

***Phytoseiulus persimilis* (Acari: Phytoseiidae) Functional Response in the Biological Control of *Tetranychus urticae* (Acari: Tetranychidae) Eggs on Rose Foliage Discs¹**

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J. Entomol. Sci. 54(3): 275–287 (July 2019)

Abstract The functional response of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) preying on *Tetranychus urticae* Koch (Acari: Tetranychidae) eggs on leaf discs of three varieties of rosebush was determined at eight time intervals (0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–24, and 0–24 h). Some differences in handling time (Th) and attack rate (a') among the three plant varieties (Royalty, Pareo, and Starlite) were observed, but the functional response on the three varieties shifted from Type II to Type III at three time intervals within the 24-h observation. The observed shift in functional response was likely due to different rates of attack and handling times linked to the prey and the plant variety.

Key Words functional response, biological control, *Phytoseiulus persimilis*, *Tetranychus urticae*

Roses, *Rosa hybrida* L. (Rosaceae), are highly decorative bushes that are extremely valuable in urban landscapes, especially at flowering. Rosebushes are the most popular perennial flowering plants in almost every country (Jaskiewicz 2006, Bidarnamania et al. 2015). In Mexico, the production of roses represents an annual economic benefit of about 1,639 million of Mexican pesos. Likewise, about 2,935 ton of roses are exported to the United States annually with a calculated value of \$8.1 million USD (SAGARPA–SIAP 2017). These plants are susceptible to the attack of pests and diseases that reduce the growth and quality of the flowers (Golizadeh et al. 2017).

Tetranychus urticae Koch (Acari: Tetranychidae) has a broad range of hosts and is an important pest of several crops worldwide (Tehri 2014). Indeed, *T. urticae* is a major pest of open-field and greenhouse crops, including ornamentals, annual plants, and perennial plants (Grbic et al. 2011). Furthermore, *T. urticae* is one of the

¹Received 6 July 2018; accepted for publication 14 August 2018.

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pests with the highest economic impact on rose production (Khajehali et al. 2011). Conventional chemical control has been the common method of control (Badii et al. 2004); however, the pest develops resistance to miticides quickly, due to its strong reproductive potential, its short generation time, its haploid-diploid sex determination system, and its low scattering capacity that limits the genetic exchange with susceptible populations (Croft and Van De Baan 1988). In rose crops, *T. urticae* has developed high levels of resistance to multiple miticides (Stavrinides and Hadjistyli 2009), fostering more research on developing new biocontrol options, such as the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), an efficient predator of *T. urticae* (Perdiki et al. 2008).

Functional response is an important aspect of the dynamics between prey and predator, and it is a major component of the population model (Berryman 1992). Likewise, the functional response helps in assessing the potential of biological pest controls (Fernández-Arhex and Corley 2003). This is important because the number of prey eaten determines the development, survival, and reproduction of predators or parasitoids (Oaten and Murdoch 1975).

According to Holling (1959), there are three types of functional response. Type I response is generally observed in filter feeders (Fernández-Arhex and Corley 2003, 2004) and describes a linear relation between the attack rate and the prey density (where the slope represents the predator prey-finding efficiency) until reaching a point from which the maximum rate of attacks remains constant. Type II response is an asymptotic curve that slows consistently as the number of prey increases, due to the time required by the predator to manipulate its prey (handling time). This type of response is typical of invertebrate predators. The asymptote reflects the maximum attack rate (Fernández-Arhex and Corley 2003, 2004). A sigmoid curve is a Type III response and is typical of vertebrate predators because this type of curve develops by learning or by the possibility of shifting from one prey to another (Fernández-Arhex and Corley 2003, 2004). In this case, the initial response is increased as the host or prey density increases, while the parasitoid or predator becomes more efficient in finding prey (the attack rate increases or the handling time decreases) (Fernández-Arhex and Corley 2003). The consequences of each type of response, in terms of population, differ (Fernández-Arhex and Corley 2003, 2004). Although a Type I response implies a rate of attack that is independent of predator density, Type II response leads to a density-dependent relationship of predation or parasitism. Likewise, Type III functional response is the only response that depends on density; therefore, when prey densities are low, interaction between prey and predators can be potentially stabilized (Hassell et al. 1977, Hassell 1978). Some predatory and parasitic arthropods exhibit Type III responses (Hassell et al. 1977, Sabelis 1981, Badii and McMurtry 1988, Schenk and Bacher 2002, Fernández-Arhex and Corley 2003, 2004).

