

Laboratory measurement of the functional and numerical responses of the predaceous mite, *Typhlodromus* (T.) *setubali* to *Panonychus ulmi* (Phytoseiidae: Tetranychidae)

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Abstract

Background The functional response of a predator is one of the fundamental methods in ecological studies. *Typhlodromus (T.) setubali* (Dosse) is an indigenous predaceous mite recently re-described in Morocco. It was reported on various crops around the Mediterranean basin. This study aims to assess the functional response of *T. (T.) setubali* to the densities of 2, 4, 8, 16, 32, 64, and 128 immature stages of *Panonychus ulmi* under the controlled conditions of 26 ± 1 °C, 65 ± 5 % RH and 16:8 h L: D photoperiod. The logistic regression was used to determine the functional response and the oviposition activity of predator females was followed to evaluate the numerical response. Thereafter, both parameters (the attack rate (a) and handling time (T_h)) were estimated and the results were entirely discussed.

Results Logistic regression analysis revealed a Holling type II functional response, indicating that the number of *P. ulmi* killed by *T. (T.) setubali* females increased as the prey density increased. The values of attack rate (a) and handling time (T_h) were 0.029 prey/ day and 0.573 prey, respectively. The oviposition of females increased above the prey density of 6.54 mites/cm² and the highest value (m) was 1.86 eggs/ day. Finally, the prey density needed to achieve half the maximum response (n) was estimated to be 2.15 prey.

Conclusions The functional response analysis is one of the most rigorous methods for understanding the ecophysiology of a predator. The proportions of prey consumed by *T. (T.) setubali* were higher at lower densities for *P. ulmi* immatures, implying that *T. (T.) setubali* should be more effective at suppressing the red spider mite populations at lower densities. Further laboratory-based studies are needed to draw biological conclusions.

Background

The functional response [1, 2] is one of the most commonly used mathematical models to describe the trophic interactions between a predator and its prey [3]. It is defined as the relationship between the prey density and the number of prey attacked by a single predator during a given time interval, for determining whether the functional response type of a predator is of type I, II, or type III. In biological control, the decision about which organism to use is strongly based on the results of such an analysis, which leads to drawing biological conclusions on a predator species [4], in combination with independent sources of information [5].

Several regression-based models describe the predation rate of a predator on various prey densities. Practically, the choice of one model to another constitutes a theoretical debate in terms of a complete analysis of a functional response data set [6]. Whenever possible, such investigation is one of the crucial methods to improve the biological control programs.

Holling (1959) [2] has defined three types of functional responses. In Type I, the number of consumed prey increases linearly to a maximum then remains constant as increasing prey density. In Type II, the number approaches the asymptote hyperbolically as prey densities increases. Whereas in Type III, the

number of prey consumed approaches it as a sigmoid curve. Therefore, a clear distinction between type II and III based on predation is rather difficult [7]. Many published results reported that type II is the commonly frequent type among predators, especially, among predaceous mites belonging to the family Phytoseiidae [8–14].

The main objective of this study is to approach the functional response of the predaceous mite, *T. (T.) setubali* Dosse (Acari: Phytoseiidae), to various densities of *Panonychus ulmi* (Koch) (Acari: Tetranychidae), whether it is of type II or type III. *Typhlodromus (T.) setubali* is an indigenous phytoseiid adapted to the local climate in Morocco [15, 16], and lacking bibliographic information on its biology and predation rate is the first challenge of this study. Therefore, fitting functional and numerical responses data on *T. (T.) setubali* can help to improve its use as a biological control agent of pest mites and other crop insects in the agricultural Mediterranean areas, as long as it was reported in Tunisia, Israel, Greece, Jordan, Spain, Portugal, Slovakia and Germany [17].

Results

Functional Response of *T. (T.) setubali*

Estimates of the logistic regression coefficients are given in Table 1. The results indicate that P_0 , P_1 , P_2 , and P_3 are significantly different from 0. Further, as the linear coefficient, P_1 is negative ($P = 0.007$), the functional response type II model is the most appropriate for modelling the functional response of *T. (T.) setubali* females when fed on immature stages of *P. ulmi* at 26 °C.

