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Article

Effects of temperature on development and reproduction of Neoseiulus bicaudus (Phytoseiidae) feeding on Tetranychus turkestani (Tetranychidae)

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Abstract

Neoseiulus bicaudus (Wainstein), a species of Neoseiulus Hughes (Acari: Phytoseiidae), was collected at Ili in the Xinjiang Uyghur Autonomous Region of China in July 2013. As Neoseiulus species are valuable predator mites, N. bicaudus could be used for biocontrol of some small pests like spider mites, whitefly, and thrips. Tetranychus turkestani (Ugarov et Nikolskii) is the main spider mite affecting agriculture and forestry in Xinjiang. The development rate and reproductive biology of N. bicaudus feeding on T. turkestani were studied at six constant temperatures: 18 °C, 22 °C, 26 °C, 29 °C, 32 °C, and 35 °C. The duration of the egg, larva, protonymph, total immature, and pre-oviposition stages all decreased as temperatures increased from 18 °C to 32 °C and then increased slightly as temperatures increased from 32 °C to 35 °C. The mean generation time (6.95 days) and the shortest time for the population to double (1.70 days) were observed at 35 °C. The intrinsic rate of natural increase (r_m) and the finite rate of increase (λ) both were larger as temperature increased, reaching their maxima at 35 °C. The net reproductive rate ($R_0 = 34.60$) also reached a maximum at 26 °C. The maximum daily fecundity (2.55 eggs/day/female) and the maximum daily female fecundity (1.69female eggs/day/female) were both observed at 26 °C. The results showed that N. bicaudus could complete its development at the six temperatures used in this study. Both the developmental duration as well as the time needed for the population to double decreased as temperature increased. As temperature increased, the duration of the oviposition period first increased and then decreased. The optimal development and reproduction temperature of N. bicaudus preying on T. turkestani is approximately 26 °C.

Key words: predatory mite, spider mite, developmental duration, intrinsic rate of natural increase, survival rate, life table

Introduction

Predatory mites have been used commercially for biocontrol of agricultural pests such as spider mites, whitefly, and thrips (Zhang, 2003; Xu et al., 2013a). Recent studies about predator mites have examined (i) the selection of efficient predatory mite species, (ii) interactions among predatory mites, plants and pests, (iii) the effects of chemical pesticides on predatory mites, and (iv) large scale production and field release of predatory mites (Smith & Papacek, 1991; Samsøe-Petersen & Hassan, 1991; Samsøe-Petersen, 1983; Ali et al., 1999; Sznajder et al., 2010; Nomikou et al., 2005; Messelink et al., 2010). Within these studies, Amblyseius swirskii (Athias-Henriot), A. largoensis (Muma) and Iphiseiodes zuluagai (Denmark & Muma) were identified as potential predators of Polyphagotarsonemus latus (van Maanen et al., 2010; Rodriguez et al., 2010; Sarmento et al., 2011). Bdella ueckermanni (Hernandes, Daud and Feres) was reported to be an efficient predator for

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controlling *Aceria guerreronis* Keifer (coconut mite) (Hernandes *et al.*, 2008; Souza *et al.*, 2012; Domingos *et al.*, 2010; Melo *et al.*, 2011; Lima *et al.*, 2012). *Phytoseiulus persimilis* (Athias-Henriot) was used to protect greenhouse tomatoes (Nihoul, 1993). *A. swirskii* was identified as a biological control agent with potential to play an important role in pest management in many greenhouse crops (Janssen & Sabelis, 2015; Midthassel *et al.*, 2013).

Several species of genus *Neoseiulus*, including *N. barkeri* (Hughes), *N. cucumeris* (Oudemans), and *N. californicus* (McGregor), have been proven to be useful predatory mites in agriculture (Fan & Petitt, 1994; Obrist *et al.*, 2006; Xia *et al.*, 2012; Yao *et al.*, 2014; Castagnoli & Simoni, 1999).

In China, *N. bicaudus* is a newly discovered native species (Wang *et al.*, 2015a). It was first named *A. bicaudus* (Wainstein, 1962). In a paper published later that year, Deleon (1962) named it *Cydnodromus comitatus*. Schuster and Pritchard (1963) used the name *Amblyseius scyphus* in reference to *N. bicaudus*. A decade later, Tuttle (1973) named it *N. comitatus* (Deleon). Finally, the mite was named *N. bicaudus* by Wainstein (1977). *N. bicaudus* mainly exists in Iran, Russia, Greece, Cyprus, and the United States (Wainstein, 1962; DeLeon, 1962; Karban *et al.*, 1995; Livshitz & Kuznetsov, 1972; Wainstein, 1975; Faraji *et al.*, 2007; Palyvos & Emmanouel, 2009; Asali *et al.*, 2011).

