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Article

Functional response and prey stage preference of *Neoseiulus barkeri* on *Trasonemus confusus*

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Abstract

The fungivorous *Tarsonemus confusus* Ewing is a tarsonemid mite causing a so call 'black-dot' symptom on the bagged apple fruit in North China, and the phytoseiid mite *Neoseiulus barkeri* Hughes is a cosmopolitan generalist predator of many agricultural and horticultural pests. In the present study, we evaluated the biological control potential of *N. barkeri* on *T. confusus*, by determining its functional response type to prey density of and prey stage preference on both active and quiescent life stages of *T. confusus* (i.e., eggs, larvae, quiescent larvae and female adults). We found that *N. barkeri* exhibited all three types of functional response. When fed on eggs and quiescent larvae of *T. confusus*, *N. barkeri* had a Type II functional response (i.e., inverse density-dependent predation rate); however, a Type I functional response (i.e., density-independent predation rate) was detected when *N. barkeri* preyed on *T. confusus* larvae and a Type III functional response (i.e., sigmoid density-dependent predation rate) when preyed on *T. confusus* adults. We further showed that the 'constant prey' functional response models fit our data better than the 'depleted prey' models, as the predator left and reencountered the previously fed prey which reduced its searching efficiency. When all active and quiescent life stages of *T. confusus* were available, *N. barkeri* significantly preferred larvae over other prey stages for feeding. Our results imply that due to its quick response to the increasing prey larval and adult densities, *N. barkeri* could consume a great number of active prey and is thus capable to decline the current fruit damage caused by prey feeding and to potentially prevent the prey population build-up later in the season. Furthermore, predators with Type II response may be efficient at low prey densities, thus augmentative release of *N. barkeri* early in the season may also suppress the egg and quiescent larva populations of *T. confusus*. Knowledge from this study provides insights into our understandings in the biological control ecology of *N. barkeri*.

Key words: phytoseiid, functional response, stage preference, prey population

Introduction

Tarsonemus confusus Ewing (Acari: Tarsonemidae), known as the confused tarsonemid mite, has a widespread distribution in North America (USA, Canada), Europe (Germany, Poland, Italy, Ireland, Crimea, Ukraine, Russia, Byelorussia), Africa (Egypt) and East Asia (Japan, Korea, China) (Nucifora & Vacante 2003; Zhang 2003). It is a primarily fungivorous species (Lindquist 1986) and present on some ornamentals (e.g. African violet, azalea, cissus, Cyclamen, Gloxinia, ivy and Pilea) in greenhouses in Europe and tomatoes in North America, but have rarely caused primary damage to its host plants (Zhang 2003).

However, the fungivorous nature of *Tarsonemus* mites play a role in fungal dispersal (Lindquist 1986; Van der Walt *et al*. 2011). In apple orchards in the Ceres area of South Africa, *Tarsonemus* mites are the dominant mites in all apple developmental stages, and the highest incidence of mites is restricted to the calyx tubes associated with the core rot diseases (Van der Walt *et al*. 2011). In China, the application of bagging technique is a prevalent strategy applied in fruit production to prevent pest damage, pathogen infection and reducing chemical application in order to improve fruit quality and increase market-access rate and value. The specific methods of bagging technique are to bag the young fruits with special double-layer paper bags 30–40 days after flowering and to remove the bags 20 days before harvest. However, in the later 1990s and early 2000s in North China, a so call 'black-dot' symptom outbroke in the peach and apple orchards where the bagging technique was applied (Wang *et al*. 1999; Hao *et al*. 2007, 2010). The field inspections from 2003 to 2005 showed that the infection rate of bagged apple was significantly higher (50–90%) than that of un-bagged fruit (1% only) (Hao *et al*. 2007). The symptoms on apple appear around the calyx and the injured pericarp usually develops to grey-brown or yellow-brown circle (≈ 1 to 6 mm) with some white powder attached sometimes, and then retracts and suberizes (Hao *et al*. 2007, 2010). Many pathogenic fungi, mainly the *Trichothecium roseum* (Pers.) Link, *Acremonium stritum* Gams and *Alternaria spp*. have been isolated from the injured pericarp, and these pathogens are weak parasitical and saprophytic fungi and cause symptoms only under moist conditions (Guo *et al*. 2005; Wang *et al*. 2014). Wang *et al*. (1999) assumed that the presence of *T. confusus* and *T. bilobatus* Sushi is a major cause of 'black-dot' symptoms on the peach fruit. Hao *et al*. (2007, 2010) further provided evidence that 'black-dot' occurrence on the bagged apple can be attributed to the presence of *T. confusus*. To reduce the impact of *T. confusus*, non-chemical control methods integrated into bagging technique are required.

