# The two spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), alternative prey for the lacewing *Dichochrysa prasina* (Neuroptera: Chrysopidae)

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**Abstract.** The predatory lacewing *Dichochrysa prasina* Burmeister can feed and reproduce on various aphid species. However, other species such as the two spotted spider mite *Tetranychus urticae* Koch may serve as an alternative prey for this predator in the field in periods when the population density of aphids is low. In peach orchards in Northern Greece *D. prasina* larvae are often found during the summer months on leaves infested with *T. urticae*. The development and reproduction of *D. prasina* fed on *T. urticae* at a series of different constant temperatures were studied. The percentages of *D. prasina* larvae that completed their development when fed on *T. urticae* ranged from 12 to 60% depending on the temperature over the range from 20 to 30°C, whereas at temperatures above 33°C no larvae survived. Longevity and egg production of *D. prasina* females fed during the larval stages on *T. urticae* were also determined and certain demographic parameters such as the intrinsic rate of increase  $(r_m)$ , mean generation time and net reproductive rate were estimated. Over the range of temperatures from 20 to 27°C, the  $r_m$  values were not significantly different and varied from 0.06629 to 0.07030. Based on the results of the present study, the possible effect of the availability of *T. urticae* as prey for *D. prasina* during summer, when its main prey species is scarce, is discussed.

### INTRODUCTION

The green lacewing *Dichochrysa prasina* Burmeister (Neuroptera: Chrysopidae) is a widespread aphid predator in the Palearctic, Oriental and African Regions (Séméria, 1984; Bozsik et al., 2002; Dong et al., 2004). In Europe, *D. prasina* is the second most abundant lacewing species after *Chrysoperla carnea* sensu lato (Canard et al., 2007). It is commonly found in warm and sunny places (Zelený, 1984), in orchards, field crops, vegetables and coniferous forests (Séméria, 1984). In Greece, *D. prasina* is recorded on the mainland and some islands in various agroecosystems such as arable crops, vegetables, fruit and olive orchards (Canard & Laudého, 1977; Santas, 1984; Souliotis & Broumas, 1994; Canard, 2001, 2003).

Adults of *D. prasina* mainly feed on honeydew and pollen grains (Bozsik, 1992, 2000) whereas larvae are generalist predators that feed on a variety of soft-bodied arthropods such as aphids, coccids, thrips, psyllids, mites and eggs of certain lepidopteran species (Principi, 1956; Babrikova, 1979; Santas, 1987; Pappas et al., 2007).

In commercial peach orchards in Northern Greece *D. prasina* is common from early May till late September, and during the summer months larvae of *D. prasina* are found on leaves infested with the two spotted spider mite *Tetranychus urticae* Koch (unpubl. data). During the summer months, when the prevailing high temperatures and arid conditions result in an abrupt population decline of the main aphid prey species *Myzus persicae* Sulzer (Pappas et al., 2007), *T. urticae* may act as an alternative

prey for *D. prasina*. However, the high mid summer temperatures may also limit the survival and population increase of *D. prasina*. The aim of the current work was to study the suitability of *T. urticae* as an alternative prey of *D. prasina* over a range of temperatures similar to those occurring in the field during summer in Northern Greece. These results may be useful for understanding the possible effect the availability of *T. urticae* has on the survival, development and population dynamics of *D. prasina* during summer.

# MATERIAL AND METHODS

## Predatory insects (D. prasina)

For the experiments approximately 80 adult females and males of D. prasina were collected with a hand net from two peach orchards in the area of Alexandria (Northern Greece). The adults were maintained in pairs in plastic cages at  $25 \pm 1^{\circ}\text{C}$  and at a photoperiod of 16L:8D, with continuous access to water and a mixture of a liquid food (a volumetric mixture of protein hydrolysate (Sigma-Aldrich®), honey, sugar and water, in ratio 1:1:1:1 (Pappas et al., 2007). For the experiments, eggs laid by the above field collected females of D. prasina within the first five days following their transfer into laboratory conditions were used.

#### Prey species (T. urticae)

Individuals of the two spotted spider mite (T. urticae) were used as prey. They originated from a laboratory colony maintained for several years on detached bean leaves ( $Phaseolus\ vulgaris\ L$ .) at  $25 \pm 1^{\circ}C$  and a photoperiod of 16L:8D, as described by Veerman & Koveos (1989).

