

## FUNCTIONAL RESPONSE OF THE COCCINELLID PREDATOR *Stethorus gilvifrons* MULSANT FEEDING ON THE TWO SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH: THE EFFECT OF DIFFERENT PREY STAGES

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### ABSTRACT

The functional response of a ladybeetle, *Stethorus gilvifrons* Mulsant, to increasing density of two spotted spider mite, *Tetranychus urticae* Koch, was of the curvilinear shape depicting Holling's type II response with larval stage being the most voracious stage when compared with nymphal and adult stages. Results of the maximum likelihood analyses confirmed this type. By plotting a type II functional response model and polynomial logistic regression model to the numbers and proportions of consumed stages of *T. urticae*, respectively, the resulting regression lines fitted the data well. A sensitivity analysis of the functional response model shows the crucial effect of prey stages on predation rates by *S. gilvifrons*. Theoretically, based on the lowest handling time for *S. gilvifrons* on larval stage of *T. urticae*, the maximum numbers of mites that could be consumed by a single female of *S. gilvifrons* within 24 h period were 77.42 larvae. Based on the inversely density-dependent predation rate for *S. gilvifrons* on different prey stages, it seems to be more effective in low prey densities and this result has to be considered in future inundative biological control programs. These results might be a better strategy for developing mass rearing of *S. gilvifrons* and biocontrol program of *T. urticae*.

### INTRODUCTION

Approximately 90 worldwide species of the tribe Stethorini Dobzhansky (genera: *Stethorus* Weise and *Parastethorus* Pang and Mao) are the only specialist mite predators in the Coccinellidae. Various species of Stethorini have received considerable attention over the last five decades because of their potential as biological control agents of spider mites. At least 12 species of Stethorini have been imported into the US for this purpose (Gordon, 1985) and many more have been purposefully redistributed throughout the world. Chazeau (1985) summarized information on Stethorini general biology and reported that 40% of the 68 species attacked spider mites of economic importance.

*Stethorus gilvifrons* Mulsant (Coleoptera: Coccinellidae) is a biological control agent of the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), which is a serious pest of different crops and ornamental plants over the world (Takafuji *et al.*, 2000; Sim *et al.*, 2003; Zhang, 2003; Alatawi *et al.*, 2005). It feeds voraciously on different growth stages of spider mites (*Tetranychidae*) and the closely related Tenuipalpidae, which are known as false spider mites or flat mites (Chazeau, 1985; Ahmed and Ahmed, 1988; Afshari, 1999).

Prior to the release of natural enemy in a biological control program, it is essential to evaluate its efficiency under laboratory conditions. One informative method for assessing the efficiency of natural enemies is the study of their foraging behavior including functional response (Fathipour *et al.* 2006; Bayoumy 2009). Functional response is the number of preys successfully consumed per predator as a function of prey density (Solomon 1949). It is a commonly measured attribute of natural enemies. Practitioners of inundative biological control may use functional response to estimate the appropriate numbers of bio-control agents to be released in order to bring about an immediate reduction in pest numbers (Mills and Laca 2004). Holling (1965) proposed three types of functional responses: type I, a linear rise to a plateau; type II, a curvilinear rise to a plateau; and type III, a sigmoid curve rising to a plateau which then levels off under the influence of handling time or satiation (Hassell 2000). Among the types of functional responses, type II and III have received the most attention (Allahyari *et al.* 2004).

Many early studies of various species of Stethorini concluded that they are “high density predators” unable to regulate spider mite populations at low densities (Fleschner, 1950; Bailey and Caon, 1986; Tanigoshi and McMurtry, 1977). Congdon *et al.* (1993) challenged this “high density predator” designation and asserted that high density spider mite populations have become common only since the advent of synthetic insecticides (Huffaker *et al.*, 1970). Although some bionomic studies on *S. gilvifrons* have been conducted by Haji-Zadeh *et al.* (1993) and Afshari (1999), no detailed study has reported on the influence of *T. urticae* stages on its functional responses. Only Sohrabi and Shishehbor (2007) reported a type III response of *S. gilvifrons* on *Tetranychus turkestanii* Ugarov and Nikolski. Therefore, the objective of this study is to quantify the effect of prey stage densities on consumption rate of *S. gilvifrons* for improving our understanding of prey-predator interaction and to know some information about the prey stage which could be maximizing mass rearing program for this predator. Thus, this study might lead to develop a better strategy for mass rearing of *S. gilvifrons* and biological control of *T. urticae*.