Table 1
Estimates of the logistic regression model adjusted of *T. (T.) setubali* to *P. ulmi* prey densities.

Coefficients	Estimate	SE	Error z value	P
Intercept P_0	4.98	0.78	5.96	< 0.0001***
Linear P_1	-0.11	0.05	-2.67	0.0075**
Quadratic P_2	$1.9 \cdot 10^{-5}$	$9.32 \cdot 10^{-6}$	2.34	0.0161*
Cubic P_3	$-2.1 \cdot 10^{-7}$	$4.49 \cdot 10^{-7}$	-2.21	0.0271*
SE Standard Error				
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.				

The logistic regression adjusting a polynomial equation of the proportion of prey attacked as function of prey density can be written as:

$$\frac{N_e}{N_0} = \frac{\exp\left(4.98 - 0.11 + 1.9 \cdot 10^{-5} N_0^2 - 2.1 \cdot 10^{-7} N_0^3\right)}{1 + \exp\left(4.98 - 0.11 + 1.9 \cdot 10^{-5} N_0^2 - 2.1 \cdot 10^{-7} N_0^3\right)}$$

The mean consumption rates of *T. (T.) setubali* females increased as prey density increased from 2 to 128 *P. ulmi* immatures, stabilizing at higher densities of 18 prey (Fig. 1a). At the low prey densities ranged from 2 to 16, more than 90% of *P. ulmi* immatures were consumed. Although the prey density increased, the proportion of prey attacked decreased and the rate of consumption tended to stabilize beyond a prey density of 64 mites (Fig. 1b).

When Rogers's (1972) [21] model for a type II response (Eq. 2) was used to describe the functional response of *T. (T.) setubali*, both parameters, the attack rate (α) and handling time (T_h) were found to be significantly different from 0 and the model was capable of explaining 95% of the total variation in data (Table 2). The maximum predation rate is found as 34.48 prey/ day. Half of the maximum predation rate is attained at a density of $D = 46.24$ prey corresponding to 6.54 prey/ cm² (Fig. 1a).

Table 2
Estimate (\pm SE) of the attack rate and handling time of *T. (T.) setubali* on immatures of *Panonychus ulmi*,

Model	Parameters	Estimates	P	95% CI	R ²
Rogers (1972)	α (prey/ day)	0.573 \pm 0.022	0.0037**	0.438–0.674	0.95
	T_h (day)	0.029 \pm 0.051	< 0.0001***	0.009–0.117	
Hyperbolic	m (eggs)	1.862 \pm 0.611	< 0.0001***	0.945–2.128	0.89
	n (prey density)	2.151 \pm 0.553	0.0235*		

Numerical Response of *T. (T.) setubali*

To assess the numerical response of *T. (T.) setubali* females, the hyperbolic model (Eq. 4) explained 89% of the total variation in daily oviposition rates (Table 2). The maximum daily oviposition (m) of a *T. (T.) setubali* female was estimated to be 1.86 eggs/ day and the prey density needed to achieve half the maximum response (n) was estimated to be 2.15 *P. ulmi* immatures (Fig. 2).

Discussion

Modeling a prey-predator system has progressed considerably to more mathematically fitted models, which correct errors related to certain classical aspects of calculation [24]. The logistic regression delivered a significantly negative linear coefficient ($P_1 < 0$) and a positive quadratic coefficient ($P_2 > 0$) for the seven classes of prey offered, suggesting that *T. (T.) setubali* performs a type II functional response to *P. ulmi* immatures (Table 1), which assumes that the predation rate increased according to the increase

in prey density, levelling off to a maximum of 18 prey. The functional response curve (Fig. 1a) showed a high proportion of prey consumed at lower prey densities, The proportion of immature prey consumed by a predator (N_e/N_0) declined with the increase of prey density (Fig. 1b). The estimated parameters were the attack rate $a = 0.573$ prey/ day and handling time $T_h = 0.029$ days per prey to Rogers's model (Table 2).