### Preimaginal survival and developmental time

Eggs laid inside the cylindrical plastic cages over 12 h intervals by field collected females of *D. prasina* were individually transferred with the help of a fine hair camel brush into plastic Petri dishes (5.5 cm in diameter) with a hole (1.5 cm in diameter) in the lid covered by gauze, for ventilation. Subsequently, the dishes with the eggs were randomly allocated among six constant temperatures (20, 25, 27, 30, 33 and 35°C) and a photopheriod of 16L: 8D. For each temperature 50 eggs of *D. prasina* were used.

Upon hatching, the larvae were each provided with more than two hundred spider mites of various developmental stages every 12 h throughout their development and the developmental stage and survival recorded.

Two-way analysis of variance (ANOVA) was used to evaluate the effect of rearing temperature and sex (main factors) on total developmental time (SPSS, 2005). Since both factors significantly affected preimaginal development, further analysis of variance (ANOVA) for each developmental stage and sex was performed. Following a significant difference, means were compared by Student-Newman-Keuls test (SPSS, 2005). Prior to data analysis, the developmental time was logarithmically transformed and homogeneity of variances subsequently tested using Levene's test (SPSS, 2005). In those cases in which the data transformation failed to meet the requirements for parametric analysis, the nonparametric Kruskal-Wallis test was used and means were subsequently separated using the Mann-Whitney-Utest (SPSS, 2005). Percentages of individuals that completed preimaginal development (i.e. survived to the adult stage) at different constant temperatures were compared using a Chi-square test. The type-I error was corrected using the Bonferroni method (Sokal & Rohlf, 1995).

## Adult longevity and fecundity

Newly emerged females and males reared at each temperature were paired on the day of emergence and maintained in cylindrical plastic cages, where they had continuous access to water and the same liquid diet as fed to the field collected adults. Survival and the number of eggs laid were recorded daily for each individual female throughout its lifespan.

If any data transformation of either females' longevity or egg production failed to meet the requirements for parametric analysis, the nonparametric Kruskal-Wallis test was used (SPSS, 2005).

# Egg hatchability and progeny sex ratio

Egg hatchability and progeny sex ratio of the lacewing individuals reared on *T. urticae* at each of the different temperatures was estimated as described by López-Arroyo et al. (1999). During the first week of the oviposition period, at all tested tem-

peratures and for each ovipositing female, 30 eggs were randomly chosen and transferred individually to plastic Petri dishes. These eggs were incubated at the same temperature and photoperiodic conditions as the parental females. Daily, the eggs were inspected and the number of newly hatched larvae recorded. Arcsine transformation of data was used to equalize variances between treatments and an analysis of variance (ANOVA) performed to compare the effect of temperature on hatchability (SPSS, 2005).

Following egg hatch the effect of rearing temperature on offspring's sex ratio was determined by rearing 50 newly hatched larvae, selected at random, to the adult stage at each of the temperatures. The lacewing larvae were maintained at the same temperature and photoperiodic conditions as their parental females and provided daily with an ample supply of *T. urticae*. The adults that emerged at each temperature were counted and sexed. Sex ratios (i.e. number of females / number of females plus males) of the offspring at the different constant temperatures were determined and compared using a Chi-square test.

## **Demographic parameters**

Life and fertility table parameters were estimated by combining data for preimaginal development, adult survival and reproduction at different constant temperatures, as described by Wang & Tsai (2001). The intrinsic rates of population increase were estimated by iteratively solving the equation given by Birch (1948):

$$\sum_{x=0}^{n} e^{-rx} l_x m_x = 1$$

where x is the mean age class,  $m_x$  the mean number of female progeny per female of age x and  $l_x$  the probability of survival to age x ( $l_x$ ). The Jacknife procedure was used to estimate a standard error for the  $r_m$  values at different temperatures (Meyer et al., 1986). The  $r_m$  values were further compared using Student-Newman-Keuls test (Sokal & Rohlf, 1995) as described by Broufas & Koveos (2001). Furthermore, for each rearing temperature the demographic parameters: net reproductive rate ( $R_0 = \Sigma l_x m_x$ , number of female offspring produced per female), mean generation time ( $T = \ln R_0 / r_m$ ) and finite rate for increase ( $\lambda = e^{rm}$ , number of times the population will multiply itself per unit of time) were estimated (Birch, 1948; Southwood, 1978).

