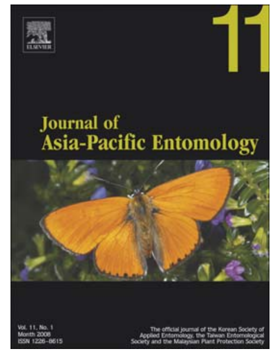

The effect of *Tetranychus turkestani* and *Eutetranychus orientalis* (Acari: Tetranychidae) on the development and reproduction of *Stethorus gilvifrons* (Coleoptera: Coccinellidae)

Z. Imani, P. Shishehbor, F. Sohrabi

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The effect of *Tetranychus turkestani* and *Eutetranychus orientalis* (Acari: Tetranychidae) on the development and reproduction of *Stethorus gilvifrons* (Coleoptera: Coccinellidae)

Z. Imani, P. Shishehbor and F. Sohrabi

Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran.

Email : shishehborpf@yahoo.com

Abstract: *Stethorus gilvifrons* Mulsant, native to the Mediterranean region, is often observed feeding on *Tetranychus turkestani* Ugarov & Nycolsky and *Eutetranychus orientalis* Klein on different host crops. Fecundity of *S. gilvifrons* on *T.turkestani* and *E. orientalis* was evaluated by placing newly emerged pairs on leaf discs infested with different developmental stages of *T. turkestani* or *E. orientalis*. They were maintained at 30ºC and changed daily until death of the female. Adult female mean longevity was 58 d on *T. turkestani* and 45 d on *E. orientalis*. Mean fecundity was 175 eggs per female on *T. turkestani* and 318 eggs per female on *E. orientalis*. No significant differences were detected in the duration of life stages between *T. turkestani* and *E. orientalis*. Mean preimaginal mortality was 20% on *T. turkestani* and 24% on *E. orientalis*, with no statistical differences. Mean generation time (T) was 21 and 23 days on *T. turkestani* and *E. orientalis*, respectively. Net reproductive rate (R_o) was significantly greater on *E. orientalis* (94) than on *T. turkestani* (45), but the estimate of intrinsic rate of increase (r_m) was not statistically different (0.193 and 0.179, respectively). Our results suggest that both *T. turkestani* and *E. orientalis* are essential prey for *S. gilvifrons* development and reproduction and that *E. orientalis* is slightly more suitable than *T. turkestani*.

Key words: *Stethorus gilvifrons,* developmental time, fecundity, life table, *Tetranychus turkestani, Eutetranychus orientalis*

Introduction

Tetranychs turkestani Ugarov & Nycolsky and *Eutetranychus orientalis* Klein (Acari: Tetranychidae) are two important pests in southwestern Iran agricultural systems. They cause significant damage to horticultural plants in both the field and in greenhouses. The strawberry spider mite, *T. turkestani*, is a polyphagous cosmopolitan pest (Jeppson et al. 1975; Mossadegh and Kocheili 2003). It is one of the best known pests in tropical ecosystems and it causes damage to cucurbitacean, leguminosae and other field and horticultural plants (Jeppson et al. 1975; Kamali et al. 2004). The citrus spider mite, *E. orientalis*, is a polyphagous species which is found in tropical regions that threatens many economically important horticultural and ornamental plants (Jeppson et al. 1975; Kamali et al. 2004).

Current control of these pests in Iran relies mainly on acaricides. Due to continuous use of pesticides, these mite species have developed resistance to most available acaricides (Shishehbor, unpublished data). In addition, public concern regarding pesticide residue in both food products and the environment encouraged researchers to look for alternative methods of managing spider mites in field crops and fruit trees. Therefore, there has been an increasing interest in controlling spider mites with biological control agents (Roy et al. 2002; Roy et al. 2003; Gotoh et al. 2004).

