: Number of prey consumed, T: Duration of keeping the predator and prey together, P: Number of predators, N: Prey density per unit area, a: Attack rate of the predator, Th: Handling time of each prey)

Numerical Response Experiments

At this stage, as soon as the larvae (L1) hatched from the eggs of *H. variegata*, they were taken into separate petri dishes. Certain numbers (5, 10, 20, 40 and 80) of 2nd and 3rd period *M. rosae* were given. All developmental stages of the predator insect were followed (L2, L3, L4, female and male) and the amount of food consumed each day was recorded and the missing ones were added. After the individuals became adults, females and males were brought together and mated. The number of eggs laid by the females was recorded. These processes continued till to the individuals died. Numerical response experiments were carried out with 50 replications. These experiments were carried out in climate rooms with a temperature of 27±1°C, 65±5% RH and long daylight (16:8/ L:D). In calculating the data obtained, the reproductive response (ECI) of female predators at different prey concentrations were calculated using the following formula:

$$\text{ECI (Efficiency of Conversion of Ingested Food)} = \frac{\text{Number of eggs laid}}{\text{Number of consumed food}} \times 100$$ (Omkar and Pervez, 2004).

Analysis of variance (ANOVA) was applied to determine the differences related to the data obtained from the functional and numerical response of *H. variegata*. The level of this significance was determined according to the TUKEY multiple comparison test, if the difference between the means was statistically significant. SPSS (Ver. 17) and Minitab (ver. 16) statistical programs were used in the analysis of the data.

Results and Discussion

Functional response of *Hippodamia variegata*

In the first phase of the study, the functional response of the predatory insect *H. variegata* on *M. rosae* at different densities was determined. Looking at the results, it was determined that the amount of food consumed increased with the development of the larval stages of *H. variegata*. It is seen that the amount of food consumed after the individuals become adults decreases compared to the fourth larval stage, and the amount consumed by male and female individuals is close (Figure 1).

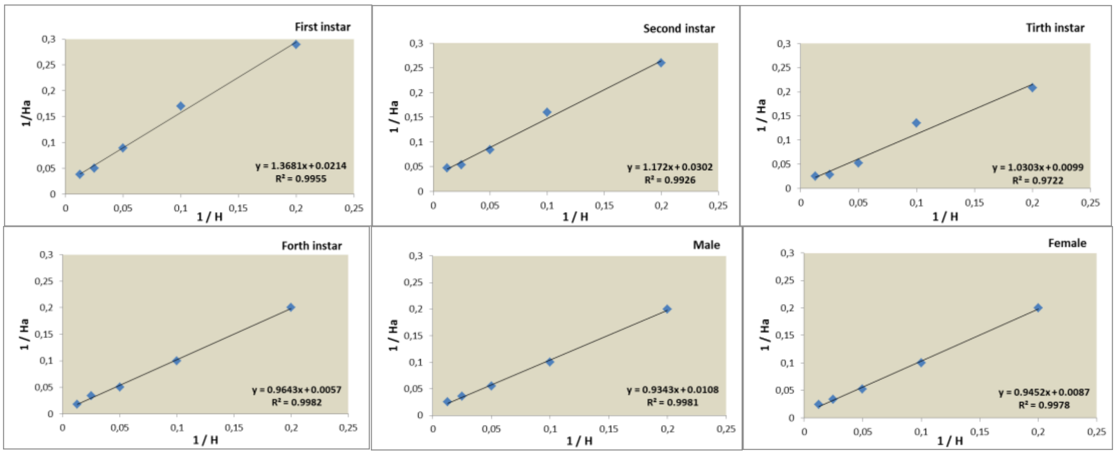

Figure 1. Functional response of *Hippodamia variegata* on *Macrosiphum rosae* at different densities

The hunting rates of *H. variegata* were also calculated. Accordingly, it has been observed that *H. variegata* individuals consume low-density foods to a large extent. Considering the data obtained, it was determined that the consumption decreased as the prey density increased. The proportional data of this are also given in Figure 2.

