Predatory performance of two Mediterranean phytoseiid species, *Typhlodromus laurentii* and *Typhlodromus rhenanoides* fed on eggs of *Panonychus citri* and *Tetranychus urticae*

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Abstract

Laboratory trials were carried out to determine the predatory capacity of two endemic to the Mediterranean area phytoseiid mites *Typhlodromus* (*Typhlodromus*) *laurentii* Ragusa et Swirski and *Typhlodromus* (*Anthoseius*) *rhenanoides* Athias-Henriot (Parasitiformes Phytoseiidae) upon the eggs of the two most important tetranychids damaging *Citrus* orchards in Sicily: *Tetranychus urticae* Koch and *Panonychus citri* (McGregor) (Acariformes Tetranychidae). The research aimed at investigating the predatory behaviour of the phytoseiids upon the prey stage considered the most vulnerable. The predation rate of the two phytoseiid species was different on the two prey eggs, both in every observation day and for the whole observation period. Both phytoseiid species have showed a better performance on *T. urticae* eggs, on which the predation rate was significantly higher for *T. laurentii* (12.53 ± 0.28 preyed eggs/female/day), than for *T. rhenanoides* (9.71±0.22 preyed eggs/female/day). On the opposite, the predation upon *P. citri* eggs was very limited, without substantial differences between the two phytoseiids: 0.54 ± 0.09 and 0.21 ± 0.04 eggs/female/day for *T. laurentii*, and *T. rhenanoides* respectively. The prey provided has also affected the oviposition rate of the two phytoseiid species. *T. laurentii* was the most prolific species with 2.13 ± 0.08 eggs/female/day, when eggs of *T. urticae* were supplied as food, while upon *P. citri* eggs only 0.31 ± 0.05 eggs/female/day was laid by this predator. The oviposition rate of *T. rhenanoides* on *T. urticae* eggs (1.31 ± 0.07 eggs/female/day) was significantly lower, if compared to that of *T. laurentii* (2.13 ± 0.08 eggs/female/day). However, no significant difference was found when the phytoseiids preyed upon *P. citri* eggs.

Key words: *Typhlodromus* (*Typhlodromus*) *laurentii*, *Typhlodromus* (*Anthoseius*) *rhenanoides*, *Panonychus citri*, *Tetranychus urticae*, predation.

Introduction

Among phytoseiids inhabiting *Citrus* in Sicily, the densities of two native species, *Typhlodromus* (*Typhlodromus*) *laurentii* Ragusa et Swirski and *Typhlodromus* (*Anthoseius*) *rhenanoides* Athias-Henriot (Parasitiformes Phytoseiidae), have constantly increased in the last decade, especially in springtime and autumn. Sometimes, their populations overpass those of the dominant phytoseiid species *Euseius stipulatus* (Athias-Henriot) (Ragusa, 1977). The above-mentioned species, like the majority of phytoseiids associated with *Citrus* in Sicily, are generalist predators and feed on pollen. They can feed and reproduce on various spider mites (Ragusa, 1981; Tanigoshi, 1982; Castagnoli and Simoni, 1990; Ragusa Di Chiara and Tsolakis, 1995), but their impact on tetranychid infestations is not comparable to that of type I and type II predators, especially on tetranychids producing a complicated web structure (McMurtry and Croft, 1997). On the other hand, the coincidence between predator females and prey eggs in the first phase of the colony growth, is comparable to type I phytoseiids (Sabelis, 1981), and their population density is inversely proportional to that of the prey (McMurtry and Croft, 1997). These facts suggest that type III and IV phytoseiids might control the tetranychid populations mainly at the beginning of their infestation, as they can maintain high densities even in the absence of tetranychids (Ragusa, 1986; Badii *et al.*, 2004).