Functional responses can provide significant information regarding the voracity of a biocontrol agent and the effects of abiotic factors (i.e., temperature) and biotic factors (i.e., hosts and prey) on the predator food-finding efficiency (De Clercq et al. 2000, Mohaghegh et al. 2001, Skirvin and Fenlon 2001, Mahdian et al. 2006, Li et al. 2007, Jalali et al. 2010). In this study, we assumed that the exposure time of the prey is a factor that contributes to the development of different functional responses of *P. persimilis* on *T. urticae*. Therefore, the aim of this assay was to analyze the

behavior of *P. persimilis* functional response at selected time intervals on *T. urticae* eggs on discs of three rose varieties.

Materials and Methods

The assay was conducted at the Universidad Autónoma Agraria Antonio Narro in Buenavista, Saltillo, Coahuila, Mexico by using *T. urticae* eggs, *P. persimilis* predatory mites, and leaf discs of three rosebush varieties (e.g., Royalty, Starlite, and Pareo). The colony of *T. urticae* from which the eggs were obtained was initially established on rosebush plants and maintained under greenhouse conditions ($27 \pm 4^\circ\text{C}$; 60–70% relative humidity [RH]). The *P. persimilis* predators were obtained from the colonies initially established from colonies provided by Berrymex Company (Mexico). The predatory mites were fed on *T. urticae* maintained on bean plants, *Phaseolus vulgaris* L., in a greenhouse at $27 \pm 4^\circ\text{C}$, $65 \pm 5\%$ RH, and a 12-h photoperiod.

The functional response assays were conducted in an environmental chamber by using the modified sand technique of Ahmadi (1983). For each rosebush variety, 2.5-cm-diameter leaf discs were placed individually, with upper surface down, on water-saturated cotton in petri dishes (5 cm). The dishes with the leaf discs were covered with felt paper with a central circular opening over the disc. The number of eggs per disc was established as 1, 2, 4, 8, 16, 32, 64, and 100. One *P. persimilis* female was introduced on each leaf disc, and predation rate was measured at time intervals of 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, and 12–24 h. Counts were ended after 24 h. Each treatment was replicated 15 times at $27 \pm 2^\circ\text{C}$, 60–0% RH, and on a photoperiod of 16:8 h light:dark.

We determined the type of functional response (shape of curve) through logistics regression analysis, adjusting the polynomial equation of rate of prey consumed (N_a/N_o) versus the number of prey offered (N_o) by using the formula:

$$\frac{N_a}{N_o} = \frac{\text{Exp}(P_0 + P_1 N_1 + P_2 N_o^2 + P_3 N_o^3)}{1 + \text{Exp}(P_0 + P_1 N_1 + P_2 N_o^2 + P_3 N_o^3)}$$

where N_a = number of prey consumed, N_o = number of prey offered, and P_0 , P_1 , P_2 , and P_3 = parameters to be estimated.

The regressions began with a cubic model, eliminating high-order coefficients that were not significantly different from zero and leaving only those coefficients that were significantly different from zero. We used a linear P_1 parameter symbol to distinguish between functional response Type II and Type III. If $P_1 < 0$, the ratio of prey consumed decreases monotonically versus the initial number of prey offered, leading to a Type II functional response. However, if $P_1 > 0$ and $P_2 < 0$, the ratio of prey consumed is positively dependent on the initial density, leading to a Type III functional response (Juliano 2001). The parameters were estimated through the generalized linear model function by using R software from R Development Core Team (2016).

