Survival and developmental characteristics of the predatory bug *Orius similis* (Hemiptera: Anthocoridae) fed on *Tetranychus cinnabarinus* (Acari: Tetranychidae) at three constant temperatures

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Abstract. Developmental characteristics of the predatory bug *Orius similis* fed on *Tetranychus cinnabarinus* were investigated at three constant temperatures (25, 28, and 31°C) under laboratory conditions (75 ± 5% relative humidity and a 14L : 10D photoperiod). The survival of nymphs was highest at 28°C (75.57%) and at this temperature female adults *O. similis* had the longest oviposition period (21.1 d), the greatest fecundity (40.3 eggs) and the highest potential intrinsic rate of increase (r_m : 0.108 d⁻¹). These results suggest that *O. similis* can maintain greater population densities at 28°C than at the other temperatures tested. In addition, the functional response indicates that the attack rate of *O. similis* (1.04) and the maximum prey capacity (30.7 spider mites per bug in 24 h) are greatest at 28°C. The results of this study provide useful information on the biology and time when to release *O. similis* in order to reduce the abundance of *T. cinnabarinus* in cotton fields.

INTRODUCTION

The carmine spider mite Tetranychus cinnabarinus (Boisduval) (Acari: Tetranychidae) is one of the most economically important pests of vegetable crops and ornamental plants around the world (Capinera, 2001). Nymphs and adults primarily cause damage by feeding on the underside of leaves. The upper surfaces of leaves often become reddish and discoulored before senescing prematurely. When examined closely, fine strands of silk are usually visible near the veins and midribs close to areas of discolouration (Sengonca, 1982; Mo & Liu, 2006). In the western region of America, more than 10 generations of T. cinnabarinus can develop on a cotton plant in a season (Falcon & Smith, 1973) and it is the most serious crop mite pest in China (Feng et al., 2011). Its high reproductive capability, short life cycle and high rate of inbreeding result in quick developing resistance to pesticides, including miticides (He et al., 2005). Since the 1980s, the level of resistance of T. cinnabarinus to pesticide has increased rapidly in China, sometimes even by as much as a 152 fold increase (Guo & Zhao, 1999). As a result, the efficacy of many pesticides has declined and the cost of crop production and level of pesticide residues have increased. Biological control of T. cinnabarinus is an alternative strategy, which has no toxic side effects compared with chemical control (He et al., 2005).

Anthocorid bugs are well-known biological control agents because they are polyphagous and eat a wide range of pest species. Previous literature is mainly on the use of *Orius* spp. for the biocontrol of thrips (Dogramaci et al.,

2011), aphids (Bulter & O'Neil, 2006; Zhou et al., 2006), and young larvae or eggs of some lepidopterous insects (Bonte & De Clercq, 2011). However, little attention has so far been devoted to using *Orius* spp. for controlling spider mites (Xu et al., 2006; Fathi, 2009).

Orius similis Zheng (1982), occurs in southern China where it is an important predator in agroecosystems. Its adults and nymphs eat a variety of pests, such as aphids (Zhou et al., 2006), flower thrips, and young larvae and eggs of some lepidopterous insects and spider mites (Zhou & Lei, 2002) on more than 20 vegetable crops. Moreover, O. similis can prey on pests from March to October when the temperature is 20°C to 37°C and produce four to eight generations per year in four provinces (Henan, Hubei, Hunan, and Guangxi province) of China (Zhou & Lei, 2002). One generation of O. similis takes approximately 30 days and one mated female adult can produce 20 to 100 offspring over a period of 10 to 30 days. Moreover, the number of O. similis can reach 260/100 cotton flowers in the field during summer and one bug can in the absence of other prey kill 500 carmine spider mites during its life time (Lei, 1997). The spider mite is one of the three preferred prey items (flower thrips, aphids and spider mites) of O. similis according to Lei (1997). However, although the life history characteristics of O. similis fed on T. cinnabarinus are unknown there are some studies of these characteristics when it is fed on aphids (Zhou et al., 2006; Ahmadi et al., 2007).