In this study, the handling time estimated for predator females of *T. (T.) setubali* was relatively short, meaning that predators spend less time in capturing, killing, and digesting the prey. For example, the value estimated for *T. (T.) setubali* is similar to that obtained for *Euseius concordis* (Chant) adult females feeding on *Oligonychus ilicis* (McGregor) at 25 °C (0.033 days) [13] and also to *Neoseiulus cucumeris* (Oudemans) on *Thrips flavidulus* (Bagnall), whereas it is significantly lower than those obtained for *Neoseiulus barkeri* (Hughes) and *Euseius nicholsi* (Ehara & Lee) at 26 °C in the same study (0.042 and 0.057 days, respectively), for which, the time taken during predation appears quite long [25].

The functional response alone cannot explain the success or failure of *T. (T.) setubali* as a biological control agent [26]. Several determining factors can affect its efficiency such as the intraguild predation [27], interactions with potential predators, properties of host plants [28], as well as abiotic factors such as temperature [29]. Although other experimental conditions of temperature and relative humidity, as well as the food diets, were not tested, *T. (T.) setubali* can capture and feed on prey without any initial delay in learning ways to circumvent difficulties.

Regardless of the host plants used in experiments, several authors have studied the functional responses of predatory mites on *P. ulmi* (Koch, 1836). For example, *Euseius (Amblyseius) finlandicus* (Oudemans) and *Amblyseius andersoni* (Chant) displayed a functional response of type II when exposed to increasing densities of larvae and adults of *P. ulmi* at 25 °C [9]. Also, *Typhlodromus (Typhlodromus) pyri* (Scheuten), a generalist predator widely used in biological control, displays a functional response type II to *P. ulmi* at 25 ± 1 °C, 75% RH, and a photoperiod of 16: 8 (L: D)h [30]. The functional response of the phytoseiid predator *Chiloseius camposi* (González y Schuster) on densities of *P. ulmi* under controlled temperature 20 ± 2 °C, relative humidity of 75 ± 15% and photoperiod 16:8 h (L:D) was found to be of type II [31]. The functional response of a predator can change from type II to III and vice versa in response to different factors described above.

Conclusions

Although this study was conducted on a predatory mite, the study of the functional response is an analytical model applicable to the whole scale of zoology in different types of Ecosystems. The present study demonstrated that the functional response of *T. (T.) setubali* is of type II. The oviposition of the predator females increased curvilinearly with increasing prey density until reaching a plateau at densities from 32 to 128 *P. ulmi* immatures, with approximately 2.15 eggs/ female (Fig. 2), indicating that the relationship between prey density and oviposition increases with the predation rate. The female fecundity of phytoseiid mites depends strongly on the number of resources invested per egg [32]. Both functional

and numerical responses of *T. (T.) setubali* females when fed on *P. ulmi* can be useful for determining the potentiality of this predatory mite and presents elementary information on this species. When more predators are candidates for use in biological control, the estimation of the functional response allows comparing the relative effectiveness of each one [33]. Therefore, our laboratory results may not exactly correspond to the situation in field, but this study will be helpful to further evaluate the feasibility of incorporating *T. (T.) setubali* in integrated pest management programs against *P. ulmi* on a wide variety of crops.

Methods

Rearing mites

Panonychus ulmi was collected from apple trees in Oulmes region, Morocco, and reared on green bean plants *Phaseolus vulgaris* L. (Leguminosae) for four generations before the beginning of the experiments. To obtain the seven densities that have been using in functional response experiments, the oviposition of *Panonychus ulmi* females was taken into account. Prey densities of 2, 4, 8, 16, 32, 64, and 128 immatures were obtained by transferring 1, 3, 5, 10, 16, 34, and 54 gravid females, respectively, onto the leaf discs. Females were allowed to lay eggs for 24 hours, then were removed. The eggs were followed daily once until to become immature forms, which will be used as food for predators.