The parameters of the functional response (e.g., handling time [Th] and attack coefficient [a']), were estimated using Holling's model (1966) for Type II functional response using the formula:

$$N_a = \frac{a' N_o T}{1 + a' N_o Th}$$

where N_a is the number of prey consumed, a' is the constant rate of attack (rate at which the predator finds its prey instantaneously), N_o is the density of prey offered, T is total available time (time intervals in this essay), and Th is the handling time.

Type III functional responses were represented in models using the previous equation, whereby a' , the attack constant, becomes a function of prey density N_o (Hassell 1978). In a useful way, a' is a hyperbolic function of N_o : $a = (d + bN_o)/(1 + cN_o)$, where b , c , and d are constants in the formula:

$$N_a = \frac{dN_o T + bN_o^2 T}{1 + cN_o + dN_o Th + bN_o^2 T}$$

In general, Type III functional responses are indicated if a' is an increasing function of N_o (Juliano 2001). Function nonlinear least square was used to estimate the parameters by using R software from R Development Core Team (2016). The Student t test ($P \leq 0.05$) was used to determine if parameters a' and Th were the same among the three rosebush varieties.

Results

The logistics regression analysis indicated two types of functional response for *P. persimilis* preying on *T. urticae* eggs on Royalty, Pareo, and Starlite rosebush varieties. Type II responses occurred at observation intervals 0–2, 2–4, 4–6, 6–8, and at 24 h, whereas Type III responses were observed at 8–10, 10–12, and 12–24 h. The linear coefficient of the logistics regression model consistently resulted in significant negative values ($P_1 < 0$), and for Type III, the quadratic and linear coefficients yielded significant negative and positive values, respectively ($P_2 < 0$, $P_1 > 0$) (Table 1; Fig. 1). At 24 h, *P. persimilis* had consumed 56 eggs on Royalty and Starlite varieties and 54 on Pareo. We observed that the predator consumed the greatest number of *T. urticae* eggs at every time interval on the Royalty variety, followed by the Pareo and Starlite varieties. The number of prey consumed by *P. persimilis* decreased as the density of offered prey increased in the three rosebush varieties (Fig. 1).

There were significant differences in predator attack rates (a') ($t = 1.66$; $df = 117$; $P \leq 0.05$) with a higher a' at time intervals 0–2, 2–4, 6–8, and 24 h in Royalty and in Starlite at 4–6 h. It was not possible to compare with equivalent parameters from intervals 8–10, 10–12, and 12–24 h due to the different types of functional response. The predator handling times (Th) were significantly different among varieties ($t = 1.65$; $df = 117$; $P \leq 0.05$) with the shortest Th observed with the Pareo variety followed by Starlite and Royalty (Table 2).

Discussion

We found that *P. persimilis* exhibited Type II and Type III functional responses in the predation of *T. urticae* eggs on three rosebush varieties at different time intervals. Chacón et al. (2017) observed a Type II response of *P. persimilis* preying on *T. urticae* eggs, larvae, nymphs, and adults on the Royal rosebush variety, also under laboratory conditions. Sabelis (1981) obtained a sigmoid function with