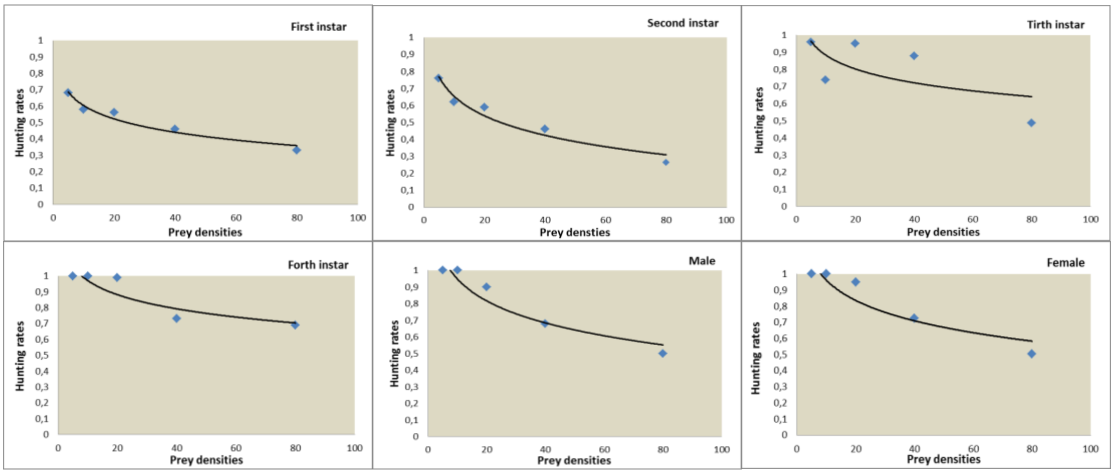

Figure 2. Hunting rates of *Hippodamia variegata* on *Macrosiphum rosae* at different densities

Using the data obtained as a result of the study, the attack rate (a) and catching times (Th) of predatory insects on different foods were calculated according to Holling (1959). Accordingly, the attack rates increased as the developmental stages of the predator insect improved. The highest value was observed in the fourth instar larva and adult individuals. When the catching time was examined, the lowest catching time was calculated in the fourth instar larva (Table 1).

Table 1. Parameters of functional response (attack rate, handling time) of *Hippodamia variegata* on *Macrosiphum rosae*

Parameters	1st instar	2nd instar	3rd instar	4th instar	Male	Female
Attack rate (*a*)	0.73	0.85	0.97	1.037	1.07	1.06
Handling time (*Th*)	0.51 h	0.72 h	0.2376 h	0.1368 h	0.26 h	0.21 h

Numerical response of *Hippodamia variegata*

In the second stage of the study, the numerical response of *H. variegata* on *M. rosae* at different densities was determined. Prey utilization efficiencies of *H. variegata* were demonstrated depending on aphid amounts at different densities. According to the data obtained, this ratio was observed to be over 90% in low prey densities (10, 20), while the efficiency was calculated as 81.1% in 40 prey densities. In the study, it was determined that the productivity was 53.3% in the highest preferred prey density (80) (Figure 3).

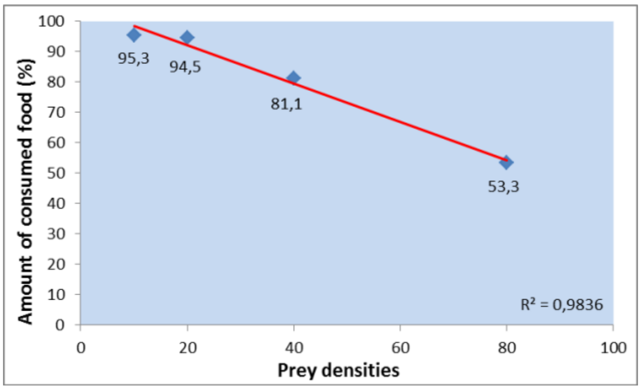

Figure 3. Amount of consumed food at different prey densities

Since the daily developments of the predatory insects were followed in numerical response experiments, the number of eggs laid by female individuals after they became adults was also calculated. Accordingly, the total number of eggs laid by *H. variegata* fed at different prey densities (5, 10, 20, 40 and 80) was calculated as 0, 68.0, 197.9, 643.6, 1224.0, respectively (Figure 4).