Both the development and the predatory action of mites are affected by environmental factors (Palyvos & Emmanouel, 2011; Weintraub *et al.*, 2007; Felton & Dahlman, 1984), including temperature, humidity, rainfall, light intensity, prey, artificial feeding, and fungicides (Domingos *et al.*, 2010; Schütte & Dicke, 2008; Shinmen *et al.*, 2010; Toyoshima *et al.*, 2009; Kawashima & Jung, 2011; Delisle *et al.*, 2014).

The mites are poikilotherms; temperature is the main abiotic factor influencing their biology, ecology, and population dynamics (Liu, 1986; Palyvos *et al.*, 2009; Ghazy *et al.*, 2012; Palyvos & Emmanouel, 2009; Mesa *et al.*, 1990; Hart *et al.*, 2002). Each mite species has its optimal temperature for development and reproduction (Palyvos & Emmanouel, 2009). Such information is useful for assessing the potential of predator mites to suppress a pest population (Midthassel, 2013). For example, after observing that *Proprioseiopsis asetus* (Acari: Phytoseiidae) can develop and reproduce at temperatures above 25 °C, Huang *et al.* (2014) concluded that *P.asetus* had significant potential for control of asparagus thrips, *Thrips tabaci*, in southern China. In contrast, Stavrnides and Mills (2011) concluded that the effectiveness of the predatory mite *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) against a common vineyard pest, *Tetranychus pacificus* McGregor, was limited because high temperature slowed the development of *G. occidentalis* but increased the development of *T. pacificus*. The predatory ability of *P. persimilis* feeding on *T. turkestani* increased as temperature increased to 27 °C and then declined (Wang *et al.*, 2013a). The searching activity of *N. californicus* on *T. turkestani* increased first and then declined as temperature increased. The searching and hunting activities both reached maxima at 28 °C (Wang *et al.*, 2014a).

The mite *T. turkestani* is a serious pest to many crops including cotton, corn, sorghum, medlar, beans, rape, peanut, hops, alfalfa, and vegetables (Wang *et al.*, 1998; Pang *et al.*, 2003; Wang *et al.*, 2013b; Guo *et al.*, 2013; Li *et al.*, 2014). The mite is mainly distributed in Russia, Kazakhstan, America, the Middle East, and Xinjiang, China. *T. turkestani* is the most important pest mite in Xinjiang, China (Yang *et al.*, 2013).

The taxonomic characteristics of *N. bicaudus* are well known; however, little is understood about its ecological and biological characteristics. We hypothesized that if the optimal temperature of the *N. bicaudus* fed on *T. turkestani* approach to the range of the optimal temperature of the *T. turkestani*, *N. bicaudus* could be an effective predator species for controlling *T. turkestani*.

We constructed life tables to investigate the developmental stages and life parameters of *N. bicaudus* feeding on *T. turkestani* at six temperatures between 18 °C to 35 °C. The objectives of this study were to develop (i) better understanding about the population growth of *N. bicaudus*, (ii) better

methods to establish the population of *N. bicaudus* in the laboratory, and then (iii) better mass-rearing technology for industrialization (IV). Eventually, we will release *N. bicaudus* in the field to control the spider mites.

Materials and methods

Stock colonies of T. turkestani and N. bicaudus

The stock colony of *T. turkestani* was initiated with individuals collected from a cotton field near Huayuan, Shihezi City, Xinjiang Uygur Autonomous Region, P. R. China in 2010. The colony was maintained on potted sword bean (*Semen canavaliae* Gladiatae). The stock colony of *N. bicaudus* was initiated with individuals collected from Ili, Xinjiang Uygur Autonomous Region, P. R. China in 2013. The colony was reared on *T. turkestani*. Both colonies were kept in a growth chamber (FLI-2000H, Eyela, Japan) at 26±1 °C, 60% relative humidity, and a 16:8 h (light:dark) photoperiod.