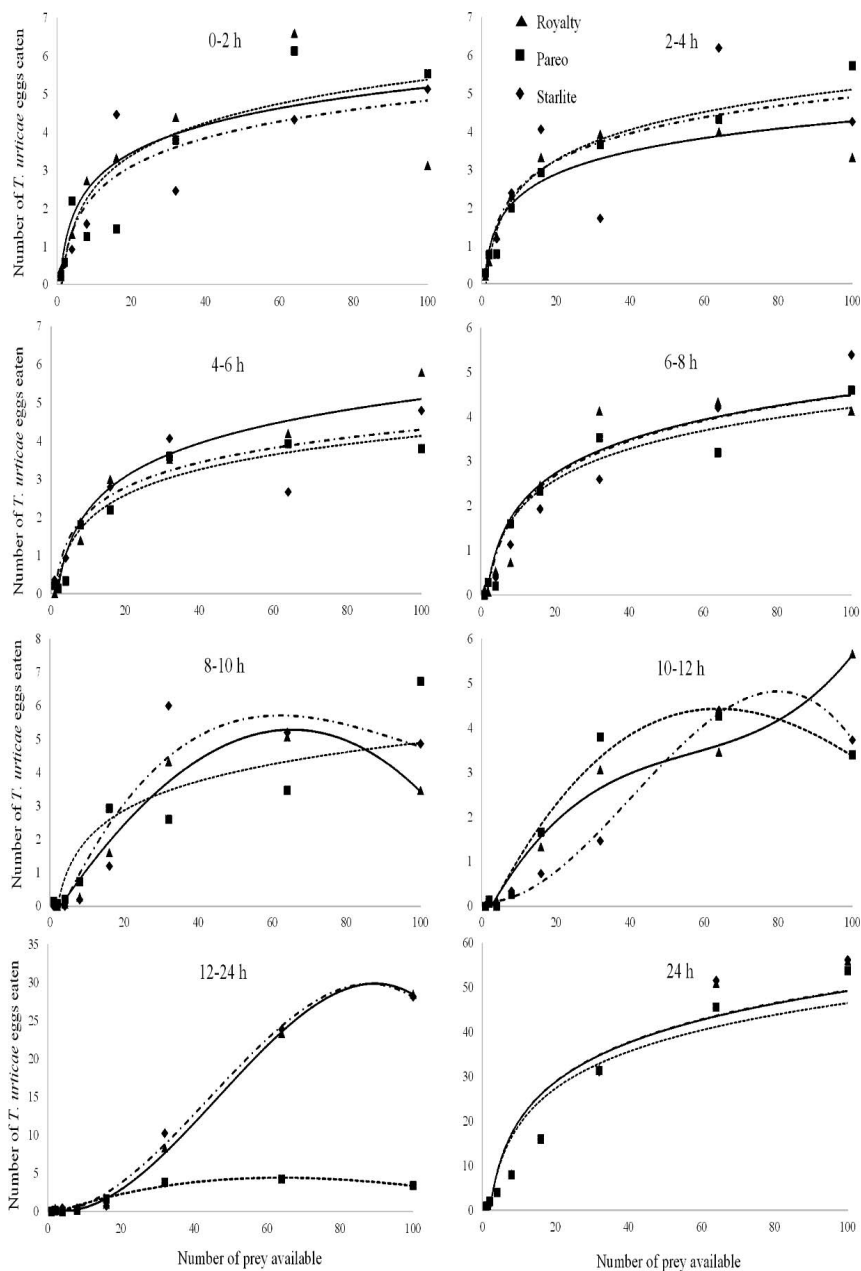


Fig. 1. The functional response of *Phytoseiulus persimilis* to eggs of *Tetranychus urticae* in eight time intervals.

Table 1. Maximum likelihood estimates from logistic regression analysis of the proportion of prey eaten by *Phytoseiulus persimilis* against initial number of eggs of *Tetranychus urticae* on three varieties of rosebush.

Time Interval	Parameters	Royalty			Pareo			Starlite		
		Estimates	S.E.		Estimates	S.E.		Estimates	S.E.	
0–2 h	Intercept (P_0)	–0.7599***	0.1025		–1.3466***	0.1118		–0.8049***	0.1588	
	Linear (P_1)	–0.0254***	0.0018		–0.0151***	0.0017		–0.0464***	0.0077	
	Quadratic (P_2)							0.0003***	6.87E–05	
2–4 h	Intercept (P_0)	–0.6944***	0.1553		–0.8442***	0.1586		–0.6711***	0.1533	
	Linear (P_1)	–0.0423***	0.0077		–0.0440***	0.0076		–0.0439***	0.0074	
	Quadratic (P_2)	0.0001*	0.0000		0.0002***	6.72E–05		0.0002**	6.68E–05	
4–6 h	Intercept (P_0)	–1.6829***	0.1222		–1.5431***	0.12255		–0.8750***	0.1634	
	Linear (P_1)	–0.0119***	0.0018		–0.0172***	0.00199		–0.0486***	0.0080	
	Quadratic (P_2)							0.0003***	7.22E–05	
6–8 h	Intercept (P_0)	–1.6569***	0.1243		–1.7172***	0.1270		–1.9942***	0.1339	
	Linear (P_1)	–0.0145***	0.0019		–0.0144***	0.0019		–0.0091***	0.0019	
	Intercept (P_0)	–3.865***	0.4447		–1.7442***	0.1960		–5.3790***	0.5834	
8–10 h	Linear (P_1)	0.131***	0.0323		–0.0260**	0.0086		0.2511***	0.0389	
	Quadratic (P_2)	–0.0024***	0.0006		0.0002*	7.37E–05		–0.0048***	0.0007	
	Cubic (P_3)	1.24E–05**	0.0000					2.58E–05***	4.23E–06	

Table 1. Continued.