## **MATERIALS AND METHODS**

### **Stock culture of *Tetranychus urticae* and *Stethorus gilvifrons***

The two spotted spider mite *T. urticae*, used as prey for the coccinellid predator *S. gilvifrons*, was reared on the whole plants of potted beans, *Phaseolus vulgaris* L. at room conditions of 26±3, 65±10% rh, and 16:8 (L:D) photoperiod. The initial population of *S. gilvifrons* was originated from castor plants (*Ricinus communis* L.) in the pupal stage at Mansoura district, Egypt. The collected pupae were kept in Eppendorf tubes and maintained in the incubator at 25±1 and 16:8 (L:D) photoperiod until adult emergence. The stock culture of *S. gilvifrons* was then started using all stages of *T. urticae* on castor leaves. One leaf disc placed upside-down on water saturated cotton (2 cm thick) in a plastic box (20×10×5 cm). Water was added daily to cover the base of the box to prevent the mites from escaping. A surplus of all stages of

*T. urticae* was brushed daily onto the leaf arenas using a soft brush and funnel. The stock culture was maintained in the incubator at  $25 \pm 1$  °C,  $65 \pm 10\%$  RH., and 16:8 (L:D) photoperiod. The leaf discs were renewed once a week. The predatory mites used in the experiment were reared for two successive generations prior to this study (*i.e.*, functional response).

#### **General experimental conditions**

Functional response test was carried out into the modified Huffaker cell (Sabelis, 1981 and Overneer, 1985) provided with castor leaf disc as an experimental arena. The tested densities of *T. urticae* for *S. gilvifrons* were transferred from potted bean leaves to castor leaf disc using a soft brush. The functional response test was conducted at  $25 \pm 1$  °C,  $65 \pm 10\%$  RH and 16:8 (L:D) photoperiod. Three days old females of *S. gilvifrons* were used in the current test. Prior to the functional response test, the predators were collected from the stock culture in their pupal stage and placed in Petri dishes. The emerged adults were provided with *T. urticae* as a source for food for 48 h, then separated individually in Eppendorf tubes and starved for another 24 h before being used in the experiment to standardize their hunger.

#### **Functional response**

In order to test the response of *S. gilvifrons* to change in the prey stage density of the two spotted spider mite, individual predators (72 h old) were exposed to ten prey density levels (*viz.*, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100) of each tested stage (*i.e.*, larva, nymph and adult) for 24 h. In case of adult mite females, newly females were transferred directly from the stock culture to the experimental arena to avoid reproduction of ovipositing females in the experimental arena. Fifteen replications of each prey density were set up simultaneously. After 24 h, the predators removed from the experimental arenas and the live mites left in the experimental arena were counted to determine the number of aphids consumed.. Only replicates with the predator surviving after 24 h exposure time were considered.

#### **Data analysis**

Data from functional response experiments were used to establish the type and parameters of functional response using the approach developed by Juliano (2001). This approach includes two sequential analyses. Initially, the shape (type) of functional response must be determined, typically by determining if the data fit a type II or III functional response. A polynomial logistic regression of the proportion of attacked preys ( $N_a/N_0$ ) vs. the initial number of prey offered ( $N_0$ ) is established for distinguishing between both types. The curves of  $N_a/N_0$  vs.  $N_0$  when the response is a type II, have the shape of a negative exponential (proportion of attacked preys decaying exponentially) and when the response is a type III have a dome-shape, with a maximal of proportion attacked and then a continuous decline. These curves may both be fit by quadratic (or higher order) polynomials expressions. In the type II curve, the linear ( $P_1$  in Eq. 1) and quadratic ( $P_2$  in Eq. 1) term would be negative (initially decreasing) whereas in the type III curve the linear term would be positive (initially increasing) and the quadratic term negative (Trexler *et al.*, 1988; Juliano, 2001). Thus, one criterion for separating type I to III functional responses is to analyze the proportion of prey attacked and to test for significant positive or negative linear coefficients from Eq. 1. Linear

term not significantly different from 0 indicates a type I functional response, a significant negative indicates a type II response, and a significant positive indicates a type III (Juliano 1993). The second part of the analyses uses a non-linear least squares method to obtain estimates of the functional response parameters (Juliano, 2001).