The mass rearing of *Orius* for release into the field or greenhouses for the biological control of pests is feasible

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and could result in the reduction in the use of pesticides (Bonte & De Clercq, 2011). However, before releasing these bugs it is important to determine the temperature at which this bug is most efficient at controlling pests and the best time to release them in the field. In the present study, the development and predatory activity of the anthocorid bug, *O. similis*, against the carmine spider mite, *T. cinnabarinus*, at three temperatures was determined. The temperatures were close to the average monthly temperatures recorded in the Yangtze River area from May to September. The aim of this study was to provide information on the potential use of *O. similis* in integrated pest management (IPM) programs.

MATERIAL AND METHDOS

Insect and mite cultures

Anthocorid bugs of the species *O. similis* were collected from cotton flowers in the grounds of Huazhong Agricultural University, Wuhan City (30.5°N latitude and 114.3°E longitude), Hubei Province, P.R. China. Stock cultures were maintained in an insectary according to the method described by Zhou et al. (2006).

T. cinnabarinus was obtained from cotton leaves at Huazhong Agricultural University and cultured on detached cotton leaves in an insectary at $26 \pm 2^{\circ}$ C, $75 \pm 5\%$ relative humidity (RH) and a 14L: 10D photoperiod. The mite was identified based on the description of Zhang & Jacobson (2000). All spider mites used as prey in this study were in the third to fourth stadia.

Experimental protocols

The mean temperatures per month in the central region of the Yangtze River ($109^{\circ}01'-121^{\circ}07'E$ and $29^{\circ}05'-31^{\circ}21'N$) from May to September (1990-2005) is 23.2, 25.7, 30.5, 28.4 and 24.5°C, respectively (http://cdc.cma.gov.cn, Meteorological Data Sharing Service System of P.R. China). Thus, three temperatures (25, 28 and 31°C) were used in this study. All experiments were conducted at a 75 \pm 5% RH and a 14L : 10D photoperiod in climate controlled cabinets (HP250GS, Ruihua Instrument & Equipment Co., Ltd., Wuhan, P.R. China) equipped with fluorescent lighting controlled by an automatic timer. The variation in temperature was less than 1°C and the light intensity was approximately 20.0 W·m $^{-2}$.

Functional response of O. similis to T. cinnabarinus

The functional response of adult *O. similis* to different population densities of *T. cinnabarinus* was measured in Petri dishes (9 cm in diameter and 2 cm in depth) lined with filter paper. A single detached cotton leaf (~50 cm²) was placed in each Petri dish as food for the mites and the petiole of the leaf was wrapped in moistened tissue, which also served as a source of water for *O. similis*. Individual mites were assigned to one of six prey densities (6, 12, 18, 24, 36 and 48 per dish) at each temperature. The predators were starved for 24 h before each assay and were 4 to 7 days old.

After 24 h, the predators were removed and the number of dead prey counted. We assumed all dead spider mites were killed by the predator as a preliminary study indicated a nearly 100% survival of mites in the absence of predators and 100% survival of *O. similis* in the absence of mites. If the predator escaped or died, the data was discarded. Dead mites were not replaced. There were ten replicates of each prey density. All *Orius* bugs used in this experiment were adult females.

The mean number of prey killed as a function of prey density was plotted and the data fitted using the random predator equation of Rogers (1972):

 $Na = N_0 \{1 - exp[-aT/(1 + aT_h N_0)]\}$

where Na is the number of prey killed, N_0 is the initial prey density, T is the total time available for attacking (24 h), a is the attack rate (h⁻¹), and T_h is the handling time (h) (Rogers, 1972). The parameters a and T_h were calculated using the PROC NLIN procedure in SAS (SAS Institute, 1996). A chi-square test for goodness of fit was used to compare the actual with the theoretical data.

Nymphal life history study

The survival and developmental traits of *O. similis* nymphs fed on different numbers of spider mites were measured based on the results of this predator's functional responses at three different temperatures (25, 28 and 31°C). The results indicated that *O. similis* consumed a maximum of 24, 31 and 30 spider mites in 24 h at 25, 28 and 31°C, respectively, when the prey density was 48. Thus, 30 spider mites were provided per day at each temperature in all the treatments.