# RESULTS

#### Preimaginal survival and developmental time

Rearing temperature significantly affected the percentage of individuals that completed their preimaginal development ( $\chi^2 = 25.26$ , df = 3, P < 0.05) (Table 1). At the highest temperatures of 33 and 35°C, all lacewing larvae failed to complete their preimaginal development

TABLE 1. Preimaginal survival	(%) of $D$ .	prasina reared on T.	urticae at six	constant temperatures.

Rearing		Number (	n <sub>i</sub> ) / percentage	(a <sub>i</sub> %) of individu	als surviving to	each developme	ntal stage	Total
temperature (°C)	n¹	L1	L2	L3	Prepupa	Pupa	Adults	preimaginal survival (%)
20	50	48 / (96%)2	34 / (68%)	29 / (58%)	22 / (44%)	21 / (42%)	20 / (40%)	40a³
25	50	47 / (94%)	41 / (82%)	40 / (80%)	33 / (66%)	33 / (66%)	30 / (60%)	60b
27	50	47 / (94%)	42 / (84%)	42 / (84%)	34 / (68%)	32 / (64%)	17 / (34%)	34a
30	50	47 / (94%)	35 / (70%)	32 / (64%)	19 / (38%)	13 / (26%)	6 / (12%)	12c
33	50	45 / (90%)	37 / (74%)	33 / (66%)	4 / (8%)	0 / (0%)	0 / (0%)	0
35	50	44 / (88%)	22 / (44%)	3 / (6%)	0 / (0%)	0 / (0%)	0 / (0%)	0

<sup>&</sup>lt;sup>1</sup> Number of eggs of *D. prasina* reared at each temperature; <sup>2</sup>  $a_i = 100 \times (n_i / n)$  %; n = 50; <sup>3</sup> Percentages followed by the same latter are not significantly different ( $\chi^2$ -test, P > 0.05).

TABLE 2. Mean developmental time (± SE) (days) of preimaginal stages of *D. prasina* reared on *T. urticae* at different constant temperatures and 16L:8D.

Temperature (°C)	n <sup>1</sup>	Egg	L1	L2	L3	Prepupa	Pupa	Egg-Adult
Females								
20	9	$7.2\pm0.2a^2$	$10.7 \pm 0.6 a$	$11.1 \pm 1.0a$	$21.1 \pm 0.9a$	$10.1\pm0.5a$	$9.7 \pm 0.5 a$	$69.9 \pm 2.1 aA^3$
25	16	$5.9 \pm 0.2b$	$8.3 \pm 0.3 b$	$7.6 \pm 0.3 b$	$12.2 \pm 0.8 b$	$8.0 \pm 0.4 b$	$6.9 \pm 0.5 b$	$48.8 \pm 1.1 bA$
27	8	$4.4 \pm 0.2 c$	$8.0 \pm 0.7 b$	$6.4 \pm 0.2 c$	$10.6 \pm 0.4 b$	$3.0 \pm 0.3 c$	$8.1 \pm 0.5 ab$	$40.5 \pm 0.8 cA$
30**	4	$3.7 \pm 0.5$	$5.7 \pm 0.5$	$4.2 \pm 0.2$	$7.2\pm0.5$	$2.3\pm0.3$	$4.3 \pm 0.3$	$27.2 \pm 0.2$
Males								
20	11	$6.9 \pm 0.2a$	$10.7 \pm 0.6 a$	$10.1\pm0.7a$	$19.3\pm1.0a$	$10.2 \pm 0.4 a$	$8.5 \pm 0.4 a$	$65.7 \pm 1.2 aA$
25	14	$5.9 \pm 0.2b$	$7.8 \pm 0.4 b$	$8.0 \pm 0.5 b$	$9.2 \pm 0.5 b$	$8.4 \pm 0.5 b$	$6.0 \pm 0.6 b$	$45.3 \pm 0.7 bB$
27	9	$4.7 \pm 0.2 c$	$7.7 \pm 0.4 b$	$5.7 \pm 0.5c$	$8.9 \pm 0.7 b$	$3.6 \pm 0.3 c$	$6.9 \pm 0.3 ab$	$37.7 \pm 0.8 \text{cA}$
30**	2	$3.5 \pm 0.5$	$5.5\pm0.5$	$4.5 \pm 0.5$	$6.5\pm0.5$	$2.5 \pm 0.5$	$4.5 \pm 0.5$	$26.5\pm2.5$