Different species of *Stethorus*, an acarophagous ladybug, including *S. loxtoni* Britton and Lee (Richardson 1977), *S. madecassus* Chazeau (Gutierrez and Chaezau 1972; Chazeau 1974), *S. picipes* Casey (Tanigushi and McMurtry 1977), *S. punctillum* Weise (Putman 1955; Skeroglu and Yigit 1992; Roy et al. 2003), *S. japonicus* (Mori et al. 2005), *S. tridents* Gordon (Fiaboe et al. 2007) and *S. gilvifrons* Mulsant (Aksit et al. 2007; Taghizadeh et al. 2008a,b) have recently received intensive studies for their effectiveness as predators on spider mites.

S. gilvifrons is a native beneficial coccinellid in Iran (Mossadegh and Kocheili 2003) and other countries in the region (McMurtry et al. 1970; Chazeau 1985; Aksit et al. 2007). It is common in fields of sugarcane (Afshari 1998), date palm (Kajbaf Vala 1991, 1999) and castor bean (Modares Awal 2001) and it is a good candidate for biological control of numerous spider mites (Chazeau 1985).

As these two pests may be simultaneously present in horticultural plants, both in fields or greenhouses, we evaluated the development and reproduction of *Stethorus gilvifrons* Mulsant feeding on both *T. turkestani* and *E. orientalis* as food sources. Although some bionomic studies of *S. gilvifrons* have been conducted (Aksit et al. 2007; Matin 2008; Taghizadeh et al. 2008a, b), no detailed study has reported its biology on *T. turkestani* and *E. orientalis*. The objective of this study is to quantify the effects of different prey species on biological characteristics of *S. gilvifrons* which could lead to the development of a better strategy for biological control of these two mite species using *S. gilvifrons.*

Materials and methods

Mites and coccinellid stock colonies

Our laboratory stock colonies of *Tetranychus turkestani*, *Eutetranychus orientalis* and *Stetorus gilvifrons* were established one month before starting the experiments with ≈ 50 adult individuals collected from wild castor bean plants on the campus of the Shahid Chamran University, Ahvaz, Iran (31º 20´ N, 48º 40´ W). Two species of mites were reared separately on young castor plants (cultivar Ahvazy) which were grown from seeds in plastic pots (20 cm diameter) in mesh covered wooden cages (60 × 60 ×120 cm).

Ladybird beetles were reared in two separate wooden cages (as described above) on a tritrophic system (host plant castor bean; prey *T. turkestani* and *E. orientalis*; predator *S. gilvifrons*). The wooden cages were kept in the laboratory condition at 20 ± 1 ºC and 50 ± 5 % R. H. The photoperiod was 14 L: 10 D, using fluorescent lamps.

Each culture was maintained by the addition of suitable castor bean seedlings at weekly intervals. Extra plants were also grown to provide additional leaves for the Petri dish experiments. The experimental arena consisted of 6.0 cm diameter cowpea and castor bean leaf discs (for *T. turkestani* and *E. orientalis*, respectively) placed upside down on a soaked foam base in individual 8.0 cm diameter Petri dishes. The Petri dishes were covered with lids ventilated with a 0.12 mm mesh. A small paint brush was used to transfer mites and coccinellids to the leaf discs in petri dishes. A stereomicroscope was used for observations.

Development and reproduction

To obtain synchronized eggs, coccinellid females (ca. 20 for each mite species) were isolated for 24 hours on cowpea and castor bean leaf discs harbouring *T. turkestani* or *E. orientalis*, respectively. Newly laid eggs of *S. gilvifrons* were then placed individually on respective leaf discs. Upon hatching, *S. gilvifrons* larvae were fed daily with excess (100) various stages of *T. turkestani* and *E. orientalis*. The Petri dishes were placed in growth chamber (30 ± 1 ºC, 60-70 % R. H. and 14: 10 L: D) and egg to adult developmental time and mortality of immature stages were recorded under each of the two different mite species. The presence of an exuvium was used as the criterion of a successful molting.

