Functional responses of two predatory bugs (Hemiptera: Anthocoridae) to changes in the abundance of Tetranychus urticae (Acari: Tetranychidae) and Bemisia tabaci (Hemiptera: Aleyrodidae)

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Abstract. Orius spp. (Hemiptera: Anthocoridae) is well-known genus of generalist predators, which feed on numerous pest insects and mites infesting crops. In this study, the functional responses of the predatory bugs, Orius laevigatus (Fieber) and Orius vicinus (Ribaut), to different densities of the eggs of the whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) and red spider mite, Tetranychus urticae Koch (Acari: Tetranychidae), were determined under laboratory conditions. Different numbers of eggs (2, 4, 6, 8, 16, 32, 64 and 128) of both species of prey were offered to females of the above predators for 24-h in a controlled environment of 25 ± 1°C, 60 ± 10% RH and under a 16L : 8D photoperiod. The parameters of the functional responses were assessed using Holling’s Disc Equation. Both predators showed a Type II response to both prey. The attack rates (a) and handling times (Th) of the predators were computed for spider mites eggs: O. laevigatus (a: 0.972, Th: 0.007) and O. vicinus (a: 1.113, Th: 0.005), and whitefly eggs: O. laevigatus (a: 1.022, Th: 0.002) and O. vicinus (a: 0.772 Th: 0.006). Furthermore, the average number of B. tabaci eggs consumed by O. laevigatus females was greater than by those of O. vicinus. In contrast, O. vicinus was a more effective predator of T. urticae eggs than O. laevigatus. Consequently, these results indicate that together these predators might be effective biological control agents in regulating populations of B. tabaci and T. urticae in agricultural ecosystems.

INTRODUCTION

The cotton whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) and the two-spotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae) are common phytophagous pests that damage many economically important agricultural crops around the world (Jeppson et al., 1975; Helle & Sabelis, 1985; Gerling et al., 2001; Aslan et al., 2004). Bemisia tabaci causes direct and indirect damage by sucking sap, virus transmission and producing honeydew that leads to rapid growth of sooty mould (Breene et al., 1992). Tetranychus urticae feeds on leaves causing damage to chlorophyll and thereafter serious loss of yield (Nachtman & Zemek, 2002). For the control of these pests, farmers mostly apply chemical treatments to keep their abundance below economic damage threshold levels (Knowles, 1997; Denholm et al., 1998; Van Leeuwen et al., 2010). However, intensive use of pesticides adversely affects the environment and human health. Moreover, it becomes ineffective due to resistance that is developed by the pest after a while and in restricting naturally occurring biological control agents (Riudavets & Castañé, 1998; Biondi et al., 2012). Therefore, alternative pest control strategies including biological control have been adopted to control whiteflies and spider mites around the world in recent years (Lester et al., 2000; Bostanian et al., 2003; Roy et al., 2005; Calvo et al., 2009; Drobnjaković et al., 2016).

To date, several studies report that whiteflies and spider mites have many predators and parasitoids belonging to various families, e.g., Aphelinidae, Phytoseidae, Miridae, Thripidae, Anthocoridae (Lopez-Avila, 1986; Raworth, 1990; Cote, 2001; Gerling et al., 2001; Gigon et al., 2016). Among them, Anthocoridae (Hemiptera: Cimicoidea), which include a number of genera (e.g., Anthocoris, Orius), are well-known as generalist predators, preying upon small bodied insects such as thrips, whiteflies, mites, scales, aphids, psyllids, psocids, bark beetles, small caterpillars and the eggs of various insects and mites (Önder, 1982; Lattin, 2002). According to field observations on species of anthocorids in Adana province of Turkey, Orius laevigatus (Fieber) and O. vicinus Ribaut are the most common species, followed by O. niger Wolff (Pehlivan & Atakan, 2020). While O. laevigatus has been commercially used for the biological control of the thrips Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) in Mediterranean regions (Chambers et al., 1993), O. vicinus is considered to be a potential biological control agent against...
various species of thrips and especially phytophagous mites (Heitmans et al., 1986). There are several studies on the biological parameters and predation abilities of these predators feeding on different prey (Alvarado et al., 1997; Cocuzza et al., 1997; Wearing & Colhoun, 1999; Pehlivan & Atakan, 2017). However, there is little knowledge of their efficiency as predators of B. tabaci and T. urticae with the view of using them as biological control agents (Venzon et al., 2002; Arnö et al., 2008).