Sicilian *Citrus* orchards are frequently infested by two tetranychid species, *Tetranychus urticae* Koch and *Panonychus citri* (McGregor) (Acariformes Tetranychidae), considered the most important mite pests on *Citrus* also in other Mediterranean countries (Garcia-Marì *et al.*, 1983; Papaioannou-Souliotis *et al.*, 1993; Vassiliou and Papadoulis, 2009). The former species is most common during the end of spring and summer, while the second one is usually observed during early spring and autumn. Preliminary laboratory tests have shown that *T. laurentiii* and *T. rhenanoides* developed well on infested leaves, when all life stages of each of the two *Citrus* pests were given. This fact indicates the potential predatory capacity of these two phytoseiid species on the two tetranychids; however additional tests are required to better characterize these predation abilities.

In the present paper we report data on the predatory performance of the two phytoseiid species, using the egg stage of the two tetranychids as prey, in order to enhance our knowledge on the predatory capacity of native phytoseiid species, and to get information on their capacity to control the tetranychid populations especially at the begining of the infestation. This is the first of a series of studies that will be carried out on the above mentioned phytoseiid species.

Materials and methods

Mite species tested

Both tetranychid and phytoseiid mites were collected on lemon trees in the province of Palermo (Sicily). *T. urticae* and the two phytoseiid species were collected

in 2009 at the end of spring, while *P. citri* was collected in autumn. The tetranychids were reared on green lemon fruits in a growth chamber, and the two phytoseiid species were kept on plexiglas arenas (Swirski *et al.*, 1970), using a mixture of pollens of *Oxalis pes-caprae* L. (Oxalidaceae) and *Carpobrotus edulis* L. (Aizoaceae) as food. All colonies were maintained at 25 ± 1 °C, 70 ± 5% RH and 16L:8D photoperiod.

Experimental units

Experimental units (EU) consisted of a lemon leaf disk (3 cm diameter) placed with its upper surface on wet cotton wool on a plastic Petri dish (100 × 10 mm). Drinking water was daily supplied to the Petri dish during the test period.

Experimental design

Only no-choice tests were carried out because the two tetranychids were not present contemporaneously in the field. To obtain young phytoseiid females for the experiments, more than 50 females from the stock colony of each species were transferred to a new arena (5 cm diameter) to deposit eggs for 24 hours and afterwards removed. After hatching, immature stages were reared on *Oxalis* pollen until they reached adulthood. Couples of young females and males (max 24 h old) were transferred to new arenas (one couple per arena), for 3 days for mating and to include the pre-oviposition period, using *Oxalis* pollen as food. Only females that began deposit eggs were used for the experiments. After the first egg was laid, each female was transferred onto a leaf disc with 20 prey eggs. Every day, each female was transferred, using a fine brush, onto a new leaf disc with the predefined number (20) of prey eggs.

To obtain fresh prey eggs, 5 to 7 tetranychid females were transferred from cultures onto a leaf disc to deposit eggs for 24 hours. Afterwards, females were removed, and only 20 eggs were left on each leaf disc. The number of prey eggs consumed was daily recorded at the same time. Each test lasted four days and it was replicated 20 times. Predator eggs were recorded and removed daily.

To calculate the mean egg weight, three groups of 300 eggs of each phytoseiid species have been weighed.

The theoretical population growth of the two phytoseiid species on different prey eggs was estimated by the instantaneous rate of increase (r_i) (Hall, 1964; Walthall and Stark, 1997), calculated according to the following equation: $r_i = \ln(N_f/N_o)/\Delta t$ (N_f is the final number of animals, N_o is the initial number of animals and Δt refers to the number of days the experiment is run). According to Walthall and Stark (1997), the instantaneous rate of increase is similar to the intrinsic rate of increase (r_m). Positive values of r_i show a growing population, $r_i = 0$ indicates a stable population while negative values of r_i indicate a declining population directed toward extinction (Walthall and Stark, 1997).

Statistical analysis

Because the assumptions of a parametric model underlying the ANOVA method are not met, the non-parametric Kruskal-Wallis test was performed in order to verify the hypothesis of equality of the population medians. The statistic H was adjusted for ties dividing it by the factor D. The multiple comparisons between tests were based on Mann-Whitney U-test, as described by Sokal and Rohlf (1995).