Approximately 500 randomly sampled fresh eggs (< 12 h) were obtained from the insectary and incubated in a Petri dish (9 cm in diameter and 2 cm in depth) lined with wet filter paper. Each newly hatched nymph was coded and placed and kept in a transparent plastic tube (15 mm in diameter and 75 mm in length) until it reached the adult stage. Each tube contained a piece of cotton leaf and 30 spider mites and was covered with water saturated cotton. The nymphs were checked twice a day (08:00 and 20:00) and transferred to new tubes daily (08:00). Thirty nymphs were used at 25, 28 and 31°C, respectively. The experiment was replicated three times. The duration of each immature stage was measured. Gender was determined immediately the adults emerged. The results for individuals that were accidentally killed were not included in the analysis.

Adult life history study

Virgin females and males were paired for mating. The pairs were observed to ensure that mating occurred and the females that remained in copulation for > 1.5 min were assumed to have been mated (Butler & O'Neil, 2006). The females were individually transferred to new cylindrical vitreous vials (2.5 cm in diameter and 14 cm in length) covered with a fine mesh nylon screen. A small tender stem of Vitex negundo L. was provided as a substrate for the female O. similis to oviposit on following the method of Zhou et al. (2006). The end of each stem was wrapped with moistened tissue. Spider mites (n = 50) were brushed into the vials to provide food for the females. The stems were examined daily under a stereomicroscope (15×) to check and count the number of eggs. Dead spider mites were replaced daily and the stems changed every four hours during the preoviposition period. After the first egg was laid the stems were changed once a day (08:00) and watered twice a day (08:00 and 20:00) to provide moisture and water for the females. Preoviposition period, oviposition period (time that elapsed between the first and last egg being laid), fecundity (total number of eggs laid over a female's lifetime) and longevity (time from adult eclosion to death) at the three temperatures were recorded. All bugs were kept and observed until they died.

Life tables

The important life table parameters, age-specific survivorship (l_x) and fecundity (m_x) schedule, were calculated for *O. similis* at each temperature. Based on previous laboratory experience and the data from previous studies on other *Orius* spp. (Chyzik et al., 1995; Cocuzza et al., 1997), we assumed a sex ratio of 1:1 in all treatments. For each temperature, daily age-specific survival and age-specific fecundity were used to calculate the intrinsic rate of natural increase (r_m) , which is expressed as the

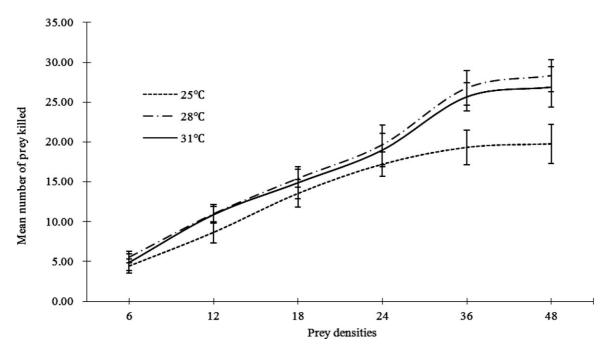


Fig. 1. Functional response of O. similis to the density of T. cinnabarinus in a Petri-dish arena recorded at three temperatures, $75 \pm 5\%$ RH and a 14L: 10D photoperiod and measured over a over a period of 24 h. Points show average number of spider mites killed by O. similis at each prey density. Error bars show standard error. Each point is the average of 10 replicates.

number of females per female per day using the following formula of Birch (1948):

$$\sum_{x=1}^{\omega} e^{-r_m x} l_x m_x = 1$$

where ω is the oldest age class, and l_x and m_x are the proportion of surviving females at age x and the number of females produced per female in the age interval x, respectively. With a stable age distribution and under given climatic and food conditions, the intrinsic rate of natural increase is useful for comparing population growth potentials (Southwood, 1978).