Experiment set-up

The experiment was conducted using plexiglass chambers (3 cm long × 2 cm wide × 0.3 cm high). The chambers were constructed by drilling a hole (1 cm diam) through solid pieces of plexiglass (Jiang *et al.*, 2015; Xu *et al.*, 2013b). A section of a sword bean leaf was placed on a strip of filter paper and then positioned over the hole at one end of the chamber. A fertilized adult *N. bicaudus* female and ten adult *T. turkestani* females (Wang *et al.*, 2015b) were transferred onto the leaf surface. The leaf section was positioned over the hole at one end of the chamber, so that the mites were inside the hole. Both ends of the chamber were then covered with thin pieces of glass to keep the mites from escaping. The chambers were placed in growth chambers at 18 °C, 22 °C, 26 °C, 29 °C, 32 °C, and 35 °C. The photoperiod in the chambers was 16:8 h (L:D). The relative humidity in the chambers was 60% because it is typical of the arid/semi-arid climate in Xinjiang (Zhang & Zhang, 2006). There were 120 replications for each temperature.

After the *N. bicaudus* eggs were laid, the adult female and all the eggs but one were removed from each chamber. The development of immature *N. bicaudus* was observed at 12 h intervals. The duration of each developmental stage was noted until all of the *N. bicaudus* reached adulthood. The leaf was not changed until the nymph stage. Afterwards, the leaf was changed daily. After reaching adulthood, the mites were sexed and males and females from the same temperature conditions were paired inside the chambers. *T. turkestani* were provided as food. The number of eggs laid by each female was recorded at 24 h intervals until each female died. After being counted, the eggs were transferred to fresh chambers. They were reared to adulthood and their sex-ratio was determined. (Huang *et al.*, 2014; Palyvos & Emmanouel, 2011; Stavrinides & Mills, 2011; El Taj & Jung, 2012).

Life history parameters and statistical analysis

The survival rate (lx, percentage of spider mites still living at age x) and daily fecundity (m_x , mean number of female eggs laid per adult female at age x) were calculated based on the number of female individuals. Population parameters were calculated according to the following formulae (Birch's, 1948):the net reproductive rate, R_o , is given by $R_0 = \Sigma l_x m_x$; the intrinsic rate of increase, r_{mv} is given by $r_m = (L_n R_o)/T$); the finite rate of increase, λ , is given by $\lambda = e^{rm}$; the mean generation time, T, is given by $T = \Sigma x l_x m_x / \Sigma l_x m_x$); and the doubling time, Dt, is given by $Dt = (L_n 2)/r_m$.

Analysis of variance of temperature effects on *N. bicaudus* at different life stages was conducted using SPSS version 17.0. The means were compared using Duncan's multiple range tests.

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Results

Immature development

The development time of immature *N. bicaudus* decreased significantly as temperature increased from 18 °C to 32 °C (Table 1). The egg to adult phase was 10.02 d at 18 °C and 4.27 d at 32 °C. There was no significant difference in development time between the 32 °C and 35 °C treatments. The duration of the egg stage decreased from 3.16 d at 18 °C to 1.17 d at 32 °C. There was no significant difference between the 32 °C and 35 °C treatments. The larval stage, which was the shortest among the immature stages, decreased from 1.14 d at 18 °C to 0.54 d at 32 °C. There was no significant difference among the 26 °C, 29 °C, and 32 °C treatments. The protonymph stage decreased from 3.05 d at 18 °C to 1.18 d at 32 °C. The difference between the 32 °C and 35 °C treatments was not significant. The duration of the deutonymph stage decreased from 2.67 d at 18 °C to 1.27 d at 35 °C. There was no significant difference between the 32 °C and 35 °C treatments.

The data in Table 1 were used to calculate linear regression equations relating the duration of the egg (R=0.981), larval (R=0.962), protonymph (R=0.968), deutonymph stages (R=0.966) and total immature period (R=0.977) to temperature (Table 2).

TABLE 1. Development time (days) of immature *N. bicaudus* feeding on *T. turkestani* at different temperatures.

Developmental	Temperatures (°C)							
stage	18	22	26	29	32	35		
Egg (d)	3.16±0.07a	2.38±0.07b	1.65±0.03c	1.38±0.03d	1.17±0.04e	1.19±0.05e		
Larva (d)	1.14±0.04a	0.93±0.07b	$0.62\pm0.02d$	0.58±0.02d	0.54±0.02d	0.73±0.05c		
Protonymph (d)	3.05±0.13a	2.10±0.12b	1.66±0.03c	1.43±0.08d	1.18±0.08e	1.24±0.07de		
Deutonymph (d)	2.67±0.12a	2.17±0.09b	1.71±0.03c	1.31±0.06d	1.39±0.07d	1.27±0.05d		
Total immature (d)	10.02±0.20a	7.58±0.13b	5.65±0.06c	4.70±0.09d	4.27±0.09e	4.43±0.10de		

Note: Data in the table are MEAN±SE. Different letter labels in the same line indicate significant difference (P<0.05).