<sup>&</sup>lt;sup>1</sup> Number of individuals surviving to the adult stage; <sup>2</sup> Means within a column followed by the same lower case letter are not significantly different (Student-Newman-Keuls test, P > 0.05); <sup>3</sup> Mean developmental times of females and males at the same temperature followed by the same upper case letter are not significantly different (t- test, P > 0.05); \*\* Due to the low number of individuals surviving this treatment was excluded from the analysis.

and were not included in the data analysis (Table 1). The survival from egg to adult emergence varied from 34 to 40%, and was not significantly different at 20 and 27°C ( $\chi^2 = 0.169$ , df = 1, P > 0.05). The highest percentage of immature survival (60%) was at 25°C (4 <  $\chi^2$  < 25.1, df = 1, P < 0.05), and the lowest (12%) at 30°C (6.85<  $\chi^2 < 25.1$ , df = 1, P < 0.05).

Further data analysis revealed that temperature significantly affected the developmental rate of the lacewing larvae. Two-way analysis of variance showed that both rearing temperature (F = 291.53, df = 2, 61, P < 0.001) and sex (F = 14.49, df = 1, 61, P < 0.001) significantly affected the total preimaginal development time of D. prasina, but that the interaction between these two factors was not significant (F = 0.057, df = 2, 61, P = 0.944).

As shown in Table 2, temperature significantly affected the duration of the preimaginal developmental stages of *D. prasina* of both females (6.629 < F < 67.175, df = 2, 30, P < 0.05) and males (8.301 < F < 51.057, df = 2, 31, P < 0.05). Furthermore, a gradual and significant decrease in mean total preimaginal developmental time for females (F = 102.251, df = 2, 30, P < 0.005) and males (F = 224.782, df = 2, 31, P< 0.05) was recorded with the increase in temperature over the range from 20 to 30°C (Table 2). Males completed their preimaginal development in a significantly shorter period than females at

25°C (t = 2.659, df = 28, P < 0.05). However, there was no significant difference in the mean total preimaginal developmental times of males and females at 20°C (t = 1.831, df = 18, P = 0.084) and 27°C (t = 1.831, df = 15, P = 0.084).

#### Adult longevity and fecundity

As shown in Table 3, with increase in the rearing temperature from 20 to 27°C there was a substantial reduction in the mean total egg production from approximately 151 to 98.8 eggs per female. However, this was not statistically significant ( $\chi^2 = 3.451$ , df = 2, P = 0.178). Similarly, the increase in rearing temperature from 20 to 27°C resulted in a substantial though not statistically significant ( $\chi^2 = 4.623$ , df = 2, P = 0.099) reduction (approximately 14 days) in mean adult longevity.

However, at 30°C females of *D. prasina* lived on average approximately 6 days and did not lay any eggs. It is, therefore, concluded that 30°C is close to the upper temperature limit for survival and reproduction in *D. prasina* (Table 3).

The age-specific oviposition rates and survival of *D. prasina* at different temperatures are shown in Fig. 1. According to these results the maximum daily oviposition rates varied from approximately 5 eggs/ female at 20°C to 9.8 eggs/ female at 27°C. Furthermore, maximum lifespan

TABLE 3. Mean total egg production (eggs / female) (±SE), mean female adult longevity (days) (±SE), egg hatchability (%) and progeny sex ratio of *D. prasina* fed on *T. urticae* at different constant temperatures and 16L: 8D.

