

Life tables of the predatory mite *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures (Acari: Phytoseiidae, Tetranychidae)

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Received: 3 November 2006 / Accepted: 23 January 2007 / Published online: 3 March 2007
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Abstract The tomato red spider mite, *Tetranychus evansi*, is reported as a severe pest of tomato and other solanaceous crops from Africa, from Atlantic and Mediterranean Islands, and more recently from the south of Europe (Portugal, Spain and France). A population of the predaceous mite *Phytoseiulus longipes* has been recently found in Brazil in association with *T. evansi*. The objective of this paper was to assess the development and reproduction abilities of this strain on *T. evansi* under laboratory conditions at four temperatures: 15, 20, 25 and 30°C. The duration of the immature phase ranged from 3.1 to 15.4 days, at 30 and 15°C, respectively. Global immature lower thermal threshold was 12.0°C. Immature survival was high at all temperatures tested (minimum of 88% at 30°C). The intrinsic rate of increase (r_m) of *P. longipes* ranged from 0.091 to 0.416 female/female/day, at 15 and 30°C,

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respectively. *P. longipes* would be able to develop at a wide range of temperatures feeding on *T. evansi* and has the potential to control *T. evansi* populations.

Keywords *Tetranychus evansi* · *Phytoseiulus longipes* · Biological control · Life tables · Temperatures

Introduction

The phytophagous mite *Tetranychus evansi* Baker and Pritchard is found mainly on solanaceous plants such as tomato, eggplant and tobacco (Bolland et al. 1998). It is reported as a severe pest in tomato crop in Africa (Saunyama and Knapp 2003). It has been more recently found in Southern Europe (Ferragut and Escudero 1999; Bolland and Vala 2000; Aucejo et al. 2003; Migeon 2005; Castagnoli et al. 2006; Palevsky, pers. comm.). Resistance to various acaricides has been reported (Blair 1989), and predators commonly used against other spider mite pests, especially *Tetranychus urticae* Koch, are inefficient to control this species (Moraes and McMurtry 1985, 1986; Escudero and Ferragut 2005). Surveys for biological control agents in the suspected area of origin of *T. evansi* (Argentina and Brazil) have been conducted (Furtado et al. 2006b, in press), and a phytoseiid mite species, *Phytoseiulus longipes* Evans, has been found in association with *T. evansi* (Furtado 2006).

Previous predation tests suggested the ineffectiveness of a South African population of *P. longipes* in controlling *T. evansi* (Moraes and McMurtry 1985) but tests conducted with the Brazilian strain suggested it to be very promising (Furtado 2006). In order to optimize the use of this species in biological control, data concerning its development at different temperatures are needed. The aim of the present study was to determine life tables of *P. longipes* feeding on *T. evansi* at four temperatures (15, 20, 25 and 30°C). The temperature range selected is common in greenhouses (Zhang 2003), and is known to be favorable to the development of *T. evansi* (Moraes and McMurtry 1987; Bonato 1999).

Material and methods

Experimental conditions

The specimens of *P. longipes* used in this study were obtained from a colony initiated with specimens collected in March 2004 in Uruguaiana (29° 32' 69'' S, 56° 32' 06'' W), State of Rio Grande do Sul (Brazil) (Furtado 2006). The colony was fed with a mixture of *T. urticae*, offered on leaves of *Canavalia ensiformis* (L.) DC., placed on a plastic sheet (PAVIFLEX[®]) laid on a piece of foam mat in a plastic tray (25 × 17 × 9 cm). The margins of the plastic plate were covered with a 1-cm-wide band of cotton wool, to prevent mite escape. Plastic trays were kept in incubators at 25°C, 80 ± 10% RH and 12:12 [L:D] photoperiod.

The *T. evansi* stock colony was initiated with specimens collected from a tomato field at Piracicaba (22° 41' 72'' S, 47° 38' 48'' W), State of São Paulo (Brazil), and reared on *Solanum americanum* Miller and *Lycopersicon esculentum* Miller in screen cages.