TABLE 2. Regression equations and correlation coefficients showing the relationship between temperature (x, °C) and the duration of each developmental stage (y, days) of immature *N. bicaudus* feeding on *T. turkestani*.

Development stage	Linear model	R	F	P
Egg	y=-0.144x+5.615	0.981	77.592	0.003
Larva	y=-0.045x+1.917	0.962	36.783	0.009
Protonymph	y=-0.128x+5.137	0.968	44.969	0.007
Deutonymph	y=-0.099x+4.367	0.966	42.336	0.007
Total immature	y=-0.417x+17.035	0.977	63.608	0.004

Adult development and reproduction

The longevity of adult *N. bicaudus* was greatest at 22 °C (50.81 d) and least at 35 °C (15.29 d). The pre-oviposition period was longest (4.55 d) at 18 °C. There was in no significant difference in the duration of the pre-oviposition period among the other temperature treatments. The duration of the oviposition period was longest (38.65) at 18 °C. The oviposition period generally decreased as temperature increased, with the greatest difference between the 22 °C and 26 °C treatments. The post-

oviposition period was longest at 22 °C (13.57 d) followed by 26 °C (11.93 d) and 18 °C (7.61 d). There was in no significant difference in the duration of the post-oviposition period among the other temperature treatments.

TABLE 3. Development time (days) of adult *N. bicaudus* feeding on *T. turkestani* at different temperatures.

Developmental	Temperatures (°C)							
stage	18 °C	22 °C	26 ℃	29 ℃	32 °C	35 ℃		
Pre-oviposition	4.55±0.39a	2.83±0.14b	2.52±0.44bc	2.71±0.22bc	1.89±0.10c	2.00±0.22bc		
Oviposition	38.65±1.47a	31.93±1.21b	16.60±0.77cd	19.32±0.98c	15.41±0.70d	11.07±0.93e		
Post-oviposition	7.61±1.12b	13.57±1.77a	11.93±1.47a	3.57±0.65c	3.30±0.52c	2.21±0.55c		
Longevity	50.81±1.57a	48.33±1.71a	31.05±1.66b	25.61±1.19c	$20.07 \pm 0.84d$	15.29±0.80e		

Note: The data in the table are MEAN±SE. Different letter labels in the same line indicate significant difference (P<0.05).

Fecundity and sex ratio

Total fecundity increased, leveled out, and then decreased as temperature increased (Table 4). Total fecundity was lowest at 18 °C (32.94 eggs/female) and highest at 29 °C (43.82 eggs/ female). There was no significant difference in total fecundity among the 22 °C, 26 °C, 29 °C, and 32 °C treatments. Daily fecundity and daily female fecundity both increased between 18 °C and 22 °C and then leveled out. Daily fecundity was highest at 26 °C (2.55 eggs/day/female). There were no significant difference among the 26 °C, 29 °C, 32 °C, and 35 °C treatments. Daily female fecundity was lowest at 18 °C (0.62 female eggs/day/female) and highest at 26 °C (1.69 female eggs/day/female). There was no significant difference in daily fecundity among the 26 °C, 29 °C, 32 °C, and 35 °C treatments.

TABLE 4. Fecundity of *N. bicaudus* feeding on *T. turkestani*.

Downerstown	Temperatures (°C)							
Parameters	18 °C	22 ℃	26 ℃	29 ℃	32 ℃	35 ℃		
Total fecundity	32.94±2.37	43.37±3.02	42.40±2.36	43.82±3.26	37.96±2.13	27.14±2.79		
(eggs/female)	bc	a	a	a	ab	c		
Daily fecundity	0.85 ± 0.05	1.46 ± 0.13	2.55±0.10	2.33 ± 0.16	2.50 ± 0.13	2.38 ± 0.11		
(Eggs/day/female)	c	b	a	a	a	a		
Daily female	0.62 ± 0.04	0.98 ± 0.08	1.69 ± 0.06	1.54 ± 0.10	1.56 ± 0.08	1.68 ± 0.08		
fecundity (Female	c	b	a	a	a	a		
eggs/day/female)								

Note: The data in the table are MEAN \pm SE. Different letter labels in the same line indicate significant difference (P<0.05).