Results

The mean predation rate on the two prey eggs was different for both phytoseiid species ($H = 263.87$, asymptotically distributed as χ^2 with $df = 3$, $P = 0.00$) (table 1). The highest predation activity was observed when eggs of *T. urticae* were supplied as food to *T. laurentii* (average rank AR = 265.0, corresponding to a standardized average rank $z = 11.66$), and the lowest when eggs of *P. citri* were supplied to *T. rhenanoides* (AR = 72.7; $z = -9.81$) in comparison to the overall average rank of 160.0. A very low predation activity on *P. citri* eggs was also observed for *T. laurentii* females (table 1). The predation rate of the latter species on *P. citri* eggs decreased through time, while *T. rhenanoides* consumed almost the same number of *P. citri* eggs (0.20-0.35 eggs/female) for the first three days of the experiment: no egg was preyed on the fourth day (table 1). On the opposite, both phytoseiid species on *T. urticae* eggs showed an increasing trend through time and the mean predation rate was statistically higher on the latter prey ($P \leq 0.01$) (table 1). Generally, these same trends were observed for the mean oviposition rate values ($H = 210.81$; $df = 3$; $P = 0.00$), even if differences in egglaying upon *P. citri* eggs were not significant between the two phytoseiids ($P = 0.38$) (table 1).

The output/input ratio (eggs laid/prey eggs consumed) on *P. citri* eggs has been calculated only for the whole experimental period, because of the scarce values registered during the observation intervals (i.e. one egg laid in a 24 hours interval, but no eggs preyed upon). On *T. urticae* eggs, the reproductive output/input ratio was low for both phytoseiids during the first two days but it significantly increased and stabilized afterwards (P < 0.01) (table 1). No significant difference was found for this parameter between the two predators during the experimental period ($P > 0.05$), showing that at least 5-6 *T. urticae* eggs are necessary to produce a predator egg. Considering that the mean egg weight of phytoseiids is 3.1 ± 0.01 μg for *T. laurentii* and 3.3 ± 0.02 μg for *T. rhenanoides*, and the egg weight of *T. urticae*, according to Sabelis (1981), is 1.2 μg, the mean biomass conversion varies from 45 to 52% for *T. rhenanoides* and *T. laurentii* respectively. It should be mentioned that no statistical difference was found in the mean biomass conversion between the two predators, as a very high individual variability was registered on both species (25-79% for *T. rhenanoides* and 37-74% for *T. laurentii*).

Differences in predation and oviposition rates between the two predators on *T. urticae* eggs, as well as similarities of prey biomass converted in predator eggs, are summarized in figure 1, where we report the relationship between the mean predation and the oviposition rate of each predator. There is a highly positive relationship ($P = 0.00$) between the two parameters for both

Table 1. Trend of predation, oviposition rate and reproductive output/intput ratio of the two phytoseiid species on eggs of *P. citri* and *T. urticae* during the observation period. Reproductive output/intput was calculated on *P. citri* only for the whole period, because less than one egg/day was preyed upon. Differences between observation intervals for each predator were calculated by Mann-Whitney test at 95% of confidence interval and are indicated by different letters. Different Greek letters indicate significant differences on the performance of each predator on the two prey eggs for the whole period. Asterisks indicate significant differences between the two predators for each observation interval. *ns* - not significant.

