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ORIGINAL ARTICLE

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 efficient 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 (Nachman & 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; Arnó 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 anthocorid 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 anthocorids to different egg densities (2, 4, 6, 8, 16, 32, 64 and 128) of two species of prey over a 24h 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 lid (5 cm diameter) and sealed with mesh cloth for ventilation and preventing escape, respectively. Adults and nymphs of these predatory bugs were fed (ad libitum) with frozen eggs of the Mediterranean flour moth, *Ephestia 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 oviposition. 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 (Nüve 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 (1m × 1m × 1m). 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 SZ51) (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 the 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 unconsumed). 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).

$$\frac{N_a}{N_0}=\frac{\exp\left(P_0+P_1N_0^1+P_2N_0^2+P_3N_0^3\right)}{1+\exp\left(P_0+P_1N_0^1+P_2N_0^2+P_3N_0^3\right)} \qquad (1)$$

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_1) is not significantly different from zero, it indicates a type I functional response. Also, if P_1 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_1 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.

$$N_a=\frac{aTN_0}{1+aT_hN_0} \quad (2)$$

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_1 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 *Orius* spp. 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. vicinus*, 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. vicinus* were 0.005 ± 0.001 h and 0.006 ± 0.002 h, respectively (Table 2).

Table 1. Maximum-likelihood estimates based on the logistic regressions of the proportion of *Tetranychus urticae* and *Bemisia tabaci* eggs eaten by *Orius laevigatus* and *Orius vicinus* at different initial densities of prey.

Predator	Prey	Coefficients	Estimates	S.E.	χ^2	p-value
O. laevigatus	*T. urticae*	Intercept	–0.914	0.153	35.42	<0.0001
		Linear	–0.021	0.006	14.133	<0.0001
		Quadratic	0.00	0.00	25.45	<0.0001
	B. tabaci	Intercept	–0.758	0.264	8.228	0.004
		Linear	–0.033	0.022	2.422	0.020
		Quadratic	0.00	0.00	1.242	0.265
		Cubic	0.00	0.00	0.400	0.527
O. vicinus	*T. urticae*	Intercept	–0.271	0.277	0.96	0.329
		Linear	–0.127	0.025	25.66	<0.0001
		Quadratic	0.002	0.00	22.21	<0.0001
		Cubic	0.00	0.00	17.99	<0.0001
	B. tabaci	Intercept	0.428	0.243	3.09	0.078
		Linear	–0.138	0.021	45.14	<0.0001
		Quadratic	0.003	0.00	47.87	<0.0001
		Cubic	0.00	0.00	47.18	<0.0001

The mean number of eggs of *T. urticae* and *B. tabaci* consumed at different densities by the *Orius* spp. 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 *Orius* spp. at egg densities 2 and 32. For *B. tabaci* eggs, each predator consumed more eggs at higher densities and generally *O. laevigatus* and *O. vicinus* 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. vicinus* 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. vicinus* 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 *Orius* preying on greenhouse pests, which report type II functional responses. For instance, *O. niger* and *Orius 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), *Orius albidipennis* (Reuter) fed eggs and 3rd instar nymphs of *B. tabaci* (Shahpouri et al., 2019), *Orius sauteri* (Poppius) fed adults of *Megalurothrips usitatus* (Bagnall) (Thysanoptera: Thripidae) (Liu et al., 2018), *O. albidipennis* fed adults of *Megalurothrips sjostedji* Trybom (Thysanoptera: Thripi-

Table 2. Estimated (mean ± SE) attack rates (a) and handling times (Th) of females of *Orius laevigatus* and *Orius vicinus* of eggs of *Tetranychus urticae* and *Bemisia tabaci.*