Relationship between proportion of prey attacked and density of prey offered were used, initially, to perform logistic regression using the polynomial function:

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

where  $N_a$  is the number of prey attacked,  $N_0$  is the initial prey density, and the parameters  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the constant, linear, quadratic, and cubic parameters, respectively, related to the slope of the curve. Maximum likelihood estimates of parameters  $P_0$  to  $P_3$  were obtained by applying logistic regression (CATMOD procedure in SAS software) (SAS, 2003) to a dichotomous variable that equaled 0 for surviving preys and 1 for attacked preys. As in many cases a cubic or higher order expression are necessary for good fits.

Once the functional response type was determined from logistic regression analyses and log likelihood-ratio tests, iterative non-linear least squares regression (NLIN procedure with Proc method of SAS) (SAS, 2003) was used to estimate the parameters of Holling's disc equation (Eq. 2) (Holling, 1959).

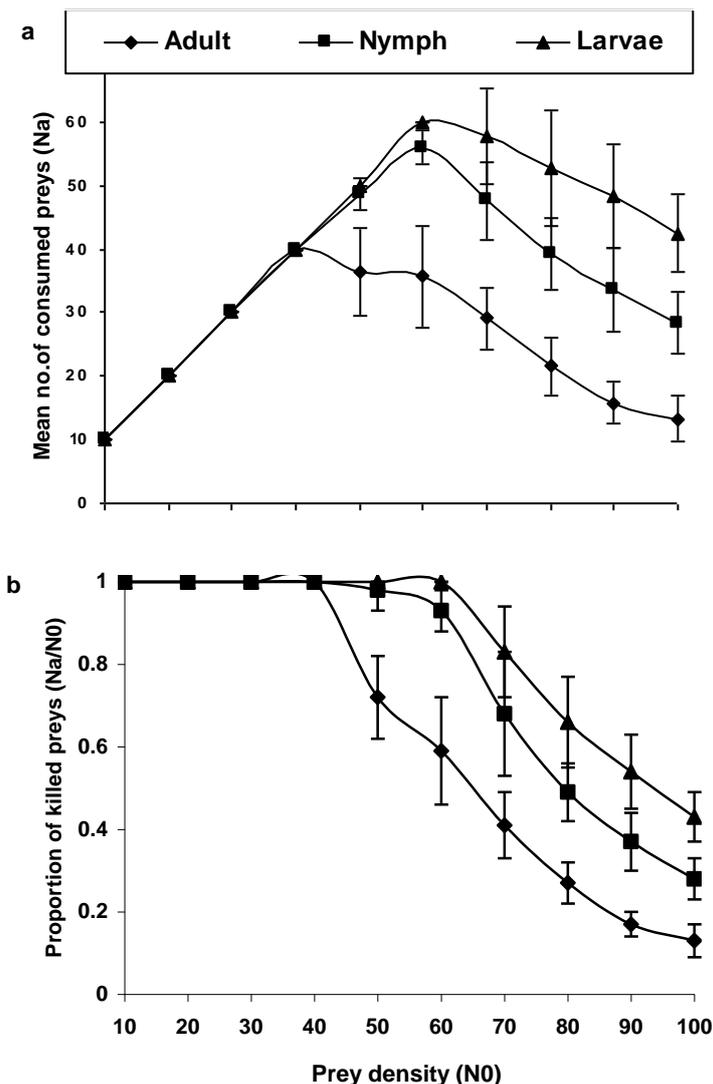
$$N_a = a T N_0 / 1 + a T_h N_0 \quad (2)$$

where  $T$  is the time of exposure (24 h),  $a$  is the rate of successful attack, and  $T_h$  is the handling time.