|	P r e y s									
	P. citri					*T. urticae*				
	No. preyed eggs/predator female					*No. preyed eggs/predator female*				
Phytoseiids	*Mean±SE*					*Mean±SE*				
	Observation intervals					Observation intervals				
	24h	48h	72h	96h	Whole period	24h	48h	72h	96h	Whole period
T. laurentii	1.30±0.25 a	0.30±0.10 b	0.30±0.11 b	0.25±0.09 b	0.54±0.09 β	10.8±0.51 a	14.0±0.52 b	12.35±0.43 b	12.95±0.59 b	12.53±0.28 γ
T. rhenanoides	0.30±0.10 a	0.20±0.09 a	0.35±0.10 a	0.00 b	0.21±0.05 β	8.75±0.45 a	9.85±0.47 ab	10.00±0.37 b	10.25±0.42 b	9.71±0.22 γ
	*	*ns*	*ns*	--	*	*	*	*	*	*
	Eggs laid/female					*Eggs laid/female*				
	Mean±SE					*Mean±SE*				
T. laurentii	0.60±0.11 a	0.05±0.05 c	0.35±0.10 ab	0.25±0.09 bc	0.31±0.05 β	1.30±0.10 a	2.20±0.12 b	2.45±0.11 b	2.55±0.13 b	2.13±0.08 γ
T. rhenanoides	0.60±0.11 a	0.10±0.07 b	0.20±0.09 b	0.09±0.07 b	0.25±0.05 β	0.85±0.08 a	1.25±0.14 b	1.55±0.13 b	1.60±0.11 b	1.31±0.07 γ
	ns	*ns*	*ns*	*ns*	*ns*	*	*	*	*	*