Time Interval	Parameters	Royalty		Pareo		Starlite	
		Estimates	S.E.	Estimates	S.E.	Estimates	S.E.
10–12 h	Intercept (P_0)	−3.741***	0.4549	−3.9050***	0.4553	−3.7340***	0.3328
	Linear (P_1)	0.1111**	0.0342	0.1315***	0.0333	0.0382**	0.0120
	Quadratic (P_2)	−0.0024***	0.0007	−0.0026***	6.78E−04	−0.0003***	9.60E−05
	Cubic (P_3)	1.40E−05***	0.0000	1.363E−05***	3.96E−06		
12–24 h	Intercept (P_0)	−3.2250***	0.2102	−3.9050***	0.4553	−3.9290***	0.4061
	Linear (P_1)	0.0776***	0.0070	0.1315***	0.0333	0.1549***	0.0278
	Quadratic (P_2)	−0.0005***	0.0001	−0.0026***	6.78E−04	−0.0022***	0.0005
	Cubic (P_3)			1.363E−05***	3.96E−06	9.25E−06**	2.98E−06
24 h	Intercept (P_0)	9.2174***	1.0918	8.7526***	0.8354	7.1351***	0.7154
	Linear (P_1)	−0.1805***	0.0288	−0.1871***	0.0222	−0.1257***	0.0195
	Quadratic (P_2)	0.0009***	0.0002	0.0010***	0.0001	0.0006***	0.0001

Parameters followed by * are significant in $P < 0.01$, ** $P < 0.001$, and *** $P < 0.0001$ (Z test).

Table 2. Parameters of the functional response Type II and III estimated with the Holling's model for *Phytoseiulus persimilis* on eggs of *Tetranychus urticae*.

Time Interval	Parameters	Royalty			Pareo			Starlite		
		Estimates*	95% CI**		Estimates*	95% CI**		Estimates*	95% CI**	
0-2 h	a'	0.5897 ± 0.2052	(0.1875, 0.9919)		0.2185 ± 0.0591	(0.1025, 0.3345)		0.3822 ± 0.1094	(0.1677, 0.5968)	
	Th	0.1852 ± 0.0229	(0.1402, 0.2301)		0.1195 ± 0.0224	(0.0755, 0.1635)		0.1846 ± 0.0221	(0.1412, 0.2280)	
2-4 h	a'	0.5877 ± 0.1646	(0.2650, 0.9103)		0.3043 ± 0.0880	(0.1317, 0.4769)		0.4703 ± 0.1308	(0.2137, 0.7268)	
	Th	0.2333 ± 0.0216	(0.1909, 0.2756)		0.1561 ± 0.0225	(0.1120, 0.2003)		0.1891 ± 0.0201	(0.1496, 0.2286)	
4-6 h	a'	0.2196 ± 0.0416	(0.1381, 0.3011)		0.2756 ± 0.0798	(0.1190, 0.4321)		0.3671 ± 0.0962	(0.1784, 0.5557)	
	Th	0.13807 ± 0.0165	(0.1055, 0.1705)		0.2054 ± 0.0273	(0.1517, 0.2590)		0.2115 ± 0.0223	(0.1677, 0.2553)	
6-8 h	a'	0.2395 ± 0.0601	(0.1215, 0.3575)		0.2337 ± 0.0657	(0.1048, 0.3626)		0.1298 ± 0.0275	(0.0759, 0.1837)	
	Th	0.1737 ± 0.0232	(0.1281, 0.2193)		0.1899 ± 0.0275	(0.1359, 0.2439)		0.1119 ± 0.0246	(0.0636, 0.1601)	
8-10 h	a'	—	—		0.1032 ± 0.0319	(0.0406, 0.1658)		—	—	
	Th	0.1692 ± 0.0259	(0.1183, 0.2201)		0.0656 ± 0.0392	(−0.0112, 0.1425)		0.2468 ± 0.0153	(0.2167, 0.2769)	
10-12 h	b	−0.0608 ± 0.0295	(−0.1187, −0.0028)		—	—		0.3171 ± 0.0477	(0.2236, 0.4107)	
	c	−0.2877 ± 0.0998	(−0.4834, −0.0920)		—	—		−0.2528 ± 0.0535	(−0.3577, −0.1479)	
	d	0.2424 ± 0.1156	(0.0158, 0.4691)		—	—		−2.4468 ± 0.3866	(−3.2046, −1.6890)	
	Th	0.3004 ± 0.0227	(0.2559, 0.3449)		0.3346 ± 0.0257	(0.2840, 0.3851)		0.4281 ± 0.0608	(0.3088, 0.5474)	
	b	0.1279 ± 0.0209	(0.0869, 0.1689)		0.2288 ± 0.0430	(0.1445, 0.3132)		0.0357 ± 0.0093	(0.0174, 0.0539)	
	c	−0.1789 ± 0.0238	(−0.2257, −0.1321)		−0.2652 ± 0.0638	(−0.3904, −0.1400)		−0.2380 ± 0.0595	(−0.3546, −0.1214)	
	d	−0.9869 ± 0.1671	(−1.3145, −0.6593)		−1.7586 ± 0.3447	(−2.4344, −1.0829)		−1.2553 ± 0.3082	(−1.8594, −0.6511)	