Predator	Prey	Parameters	Estimate ± SE	Asymptotic 95% CI		R^2
				Lower	Upper	
O. laevigatus	*T. urticae*	a	0.972 ± 0.073	0.790	1.154	0.90
		T_h	0.007 ± 0.001	0.005	0.008	
	B. tabaci	a	1.022 ± 0.085	0.806	1.239	0.88
		T_h	0.002 ± 0.001	0.001	0.006	
O. vicinus	*T. urticae*	a	1.113 ± 0.083	0.917	1.310	0.925
		T_h	0.005 ± 0.001	0.003	0.007	
	B. tabaci	a	0.772 ± 0.147	0.540	1.003	0.784
		T_h	0.006 ± 0.002	0.004	0.009	

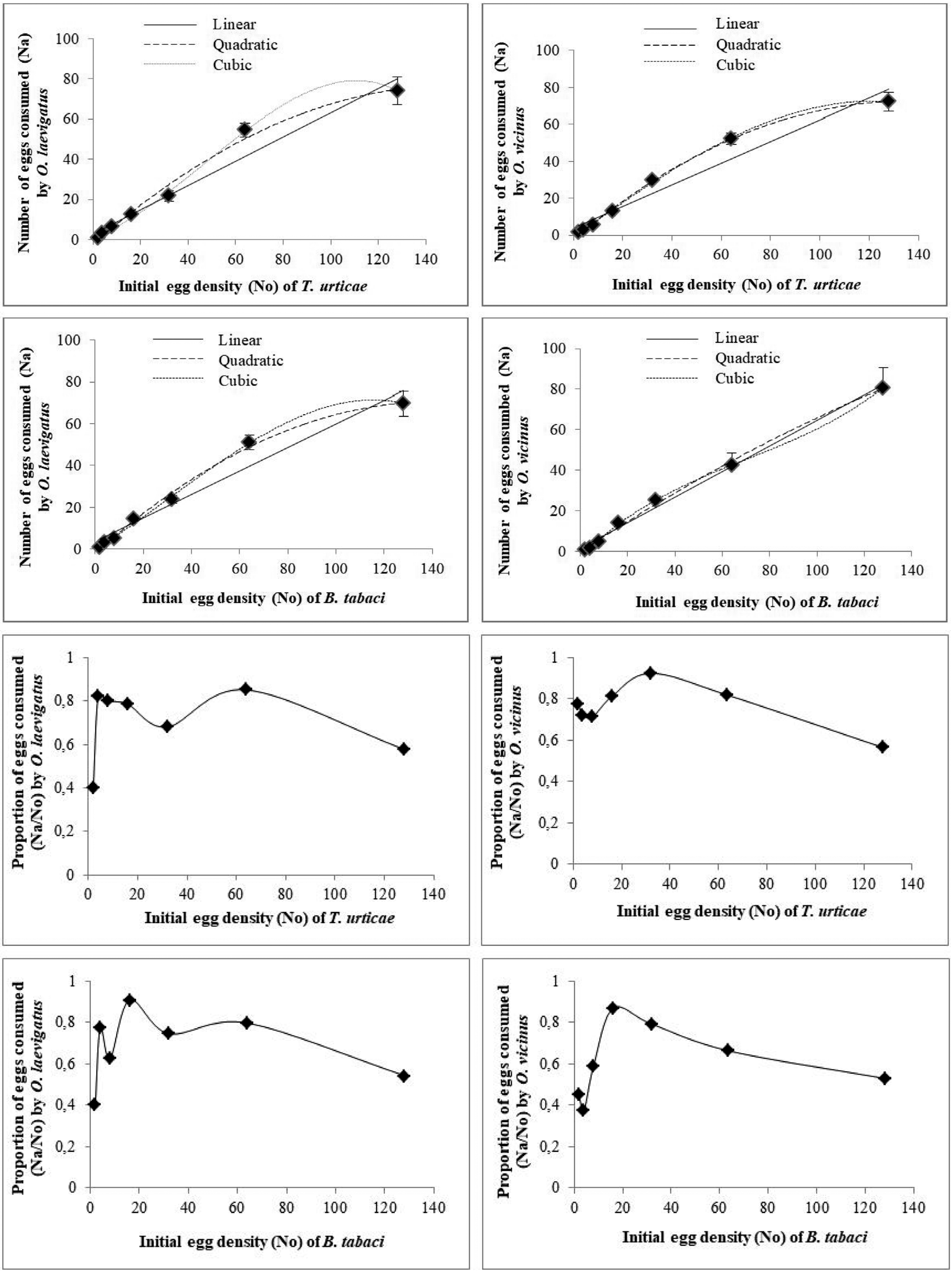

Fig. 1. Type II Functional responses and proportion of eggs consumed by *Orius laevigatus* and *Orius vicinus* when provided with different densities of the eggs of *Tetranychus urticae* and *Bemisia tabaci*.