Newly molted adult female coccinellid beetles (age <24 h old) reared from larva to adult on each of the two mite species were maintained individually on a leaf disc harbouring respective *T. turkestani* or *E. orientalis* in a Petri dish with a young adult male (age <24 h old). Egg laying and mortality were recorded daily. Males that died or escaped from the experimental unit were replaced by the young ones (age <24 h old). Females that were trapped in the wet sponge or died because of improper handling were excluded from data analysis. At the onset of reproduction, females and males were transferred daily to fresh leaf discs and longevity (mean total adult life span) and fecundity (mean daily and total number of eggs laid per female) on the different mite species were recorded. All eggs were transferred to new leaf discs until adult eclosion and the numbers of male and female coccinellid beetles were recorded to determine the sex ratio.

Data analysis

Where appropriate, parameters such as development time, preoviposition time, oviposition time, postoviposition time, longevity and fecundity were subjected to either one- or two-way analysis of variance and mean separation by Fisher's protected LSD test ($P < 0.05$) (SAS 2001). A series of Chi-square tests were conducted to determine if there were any significant differences in stage mortality for ladybirds reared on *T. turkestani* or *E. orientalis*.

Life and fecundity table parameters were estimated by combining data from the preimaginal development and adult survival and reproduction experiments on different prey species. The intrinsic rate of population increase was determined by interactive substitution of r_m values into the Lotka-Euler equation (Lotka, 1924; Birch, 1948; Southwood, 1978) as follow: $\sum e^{-r_m x} l_x m_x = 1$

where x is the mean age class, m_x is the mean number of female progeny per female of age x, and l_x is probability of survival to age x. The sex ratio of 1 female:1 male was used to calculate life table parameters. A trial number of values for r_m were substituted into the equation until the r_m value for which the sum of the left side of the equation approximated unity. Net reproductive rate ($R_o = \sum l_x m_x$, the total number of female offspring produced per female), mean generation time ($T = \ln R_o / r_m$), doubling time ($DT = \ln 2 / r_m$, number of days required for the population to double its number when the population reach the stable age distribution), and finite rate of increase ($\lambda = e^{r_m}$, number of times the population will multiply itself per unit of time) were also calculated (Chi and Su 2006).

Results

Development time of immatures

No statistical differences in developmental time of the immature stages of *S.gilvifrons* were found between either prey species (df= 3, 47; F= 1.42; P= 0.2391) or sex (df=3, 42; F= 2.53; P= 0.1186) (Table 1). No interaction was found between prey species and sex (df= 3, 47; F= 0.99; P= 0.3255).

Mortality of Immatures

No statistical differences were observed in mortality of predator eggs, larvae and pupae between prey species (Table 2). Mortality from egg to adult tended to be lower on *T. turkestani* (20%) compared to *E. orientlis* (24%), although the differences were not significant.

Sex ratio

Sex ratio of *S. gilvifrons* feeding on *T. turkestani* and *E. orientalis* was 44.4 and 47.5% respectively, which was not significantly different (df = 1, 8; F = 2.46; P = 0.1558).

Reproductive parameters

Prey species had no significant effect on either preoviposition (df= 1, 34; F= 0.000; P= 1.00) or oviposition period (df= 1, 34; F= 0.06; P= 0.803) (Table 3). However, postoviposition period was significantly affected by prey species (df= 1, 34; F= 29.62; P= 0.0001).

No statistical differences in male longevity of *S. gilvifrons* were found between prey species (df= 1, 13; F= 0.02; P= 0.888) (Table 3). However, ANOVA indicated significant differences between female longevity on two prey species (df= 1, 34; F= 12.75; P= 0.0011).

Mean fecundity was 175.00 eggs per female on *T. turkestani* and 316.00 eggs on *E. orientalis*, and mean oviposition rate (eggs/days) was 6.64 eggs on *T. turkestani* and 10.94 eggs on *E. orientalis*. These differences were statistically significant (df= 1, 34; F= 20.24; P= 0.0001 and df= 1, 34; F= 20.07; P= 0.0001, respectively) (Table 3).

Demographic parameters

Calculated daily intrinsic rate of natural increase (r_m) of *S. gilvifrons* was 0.179 and 0.193 on *T. turkestani* and *E. orientalis*, respectively (Table 4). Net reproductive rate (R_o) was greater on *E. orientalis* (94.53) than on *T. turkestani* (45.89), reflecting higher reproduction of *S. gilvifrons* on *E. orientalis* than on *T. turkestani* (Figure 1).