The initial population of *T. (T.) setubali* was obtained from Riyad-Fruit orchard located at Tiddas, Morocco. The colony was kept in rearing unit and individuals were fed on immature stages of *P. ulmi* offered on been leaves. The rearing unit consists of black plastic tiles placed on a floating sponge into a plastic dish (40×27×17 cm) full of water. The bordering of the black plastic support was covered with a wet wide band of Kleenex to prevent mite escape. Been leaves infested by *P. ulmi* were added daily. Every week, bean leaves and water were exchanged with new ones. Rearing units were kept in growth chamber at 26 ± 1 °C, 65 ± 5 % RH, and 16:8 h (L: D) as photoperiod.

To obtain females of the same age, a total of fifty gravid females were transferred from the stock colony to a *P. ulmi*-infested been leaves placed in Petri dishes. The females were allowed to lay eggs for 12h and then were removed. Newly emerged predators were of the same age.

Functional and Numerical Response Experiments

To assess the response of *T. (T.) setubali* to immatures of *P. ulmi*, 24 h starved predator females were assigned individually to one of seven prey densities (2, 4, 8, 16, 32, 64 and 128 prey), corresponding to 0.28, 0.56, 1.13, 2.26, 4.52, 9.05 and 18.11 mites/ cm², respectively. After 24h, the predator females were removed and the number of prey killed was counted. Collected data were fitted by using Logit-model.

The commonly used models do not detect the initial increase in attacks at the lower prey densities, because the predatory mites spend more time searching and handling prey even if confined into a smaller space such as a leaf disc [6]. For overcoming this problem, our experimental design involved ten

replicates for mite densities of 0.28, 0.56, 1.13 mites/ cm², seven replicates for 2.26, and 4.52 mites/cm², and five for mite densities of 9.05 and 18.11 mites/cm². All experiments were conducted at 26 ± 1 °C, 65 ± 5 % RH, and 16:8 (L: D) h photoperiod.

To evaluate the numerical response of *T. (T.) setubali*, the oviposition activity of females was followed for 4 consecutive days. The number of eggs deposited per female was recorded every 24 h in each replicate.

Data analysis

Functional response data set were analysed according to Juliano's procedure [18] using R Commander, a graphical user interface in conjunction with R program ver. 3.5.3. [19]. The logistic regression adjusting a polynomial equation (1) of the proportion of prey attacked (N_e) as a function of the initial prey density (N_0), was used to estimate the linear, quadratic and cubic coefficients and therefore, determined the shape of the functional response curve of *T. (T.) setubali* to immature stages of *P. ulmi*. The type of response was determined by the signs of the linear and quadratic coefficients (P_1 and P_2). If the linear coefficient is negative ($P_1 < 0$), it describes a type II functional response. If $P_1 > 0$ and $P_2 < 0$, it presents a type III functional response. Equation of the logit model includes an error ϵ , assuming to be distributed according to the binomial distribution [20]. (see Equation 1 in the Supplemental Files)

Where N_0 is the initial number of prey, N_e is the number of prey eaten, N_e/N_0 is the probability of being attacked and P_0 , P_1 , P_2 , and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively.

Regardless of the results obtained, the attack rate (a) and handling time (h) can be determined by using the Holling disc equation or Rogers's random predator equations of type II (2) and type III (3) [21], known as RRPE-II and RRPE-III. Rogers's random predator equations include an attack exponent (q) to describe the per capita prey consumption in low prey densities and overcome the prey depletion at the end of experiments. Even if the number of attacked prey (N_e) appears on both sides of equation (2), the fit of data is performed by using iterative Newton's method as an alternative to LambertW function one allows an explicit solution of the implicit RRPE-II [22]. For the functional response type III, a simplified version of the original model was presented by Hassell et al. (1977) [23] after a long series of research. The equations (2) and (3) makes it possible to predict how prey will be depleted over time during functional response experiments. (see Equations 2 and 3 in the Supplemental Files)

where N_e is the number of prey consumed per predator during an exposure time T (24 h), N_0 is the initial number of prey, a is the attack rate, T_h is the handling time of prey by the predator and P as the number of predators involving in experiments.