Values of  $T_h$ , estimated by non-linear least squares regression, were used to calculate maximum attack rate as  $T/T_h$  (Hassel, 2000), that represent the maximal number of preys that could be consumed by a predator of *S. gilvifrons* during 24 h.

## RESULTS

Prey consumption by *S. gilvifrons* increased from 10 to 60, 10 to 55.93, and 10 to 35.73 individuals with increase in density of larvae, nymphs, and adults of *T. urticae* from 10 to 60, 10 to 60, and 10 to 50, respectively. In contrast, the proportion of killed preys by *S. gilvifrons* decreased from 1 to 0.43, 1 to 0.28, and 1 to 0.13 with increase in density of larvae, nymphs, and adults of *T. urticae* from 10 to 100, respectively. The increase in prey consumption and the decrease in proportion of consumed preys by the above predator with increased densities of prey stages were curvilinear in shape (fig. 1).



**Fig. 1: Mean numbers (a) and proportions (b) of different stages of *T. urticae* consumed by a single female of *S. gilvifrons* ( $N_a/P$ ) in relation to different prey densities ( $N_0/P$ ) determined in the experiments.**

The accelerated decrease in the proportion of consumed stages of *T. urticae* in relation to their density best-fitted the description of a type II functional response. Results of the maximum likelihood analyses confirmed this observation (Table 1).

**Table 1: Maximum likelihood estimates from logistic regression of the proportion of different stages of *T. urticae* consumed by *S. gilvifrons* as a function of initial prey density.**

Prey stages	Parameter	Estimate	SE	Chi-Square ( $\chi^2$ )	P-value
Larval	Intercept	131.2	17.23	58.01	<0.0001
	Linear ( $N_0$ )	-4.426	0.62	50.44	<0.0001
	Quadratic ( $N_{02}$ )	0.0501	0.0075	45.24	0.002
	Cubic ( $N_{03}$ )	-0.00019	0.000029	41.67	0.02
Nymphal	Intercept	31.2786	5.6602	30.54	<0.0001
	Linear ( $N_0$ )	-0.8991	0.2203	16.65	<0.0001
	Quadratic ( $N_{02}$ )	0.00865	0.00282	9.43	0.002
	Cubic ( $N_{03}$ )	-0.00003	0.000012	5.92	0.02
Adult	Intercept	21.6752	2.1967	97.36	<0.0001
	Linear ( $N_0$ )	-0.7456	0.0952	61.36	<0.0001
	Quadratic ( $N_{02}$ )	0.00856	0.00134	40.86	<0.0001
	Cubic ( $N_{03}$ )	-0.00003	0.000006	32.15	<0.0001

A significant negative estimate for the parameter  $N_0$  indicates that the slope of the functional response curve is declining, thus a type II functional response