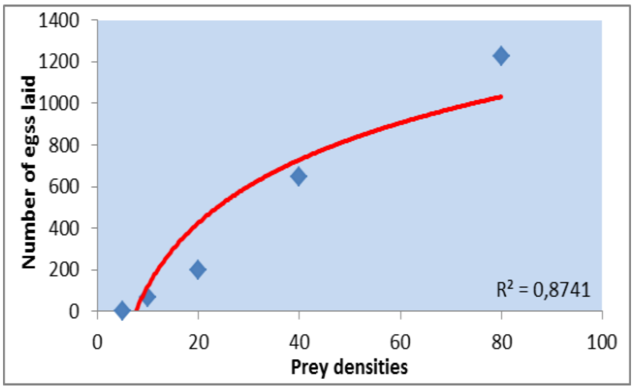

Figure 4. The number of eggs laid by female individuals at different prey densities

According to the data of the study, the reproductive responses (ECI) of female individuals of predatory insects were also determined. Looking at the data obtained, it was determined that adults did not occur at 5 prey density. In other prey densities, the reproductive response also increased as the prey density increased (Figure 5).

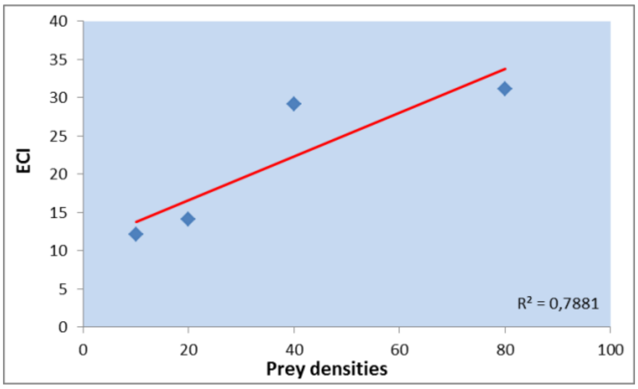

Figure 5. The reproductive responses (ECI) of female individuals of *Hippodamia variegata*

Considering the food experiments on coccinellids, it was observed that especially the last stage larvae consume a high amount of food (Bayoumy, 2011; Lee and Kang, 2014; Moura et al., 2006; Omkar and Pervez, 2004). It is thought that this is due to the need for high energy for development and the weight required for the pupal stage (Hodek and Honěk, 1996). It was reported that the food consumed in the larval stages of *Scymnus apetzi* (Mulsant) (Coleoptera: Coccinellidae) feeding on *Hyalopterus pruni* (Hemiptera: Aphididae) increased depending on the amount of prey in the environment (Kaydan and Yaşar, 1999). In a study on the same food, it was determined that consumption data of *Scymnus subvillosus* (Goeze) (Coleoptera: Coccinellidae) showed Holling's type 2 functional response (Atlıhan and Güldal, 2008). Another study examining the functional response of *Harmonia eucharis* (Mulsant) (Coleoptera: Coccinellidae) on *Aphis pomi* (Hemiptera: Aphididae), it was observed that the response obtained was of the second type. According to the data obtained in the study, it was determined that individuals in the fourth larval stage were hunted in a shorter time and in higher amounts compared to other developmental stages (Khan, 2010). According to Madadi et al. (2011) used pea aphid and cotton aphid in their study with *H. variegata* and reported that the amount of prey consumed decreased due to the intense increase in the population of these pests. Determined that the functional response type of *H. variegata* exhibited Type-II due to the decrease in the amount of food consumed. In a study on third instar nymphs of *Aphis gossypii* (Hemiptera: Aphididae), the functional response of female *H. variegata* was investigated. According to the data obtained in the study, it was reported that the ratio and viability of the remaining *H. variegata* female individuals at the end of the trials depend on the prey density (Dehkordi and Sahragard, 2013). Saleem et al. (2014) investigated the predatory effect of *Menochilus sexmaculatus* (Fab) (Coleoptera: Coccinellidae) on *M. rosae* (rose aphid) in their experiments. According to the data they obtained, it was determined that the amount consumed by the late stage larvae was higher than the other developmental stages. Zarghami et al. (2014) investigated the functional response of *Nephus arcuatus* (Kapur) (Coleoptera: Coccinellidae) on mealybug *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae). It was determined that the data obtained as a result of the study were suitable for the III Type functional response. In a different study, it was tried to determine the preference and hunting potential of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on cotton mealybug *Phenacoccus solenopsis* and *A. gossypii*. It has been observed that the predator insect can be effective in the biological control of both pests (El-Zahi, 2017). Bayoumy and Awadalla (2018) investigated the effects of *C. septempuctata* and *H. variegata* in the fourth period on two different foods [*Myzus persicae* Sulzer and *Aphis craccivora* Koch (Hemiptera: Aphididae)] at different densities. It was observed that the functional response type was Type-II. In a different study on the feeding of *Scymnus syriacus* (Coleoptera: Coccinellidae), the functional response of the predator insect *Aphis spiraecola* and *Aphis gossypii* (Hemiptera: Aphididae) was determined. It was determined that the response type was Type-II. In addition, it has been observed that especially the late larvae and adults of the predator insect have a high attack rate on both foods in a short time (Moradi et al., 2020). In a study on *Bemicia tabaci* (Hemiptera: Aleyrodidae) eggs of different densities, the functional response of *Delphastus catalinae* and *D. pallidus* (Coleoptera: Coccinellidae) was determined. Accordingly, it was determined that both predatory insects showed a Type-II functional response (Kumar et al., 2020). In our study, it was determined that especially the late larvae and adult individuals (male, female) of *H. variegata* reached a high attack rate on M. rosae in a short time. When the data obtained were examined, it was observed that the functional response type was Type-II, as in other predatory insects. When examined in terms of consumption amounts, it was determined that especially low-density foods were consumed quickly. In addition, as in other studies, it was determined that the consumption decreased with the increase in the prey density in the environment.