Table 2. Continued.

Time Interval	Parameters	Royalty		Pareto		Starlite	
		Estimates*	95% CI**	Estimates*	95% CI**	Estimates*	95% CI**
12–24 h	<i>Th</i>	0.0607 ± 0.0044	(0.0520, 0.0693)	0.3346 ± 0.0257	(0.2840, 0.3851)	0.0079 ± 0.0034	(0.0010, 0.0147)
	<i>b</i>	0.2724 ± 0.0378	(0.1983, 0.3466)	0.2288 ± 0.0430	(0.1445, 0.3132)	−0.0210 ± 0.0044	(−0.0298, −0.0122)
	<i>c</i>	−0.2263 ± 0.282	(−0.2816, −0.1710)	−0.2652 ± 0.0638	(−0.3904, −0.1400)	−0.0561 ± 0.0054	(−0.0668, −0.0455)
	<i>d</i>	−9.0907 ± 1.1610	(−11.3664, −6.8150)	−1.7586 ± 0.3447	(−2.4344, −1.0829)	0.3412 ± 0.0682	(0.2075, 0.4749)
	<i>a'</i>	1.3730 ± 0.0631	(1.2494, 1.4967)	1.3402 ± 0.0720	(1.1989, 1.4815)	1.3542 ± 0.0539	(1.2484, 1.4601)
24 h	<i>Th</i>	0.0098 ± 0.0004	(0.0088, 0.0107)	0.0107 ± 0.0006	(0.0095, 0.0119)	0.0095 ± 0.0004	(0.0087, 0.0104)

* Parameter ± standard deviation.
** Values in parentheses indicate confidence interval 95%.

respect to the response of *Metaseiulus occidentalis* Nesbitt (Acari: Phytoseiidae) preying on *T. urticae* eggs on rosebush leaves.

In our study, the graphical response of the percentage of prey versus prey density suggested a Type II functional response at time intervals 0–2, 4–6, and 6–8 h on all three varieties, at the 8–10 h interval on Pareo, and at 24 h on the three varieties. The percentage of prey consumed decreased in a monotonic function as prey density increased with a negative linear coefficient (Pervez and Omkar 2005). The Type III functional response we observed at intervals 8–10 h on Royalty and Starlite and intervals 10–12 h and 12–24 h on Royalty, Pareo, and Starlite was a polynomial function a positive relationship between egg consumption with egg density with concomitant positive linear coefficients (Pervez and Omkar 2005).