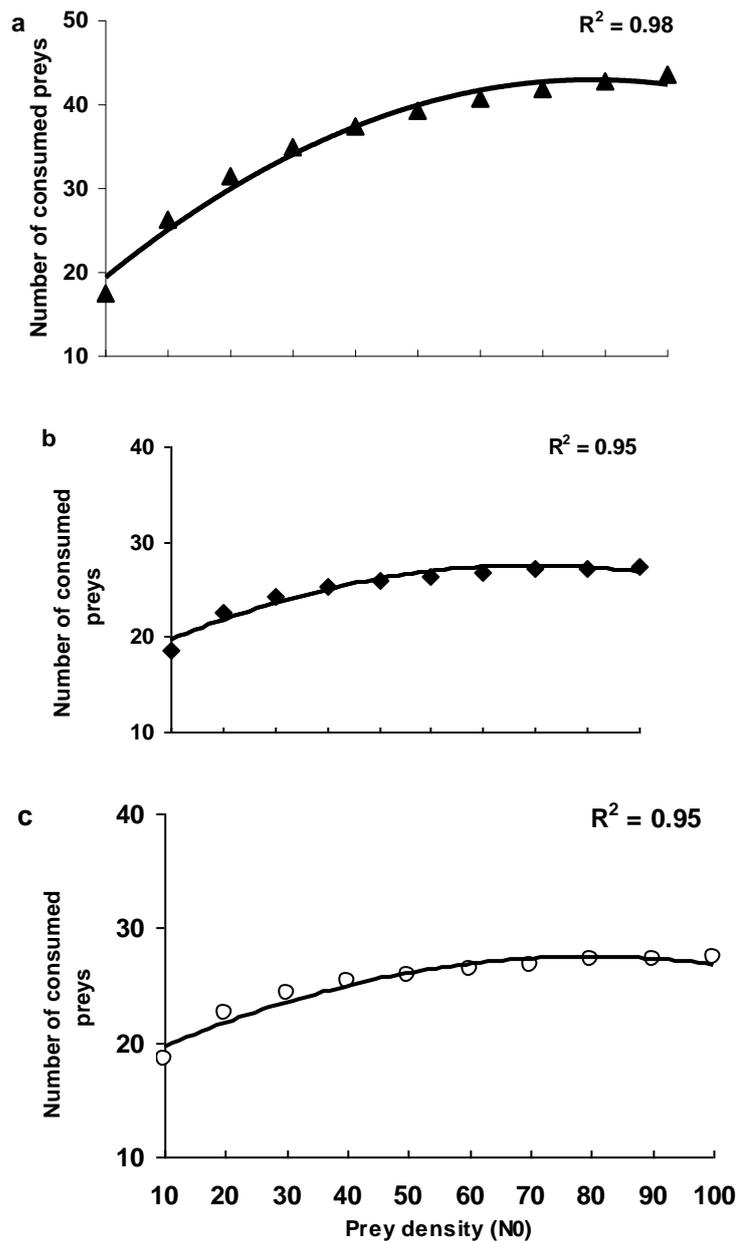
For *S. gilvifrons* consuming different stages of *T. urticae*, the significantly negative parameters of  $N_0$  suggested that the slopes of the functional response curves were declining, which is characteristics of a type II functional response. By plotting a type II functional response model and polynomial logistic regression model to the number and proportions of consumed stages of *T. urticae*, respectively, the resulting regression lines fitted the data well (fig. 2).

The highest instantaneous rate of attack ( $a$ ) and the lowest handling time ( $T_h$ ) for *S. gilvifrons* were correlated with adult and larval stages of *T. urticae*, respectively (Table 2).

**Table 2: Parameter estimates (95% confidence interval) of the attack constant [ $a$  ( $h^{-1}$ )], handling time [ $T_h$  (h)], and maximum number of killed preys ( $T/T_h$ ) for *Stethorus gilvifrons* females preying different stages of *Tetranychus urticae* derived from Holling's disc equation.**

Prey stages	a	Asymptotic 95% CI		$T_h$	Asymptotic 95% CI		$T/T_h$
		lower	upper		lower	upper	
Larval	0.08±0.008	0.0673	0.1003	0.31±0.02	0.2752	0.3528	77.42
Nymphal	0.11±0.02	0.0753	0.1493	0.46±0.03	0.4050	0.5182	52.17
Adult	0.214±0.09	0.0432	0.3843	0.83±0.05	0.7250	0.9264	28.92

Theoretically, based on the lowest handling time for *S. gilvifrons* on larval stage of *T. urticae*, the maximum numbers of *T. urticae* that could be consumed by a single female of *S. gilvifrons* within 24 h period were 77.42 larvae ( $24 h/T_h$ ).



**Fig. 2:** Functional response of *S. gilvifrons* to different densities ( $N_0/P$ ) of *T. urticae* (a: larva; b: nymphal I; c: adult stage) predicated by the Holling's model. The lines represent the best-fitted type II functional response curves of the predator feeding on larval, nymphal and adult stages ( $r^2=0.95, 0.98, \text{ and } 0.95$ , respectively).