The phytoseiid mite *Neoseiulus* (*Amblyseius*) *barkeri* Hughes (Acari: Phytoseiidae) is a cosmopolitan species, widespread on all continents (De Moraes *et al*. 2004) including China (Xin 1988; Wu *et al*. 1997; Zhang 2003; Wu *et al*. 2014) and a common species in many agroecosystems (Xin 1988; Fan & Petitt 1994a; Wu *et al*. 1997; Beard 2001). *N. barkeri* is a generalist predatory mite, which feeds on a wide range of pests such as the storage mites, spider mites, broad mites, thrips, and whitefly immatures (e.g., Ramakers & Van Lieburg 1982; Fan & Petitt 1994a, 1994b; Nomikou *et al*. 2001; Zhang 2003; Wu *et al*. 2014; Hekmat *et al*. 2017). *N. barkeri* may develop and reproduce on pollen grains of various plants (Van Rijn & Van Houten 1991; Rezaie & Askarieh 2016) which enable *N. barkeri* to subsist in environments of prey scarcity (Fan & Petitt 1994b). This species has been commercially produced worldwide (Ramakers & Van Lieburg 1982; Gerson *et al*. 2003; Li *et al*. 2015) and applied in augmentative biological control programmes to control various pests of different agroecosystems (Karg 1987; Hansen 1988; Bonde 1989; Xu & Wang 2007; Jafari *et al*. 2012a, Fang *et al*. 2013; Fan & Petitt 1994b).

Many studies have evaluated the potential of *N. barkeri* in biological control of pests by determining its demographic response to prey density (e.g., Fan & Petitt 1994a, 1994b; Jafari *et al*. 2012b; Fang *et al*. 2013; Wu *et al*. 2014; Wu *et al*. 2015; Li *et al*. 2017; Fathipour *et al*. 2018). However, these studies focus on the egg and active nymphal or adult stages but ignore the quiescent larvae and nymphs, making it difficult in fully understanding the general biological control ecology of *N. barkeri*. Before or even after the published work of Juliano (2001), many authors fit their data directly to the conceivable models without testing the appropriateness of selected models (e.g., Fan & Petitt 1994a, 1994b; Wu *et al*. 2014; Wu *et al*. 2015; Li *et al*. 2017), which may mislead the nature of the response type of predator. Moreover, studies on the response of predators to prey density unassailably prefer the "depleted prey" functional response models where prey are gradual depleted during each time interval (Rogers 1972; Hassell 1978). However, we argue that if the prey

are not 'completely' consumed and their residues left in situ on the arena, predators may subsequently reencounter the previously fed prey, which will alter their searching behaviors. In this case, the functional response models designed for parasitoids ("constant prey" model, prey/hosts are reencountered during searching) (Holling 1966; Hassell 1978) may be more appropriate which is warranted for further test.