Kaydan and Yaşar (1999) studied both the numerical and functional responses of *S. apetzi* on *H. pruni*. Accordingly, they determined that the amount of food consumed in the larval stages increased depending on the amount of food in the environment. When the data obtained are examined, it is observed that there is a linear relationship between the number of prey and the amount of food consumed. In a different study, the amount of *Macrosiphum euphorbiae* (Hemiptera: Aphididae) consumed by *C. septempunctata* in larval and adult stages was determined. Accordingly, it has been observed that it consumes a high amount of food, especially in the last period of larvae and adults (Yoldaş and Sanjrani, 1999). Stathas (2000) investigated the predator's reproductive capacity as well as the daily and total food consumption of *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) feeding on *Aspidiotus nerii* (Hemiptera: Diaspididae). According to the data obtained, it was determined that the consumption amount of the larvae, especially in the last period, increased. In addition, he reported that female individuals consume more food than males. In addition to these studies, the effects of different predator insects that share the same habitat with coccinellids on aphids were determined. Accordingly, it was observed that the amount consumed by predator insects as their larval stages progressed (Atlıhan et al., 2004; Huang and Enkegaard, 2010; Batool et al., 2014; El-Zahi, 2017; Rana et al., 2017; Kayahan, 2020). Considering the results obtained from the numerical response experiments in our study, it was determined that the efficiency of prey consumption was high at low densities, as in the literature, and the consumption increased as the density increased. However, when these data are evaluated in terms of catch use efficiency, the opposite is the case. In other words, it was determined that the consumption efficiency decreased with the increase in the prey density. Yaşar and Özger (2005) determined the development, feeding and reproductive responses of *Adalia fasciatopunctata revelierei* (Mulsant) (Coleoptera: Coccinellidae) on *H. pruni* in their study. When the egg laying numbers of the predator insect were examined, it was determined that this number increased in direct proportion to the prey density. In another study, the effect of *Nipaecoccus viridis* (adult females and 1st, 2nd and 3rd instar nymphs of *N. viridis*) on the numerical response of *Nephus arcuatus* (Coleoptera: Coccinellidae) at different periods and at different intensities was investigated. According to the results they obtained, it was determined that as the prey density increased, the number of eggs laid by the females increased nonlinearly (Ramezani and Zargami, 2017). Considering the studies on different predator insects, it has been determined that there is an increase in the daily and total number of eggs given depending on the increase in the prey density in the environment (Ambrose and Claver, 1997; Atlıhan et al., 2004; El-Zahi, 2017, Fathipour et al., 2020; Kayahan, 2020). In our study, it was observed that the number of eggs laid by *H. variegata* increased with the increase in prey density. One of the results of numerical response studies is the Reproductive Response (ECI). According to the results obtained in our study, it was determined that the reproductive response increased as the prey density increased depending on the number of eggs laid. Considering the studies on aphids, it is reported that there is a linear increase in the reproductive response value, especially depending on the aphid density (Khan and Zaki, 2008; Kayahan, 2020).

Conclusion

When the results obtained from functional and numerical response experiments were examined, it was observed that *H. variegata* was effective on *M. rosae*. Accordingly, it is thought that the predator insect may be effective in controlling the population of this type of pest. However, since this study is a laboratory study, it was concluded that experiments should be carried out in field conditions in order to obtain more efficient results. For this reason, it is very important to reveal the numerical response of the species in determining the impact power of a predator that has an effect on aphids.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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