## DISCUSSIONS

The exactness of functional response as a comparison tool in above mentioned fields is highly related to models and data analysis; the use of inappropriate models and methods of analysis may result in an incorrect estimation. The response of *S. gilvifrons* to stages was characterized by a reduction in the proportion of prey consumed with increasing prey density, reaching a minimum consumption of ca. 0.43, 0.28, and 0.13 for larvae, nymphs, and adults of *T. urticae* at the highest prey density (100 *T. urticae*). Decreasing in proportion of prey consumed with increasing prey density is common for arthropods predator as a result of satiation level and handling time required for each prey item consumed (Holling, 1961).

For each density, predation by *S. gilvifrons* varied between the stages of *T. urticae*. At density of 10, 20, 30, and 40 *T. urticae*, all individuals were consumed, which suggests that prey density was below the level necessary for survival. At prey density of 50, the proportion of prey consumed was 0.82 and 0.98, and 0.73 on the larvae, nymphs, and adults of *T. urticae*, respectively. Each adult female may consume 30–60 mites per day for *Stethorus madecassus* Chazeau (Chazeau, 1974a), *S. punctum* (LeConte) (Tanigoshi and McMurtry, 1977), *S. punctillum* Weise (Roy *et al.*, 2003), *S. japonicus* Kamiya (Mori *et al.*, 2005), and *S. tridens* Gordon (Fiaboe *et al.*, 2007). According to Houck (1991), the feeding behaviour of *Stethorus* varies with prey density and the obtained results found agreed with this statement. At higher prey densities, the predators spend less time searching the prey resulting in higher prey consumption than in lower densities but predators require time to attack the prey.

The type of functional response and estimated parameters for a natural enemy could be affected by some factors such as host plant. Temperature and type of prey or host and prey stages (Eveleigh and Chant, 1981; Coll and Ridgway, 1995; Nwilene and Nachman, 1996; Wang and Ferro, 1998; De Clercq *et al.*, 2000; Mohaghegh *et al.*, 2001). Although the type of functional response for *S. gilvifrons* did not alter among the different stages of *T. urticae* in this study, the effects of these factors are reflected in the values of the attack rate and the handling time.

Although the logistic regression determined the shape of the functional responses of *S. gilvifrons* to be a type II response and significant models could be fitted, the parameter estimates of these functional responses were not always significantly different from zero. The lack of significant parameters complicates ecological interpretation of the estimated functional responses. According to Juliano (2003) the best guide to distinguish between the type II and type III response is to observe the shape of the fitted curve. In the current study, the shapes of the type II functional response models fitted the data very well. In all type II response models a plateau appears to be reached at the higher prey densities. However, the number of prey consumed at this plateau was quite low. This low predation rate may be related to the long handling times.

A type II functional response is the most common type reported for most coccinellid predators (Isikber, 2005). The significant negative values for the linear parameter obtained in this study confirm the type II response for *S. gilvifrons* on all tested prey stages. The obtained, type II functional response for *S. gilvifrons* was similarly reported for *Stethorus vagans* (Blackburn) (Ullah, 2000), *S. punctum* (Hull *et al.*, 1977), *S. japonicus* (Gotoh *et al.*, 2004), and *S. tridens* (Britto *et al.*, 2009), all preying on *T. urticae*.

Although Holling's disc equation (1959) is widely used to estimate parameters of type II functional response, some authors emphasize on the limitation of Holling's disc equation, and suggest Roger's random predator equation (1972) as an alternative, which is more appropriate when re-encounter (for a parasitoid) or prey depletion (for a predator) may be occurs during the experiment (Juliano, 2001). Holling's disc equation can be used only when Roger's model does not enable the researcher to estimate valid parameters ( $b$  and  $T_h$ ). For example, Mohaghegh *et al.* (2001) used Holling's model because Rogers's model provides invalid parameters for their data set. Similarly, Holling's model provided valid parameters for our data set. We first used the Roger's random equation but the parameters estimated using this model were theoretically unappreciated ( $b$  and  $T_h$  were overestimated).