In addition, net reproductive rate (R_0 or the number of female offspring produced per female), mean generation time (T) and intrinsic rate of increase (r_m) were calculated as follows:

$$R_0 = \sum l_x m_x$$

$$T = \sum l_x m_x X / \sum l_x m_x$$

$$r_m = l_n R_0 / T$$

where X is the age interval, l_x is the survival of the immature and adult stages of the females at age X, and m_x is the number of female progeny produced per female in the age interval X.

Statistical analysis

Data were tested for significance using the one-way analysis of variance in SPSS for Windows version 11.5 (SPSS Inc., Chicago, Illinois). The mean values were compared using Tukey's test with P=0.05. The cumulative survival data for the nymphs were subjected to arcsine square root transformation before analysis.

RESULTS

Functional response of O. similis to T. cinnabarinus

The number of prey killed by *O. similis* generally increased as the prey density increased. However, at a prey density of 36 spider mites per dish the curve leveled off at all the three temperatures (Fig. 1). At 28 and 31°C, there were no significant differences between the average

numbers of spider mites killed by *O. similis* at each prey density (Fig. 1). As shown in Table 1, the attack rate (a) of *O. similis* was highest at 28°C (1.04). The number of prey killed by *O. similis* was closely correlated with the density of prey, and the χ^2 values (1.1247, 0.2917, and 0.5342) were far less than $\chi^2_{0.01,5}$ (15.09).

Survival and duration of development of nymphs

The greatest cumulative survival of the nymphs (75.57%) was recorded at 28°C (Table 2) and the percentage survival at 28 and 31°C (75.57% vs. 64.43%) did not differ significantly (d.f. = 2.8, F = 5.027, P = 0.052). Survival was above 50% at all three temperatures.

The analysis of the variance in the average durations of the immature stages of O. similis at 25, 28 and 31°C is recorded in Table 2. The average duration of development of both females and males were significantly affected by temperature (d.f. = 2, 96, F = 66.725, P < 0.001 and d.f. = 2, 96, F = 37.874, P < 0.001 respectively). That is, it decreased with increase in temperature, with the duration of development decreasing from 23.8 to 15.6d for females and from 21.2d to 14.8d for males when the temperature increased from 25 to 31°C.

Table 1. Effect of temperature on the handling time (T_h) and attack rate (a) of *O. similis* attacking *T. cinnabarinus* at $75 \pm 5\%$ RH and a 14L: 10D photoperiod.

Temp. (°C)	T _h (h)	a (h ⁻¹)	χ^2
25	0.0159	0.8676	1.1247
28	0.0130	1.0400	0.2917
31	0.0115	0.9569	0.5342

Notes: χ^2 values were the results of chi-square test with P < 0.05

Table 2. Developmental parameters of nymphs of O. similis fed daily on T. cinnabarinus at three constant temperatures, $75 \pm 5\%$ RH and a 14L:10D photoperiod.

Temp. (°C)	Avamaga aumaylativa aumyiyal (0/)	Duration of immature stages (days)		
	Average cumulative survival (%)	female	male	
25	$57.80 \pm 5.88(3)b$	$23.8 \pm 0.61(33)a$	$21.2 \pm 0.61(27)$ a	
28	$75.57 \pm 1.13(3)$ a	$18.3 \pm 0.39(34)b$	$18.2 \pm 0.44(37)b$	
31	$64.43 \pm 4.03(3)$ ab	$15.6 \pm 0.50(30)$ c	$14.8 \pm 0.48(33)$ c	
Results of ANOVA	d.f. = 2.8, F = 5.027, P > 0.05	d.f. = 2, 96, F = 66.725, P < 0.001	d.f. = 2, 96, F = 37.874, P < 0.001	

Notes: Figures in parentheses indicate the number sampled. Values (means \pm SE) followed by different letters within a column are significantly different based on Tukey's test with P < 0.05.

Table 3. Average pre-oviposition period, reproductive period, longevity and total fecundity of O. similis adults fed daily on T. cinnabarinus at three constant temperatures, $75 \pm 5\%$ RH and a 14L: 10D photoperiod.