Abrams (1982) stated that the shape of functional response curves may vary according to environmental conditions. Those conditions include habitat heterogeneity (Lipcius and Hines 1986), light intensity (Koski and Johnson 2002), host plants (Messina and Hanks 1998, De Clercq et al. 2000, Skirvin and Fenlon 2001), temperature (Mohaghegh et al. 2001, Mahdian et al. 2006, Li et al. 2007, Jalali et al. 2010), and prey refuge (Lipcius and Hines 1986, Messina and Hanks 1998). Other predatory mites have demonstrated a Type III functional response on different plants, including *Iphiseius degenerans* Berlese and *Neoseiulus teke* L. (Acari: Phytoseiidae) preying on eggs of *Mononychellus tanajoa* (Bondar) Flechtmann & Baker (Acari: Tetranychidae) on leaf discs of cassava, *Manihot esculenta* Crantz (Nwilene and Nachman 1966) and *P. longipes* Evans preying on eggs of *T. pacificus* (McGregor) on bean, *Phaseolus vulgaris* L. (Badii and McMurtry 1988).

Rosa sp. leaves do not have trichomes (Sabelis 1981); therefore, the mobility of *P. persimilis* on rose foliage is not linked to the density of trichomes (Krips et al. 1999). In fact, Workman and Martin (2000) reported greater *P. persimilis* mobility on *Rosa* sp., leaves than on other cut flowers. Skirvin and Fenlon (2001) also found high efficiency of *P. persimilis* on glabrous leaves, which suggests that the functional response of this predator is favored by the absence of physical structures on the leaves, such as trichomes (Forero et al. 2008). Therefore, the attack rate (a') may be a function of prey density (N_0) (Hassell 1978) in certain periods of time, further explaining why we saw *P. persimilis* exhibit two types of functional responses (Types II and III) within a 24-h period.

The observed shift of *P. persimilis* functional response on the three rosebush varieties from Type II to Type III within 24 h was probably due to the low attack rates with low prey densities, leading to low predation potential. Likewise, changes in the handling time (Th) associated with prey density in the observational time intervals increased the stability of prey population, resulting in a Type III functional response. Similarly, this shift may reflect increased *P. persimilis* predation efficiency on *T. urticae* eggs within the 24 h (Fig. 1; Table 2). The stability of a population increases when functional response is Type II; however, the increase of such stability is even greater when the functional response is Type III (Hammill et al. 2010).

At set time intervals, we observed that the time spent by the predator in catching, killing, consuming, and digesting *T. urticae* eggs was shortest on the Pareo variety, followed by Starlite and Royalty. However, after 24 h of observation, we found the handling time (Th) was shortest on the Starlite variety, followed by Royalty and Pareo. Estimates of the attack rate (a') in the four time intervals, as well as the entire 24-h period, showed that *P. persimilis* encountered *T. urticae* eggs more

frequently on the Royalty variety, followed by Starlite and Pareo. These observed differences in responses on the rosebush varieties might be attributed to differences in chemical composition among those plant varieties, which, in turn, impacts natural enemy populations indirectly through sequestering toxins by prey species or, directly, via the production of attractants (Price et al. 1980). Based on our results, we believe that rosebush varieties directly impact handling time (Th) and, thus, the attack rate (a'), with the predator spending more time in handling its prey on the Royalty variety. We further believe this may impact the prey-predator dynamics under field and/or greenhouse conditions (Chacón et al. 2017).

The results of our assay provide information on the way in which *P. persimilis* responds to prey density under laboratory conditions. After observing predator response to prey at eight time intervals, we concluded that *P. persimilis* is a powerful biocontrol agent for *T. urticae*, although a larger number of similar assays under field and greenhouse conditions are required for confirmation. From the functional response curves, attack rates, and handling times at different time intervals, we can predict that on the three varieties of rosebush, this predator can be an efficient biocontrol agent, supporting the hypothesis that the predator presents different functional responses (Type II and Type III).

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