Handling time of *S. gilvifrons* estimated in this study on *T. urticae* larvae (0.31 h) is shorter than that of 0.46 and 0.83 h on *T. urticae* nymphs and adults. However, the highest attack rate for *S. gilvifrons* was found on *T. urticae* adults. This may be due to *S. gilvifrons* at high prey densities continued to feed even when satiated and continued to attack mites, initiate feeding, but then subsequently abandon the prey with minimal attack of bodily parts. However, it takes a long time in dealing with each adult individual of prey. This may be due to the bigger size and faster movement of prey adult than other immature stages. The ratio between exposure time and handling time ( $T/T_h$ ) is an indicator of maximum predation. Based on the lowest handling time for *S. gilvifrons* on larval stage of *T. urticae* the maximum number of consumed preys (per day) by *S. gilvifrons* is 77.42 larvae of *T. urticae*. The handling time and the maximal number of consumed preys ( $T/T_h$ ) of *T. urticae* nymphs by *S. gilvifrons* in our study is similar to that reported for the same predator on *T. turkestanii* nymphs (Sohrabi and Shishehbor, 2007).

According to Berry *et al.* (1988) several problems are associated with estimating functional responses in the laboratory. Predators are not allowed to leave a patch in search of higher densities where prey location is more efficient, and as a consequence predators consume more prey at low density than they would probably do in nature (Van Lenteren and Bakker, 1976). Moreover, the length of the experimental period may be critical (Eveleigh and Chant, 1981). Such studies are, however, useful in providing the first step to compare the efficiency of a natural enemy against different prey species or stages and to provide a valid means of comparing host finding abilities of candidate natural enemies (Munyaneza and Obrycki, 1997)

Hence, releasing the predator early in the beginning of growing season on low prey populations might be contributed toward maximizing the efficiency of pest control. The failure of coccinellids in controlling the mites at

higher population densities could be attributed to the low oviposition rate of predator at these densities, which reflects the desire to reduce intraguild competition and leads to lower chances of establishment of a viable colony of the predator at higher prey densities (Mandour, unpublished).

The results show that *S. gilvifrons* females increase predation rate as function of *T. urticae* larva densities. This fact, together with lower handling time for the coccinellid predator on prey larvae indicates that *S. gilvifrons* could be consumed high numbers of prey larvae per day. Therefore, the best time to release the predator in the field is being when the dominate prey population is larvae.

#### **Acknowledgements**

I would to thanks Prof Dr. S. A. Juliano (USA) and M. H. Bayoumy (Economic entomology Dept. for helping in statical analyses. Also, I thank Prof Dr. A. A. Osman (Economic entomology & Agric. Zoology Dept.) Menofia University for helpful insights and critical reviews of the manuscript.

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**الاستجابة الوظيفية للمفترس الحشري *Stethorus gilvifrons* عند تغذيته على العنكبوت الأحمر : تأثير الأطوار المختلفة من العنكبوت الأحمر**  
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الاستجابة الوظيفية للمفترس الحشري *Stethorus gilvifrons* لزيادة تعداد العنكبوت الأحمر أعطت منحنى خطى يوضح الاستجابة من النوع الثانى الخاص بهولنج للمفترس الحشري مع تفضيل لطور اليرقة مقارنة بطور الحورية والطور الكامل. نتائج تحليل الاحتمال أكدت ذلك النوع من الاستجابة وتطبيق النموذج الرياضى الخاص بالاستجابة الوظيفية من النوع الثانى والانحدار المتعدد على أعداد ونسب الأطوار المفترسة بواسطة المفترس على التوالي ، فان خطوط الانحدار الناتجة أظهرت النتائج والنموذج المتصل عليه بصورة جيدة. والتحليل الدقيق للاستجابة الوظيفية أوضح التأثير الحرج لأطوار الفريسة على معدلات الافتراس بواسطة المفترس الحشري . نظرياً بناء على وقت الاداء المنخفض للمفترس الحشري على طور اليرقة للعنكبوت الأحمر فان أقصى عدد من العنكبوت يمكن أن يستهلك بواسطة أنثى واحدة من المفترس الحشري خلال 24 ساعة كان 77.42 يرقة. وهـه النتائج ربما تكون استراتيجية مفيدة لتطوير برامج الانتاج الكمى للمفترس الحشري أو المكافحة الحيوية للعنكبوت الأحمر دوالبقعتين

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