The *T. urticae* stock colony initiated with specimens collected in Piracicaba (22° 41' 72'' S, 47° 38' 48'' W), State of São Paulo (Brazil), was reared on *C. ensiformis* in screen cages.

Immature development

The following steps were performed at 15, 20, 25 or 30°C, at 80 ± 10% RH and with a 12:12 [L:D] photoperiod. Groups of five to eight *T. evansi* females were placed in experimental units, consisting of a leaf disk of *S. americanum* (2 cm in diameter) placed underside up onto a moist disk of filter paper inside a Petri dish (2 cm in diameter, 1 cm high) using a thin paintbrush. Two days later, when 20–30 eggs of *T. evansi* had been laid, an egg of *P. longipes* (between 0 and 6 h old) was taken from the intermediate stock colony (consisting in 30–50 females reared on a leaf of *C. ensiformis* placed in a plastic tray as described above) and transferred to each experimental unit every 6 h. Each unit was then closed with a transparent plastic film. To maintain humidity, distilled water was added on the filter paper every day. Periodically (from 2 to 3 days at 30°C to 7 days at 15°C) *P. longipes* individuals were transferred to new leaves infested with *T. evansi* as previously reported.

Observations have been carried out every 8 h to determine the duration and the survivorship for each stage.

Lower thermal threshold (TD) was calculated as a/b , where a and b are determined by the following linear regression: $DR = a + bT$, where DR is the development rate per day, T the temperature in °C and a and b the regression coefficients (Bonato 1999).

Reproduction

Recently emerged adult *P. longipes* females obtained were transferred to new experimental units. A male taken from the stock colony was then added to each unit containing one female. At least 30 couples were observed at each temperature. Daily observations were conducted to determine female fecundity and survivorship. The eggs laid were placed together daily in a single unit and reared to adulthood to determine the secondary sex ratio (female percentage of the studied female cohort offspring).

Life table

The life table was constructed considering the females of the cohort studied. The net reproductive rate (R_0), the mean generation time (T), the intrinsic rate of increase (r_m), the doubling time (D_t), and the finite rate of increase (λ) were calculated using the method recommended by Birch (1948):

- $R_0 = \sum (l_x \times m_x)$
- $T = \sum (x \times l_x \times m_x) / \sum (l_x \times m_x)$
- $r_m = \text{Ln}(R_0) / T$
- $D_t = \text{Ln}(2) / r_m$
- $\lambda = \exp(r_m)$

Here x is age (with 0.5 for the day when eggs had been laid), l_x , the cumulative female survivorship, and m_x , the number of female descendants per female at x .

Calculation of a corrected r_m value was performed by iteration. The method, aiming to find r_m for which $(1 - \sum \exp(-r_m \times x) \times l_x \times m_x)$ is minimal, was given by Maia et al. (2000).

Analysis of variance (ANOVA) and related Tukey HSD mean comparison tests were performed to determine differences between duration of the immature phases and adult stages at the four temperatures tested (R project 2006).

Results

Immature development

Increasing temperatures significantly decreased the duration of all stages. The egg stage was the longest for each temperature tested, decreasing from 5.9 days at 15°C to 1.1 days at 30°C. The larval stage was the shortest, varying from 1.5 day at 15°C to 0.4 day at 30°C. The durations of the protonymphal and deutonymphal stages were about the same, within each temperature tested. Duration of the whole immature phase (egg to adult emergence) decreased from 15.4 days at 15°C to 3.1 days at 30°C (Table 1).

At 15 and 20°C, 96 and 95% of the immatures survived, respectively. The lowest mortality occurred at egg stage. At 25°C, all mites reached the adult stage. The lowest immature survival rate was observed at 30°C (88%) and concerned all immature stages. Lower thermal thresholds (TD) were calculated for each immature stage and for the whole immature phase (Table 2). TD ranged from 10.2°C for the larval stage to 12.6°C for the egg stage.