Prey stage preference is critical in the success of a biological control program. Predation on mites such as *T. confusus*, having both active and quiescent stages, predators preferring the active immatures prey and adults will reduce the current damage caused by the prey feeding, and preferring adults may decline pests' population size later in the season. In the phytoseiid-spider mite system, many studies have assessed the prey stage preference by simultaneously offering two or more prey stages to a predator (e.g., Burnett 1971; Takafuji & Chant 1976; Fernando & Hassell 1980; Blackwood *et al.* 2001, 2004; Carrillo & Peña 2012; Moghadasi *et al.* 2013; Naeem *et al.* 2017). However, in these studies the quiescent (larva and nymph) stages are usually omitted for test, failing to address truly preference in the natural environments. So far, the influence of quiescent larval and nymphal stages on prey stage preference of predators is largely unknown.

In the present study, we evaluated the potential efficiency of *N. barkeri* in biological control of *T. confusus*. We firstly investigated the predation ability of *N. barkeri* in response to the increasing density of different life stages of *T. confusus* and compared the fitness of "constant prey" and "depleted prey" functional models. We then examined the prey stage preference of *N. barkeri* by providing all life stages of *T. confusus* in an experimental arena. The implications of results from this study in the implement of biological control programmes were discussed.

Materials and methods

Breeding colony and mite preparation

The breeding colony of *N. barkeri* started from a mixed population of nymphs and adults collected from an apple orchard in 2008, in Funing County, Hebei Province, China. A storage mite, *Aleuroglyphus ovatus* Troupeau produced on wheat bran was served as prey of *N. barkeri*. The *T. confusus* colony reared on wheat bran was established from adults collected from an apple orchard in 2012, in Changli County, Hebei Province, China. The mite colonies were maintained in two separate climate chambers (PRX-250C, Ningbo Saifu Experimental Instrument Co., Ltd.) at 25 ± 1ºC and 80 ± 5% RH, with a photoperiod of 16:8 h (light:dark) for *N. barkeri* and dark for *T. confusus*. All experiments were carried out in the Plant Protection Laboratory, Changli Institute of Pomology, Hebei Academy of Agricultural and Forestry Sciences, China at the same environmental conditions as that for *T. confusus* colony.

To obtain the female adult predators for experiments, five female adults were randomly selected from the colony and transferred onto an experimental arena by a fine brush. The experimental arena was a chamber (16 mm diameter × 4 mm depth) which was made by digging at the center of a piece of plexiglass (76 mm length × 25 mm width × 5 mm height). After adults were transferred, the chamber was covered by a piece of glass (76 mm length × 25 mm width × 1 mm height). The plexiglass and glass cover were clipped by two clamps to prevent mites from escaping. *N. barkeri* adults were provided with sufficient *A. ovatus* eggs as food, and allowed to oviposit for 24 hours before removal. Eggs laid were maintained in the same chamber, and hatched larvae were daily supplied with *A. ovatus* eggs as food until they developed to adults. Thirty chambers were set up. Newly emerged adults were sexed and female adults were individually transferred into a clean chamber and starved for 24 hours before used for experiments.

To prepare prey of desired life stages (i.e., egg, larva, quiescent larva and adult) for experiments, 20 female and 3 male adults of *T. confusus* were randomly selected from the colony and reared in a Petri dish (9 cm diameter × 1 cm height) maintaining 1 g wheat bran (added 0.2 g weekly). The Petri dish was placed upon a plastic block (6.0 cm diameter × 2.5 cm height) in a plastic box (42 cm length × 28 cm width × 16 cm height) filled with tap water (2 cm depth) to prevent contamination. Twenty Petri dishes were set up.

Response to prey density

To determine the functional response of *N. barkeri* to prey density of different life stages, 20 combination treatments were set up: five prey densities (10, 20, 30, 40 and 50) × four prey life stages (i.e., egg, larva, quiescent larva and adult), with five replicates for each treatment. For each treatment, a 24 h-starved *N. barkeri* female was transferred onto an above mentioned experimental chamber maintaining prey of a test density of a test life stage. Before mites were transferred, one drop (0.05 ml) of PDA culture medium (i.e., 16.13% potato, 1.61% dextrose, 1.61% agar and 80.65% sterilizing water, and sterilized at 120 ºC for 20 minutes) was dropped onto the chamber to serve as food for prey and to maintain humidity. The female predator was allowed to feed for 24 hours and the number of prey fed by the predator was counted under a stereomicroscope (Leica EZ4, Germany).