Temp. (°C)	Pre-oviposition period	Oviposition period	Adult longevity(days)		Total fecundity
remp. (C)	(days)	(days)	female	male	(eggs)
25	$6.2 \pm 0.24(18)$ a	$15.5 \pm 0.78(13)$ b	$35.9 \pm 2.37(13)$ a	$33.1 \pm 1.75(15)$ a	$21.2 \pm 1.00(13)$ b
28	$5.8 \pm 0.21(19)a$	$21.1 \pm 0.64(16)a$	$30.6 \pm 1.42(16)ab$	$29.5 \pm 1.40(16)ab$	$40.3 \pm 2.05(16)a$
31	$5.5 \pm 0.20(22)a$	$18.8 \pm 0.79(16)$ a	$27.6 \pm 1.31(16)b$	$26.9 \pm 0.91 (15) b$	$34.3 \pm 1.85(16)a$
Results of ANOVA	d.f. = $2,58$, F = 2.240 , P > 0.05	d.f. = 2,44, F = 13.448, P < 0.001	d.f. = 2,44, F = 5.935, P < 0.05	d.f. = 2,45, F = 4.852, P < 0.05	d.f. = $2,44$, F = 28.281 , P < 0.001

Notes: Figures in parentheses indicate the number sampled. Values (means \pm SE) followed by different letters within a column are significantly different based on Tukey's test with P < 0.05.

Longevity and fecundity of adults

There were no significant differences in the preoviposition period recorded at the three temperatures (d.f. = 2,58, F = 2.240, P = 0.116, Table 3). However, the oviposition periods did differ significantly (d.f. = 2,44, F = 13.448, P < 0.001, Table 3). At 25°C, it was significantly shorter (15.5d) than that of those fed on *T. cin*nabarinus at 28°C (21.1d) and 31°C (18.8d). The longevities of adults of both female and male *O. similis* declined with increase in temperature (35.9d to 27.6d for females and 33.1d to 26.9d for males). However, there were no significant differences in the fecundity recorded at 28 and 31°C (female: d.f. = 2,44, F = 5.935, P = 0.005; male: d.f. = 2,45, F = 4.852, P = 0.013, Table 3). The greatest fecundity was recorded at 28°C (40.3 eggs).

Life table parameters of adults

Life table parameters of O. similis fed on T. cinnabarinus at the three temperatures are presented in Table 4. The value of net reproductive rate (R_0) increased from 13.50 at 25°C to 25.76 at 28°C and then decreased to 22.12 at 31°C. Mean generation time (T) declined from

Table 4. Life table parameters of mated *O. similis* females fed daily on *T. cinnabarinus* at three constant temperatures, 75 \pm 5% RH and a 14L : 10D photoperiod. * R_{0} , T, r_{m} are the net reproductive rate, mean generation time in days and intrinsic rate of increase (day $^{-1}$), respectively.

Temp. (°C)	R ₀ -value*	T-value*	r _{m-} value*
25	13.50	30.32	0.086
28	25.76	30.12	0.108
31	22.12	25.87	0.120

30.32 d to 25.87 d with increase in temperature from 25°C to 31°C. This finding is in accordance with the result that the duration of immature stage was shortest at 31°C (15.6 d and 14.8 d for females and males, respectively) (Table 2). The intrinsic rate of increase (r_m) increased from 0.086 day⁻¹ to 0.120 day⁻¹ with increase in temperature from 25°C to 31°C (Table 4).

DISCUSSION AND CONCLUSIONS

The number of T. cimnabarinus eaten by O. similis was affected by both temperature and prey density. The consumption of O. similis reached a plateau at a prey density of 48 spider mites at all three temperatures (Fig. 1), indicating that at this prey density the predator is satiated, which could serve as a reference when deciding the number and when to release them in the field. The low χ^2 values indicate that the number eaten is in accordance with the theoretical prediction for all three temperatures.