Reproduction

As previously observed for immature development, the longevity of adults was highest at 15°C and decreased as the temperature increased (Table 3). The pre-oviposition period ranged from 3.1 to 0.5 days (maximum was 3.3 at 20°C), oviposition from 17.0 to 6.8 days, post-oviposition from 6.2 to 1.3 days and longevity from 43.8 to 13.1 days, at 15 and 30°C, respectively.

Table 1 Mean duration (\pm Standard Error) in days of the immature instars of a Brazilian strain of *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four constant temperatures, number of replicates (n) and immature survival

Stage	Temperature (°C)				$P < F_{(df1, df2)} (\alpha = 0.01)$
	15	20	25	30	
Egg	5.9 (0.6) a	3.1 (0.2) b	2.0 (0.2) c	1.1 (0.1) d	$P < F_{(3, 186)} = 1706.30$
Larva	1.5 (0.2) a	0.8 (0.2) b	0.5 (0.2) c	0.4 (0.2) d	$P < F_{(3, 186)} = 370.70$
Protonymph	3.8 (0.9) a	2.1 (0.5) b	1.0 (0.2) c	0.7 (0.3) d	$P < F_{(3, 186)} = 328.29$
Deutonymph	4.3 (0.7) a	2.2 (0.5) b	1.3 (0.4) c	0.9 (0.3) d	$P < F_{(3, 186)} = 468.33$
Egg to adult	15.4 (1.5) a	8.2 (0.8) b	4.9 (0.4) c	3.1 (0.3) d	$P < F_{(3, 186)} = 1988.60$
N	39	40	56	54	
Immature survival (%)	96	95	100	88	

$P < F$ (ANOVA, $df1 = 3$, $df2 = 119$, $\alpha = 0.01$) means that there are significant differences between means for a stage. Means followed by the same letter for a stage are not significantly different (Tukey HSD test, $\alpha = 0.01$)

Table 2 Lower thermal thresholds (TD) for development for all immature stages and “egg to adult” development of a Brazilian strain of *Phytoseiulus longipes* feeding on *Tetranychus evansi*, linear regression coefficient (R^2), number of replicates (n)

Stage	Regression equation	R^2	TD (°C)
Egg	$y = 0.2384x - 0.1226$	0.9335	12.6
Larva	$y = 0.6613x - 0.0279$	0.9981	10.2
Protonymph	$y = 0.3905x - 0.1972$	0.9768	12.5
Deutonymph	$y = 0.2932x - 0.0843$	0.9949	11.4
Egg to adult	$y = 0.0856x - 0.0348$	0.9777	12.0

$n = 189$

Table 3 Mean durations (\pm Standard Error) of adult phases, longevity and ovipositional rates of the Brazilian strain of *Phytoseiulus longipes*, number of replicates (n)

Stage	Temperature (°C)				$P < F_{(df1, df2)}$ ($\alpha = 0.01$)
	15	20	25	30	
Pre-oviposition in days	3.1 (1.1) a	3.3 (1.9) a	2.3 (1.9) a	0.5 (1.4) b	$P < F_{(3, 119)} = 20.95$
Oviposition in days	17.0 (12.3) a	11.0 (7.6) ab	11.1 (6.0) ab	6.8 (5.3) b	$P < F_{(3, 119)} = 7.99$
Post-oviposition in days	6.2 (9.1) a	2.0 (1.6) b	2.0 (1.9) b	1.3 (1.2) b	$P < F_{(3, 119)} = 6.99$
Longevity in days	43.8 (17.9) a	27.2 (9.0) b	20.8 (5.8) bc	13.1 (5.5) c	$P < F_{(3, 119)} = 45.68$
Eggs per female per day	0.3 (0.3)	0.5 (0.5)	0.9 (0.5)	1.7 (0.8)	
Total eggs per female	14.9 (10.6)	17.9 (12.4)	22.7 (14.1)	26.4 (21.4)	
N	30	30	30	31	

$P < F$ (ANOVA, $df1 = 3$, $df2 = 119$, $\alpha = 0.01$) means that there are significant differences between means for a stage. Means followed by the same letter for a stage are not significantly different (Tukey HSD test, $\alpha = 0.01$)

A reverse tendency was observed for daily oviposition and fecundity rates, which ranged from 0.3 to 1.7 and 14.9 to 26.4 eggs per female at 15 and 30°C, respectively.