To describe the numerical response, the relationship between the fecundity of *T. (T.) setubali* females and the prey density available was fitted by using a hyperbolic model (4), including the maximum daily

oviposition (m) and the density necessary for the predatory mite to oviposit half the maximum response (n). (see Equation 4 in the Supplemental Files)

Declarations

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Authors' contributions

AL performed the experimental design. SO analyzed and interpreted the results, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This research was conducted in compliance with the regulations of the National Center for Scientific and Technical Research and the Moroccan Institute of Scientific and Technical Information.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

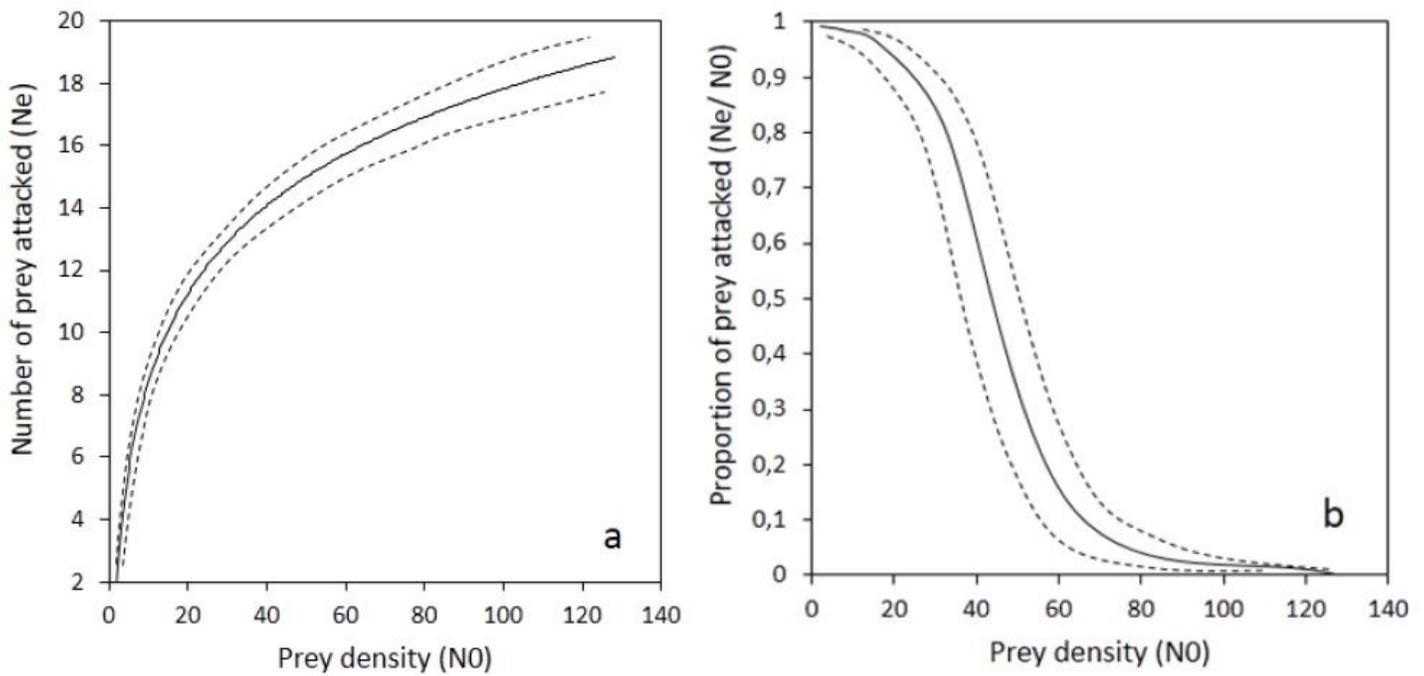


Figure 1

a: Mean number of *Panonychus ulmi* immatures killed per day by a female *Typhlodromus (T.) setubali* (Ne), based on Rogers's random disc equation. b: Mean proportion of *Panonychus ulmi* attacked by a *Typhlodromus (T.) setubali* female (Ne/N0) at increasing densities of *Panonychus ulmi* immatures. In both illustrations, the dashed lines are the 95 % confidence limits.