Their efficiency in regulating pest populations depends on different biological and behavioural traits. One of the most important methods for evaluating their effectiveness in biological control programs is to determine their response to changes in prey species and densities, namely, their functional responses (Rogers, 1972; Houck & Strauss, 1985). Holling (1965) identified three types of functional response: (i) increasing linear response to increasing prey density, type I response, (ii) initial linear response that reaches a plateau, type II response, and (iii) sigmoidal shaped response with a slow start, type III response. There are functional response studies on anthuricoli bugs to some stages of pests indicate that a type II response is the most often recorded for species of Orius (Coll & Ridgway, 1995; Alvarado et al., 1997; Montserrat et al., 2000; Rutledge & O’Neil, 2005). However, there is no data on the functional responses of O. laevigatus and O. vicinus to different egg densities of B. tabaci and T. urticae.

The aim of this study was to determine the functional response of two species of anthuricoli bugs to different egg densities (2, 4, 6, 8, 16, 32, 64 and 128) of two species of prey over a 24 h period under laboratory conditions. The following topics were addressed: (1) Do the predators in same genus show different types of functional response when feeding on the same prey? (2) How does the functional response of each predator change when they feed on different prey? (3) What is their potential for suppressing pest populations in biological control programs? The results and further investigations might be helpful in estimating the predatory ability of O. laevigatus and O. vicinus when attacking B. tabaci and T. urticae and their value as biological control agents.

**MATERIAL AND METHODS**

**Insect rearing**

Cultures of the predators O. vicinus and O. laevigatus were established in the Laboratory of Entomology (hereafter referred as laboratory) of Cukurova University, Adana, Turkey. These predators were collected from pepper and eggplant crops grown in open fields in the Adana Province, Turkey in 2016. The predators were identified using the identification key of Péricart (1972) and later reared separately in plastic jars (1 l) with a perforated (5 cm diameter) lid and sealed with a mesh cloth to prevent genetic degeneration. Adults and nymphs of these predatory bugs were fed (ad libitum) with frozen eggs of the Mediterranean flour moth, Ephesia kuehniella Zeller (Lepidoptera: Pyralidae) and pollen of Typha latifolia L. (Typhaceae). The moth culture was obtained from the Biological Control Research Institute in Adana in 2016 and maintained in the laboratory. Bean pods of Phaseolus vulgaris L. were provided every other day as a substrate for ovi-position. The bean pods were replaced with fresh ones every two days and the old pods (after oviposition) were transferred to new containers to start a new generation. The predators were reared in a climatic chamber (Nıve TK120) at 25 ± 2°C, 65 ± 5 RH% and under a photoperiod of 14L : 10D.

Nymphs and adults of B. tabaci reared on cotton plants in a controlled room (at 25 ± 2°C, 65 ± 5 RH%, photoperiod of 14L : 10D) in the Department of Plant Protection were used. This culture of B. tabaci was maintained for about 5 years, and twice a year field collected B. tabaci were added to the culture in order to prevent genetic degeneration.

Adults of the red spider mite, T. urticae were collected from pesticide-free strawberry in a semi greenhouse in the Agricultural Research and Implementation Area of Cukurova University, Adana, Turkey. These mites were reared on potted bean plants in wooden-framed mesh cloth cages (1 m × 1 m × 1 m). Potted bean plants in the cages were replaced when necessary. The cages were kept in a controlled room (25 ± 2°C, 65 ± 5 RH%, 14L : 10D photoperiod). *Tetranychus urticae* culture has been maintained in the laboratory for two years.

**Functional response experiments**

Cups (5 cm × 2 cm) were used as experimental units. Fresh bean (*Phaseolus vulgaris* L.) leaf discs (2.5 cm diameter) were placed upside down on wet cotton in the bottom of cups to keep the leaf disc fresh. To ensure maximum predation rates the numbers of eggs of the prey provided were determined in a preliminary experiment. With a fine camel’s hair brush and under a stereomicroscope (Olympus SZS1) (X40), 2, 4, 8, 16, 32, 64 and 128 eggs of both species of prey were transferred onto the leaf discs in separate cups. Within five minutes of transferring the prey, a one-day-old female predator, which had been starved for 24 h was placed on the leaf disc in each cup. The cups were covered with a perforated (2 cm) lid and sealed with a mesh cloth. The cups were randomly placed in the rearing chamber and kept at 25 ± 2°C, 65 ± 5 RH% and under a photoperiod of 14L : 10D. There were between 10–15 replicates per treatment (prey-predator). In total, there were 4 treatments (prey-predator): (i): T. urticae – O. laevigatus, (ii): T. urticae – O. vicinus, (iii): B. tabaci – O. laevigatus and (iv): B. tabaci – O. vicinus. Twenty-four hours later, the number of eggs consumed (Na) were counted with the aid of a stereomicroscope (X40).