The secondary sex ratio was 0.85, 0.83, 0.78 and 0.82 at 15, 20, 25 and 30°C, respectively.

Life table

Calculated life table parameters are given in Table 4. Concurrently with the tendency observed for duration of immature and adult stages, a trend towards lower values of mean generation time and doubling time was observed from the lowest to the highest temperatures. Congruently with those trends and with the observed higher rates of oviposition at higher temperatures, intrinsic rates of increase (iterative method) and finite rates of increase raised from 0.091 to 0.416 female/female/day and from 1.09 to 1.53, from 15 to 30°C, respectively. The net reproductive rate increased progressively from 15 to 25°C, and remained about the same at 25°C and 30°C.

Discussion

Up to 2005, data on the biology of *P. longipes* concerned a strain from South Africa (Badii 1981; Badii and McMurtry 1983; Takahashi and Chant 1992, 1994), reported as not effective to control *T. evansi* (Moraes and McMurtry 1985). Furtado et al.

Table 4 Demographic parameters of *Phytoseiulus longipes* feeding on *Tetranychus evansi* at four temperatures: net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), doubling time (D_t), and finite rate of increase (λ)

Temperature (°C)	Demographic parameter				
	R_0	T	λ	D_t	r_m
15	9.74	26.44	1.09	8.05	0.091
20	10.01	18.65	1.13	5.61	0.123
25	13.88	12.92	1.23	3.40	0.293
30	13.84	8.17	1.38	2.15	0.416

(2006a, submitted) reported for the first time data on the biology of the strain from Brazil.

The present study confirms previous observations about the ability of the Brazilian strain of *P. longipes* to develop feeding on *T. evansi* (Furtado 2006). The larvae did not feed, thus no food was required to reach the protonymphal stage, as it is the case in many Type I phytoseiids as defined by McMurtry and Croft (1997), especially species of the genus *Phytoseiulus*. It has been observed, as previously by Furtado et al. (2006b, in press), that other stages of this species are neither hampered by *T. evansi* webbing nor by tomato trichomes. These observations could make *P. longipes* a preferential biocontrol agent against *T. evansi* populations.

Generally, demographic parameters from different studies are difficult to compare, as differences could be due to strains as well as to experimental methodology variations, for instance size and type of arenas, food provided, relative humidity, photoperiod and differences in calculations method.

The duration of the immature stages of *P. longipes* feeding on *Tetranychus pacificus* (McGregor), *Oligonychus punicae* (Hirst) and *Panonychus citri* (McGregor) were lower at 20, 25 and 30°C for the South African strain than for the Brazilian (Badii 1981; Badii and McMurtry 1983; Takahashi and Chant 1992). The latter seems to have a greater development ability than the South African populations at these temperatures, feeding on *T. evansi*. Development durations mentioned by Furtado et al. (2006a, submitted) were very close to those reported in this paper, for all immature stages.

The egg stage was the most sensitive to low temperatures, as it has the highest TD value (12.6°C). Lower thermal threshold values are similar to those of other phytoseiids. For instance, Gotoh et al. (2004) reported a TD of 10.3°C from egg to oviposition for *Neoseiulus californicus* (McGregor), while in the present work TD for immature development was 12°C. *Phytoseiulus longipes* has been reported from various regions of the world where low temperatures are very common during the winter, such as North Argentina, North Chile, South Africa, Zimbabwe (Moraes et al. 2004) and now Southern Brazil (Furtado 2006). The rapid development of the Brazilian strain of *P. longipes* feeding on *T. evansi* and its ability to develop during cold periods suggest that it would perhaps survive in temperate climates in Africa and Europe.

As for immatures, important differences were also found between previous data and the present results concerning the duration of the adult phases of *P. longipes*. At 25°C, Badii (1981) reported for the South African population an oviposition phase of 20.8 days feeding on *T. pacificus* versus 11.1 days in the present study. Takahashi

and Chant (1992) reported an oviposition phase of 10.9 at 26°C feeding also with *T. pacificus* as prey.