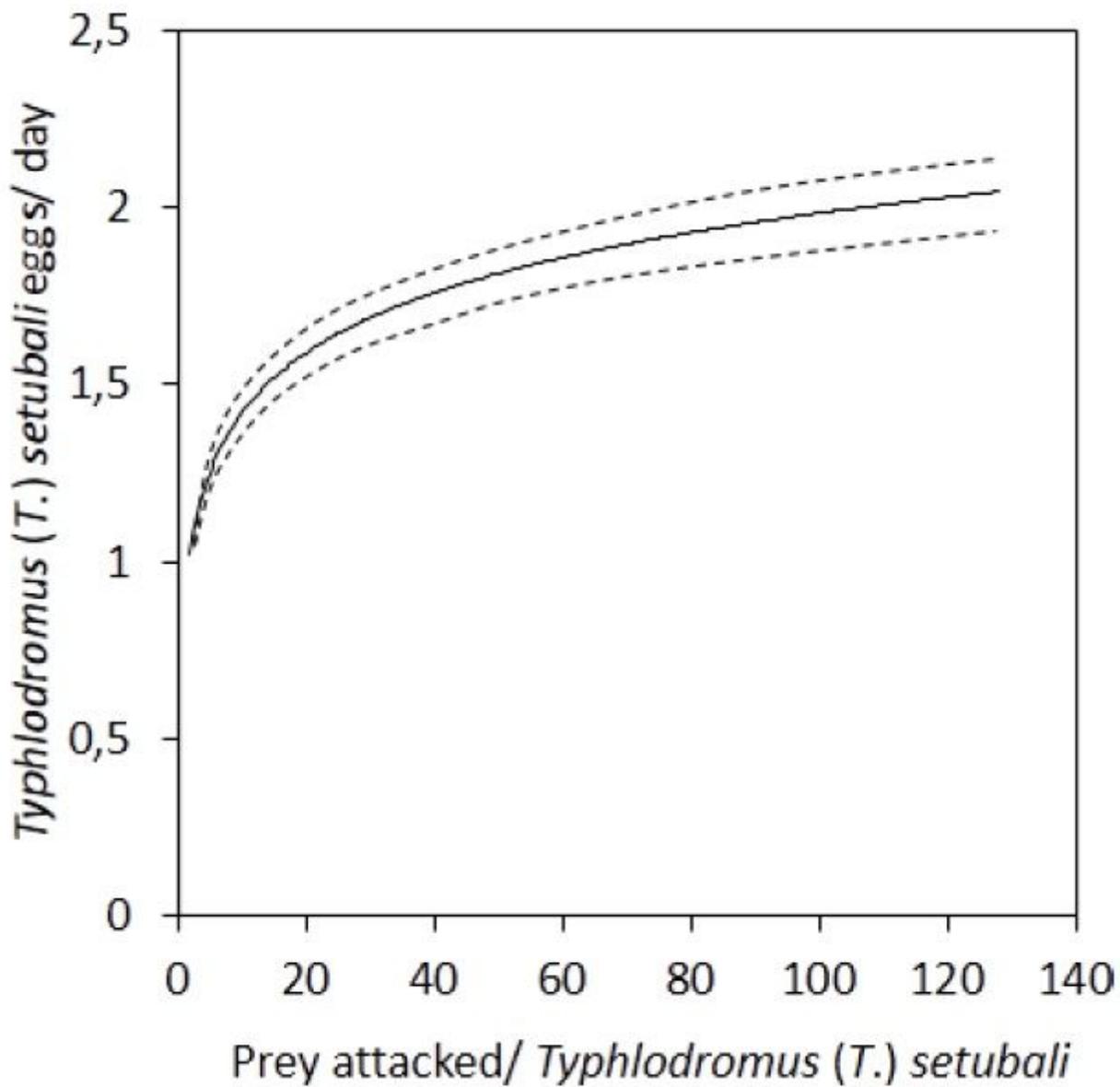


Figure 2

Mean (\pm SE) number of eggs produced per day by a *Typhlodromus (T.) setubali* female when exposed to increasing densities of *Panonychus ulmi* immatures. The line shows the predicted relationship based on a hyperbolic model (equation 3) with 95% confidence intervals as dashed lines.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Equations.pdf](#)