Prey stage preference

To investigate prey stage preference of *N. barkeri*, a 24 h-starved adult female was transferred into an experimental chamber maintaining five prey of each life stage (i.e., 20 prey in total). Other procedures were the same as mentioned above. The number of prey fed was counted under the stereomicroscope after 24 hours. All test female predators were used once only. There were 20 replicates for this experiment.

Functional response determination and modeling

A polynomial logistic regression model (Juliano 2001) was initially used to distinguish the functional response types of *N. barkeri* by taking the proportion of prey fed (N_f/N_0) as a function of the initial density of prey (N_0). A cubic logistic regression model is usually sufficient for a biological study:

$$N_e/N_0 = \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)/[1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)] \quad (\text{eq. 1})$$

where N_e is the number of prey eaten, N_0 is the initial number of prey, P_0 is the intercept, and P_1, P_2, and P_3 are linear, quadratic and cubic coefficients, respectively, related to the slope of the curve. The slope of the proportion of fed prey near the lowest host density is characteristic of the functional response (Juliano 2001): $P_1 = 0$ indicates a Type I functional response; a significant negative linear coefficient of $P_1 < 0$ demonstrates a Type II functional response, and a significant positive linear coefficient of $P_1 > 0$ with a significant negative quadratic coefficient of $P_2 < 0$ indicates a Type III functional response. If cubic logistic regression model may yield a non-significant cubic parameter, or even if all other parameters are non-significant, then a reduced model by eliminating the cubic term from eq. 1 is requited to retest other coefficient (Juliano 2001). In this case, a quadratic model may sufficient to test the Type II functional response, while a cubic model is used to determine the Type III functional response (Juliano 2001). Both quadratic and cubic models were tested and the statistical results were compared for each life stage of *T. confusus*.

In the present study, a Type I functional response was detected on *T. confusus* larvae, Type II on eggs and quiescent larvae and Type III on female adults (see Table 1 and Figure 1). Numerous mechanistic and phenomenological models have been developed to describe functional response. The Nicholson-Bailey model (Nicholson & Bailey 1935), describing a rectilinear rise in the number of prey eaten per predator as prey density increases, was applied to fit the Type I functional response of *N. barkeri* to *T. confusus* larval density:

$$N_e = aTN_0 \qquad\qquad\qquad (eq. 2)$$

where a is the attack rate (or instantaneous search rate, prey h^{-1}), and T is the total time (i.e., 24 h) available for search. Holling (1966) modeled a Type II functional response by assuming that (1) encounter rate with prey is linearly to prey density ($= aN_0$), (2) predators cannot make additional captures when they are handling prey, and (3) prey density is constant:

$$N_e = aN_0T/(1 + aN_0T_h) \qquad\qquad (eq. 3)$$

in which T_h is handling time per prey. While if the attack constant is a function of prey density: $a = bN_0/(1 + cN_0)$, where b and c are constant, then a Type III functional response is modeled by substituting the a equation into eq. 3 (Hassell 1978):

$$N_e = bN_0^2 T/(1 + cN_0 + bN_0^2 T_h) \qquad\qquad (eq. 4)$$

Many investigators (e.g., Juliano 2001; Kidd & Jervis 2005) argued that if predation experiments are done without replacement of prey, employing the above Type II and III functional response models to analyse the data is inappropriate, as prey eaten are not reencountered by the predators. Rogers (1972) and Hassell (1978) have respectively improved the Type II and III functional models (eqs. 5–6, respectively) for predators by taking account of gradual prey depletion during each time interval:

$$N_e = N_0\{1 - \exp[a(N_eT_h - T)]\} \qquad\qquad (eq. 5)$$
$$N_e = N_0\{1 - \exp[bN_0(N_eT_h - T)/(1 + cN_0)]\} \qquad (eq. 6)$$

However, our pre-experimental observations show that a *N. barkeri* adult did not 'completely' consume its prey but left the prey residue on the arena, and thus frequently reencountered the fed prey. *N. barkeri* reacted to the fed prey by walking slower when closed to or passed by the prey residue, indicating an impact of fed prey on its subsequent searching efficiency. To determine the appropriateness of models selected to describe the functional response, we applied both 'constant prey' and 'depleted prey' models (eqs. 3–6) to fit our data. Models with higher fitness were finally used.