Temperature	$\mathbf{n}^1$	Mean total egg production (eggs / female) <sup>3</sup>	Longevity (mean lifespan in days) <sup>3</sup>	Egg hatchability (%) <sup>4</sup>	Progeny sex ratio <sup>5</sup>
20°C	9 (2)2	$151.1 \pm 38.8a$	$46.4\pm7.5a$	$77.1 \pm 2.5a$	0.53
25°C	16 (0)	$150.8\pm15.2a$	$44.0 \pm 3.3 a$	$80.4 \pm 2.1a$	0.46
27°C	8 (1)	$98.8 \pm 22.1a$	$32.3 \pm 2.3a$	$81.9 \pm \mathbf{2.8a}$	0.55
30°C	4* (4)	$0.0 \pm 0.0$	$6.0 \pm 0.4$	_	_

<sup>&</sup>lt;sup>1</sup> Number of individuals tested; <sup>2</sup> Number of individuals that did not lay eggs; <sup>3</sup> Means within a column followed by the same letter are not significantly different (Nonparametric Kruskal-Wallis); <sup>4</sup> Means within a column followed by the same letter are not significantly different (Student-Newman-Keuls test, P > 0.05); <sup>5</sup> Sex ratio = females / females + males; \* Due to the low number of individuals surviving this treatment was excluded from the analysis.

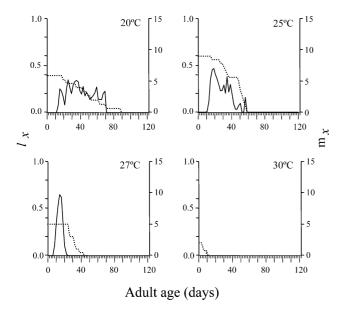


Fig. 1. Age-specific oviposition rates ( $m_x$ , mean number of eggs laid per day per female) (solid line) and survival ( $l_x$ ) (dotted line) of D. prasina females reared on T. urticae at different constant temperatures and a photoperiod of 16L:8D.

gradually declined from approximately 90d at 20°C to 50 and 42d at 25 and 27°C, respectively, whereas at the high temperature of 30°C the adults lived on average 6 days.

## Egg hatchability and progeny sex ratio

The temperatures at which the parental generation were reared did not significantly affect egg hatchability (F = 0.718, df = 2, 27, P = 0.497), which was high and ranged from 77.1 to 81.9%. Furthermore, the sex ratios of the offspring produced at the different temperatures did not differ significantly ( $\chi^2 = 0.381$ , df = 2, P > 0.05) and ranged from 0.46 to 0.55 (Table 3).

# **Demographic parameters**

Further data analysis was used to estimate the demographic parameters (i.e. intrinsic rate of increase, net reproductive rate, mean generation time and finite rate of increase) of D. prasina at different temperatures (Table 4). The estimated values of the mean generation time at the different temperatures varied from approximately 47 to 73 days. Temperature substantially affected the estimated values of net reproductive rate ( $R_0$ ), which

increased from approximately 26 female offspring/female at 20°C to 42.5 female offspring/female at 25°C. At higher temperatures there was an abrupt decline in the  $R_0$  value. The highest  $r_m$  value was recorded at 27°C. The estimated  $r_m$  values were similar over the range of temperatures from 20 to 27°C and varied from 0.06629 at 20°C to 0.07030 at 27°C (Table 4).

#### DISCUSSION

Temperature has a significant effect on the development, longevity and reproduction of chrysopid species. In several chrysopid species, such as Mallada desjardinsi (Navas), Chrysoperla nipponensis (Okamoto) (Nakahira et al., 2005) and Chrysopodes lineafrons Adams & Penny (Silva et al., 2007), reared on Ephestia kuehniella (Zeller) eggs, as well as for three Ceraeochrysa species (C. cincta (Schneider), C. smithi (Navàs) and C. cubana (Hagen) reared on Sitotroga cerealella (Olivier) eggs and Acyrthosiphon pisum Harris (López-Arroyo et al., 1999), high temperatures (from 28.5 to 30°C) retard preimaginal development and result in an increased larval mortality (Canard & Volkovich, 2001). Our results show that a low percentage of D. prasina larvae completed their development at 30°C and none at 33°C. The optimum temperature for development and reproduction in D. prasina is 27°C. When D. prasina is reared on E. kuehniella eggs or Myzus persicae (Sulzer) nymphs, survival and egg production at 30°C are much higher than in the present study, which indicates that prey quality may affect development a high temperatures (Pappas et al., 2008, Pappas et al., unpubl.).