Values of r_m obtained by Badii (1981) and Takahashi and Chant (1994) for the South African strain of *P. longipes* were higher than in the present study, with 0.366 at 25°C and 0.465 at 26°C, respectively. Along with the origin of the *P. longipes* strains tested, the methodology and the food provided, the calculation of the secondary sex ratio could explain those differences. Badii (1981) and Takahashi and Chant (1994) based their estimation of the sex ratio on the generation considered in their studies for immature development, while in the present study calculations were made from the offspring of the generation considered for immature development. This difference leads to an underestimation of the r_m in the present paper compared to the others cited above.

Similar values of r_m as obtained in this paper are reported in the literature for other phytoseiid species (Sabelis 1985), for instance for *Amblyseius longispinosus* (Evans) (Kolodochka 1983, in Sabelis, 1985), for *Amblyseius deleoni* Muma and Denmark (Saito and Mori 1981, in Sabelis 1985) and for *N. californicus* (Ma and Laing 1973). On the other hand, they are very low compared to other Type I species as defined by McMurtry and Croft (1997). At 26°C, for *Phytoseiulus persimilis* Athias-Henriot, *Phytoseiulus macropilis* (Banks) and *Phytoseiulus fragariae* Denmark and Schicha, Takahashi and Chant (1994) reported r_m -values of 0.4282, 0.3862 and 0.3263, respectively. Despite these differences, the values obtained are relatively high and could be considered as sufficient to control *T. evansi* populations efficiently (Gerson et al. 2003), even if more information about the specific predator/prey relation between *P. longipes* and *T. evansi* are needed to prove *P. longipes* effectiveness at a field scale (Janssen and Sabelis 1992).

The temperature for optimum development and reproduction of *T. evansi* was estimated to be 34°C ($r_m \approx 0.4$) (Bonato 1999). The optimum for *P. longipes* could not be determined in this study, but seems to occur at more than 30°C. It could thus be stressed that *P. longipes* would reproduce well at a wide range of temperatures in presence of *T. evansi*. It seems to perform better in warm environments, at temperatures at which *T. evansi* also develops better.

Furtado et al. (2006a, submitted) worked for the first time on the Brazilian *P. longipes* strain biology. Three major differences can be found between their study and the results reported here. Firstly, the adult phases are always longer in their work than in the present. Then, the secondary sex ratio is also higher, 0.90 vs. 0.78 between Furtado et al. (2006a, submitted) and the present values, respectively. Finally, all the reproductive parameters are greater, due to a major difference in the daily oviposition rates obtained in this previous study. Those differences could be explained mainly by two reasons. In Furtado et al. (2006a, submitted), the *P. longipes* stock colony was reared on tomato and fed on *T. evansi*. In the present paper, it was reared on *C. ensiformis* and fed *T. urticae* for many generations before the tests. The second point is that biology tests were performed in this paper on *S. americanum* instead of *L. esculentum* in the other work. Despite these differences, both papers reported that the life table parameters values would support the effectiveness of the Brazilian strain of *P. longipes* for *T. evansi* biocontrol. These promising results should lead to further work, for instance to compare variations of life table parameters, and especially oviposition, depending on the food provided and the vegetal support on which experiments are conducted.

Phytoseiulus longipes has excellent potential as a biocontrol agent for *T. evansi*, in European tomato greenhouses as well as in small-scale tomato production in Africa. The temperatures at which it develops and reproduces well lead to promising perspectives for its use in solanaceous greenhouses. Further experiments on its effectiveness and uses in biocontrol will be conducted in order to set the right conditions in which *P. longipes* would be the most efficient.

Acknowledgements This collaborative research between the Red Spider Mite Project at ICIPE, ESALQ/USP and ENSAM/INRA was funded by a grant of the German Federal Ministry for Economic Cooperation and Development (BMZ) to ICIPE.

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