Life table parameters

The net reproductive rate (R_0) ranged from a maximum of 34.60 at 26 °C to a minimum of 16.87 at 35 °C (Table 5). As temperature increased, the mean generation time (T) decreased from 20.75 to 6.95, the doubling time (Dt) decreased from 4.57 to 1.70, the intrinsic rate of natural increase (r_m) rose from 0.15 to 0.40, and the finite rate of increase (λ) rose from 1.16 to 1.50.

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TABLE 5. Life table parameters of *N. bicaudus* feeding on *T. turkestani*.

	Temperatures (°C)						
Parameter	18 ℃	22 °C	26 ℃	29 ℃	32 ℃	35℃	
Net reproductive rate (R_0)	23.20	28.52	34.60	27.35	22.56	16.87	
Mean generation time $(T, in days)$	20.75	15.85	14.46	10.64	7.85	6.95	
Intrinsic rate of natural increase (r_m)	0.15	0.21	0.24	0.31	0.39	0.40	
Doubling time for population (Dt)	4.57	3.27	2.83	2.23	1.74	1.70	
Finite rate of increase (λ)	1.16	1.23	1.27	1.36	1.48	1.50	

Age-specific survival rate (l_x) and age-specific fecundity (m_x) of adult females of N. bicaudus

The population survival rate of *N. bicaudus* gradually declined as the temperature rose from 18 °C to 35 °C (Fig. 1). The survival rate decreased significantly in the later developmental stages, especially at temperatures between 29 °C and 35 °C. The oviposition period of *N. bicaudus* was significantly shorter in the 29 °C to 35 °C range than in the 18 °C to 26 °C range. The total fecundity of *N. bicaudus* increased and then decreased as temperature increased. The total fecundity was greatest at 26 °C. In the 18 °C treatment, the female mites began dying on 44 d. All of the female mites died by 63 d (Fig. 1). In the 35 °C treatment, the female mites began dying on 5 d and all of them died by 23 d. The survival rate declined quickly as temperature increased (Fig. 1). At 18 °C, the daily female fecundity reached a maximum (0.88 female eggs/day/female) on 19 d. At 35 °C, the daily female fecundity reached a maximum (2.24 female eggs/day/female) on 5 d. When temperatures were low, the daily fecundity of *N. bicaudus* was low but the oviposition period was long. The opposite trend was observed when temperatures were high. The pre-oviposition period and longevity both became gradually shorter as temperature increased. The pre-oviposition period was shortest at 32 °C. The post-oviposition period was significantly shorter at high temperature than at low temperature. The shortest post-oviposition period was at 35 °C.

Discussion

The use of predatory mites to control crop pests such as whitefly, thrips, and aphids in the field is an important part of biological control. Predatory mites have been produced commercially for use as biological control agents against pest insects (Xu *et al.*, 2013a). Life tables of both predator and prey allow the control effects of predatory mite species on harmful mites to be objectively evaluated, thus providing a theoretical foundation for efficient biological control (Prischmann *et al.*, 2005).

At least three predatory mite species (*P. persimlis*, *N. cucumeris* and *N. californicus*) have been introduced in Xinjiang Uygur Autonomous Region for use in pest control. Laboratory populations of all three predatory mites species feeding on *T. turkestani* have been established. *N. cucumeris* has been used to control pest mites on pear trees (Zhang *et al.*, 2006a). However, summer-time temperatures are high in Xinjiang and humidity is low. These conditions make it difficult for nonnative mite species to survive (Escudero & Ferragut, 2005; Weintraub & Palevsky, 2008). Native species of predator mite have many advantages for pest control.

Temperature had significant influence on the development and reproduction of *N. bicaudus* fed on *T. turkestani*. *N. bicaudus* developed successfully between 18 °C to 35 °C. The effects of temperature varied significantly depending on the life stage. However, the optimum temperature was

about 26 °C. This finding suggests that *N. bicaudus*, like many predatory mites, has a potential to develop over a wide range of temperatures (Broufas & Koveos, 2001; Hamamura *et al.*, 1976).