	Reproductive output/input ratio					*Reproductive output/input ratio*				
	Mean±SE					*Mean±SE*				
T. laurentii	--	--	--	--	0.49±0.058 β	0.12±0.008a	0.16±0.008a	0.20±0.011b	0.20±0.010b	0.17±0.003 γ
T. rhenanoides	--	--	--	--	0.87±0.13 β	0.10±0.012a	0.13±0.014a	0.16±0.015b	0.16±0.015b	0.14±0.004 γ
					ns	*ns*	*ns*	*ns*	*ns*	*ns*
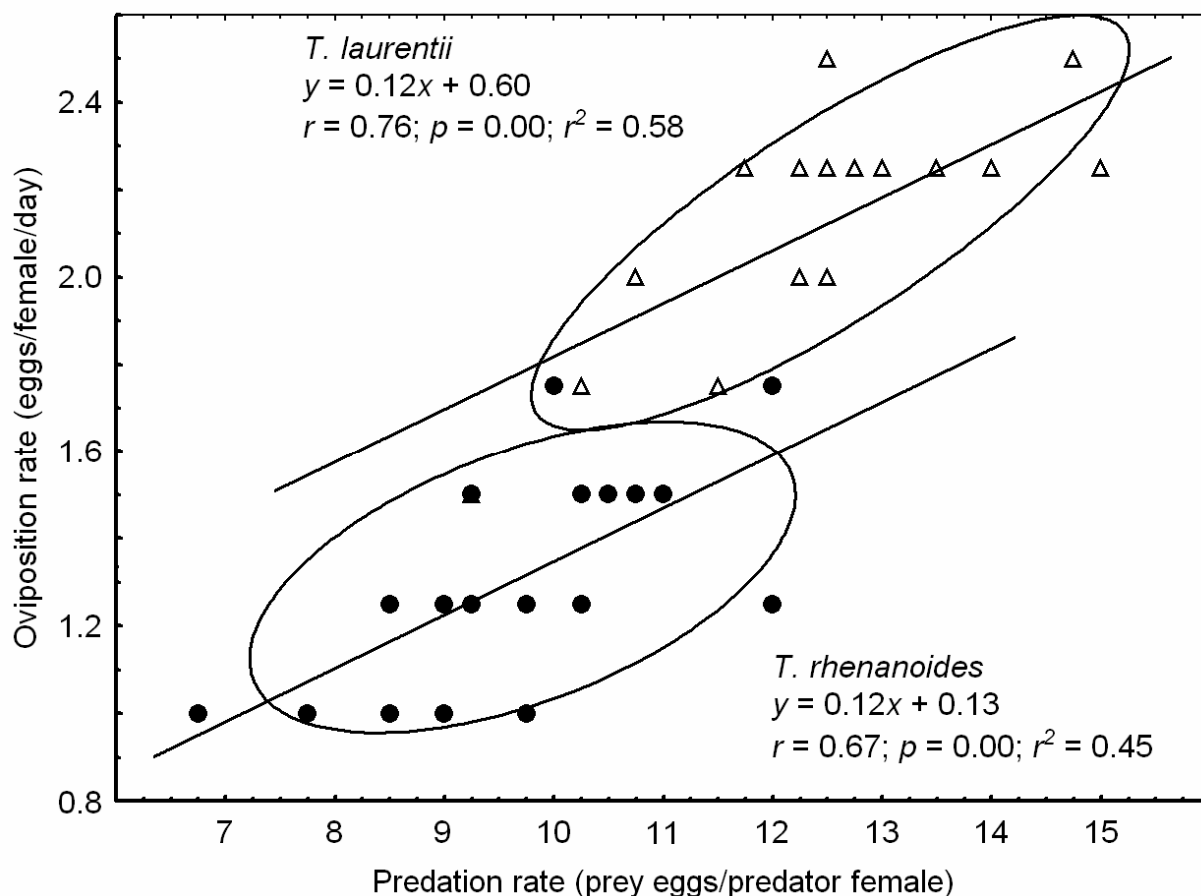
Figure 1. Relationship between predation and oviposition rates of *T. laurentii* and *T. rhenanoides*. Dots (*T. rhenanoides*) and triangles (*T. laurentii*) represent mean daily rate of one female observed during the whole observation period. Circles represent the 95% of confidence interval.
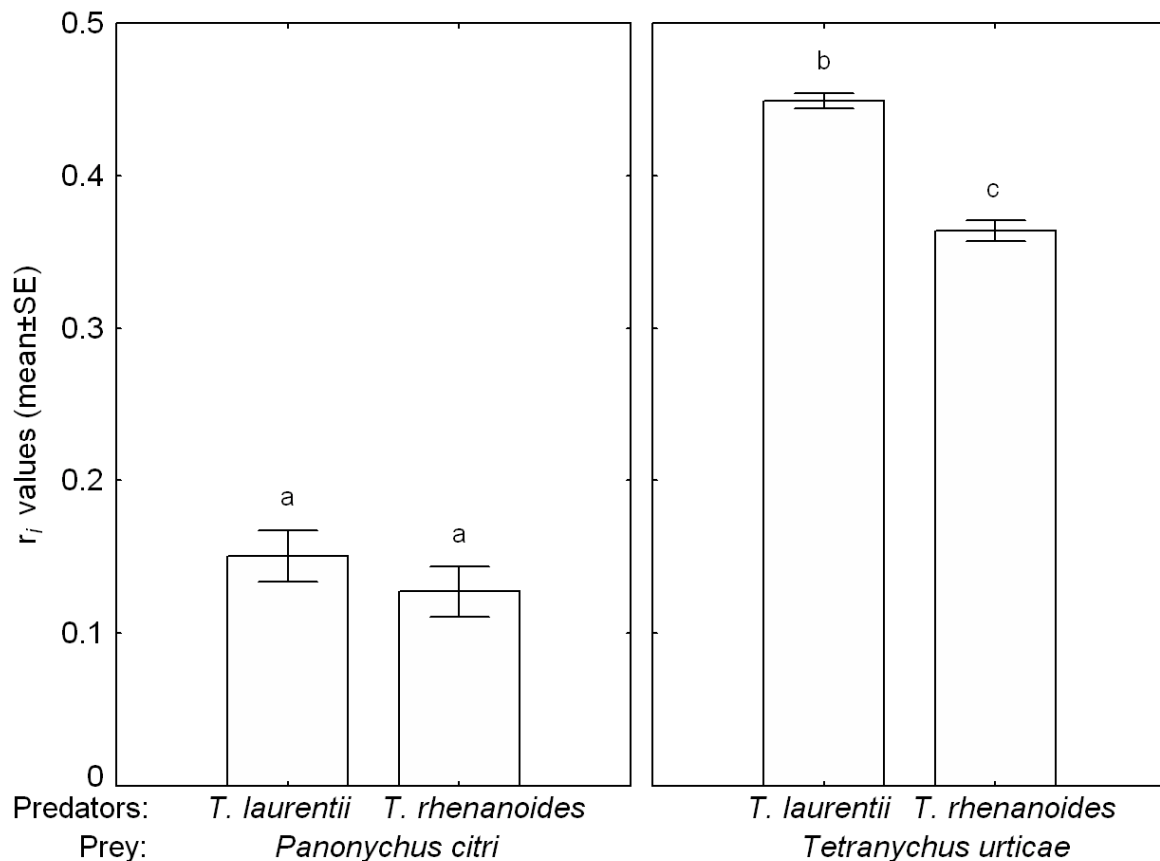
Figure 2. Instantaneous rate of increase (r_i) (mean $\pm$ SE) of the two phytoseiid mites fed upon eggs of *P. citri* and *T. urticae*. Different letters denote significant differences among phytoseiids and prey species for $P < 0.05$. The Mann-Whitney test was performed on the data.