Data analysis

Data analyses were performed using SAS software (SAS 9.4, SAS Institute Inc., NC, USA). A logistic regression analysis of maximum likelihood (CATMOD Procedure) was performed to determine the type of functional responses (Table 1). Nonlinear least square regression (NLIN Procedure) was used to estimate the attack rate (a) handling time (T_h), and constant b and c in models (Figure 1, Table 3). The estimated parameters of the nonlinear least square regressions are significantly different from 0 if the 95% confidence limits (CL) do not include 0 (i.e., both upper and lower CL > 0 or < 0) (Juliano 2001). Juliano' (2004) methods were used to compare parameters in Table 3, where there is no significant difference in a given parameter between prey life stages if the 83.4% CL overlap. The 83.4% CL is sufficient to compare the difference in a parameter (Juliano 2004). The coefficient of determination (R^2) of a nonlinear least square regression was calculated as: 1 – (residual sum of square/corrected total sum of square) (Tahriri *et al.* 2007). Data on prey stage preference (Figure 2) were not normally distributed (Shapiro-Wilk test, UNIVARIATE Procedure) and thus analysed using non-parametric analysis of variance (ANOVA, GLM

Procedure) followed by a Bonferroni (Dunn) test for multiple comparison.

Results

Determination and modeling functional response

As shown in Table 1, a quadratic logistic regression analysis shows a significant negative linear coefficient for quiescent larvae, indicating a Type II functional response (Figure 1e–f). Similarly, a cubic logistic linear model generalized a significant positive linear and a significant negative coefficient for adults, demonstrating a Type III functional response (Figure 1g–h).
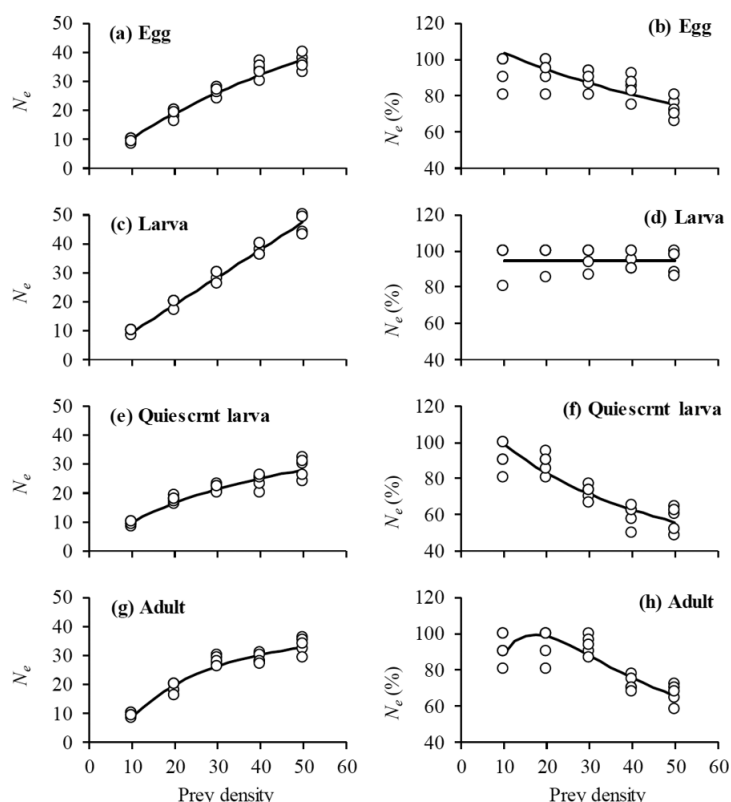