Discussion

Few differences between life history of *S. gilvifrons* feeding on *T. turkestani* or *E. orientalis* were observed. Development time of female *S. gilvifrons* was approximately 10.00 days on both spider mites which is similar to the findings of Ahmad and Ahmad (1988) on the same prey species at the same temperature. However, longer developmental time of 12.53 days was reported by Hajizadeh (1995) on *T. urticae,* 12.01 by Aksit et al. (2007) on *T. cinnabarinus*, 11.03 by Matin (2008) on *O. afrasiaticus* and 12.49 days by Taghizadeh et al. (2008a) on *T. urticae* at the same temperature. The differences may be explained by disparities in prey-insect suitability of the tetranychid mites to *S. gilvifrons*, in addition to differences in the experimental conditions (photoperiod, relative humidity, and host plant species).

The egg to adult developmental time of males *S. gilvifrons* on both prey species was very close to the respective time of females. A similar trend has also been reported for *Stethorus picipes* Casey feeding on *Oligonychus punicae* (Tanigushi an McMurtry, 1977) and *Stethorus japonicus* Kamiya feeding on *T. urticae* (Mori et al., 2005).

In the only study available, Aksit et al. (2007) reported *S. gilvifrons* mortality at 39 and 65 % under short-day (8 h light) and long-day (16 h light) photoperiod, respectively, which is higher than our findings.

In our study, *S. gilvifrons* females longevity was at 58.0 and 45.05 days on *T. turkestani* and *E. orientalis,* respectively. Similar results were also obtained on *O. afrasiaticus* by Matin (2008) and on *T. atlanticus* by Georgis et al. (1974), but not by Aksit et al. (2007) and Taghizadeh et al. (2008b) who reported longevity of 8.29 and 11.40 days at 30 ºC on *T. cinnabarinus* and *T. urticae*, respectively.

According to our results the longevity of males on both prey species was longer than the respective time of females. A similar trend has also been reported for *S. givifrons* feeding on *T. urticae* (Taghizadeh et al., 2008b; Hajizadeh, 1995) and *Stethorus loi* Sasaji feeding on *Tetranychus kanzawai* Kishida (Shieh et al., 1991). The reverse trend has been reported for *S. gilvifrons* feeding on *Oligonychus sacchari* (Afshari, 1998), *S. gilvifrons* feeding on *Oigonyshus afrasiaticus* (Matin, 2008), *Stethorus siphonulus* Kapur feeding on *T. cinnabarinus* (Raros and Haramoto, 1974) and *Stethorus punctillum* feeding on *Tetranychus viennensis* (Kasap and Aktug, 2003).

In our study, total fecundity was 175.14 and 318.00 eggs per *S.gilvifrons* female on *T. turkestani* and *E. orientalis*, respectively. These data are in line with the results of Matin (2008) and Taghizadeh et al. (2008b), who reported 151.40 and 150.90 eggs for *S. gilvifrons* on *O. afrasiaticus* and *T. uricae*, respectively. In contrast, Aksit et al (2007) reported only 28.60 eggs per female on *T. cinnabarinus*. We can only attribute these differences to differing experimental conditions.

Similar to our results, the sex ratio of *S. gilvifrons* feeding on *T. cinnabarinus* was reported to be around 1: 1 (Aksit et al. 2007).

Other laboratory studies have reported a variety of r_m values for this coccinellid species: 0.152 (Aksit et al. 2007) with *T. cinnabarinus* as prey, 0.189 (Matin, 2008) with *O. afrasiaticus* as prey and 0.191 (Taghizadeh et al. 2008b) with *T. urticae* as prey at 30 ºC (Table 5).

The intrinsic rate of increase (r_m) for *S. gilvifrons* feeding on *E. orientalis* in this study (0.193) is higher than previously reported values for this cocinellid beetle on other tetranychid species at the same temperature. This reflects lower juvenile mortality, higher fecundity and longer adult life span of *S. gilvifrons* when feeding on *E. orientalis.* Differences in the ecological factors viz. tetranychid prey species, strain of coccinellid beetle, host plant as well as measurement methods may provide an explanation for higher r_m value for *S. gilvifrons* on *E. orientalis* than on other tetranychid species.