The regional climate in the area of the Yangtze River is subtropical. The *Orius* spp. in this area are likely to be well adapted to high temperatures, based on the results of a study on *O. albidipennis* (Cocuzza et al., 1997). The high cumulative survival (64.43%) recorded at 31°C indicates that *O. similis* is able to thrive at relatively high temperatures, although its survival at this temperature was lower than at 28°C (75.57%). Together with the short duration of the immature stages, around 18d at 28°C and 15d at 31°C, this indicates *O. similis* could be used for controlling *T. cinnabarinus*, which flourishes at temperatures above 25°C during the months of June, July and August in the area of the Yangtze River (Lei, 1997).

Adult females of *O. similis* need 5–6 days to complete the development of their reproductive system after

mating, which is shorter than that recorded for O. insidious feeding on aphids (8-10d) and the oviposition period is also relatively shorter (average 20 vs 30) (Butler & O'Neil, 2007). The longevity of adult O. similis fed on T. cinnabarinus decreased with increasing temperature from 25°C to 31°C. This result is in accordance with that recorded for O. albidipennis adults fed on the western flower thrips, Frankliniella occidentalis Pergande (Cocuzza et al., 1997). In addition, the longevity of O. similis fed on T. cinnabarinus at each temperature was three to five times longer than that of O. similis fed on Aphis gossypii Glover (Zhou et al., 2006). Butler & O'Neil (2007) conclude that aphids are a poorer prey for O. insidious than other prey items, such as soybean thrips, because aphids were less often attacked by this bug when other types of prey were present. Our field observations reveal that O. similis has similar predatory tendency to O. insidiosus (S. Zhang, pers. observ.), which suggests that O. similis might be more effective in controlling spider mites than cotton aphids.

Egg production of O. similis fed on T. cinnabarinus (maximum of 40 eggs per female) was lower than that of Orius albidipennis fed on the thrips, F. occidentalis (with a range of 47-71 eggs per female) (Cocuzza et al., 1997) and on the spider mite T. urticae (approximately 100 eggs per female at most) at 25°C (Chyzik et al., 1995). It is unknown whether subtropical species of *Orius* are more likely to prey on thrips or *T. urticae* than *T. cinnabarinus*. However, fecundity and cumulative survival of nymphs was highest, oviposition period longest and the value of net reproductive rate (R₀) greatest for O. similis at 28°C, which indicates that this species is potentially capable of maintaining a higher population at 28°C. The average temperature in August (28.4°C) in Yangtze River region is close to 28°C and in August the damage caused by *T.cinnabarinus* is the most serious (Lei, 1997). But it is uncertain whether this bug could maintain a greater population density and control spider mites in the field in August because unlike in the laboratory the temperatures in the field are very variable fluctuating from day to night and day to day. Moreover, the predatory activity of O. similis in fields can be affected by other factors, such as the presence of competitors, other prey items, variation in climate and human activity.

T. cimnabarinus females can develop to sexual maturity in 9 d, and mated females can lay an average of eight eggs per female per day (approximately 60 eggs over a seven-day oviposition period) (Kazak et al., 2003). These findings indicate that the rate of population increase of O. similis is less than that of spider mites. Moreover, as an omnivore, O. similis is probably unlikely to prey on a single item, as in the present study. Furthermore, the predatory activity of O. similis can be significantly affected by competition with ladybird beetles, predatory mites and lacewings. In addition, O. similis is also killed and eaten in the field by bigger and more dominant predators, such as Geocoris, Nabis, Zelus, Chrysoperla and spiders (Rosenheim, 2001). The result of the survey conducted by Ge & Ding (1996) in fields of cotton plants

show that only 11.61% of pests killed by natural enemies are killed by anthocorid bugs. Therefore, attempts to control *T. cinnabarinus* in fields of cotton plants using only *O. similis* may not be effective. It is now important to determine the ratio of *O. similis* to *T. cinnabarinus* that is needed to bring about a reduction in the abundance of the mite. The results of this study provide useful information on the biology and when best to release *O. similis* in order to control the abundance of *T. cinnabarinus* in cotton fields.

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