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-

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

Prey	Density (No.)	*O. laevigatus*	*O. vicinus*	t-test value	*P* values
T. urticae	2	0.80 ± 0.30	1.55 ± 0.158	–2.118	0.048
	4	3.29 ± 0.24	2.89 ± 0.47	0.808	0.428
	8	6.40 ± 0.38	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

zand et al., 2012; Tuan et al., 2016) and *O. albidipennis* fed eggs of *T. absoluta* (Salehi et al., 2016) exhibit type III functional responses. This may be a consequence of using different species of prey, or *Orius,* or host plants in these studies. In addition, Jalalizand et al. (2012) report that *O. albidipennis* shows a type III functional response when fed *T. urticae* eggs, but these authors used two different host plants, strawberry and cucumber, which could affect predator foraging behaviour. Furthermore, when *T. absoluta* eggs are provided to two different species of *Orius*, *O. tristicolor* and *O. albidipennis,* they exhibit type II and type III functional responses, respectively (Queiroz et al., 2015; Salehi et al., 2016).

Predators with high attack rates (a) and low handling times (T_h) are considered to be more efficient biological control agents of pests (Fathi & Nouri-Ganbalani, 2010; Salehi et al., 2016). Our results indicate that *O. laevigatus* is more effective than *O. vicinus* in terms of the number of eggs of *B. tabaci* eaten, whereas *O. vicinus* had a higher predation rate when fed eggs of *T. urticae*. According to Heitmans et al. (1986) *O. vicinus* is considered to be a potential biological control agent of phytophagous mites. In addition, some researchers report that *O. laevigatus* prefers mixed stages of *T. urticae* and *B. tabaci* to species of thrips (Venzon et al., 2002; Arnó et al., 2008). This could be due the mites and whiteflies having stages (e.g. eggs, larvae or nymphs) that are more vulnerable to attack by predators. When prey are immobile, such as eggs or pupae, the predation rates can be high under natural field conditions (Andow, 1990; Cook et al., 1996) . In this study, the mean consumption rates of *O. vicinus* were higher than *O. laevigatus* at eggs densities of *T. urticae* of 2 and 32, whereas *O. laevigatus* consumed more *B. tabaci* eggs when provided with 4 eggs. Tuan et al. (2016) report that *O. strigicollis* show similar consumption rates of *T. urticae* eggs at the highest densities. In contrast, *O. albidipennis* consume fewer *T. urticae* (Jalalizand et al., 2012) and *B. tabaci* eggs (Shahpouri et al., 2019). The differences may be due to the different morphologies of the host plants. Many authors indicate that plant morphology, such as leaf hairs and trichome density, greatly affect the searching ability of predators (De Clercq et al., 2000; Cedola et al., 2001; Madadi et al., 2007; Jalalizand et al., 2012; Banihashemi et al., 2017).

In conclusion, this is the first report of the functional responses of *O. laevigatus* and *O. vicinus* to the abundance of two important species of pests, *B. tabaci* and *T. urticae*. In Turkey, the parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) and the predators *Macrolophus melanotoma* (Wagner) and *M. pygmaeus* (Rambur) (Hemiptera: Miridae), are considered to be important natural 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 mainly attack their eggs. The reproduction and foraging abilities 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 arenas that are very different from natural conditions. Thus, further studies regarding the biological parameters and behavioural responses of these predators when attacking these preys are needed in order to clearly understand their potential capacity in terms of biological control.

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