**Data analyses**

**t-test**

An independent t-test was used to evaluate differences in the number of eggs consumed by O. laevigatus and O. vicinus at each egg density. The analyses were done using the statistical Package Social Science SPSS (IBM Corp., 2015).

**Functional response analyses**

A logistic regression equation was used to determine the shape of the functional response recorded in each prey-predator interaction. The logistic regression model is suitable for these analyses since the output variable is dichotomous (consumed or uncon-sumed). In addition, the distribution of the error terms of this variable is often binomial rather than normal (Trexler et al., 1988).

This logistic regression equation (1) determined the proportion of prey consumed (Na/No) as a function of the initial prey density (No) (Juliano, 2001).

\[
\frac{N_a}{N_0} = \frac{\exp\left(\sum_{i=1}^{4} P_i N_0^{P_i} + P_0\right)}{1+\exp\left(\sum_{i=1}^{4} P_i N_0^{P_i} + P_0\right)}
\]

The maximum likelihood test was used to estimate the parameters \(P_0\), \(P_1\), \(P_2\), and \(P_3\), which are the intercept, linear, quadratic...
and cubic coefficients, respectively. According to Juliano (2001), if the linear term (\( P \)) is not significantly different from zero, it indicates a type I functional response. Also, if \( P \) is significant <0, this implies that the proportion of prey consumed declines monotonically with \( N_0 \) indicating a type II functional response. On the other hand, if \( P \) is significant >0, then the proportion of prey consumed is positively density-dependent, hence a type III function response.

Knowing the shapes of the functional response curves of the predators, the next step was to determine the parameters of Holling’s disc equation (2). This equation is suitable for estimating the predator attack rate or instantaneous searching rate (\( a \)) and the handling time (\( T_H \)) since the initial egg densities of prey were depleted without replacement (Rogers, 1972). A nonlinear least square regression (NLIN procedure in SPSS ver. 23) was used to estimate these parameters.

\[
N_a = \frac{aTN_0}{1+aTN_0} \tag{2}
\]

Where \( N_a \) is the number of eggs consumed, \( N_0 \) is the initial density of eggs, \( a \) is the predator attack rate or instantaneous searching rate, \( T \) is the time of exposure of predator to prey (\( T = 24 \) h) and \( T_H \) is the handling time. It is worth noting that since all functional response curves were type II, no adjustments to equation 2 were necessary in order to estimate the parameters for type III prey-predator functional response curves.

**RESULTS**

The results obtained from the logistic regression analysis of the functional response experiments had significantly negative \( P \) values indicating that the functional responses of both species of Orius to different densities of the eggs of \( T. urticae \) and \( B. tabaci \) were of type II (Table 1). The proportion of eggs of \( T. urticae \) and \( B. tabaci \) consumed by each predator declined and plateaued above a density of 64 eggs (Fig. 1).

Following the determination of the Type II functional responses, attack rate (\( a \)) and handling time (\( T_H \)) of \( O. victinus \) were estimated using Holling’s Disc Equation (Holling, 1959). The attack rates of \( O. laevigatus \), when fed eggs of \( T. urticae \) and \( B. tabaci \) were 0.972 ± 0.073 h⁻¹ and 1.022 ± 0.085 h⁻¹, respectively, and the handling times (\( T_H \)) were 0.007 ± 0.001 h and 0.002 ± 0.001 h, respectively. For \( O. victinus \), the attack rates were 1.113 ± 0.083 h⁻¹ on \( T. urticae \) and 0.772 ± 0.147 h⁻¹ on \( B. tabaci \) and handling times (\( T_H \)) of \( O. victinus \) were 0.005 ± 0.001 h and 0.006 ± 0.002 h, respectively (Table 2).

The mean number of eggs of \( T. urticae \) and \( B. tabaci \) consumed at different densities by the \( O. victinus \) is given on Table 3. The number eggs of \( T. urticae \) eaten by predators increased and reached a plateau, and there were statistical differences in numbers eaten by the two species of \( O. victinus \) at egg densities 2 and 32. For \( B. tabaci \) eggs, each predator consumed more eggs at higher densities and generally \( O. laevigatus \) and \( O. victinus \) consumed similar numbers of eggs at each of the densities (except 4 eggs). Our findings indicate that predators are not able to consume an infinite number of prey as prey density increases. Consequently, beyond a certain level of prey abundance more predators will be required to keep prey numbers below the economic threshold level.