phytoseiids, but the conversion of prey eggs consumed into number of eggs laid is not the same for the two predators: 95% of *T. rhenanoides* preyed on 7-10 eggs/female/day and laid 0.9-1.5 eggs/female/day, while *T. laurentii* for the same confidence interval preyed on 9 to 13 eggs/female/day and laid 1.7 to 2.5 eggs/female/day. Regression lines have the same slope, but the intercept of *T. laurentii* values is higher. The majority of the data falls inside the area where the 95% of data are expected to be, assuming a normal bivariate distribution; so this model is well supported.

As said above, feeding activity was very limited on *P. citri* eggs, and this of course was reflected in the reproductive potential of both species. However, this low predation activity positively affected the theoretical population growth of the two phytoseiid species (figure 2). On the other hand, the influence of *T. urticae* eggs was more positive for both phytoseiid species, but the growth rate of *T. laurentii* was significantly higher than that of *T. rhenanoides* (figure 2).

Discussion

It is evident from our data that *P. citri* eggs do not represent a suitable food for the two phytoseiid species herein considered. Does this depend on the food quality or on the defence strategy of the prey stage? Sabelis

(1981) discussed the nutritive quality among the various prey stages, considering the number of eggs laid by the predator female as the principal output measurement parameter; the author also reported the difficulty to determine this parameter because it mainly depends on the energy spent to capture a prey. Different authors showed a variety of behaviours among Phytoseiidae, but the preference for eggs and juveniles seems to be the most common (Burnett, 1971; Fernando and Hassell, 1980; Pickett and Gilstrap, 1986; Ragusa *et al.*, 2000; Blackwood *et al.*, 2001). However, the egg protection strategy is different among the various tetranychid species. *P. citri* is considered a poor weaver tetranychid (Gerson 1979; Saito, 1983); it lays an onion-like egg, surmounted by a characteristic spike, from which, the female, after ovipositing, attaches up to a dozen silk "guy ropes" to the leaf surface, as reported by Beament (1951) for *Panonychus ulmi* (Koch). According to the latter author and to Gerson (1979), the egg covering with silk might have a deterrent effect against predators, but Saito (1983) stated that there was no evidence of this function.

It is known that the success of a predator in capturing and consuming a prey depends both on the attack strategy of the predator and the defence strategy of the prey (Sabelis, 1985). There is a wide range of combinations of these aspects among phytoseiid mites. According to Gotoh *et al.* (2006), for example, *Cydnodromus californicus* (McGregor) preys on the same number of *T. urti-*