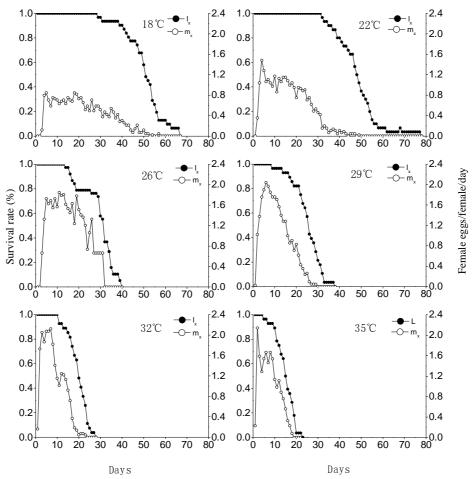


FIGURE 1. Effect of temperature on the survival and daily fecundity of *N. bicaudus* females feeding on *T. turkestani*. Abbreviations: lx, age-specific survival rate; m_x , age-specific fecundity.

The immature stages of predatory mites can be affected by temperature. Kolodochka (1985) reported that *N. barkeri* fed on *Tetranychus* sp. required 9.2 d to complete juvenile development at 26 °C and 90–95% relative humidity. This was much longer than the juvenile development time of 5.65 d *N. bicaudus* for our observation in this study. The duration of the egg, larva, protonymph, total immature, and pre-oviposition stages all decreased as temperatures increased from 18 °C to 32 °C and then increased slightly as temperatures increased from 32 °C to 35 °C the same as Gotoh et al (2004). This is consistent with previous observations that insect development rates increase as temperature rise and then slow down when temperatures become too high (Sohrabi & Shishehbor, 2008; Liu, 1986; Yue *et al.*, 2009).

The longevity of *N. bicaudus* decreased as temperature increased within the temperature range in this study. Total fecundity was higher at 22 °C, 26 °C, and 29 °C than at either 18 °C or 35 °C. Gotoh *et al.* (2004) reported that at the optimum temperature (25 °C), the maximum fecundity of *N. bicaudus* was slightly greater than that reported for *N. californicus*. Both daily fecundity and daily female fecundity were significantly higher at 26 °C, 29 °C, 32 °C, and 35 °C than at either 18 °C or 22 °C (P<0.05).

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Daily fecundity and daily female fecundity both reached peaks at 26 °C. The net reproductive rate (R_0) of N. bicaudus increased to a maximum of 34.609 at 26 °C and then decreased. This indicated that the optimal temperature range of N. bicaudus was about 26 °C. A temperature of 26 °C could be used for the development and rearing of N. bicaudus colonies in the laboratory. Net reproductive rate (R_0) of N. bicaudus at 26 °C was slightly lower than N. californicus at the optimum temperature (El Taj & Jung, 2012).

The r_m value is an imperative factor for relating the growth potential of a population under certain climatic, food provisions and as it reflects their general effects on development, reproduction, and survival (Southwood & Henderson 2000). The intrinsic rate of increase recorded in the current experiment for N. bicaudus at 26 °C and 35 °C (0.24 and 0.40, respectively), were considerably higher than the values obtained by Fouly (1997) and Emmert et al. (2008) for Proprioseiopsis asetus at 26 °C and 35 °C (0.18–0.28 and 0.17–0.32, respectively).

The mean generation time and the doubling population time of *N. bicaudus* fed on *T. turkestani* were both relatively short at 26 °C compared to values reported for several other predatory mite species (Wang *et al.*, 2014b; Zhang *et al.*, 2012a,b). This suggested that *N. bicaudus* could be effectively used to control pest mites.

During summers in Xinjiang, the mean daily temperature is about 25 °C and the relative humidity is about 60% (Zhang & Zhang, 2006). Our results show that these conditions are conducive for the development and reproduction of *N. bicaudus*. The optimal temperature of *T. turkestani* range from 25–30 °C (Zhang *et al.*, 2006b). The average preying number of *N. bicaudus* fed on *T. turkestani* were about 64.4 eggs, 39.4 larvas, 45.0 nymphs, 5.6 adults per day (Wang *et al.*, 2015b).which will in turn be favorable for the management of *T. turkestani*. These results indicate that *N. bicaudus* could be a helpful biological control agent for *T. turkestani* on crops under most of the prevailing weather conditions in Xinjiang.

Other environmental factors such as light and humidity can affect the growth and development of predator mites. The type of prey can also have great effect on the development and reproduction of *N. bicaudus* (Ferrero *et al.*, 2014; Rasmy *et al.*, 2011). The highest temperatures in Xinjiang range from >35 °C in summer and the lowest temperatures < -23 °C in winter. The development of *N. bicaudus* may be hindered by these temperature extremes. The effects of brief exposure to very hot or very cold conditions is not known. Additional studies should be done about the effects of these factors in order to make full use of *N. bicaudus* for biological control in the field.

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