**DISCUSSION**

Many predators that have been successfully used as biocontrol agents for important pests in greenhouses exhibit a type II response to their prey (Pervez & Omkar, 2006; Xiao & Fadamiro, 2010). Our results clearly indicate that the functional responses of \( O. laevigatus \) and \( O. victinus \) to different densities of the eggs of \( T. urticae \) and \( B. tabaci \) are of type II. There is no study on the functional response of \( O. victinus \) in the literature to the best of our knowledge. Monsterrat et al. (2000) report that \( O. laevigatus \) exhibits Type II responses when fed nymphs of \( T. vaporariorum \) and \( F. occidentalis \). In the literature, there are many studies on different species of \( O. victinus \) preying on greenhouse pests, which report type II functional responses. For instance, \( O. niger \) and \( O. minutus \) (L.) exhibit type II functional responses when fed adults of \( T. urticae \) and 2nd instar individuals of the onion thrips, \( Thrips tabaci \) Lindeman (Thysanoptera: Thripidae) (Fathi & Nouri-Ganbalani, 2010), \( O. albipennis \) (Reuter) fed eggs and 3rd instar nymphs of \( B. tabaci \) (Shahpouri et al., 2019), \( O. victinus \) and \( O. sauteri \) (Poppius) fed adults of \( Megalurothrips susitalis \) (Bagnall) (Thysanoptera: Thripidae) (Liu et al., 2018), \( O. albipennis \) fed adults of \( Megalurothrips sjostedti \) Trybom (Thysanoptera: Thripidae) (Shahpour et al., 2019), and \( O. sauteri \) fed adults of \( Megalurothrips sjostedti \) Trybom (Thysanoptera: Thripidae) (Shahpour et al., 2019).
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dae) (Gitonga et al., 2002) and *Orius tristicolor* (White) fed eggs of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Queiroz et al., 2015). In contrast, *O. albidipennis* and *Orius strigicollis* (Poppius) fed eggs of *T. urticae* (Jalali-
times (T_h) are considered to be more ef
type III functional responses, respectively (Queiroz et al.,
O. tristicolor.

Prey Density (No.) T. urticae O. laevigatus O. vicinus T-test value P values
2 0.80 ± 0.30 1.55 ± 0.168 -2.118 0.048
4 3.29 ± 0.24 2.89 ± 0.47 0.808 0.428
8 6.40 ± 0.36 5.70 ± 0.71 0.958 0.348
16 12.58 ± 0.83 13.0 ± 0.94 -0.325 0.748
32 21.78 ± 2.58 29.60 ± 1.07 -2.819 0.012
64 54.50 ± 3.19 52.38 ± 3.17 0.457 0.654
128 74.00 ± 6.71 72.30 ± 5.11 0.200 0.844

B. tabaci
2 0.80 ± 0.24 0.90 ± 0.23 -0.293 0.773
4 3.10 ± 0.26 1.50 ± 0.36 3.446 0.003
8 5.00 ± 0.62 4.73 ± 0.74 0.277 0.784
16 14.55 ± 0.51 13.91 ± 0.49 0.864 0.398
32 23.90 ± 1.77 25.36 ± 1.82 -0.541 0.595
64 51.10 ± 3.54 42.42 ± 6.14 1.155 0.262
128 69.60 ± 5.95 70.07 ± 9.69 -0.969 0.345

Our results indicate that plant morphology, such as leaf hairs and tri-
chome density, greatly affect the searching ability of preda-
tors (De Clercq et al., 2000; Cedola et al., 2001; Madadi et al.,
Jalalizand et al., 2012; Banihashemi et al., 2017).

In conclusion, this is the first report of the functional re-
responses of O. laevigatus and O. vicinus to the abundance of
two important species of pests, B. tabaci and T. urti-
cae. In Turkey, the parasitoid Eretmocerus mundus Mercet
(Hymenoptera: Aphelinidae) and the predators Macro-
plus melanotoma (Wagner) and M. pygmaeus (Rambur
(Hemiptera: Miridae), are considered to be important natu-
enemies of B. tabaci (Karut et al., 2016). In addition,
there are many predatory mites, especially those belonging
to family Phytoseiidae (Şekeroglu & Kazak, 1993; Attia et al.,
2013), which have been released against T. urticae.

With respect to our results, the expectation is that both of
these predators can be used as biological control agents of
whiteflies and two spotted mites as they are likely to main-
ly attack their eggs. The reproduction and foraging abili-
ties of these predators when attacking the above mentioned
pests have not been extensively investigated. In addition,
these experiments were done in the laboratory in small are-
nas that are very different from natural conditions. Thus,
other studies regarding the biological parameters and
behavioural responses of these predators when attacking
these pests are needed in order to clearly understand their
potential capacity in terms of biological control.

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Table 3. Mean (±SE) numbers of eggs of Tetranychus urticae and
Bemisia tabaci consumed by Orius laevigatus and Orius vicinus.

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