Apart from temperature other factors such as the nutritional quality or availability of prey may also significantly affect the developmental parameters and population dynamics of chrysopids under field conditions (Canard & Principi, 1984). In the case of *D. prasina*, *T. urticae* is a prey of lower nutritional quality compared to certain aphid species such as *M. persicae* and *Hyalopterus pruni* (Geoffroy) (Pappas et al., 2007). According to Şengonca et al. (1987), for another chrysopid, namely *C. carnea*, *T. urticae* is also the least appropriate prey when compared to other prey species such as the eggs of *Mamestra brassicae* (L.) and the aphids *M. persicae*, *Aphis fabae* Scopoli and *Brevicorne brassicae* (L.). *C. carnea* larvae need to consume more than 12000 eggs of *T. urticae* in order to complete their development (Şengonca & Coeppicus

Table 4. Life table statistics of D. prasina reared on T. urticae at different constant temperatures and 16L: 8D.

Temperature	$\mathbf{n}^1$	$r_{\rm m}$	95%	6 CI <sup>2</sup>	$R_0$	T	λ
20°C	7	0.06629b	0.05694	0.07562	26.8	71.1	1.069
25°C	16	0.06683b	0.06431	0.06934	42.5	73.1	1.069
27°C	7	0.07030b	0.06040	0.00198	14.6	47.0	1.073
30°C	-	_	_	_	_	_	
33°C	_	_	_	_	_	_	
35°C	_	_	_	_	_	_	

<sup>&</sup>lt;sup>1</sup> Number of individuals tested;  $r_m$  values within each temperature followed by the same letter are not significantly different at P = 0.05 using the Student-Newman-Keuls test; <sup>2</sup>95% confidence intervals;  $r_m =$  intrinsic rate of natural increase (d<sup>-1</sup>);  $R_0 =$  net reproductive rate (female offspring per female); T = mean generation time (d);  $\lambda =$  finite capacity for increase (d<sup>-1</sup>).

1985). Thus, in mid summer in the absence of more suitable prey (aphids) the presence of *T. urticae* may favour the survival and development of *D. prasina*. Other studies have shown that in apple and peach orchards during summer certain spider mite species such as *Panonychus ulmi* Koch, *T. urticae* and *Bryobia arborea* Morgan and Anderson may serve as alternative food for certain chrysopids such as *Chrysoperla carnea* (Stephens), *Chrysoperla rufilabris* (Burmeister) and *Chrysopa oculata* Say (Szentkirályi, 2001 and references therein).

The estimated values of the intrinsic rate of increase  $(r_m)$  for D. prasina fed on T. urticae ranged from 0.06629 at 20°C to 0.07030 at 27°C. Even though these values are substantially lower than those reported when D. prasina is fed on aphids (Pappas et al., 2007) they indicate that D. prasina populations reared on T. urticae may increase at temperatures from 20°C to 27°C. Furthermore, these values for D. prasina fed on T. urticae are substantially higher than those reported for Chrysopa perla (L.) reared on different aphid species, which ranged from 0.0015 to 0.0227 at 20°C (Canard, 1970). Therefore, we may assume that in northern Greece the population increase of D. prasina may be more limited by high ambient field temperatures than the availability of prey.

In conclusion, our results show that D. prasina may successfully complete its development and reproduce over a wide range of temperatures when reared on T. urticae. However, temperatures of or higher than 30°C may adversely affect both the survival and reproduction of D. prasina. In peach orchards during summer, increasing temperatures and low relative humidity result in a gradual decline in aphid and increase in *T. urticae* populations. Therefore, T. urticae could serve as a nutritionally sufficient alternative food for larvae of D. prasina during summer. In addition, the population decline of *D. prasina* during summer may be more due to high summer temperatures than availability of prey. Our experiments were done at constant temperatures. However, fluctuating field temperatures may significantly affect life history traits of insects and eventually their intrinsic rate of increase and population dynamics (Davis et al., 2006). Thus, further experiments are required in order to evaluate the importance of T. urticae as an alternative food for D. prasina during summer at fluctuating temperatures.

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