These results lend credence to reports that augmentative biological control of tetranychid mites with *Stethorus* species can be effective in fruit trees and field crops (Obrycki and Kring 1998; Roy et al. 2003; Fiaboe et al. 2007).

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Table1. Mean ± SE development time (days) of *S. gilvifrons* feeding on *T. turkestani* and *E. orientalis*

Sex	Stage	*T. turkestani*	*E. orientalis*
Male	Egg	3.00 ± 0.00	2.11 ± 0.26
	L1	1.60 ± 0.16	1.33 ± 0.16
	L2	1.33 ± 0.21	1.00 ± 0.00
	L3	1.16 ± 0.16	1.11 ± 0.11
	L4	2.00 ± 0.00	1.88 ± 0.10
	PP+P	3.00 ± 0.00	2.44 ± 0.17
	Total	11.0 ± 0.82	9.88 ± 0.35
	n	6	10
Female	Egg	2.55 ± 0.10	2.11 ± 0.15
	L1	1.05 ± 0.04	1.27 ± 0.10
	L2	1.00 ± 0.00	1.11 ± 0.11
	L3	1.11 ± 0.06	1.00 ± 0.00
	L4	1.88 ± 0.06	1.83 ± 0.09
	PP+P	2.88 ± 0.06	2.66 ± 0.11
	Total	10.43 ± 0.07	10.0 ± 0.21
	n	18	18

Table 2. Percent mortality in immature stages of *S. gilvifrons* feeding on *T. turkestani* and *E. orientalis*

Stage	*T. turketani*	*E. orientalis*
Egg	0 (0)	0 (0)
L1	0 (0)	5.4 (2)
L2	2.33 (1)	2.7 (1)
L3	3.44 (1)	8.1 (3)
L4	14.28 (4)	8.1 (3)
Pupa	0 (0)	2.7 (1)
Total	20 (30)	24.32 (27)

Sample size (n) in parenthesis is number dying in each stage except for total which is the initial number of entering the egg stage

Table 3. Adult longevity and reproductive parameters of *S. gilvifrons* feeding on *T. turkestani* and *E. orientalis*

Parameters	*Prey*	
	T. turketani	*E. orientalis*
	n=9 male, 18 female	n= 6 male, 18 female
Longevity		
females	58.00 ± 0.53	45.05 ± 3.36
Males	46.30 ± 1.18	47.11 ± 4.08
Preoviposition period	2.85 ± 0.09	2.88 ± 0.21
Oviposition period	29.19 ± 0.56	28.44 ± 2.34
Postoviposition period	26.28 ± 0.28	12.38 ± 1.61
Fecundity		
Daily	6.64 ± 0.15	10.94 ± 0.82
Total	175.14 ± 3.19	318.00 ± 32.57
Sex ratio (female %)	0.444	0.475

Table 4. Population growth parameters of *S. givifrons* feeding on *T. turkestani* and *E. orientalis*

Parameters	*Prey*	
	T. turketani	*E. orientalis*
R_o	97.6 ± 15.86	154.08 ± 30.56
r_m	0.171 ± 0.007	0.221 ± 0.011
λ	1.186 ± 0.008	1.247 ± 0.014
T	26.76 ± 0.62	22.83 ± 0.84
GRR	122.28 ± 16.33	225.84 ± 38.72

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Table 5. Population growth parameters for *S. gilvifrons* feeding on different tetranychid mites at 30 ºC

Prey species	R_o	r_m	λ	T	DT	Ref.
T. cinnabarinus	13.83	0.152	-----	17.06	-----	Axit et al., 2007
O. afrasiaticus	70.01	0.189	1.20	22.38	3.66	Matin, 2007
T. urticae	47.54	0.191	1.211	20.17	3.62	Taghizadeh et al., 2008b