cae and *P. citri* eggs (about 15), but the oviposition rate is slightly lower on *P. citri* eggs. Diversely, Katayma *et al.* (2006), reported for the same phytoseiid species a higher daily consumption on *P. citri* eggs (17.2 eggs/predator female) and a lower predation on *T. urticae* eggs (11.7 eggs/predator female), but the same oviposition rate on both prey (2.5 eggs/female/day). On the other hand, *E. stipulatus*, a very common species in Mediterranean citrus orchards, fed on all juvenile stages and adults of *P. citri* but it was unable to prey on eggs, while *Typhlodromus* (*Typhlodromus*) *phialatus* Athias-Henriot indifferently preyed on various stages of the tetranychid (Ferragut *et al.*, 1992). Both phytoseiids used in our experiments showed a very scarce consumption of *P. citri* eggs and laid few eggs during the whole experimental period. Assuming that: 1) at least the same amount of prey egg biomass ingested is equal to the predator egg biomass produced; 2) about 60-70% of food ingested is utilized for egg production (Sabelis, 1981); 3) that the egg weight of the above mentioned phytoseiids varies from 3.1 to 3.3 µg/egg, it is evident that females used part of their own reserve energy to oviposit as they were unable to prey on sufficient *P. citri* eggs. As a matter of fact, after four days, females of both species showed a thinner body, in same cases transparent, but it should be mentioned that they were still active in searching, though this behaviour was not quantified.

On the contrary, both phytoseiid species showed a round and fat body when fed on *T. urticae* eggs. On this prey, phytoseiids needed two days of adaptation before reaching the maximum predation and oviposition rates. The low reproductive output/input ratio values during the first two days could suggest the need of females to fortify their own bodies before expending energy on egg production. However, the low conversion values registered for both predators (45 to 52% for *T. rhenanoides* and *T. laurentii* respectively), in comparison to those reported in the literature, 60-70% (Sabelis, 1981), suggest that a greater amount of energy was needed for both phytoseiid species to find and prey on a *T. urticae* egg, or that not all of the content of prey egg was extracted by each phytoseiid female. As a matter of fact, *T. urticae* weaves a complicated and irregular web and lays eggs on silk threads, especially at high population density (Saito, 1983), but it oviposits directly on the leaf surface at the beginning of the infestation, without silk covering. According to Sabelis (1981), during the first phase of colony growth, the coincidence prey/predator is independent of the predator species involved. These data lead us to opt for the second hypothesis that is both species did not consume all of the prey egg content. On the other hand, Ferragut *et al.* (1992) found a very low ingestion rate for *E. stipulatus* (30% of the prey killed) and values similar to those registered in the present work for *T. phialatus* (from 40 to 100% of the eggs were consumed).

The food conversion into eggs laid directly influences the population growth and it is directly linked to the control of phytophagous mites populations. The evaluation of this influence on phytoseiid densities is well illustrated by the intrinsic rate of increase (r_m) (Castagnoli

and Simoni, 1990; Tsolakis and Ragusa, 1994; Vantornhout *et al.*, 2005). However, some authors adopted the instantaneous rate of increase (r_i), as defined by Hall (1964), as a valid alternative to the r_m, especially in short-term ecotoxicological experiments (Walthall and Stark, 1997; Tsolakis and Ragusa, 2008). On the other hand, Ragusa *et al.* (2009) used the instantaneous rate of increase as a population-level endpoint to evaluate the influence of different kinds of foods on the population growth of *C. californicus* in laboratory experiments. Our data suggest that eggs of both tetranychids positively influenced the population growth. However, *T. urticae* eggs allow a rapid population increase, especially for *T. laurentii*.

In conclusion, the performance of the above mentioned not specialized predators is different when the eggs of the two tetranychids were supplied; this fact could be relevant for the control of the phytophagous population. As a matter of fact, *P. citri* eggs were less vulnerable to the predator attacks, while those of *T. urticae* suffered from a high predation activity. This fact might be inverted during the increasing of the infestation because the web covering, protects the young stages of *T. urticae*, while the young stages of *P. citri* are more vulnerable because they are not covered by the web. Further investigations are needed in order to verify the impact of the above phytoseiid species on tetranychid population at different phases of infestation.

Acknowledgements

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