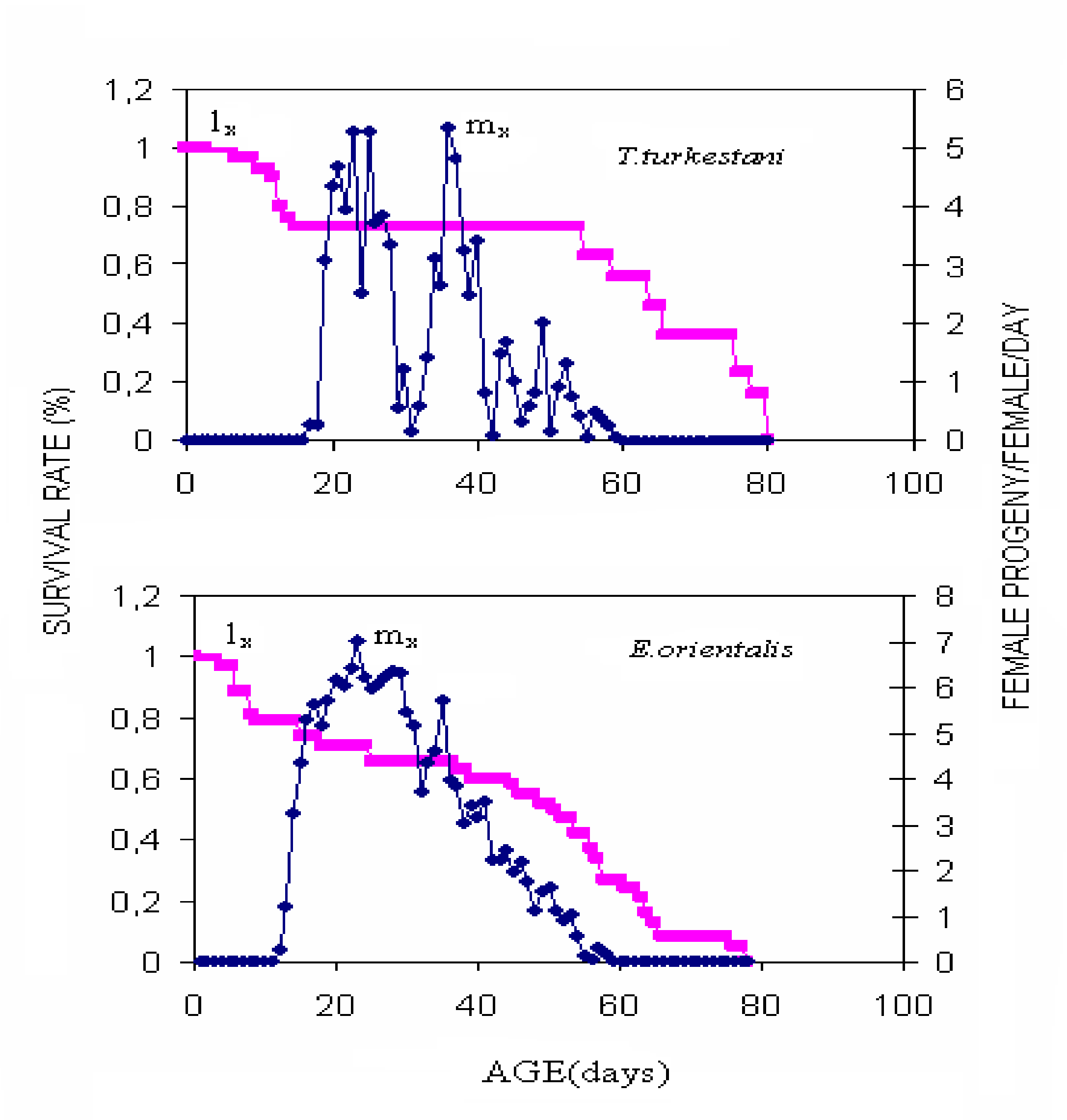

Figure 1- Daily proportion of female progeny per female (m_x) and survival rate (l_x) of *Stethorus gilvifrons* feeding on *T. turkestani* and *E. orientalis*