FIGURE 1. Functional response of *N. barkeri* to *T. confusus* density of different life stages: (**a–b**) egg, N_e = $1.1448N_0/(1+0.0105N_0)$ (R^2 = 0.9583, $F_{2,23}$ = 1948.70, P < 0.0001); (**c–d**) larva, N_e = $0.9480N_0$ (R^2 = 0.9812, $F_{1,24}$ = 7096.97, P < 0.0001); (**e–f**) quiescent larva, N_e = $1.2240N_0/(1+0.0238N_0)$ (R^2 = 0.9120, $F_{2,23}$ = 1273.43, P < 0.0001); (**g–h**) adult, N_e = $0.1440N_0^2/(1 + 0.0268N_0 + 0.0035N_0^2)$ (R^2 = 0.9490, $F_{3,22}$ = 1184.61, P < 0.0001). Estimated attack rate (*a*), handling time (T_h) and constant *b* and *c* used in the models are also shown in Table 2.

When preyed on eggs, both quadratic and cubic logistic linear models estimated a negative linear coefficient but they were not significant (Table 1); however the non-significant likelihood rate detected from both models (χ_{22}^2 = 27.89, P = 0.1792 for quadratic model; χ_{21}^2 = 27.84, P = 0.1448 for cubic model) suggested the validity of these two models (Juliano 2001). Therefore, a Type II functional response could be confirmed for eggs (Figure 1a–b).

When preyed on larvae, both quadratic and cubic logistic linear models estimated a positive linear and a negative quadratic coefficient (Table 1). However, these results did not indicate a Type

III functional response, as both linear and quadratic coefficients were not significant (Table 1), and the significant likelihood x rates (χ^2_{22} = 57.33, P < 0.0001 for quadratic model; χ^2_{21} = 57.19, P < 0.0001 for cubic model) suggested the lack of fit of these two logistic regression models (Juliano 2001). In fact, a linear relationship between the number of larvae eaten and larval density indicated a Type I functional response (Figure 1c–d).

TABLE 1. Maximum-likelihood estimates of the proportion of *T. confusus* of different stages fed by *N. barkeri* as a function of initial prey density from logistic regressions.

Prey stage	Parameters	Estimate	SE	x^2	P
Egg	Quadratic regression				
	P_0	2.8356	0.9455	8.99	0.0027
	P_1	-0.0017	0.0564	0.00	0.9761
	P_2	-0.0007	0.0079×10^{-1}	0.79	0.3749
	Cubic regression				
	P_0	3.2472	2.1046	2.38	0.1229
	P_1	-0.0510	0.2303	0.05	0.8249
	P_2	0.0098×10^{-1}	0.0762×10^{-1}	0.02	0.8983
	P_3	-0.0002×10^{-1}	0.0078×10^{-2}	0.05	0.8248
Larva	Quadratic regression				
	P_0	3.2884	1.3252	6.16	0.0131
	P_1	0.0816×10^{-1}	0.0834	0.01	0.9221
	P_2	-0.0004	0.0121×10^{-1}	0.10	0.7507
	Cubic regression				
	P_0	2.3463	2.7909	0.71	0.4005
	P_1	0.1207	0.3261	0.15	0.6969
	P_2	-0.0456×10^{-1}	0.0112	0.17	0.6835
	P_3	0.0044×10^{-2}	0.0012×10^{-1}	0.14	0.7075
Quiescent larva	Quadratic regression				
	P_0	4.3481	0.8694	25.01	< 0.0001
	P_1	-0.1615	0.0503	10.29	0.0013
	P_2	0.0016	0.0069×10^{-1}	5.32	0.0211
	Cubic regression				
	P_0	2.6226	1.7566	2.23	0.1354
	P_1	0.0337	0.1861	0.03	0.8561
	P_2	-0.0488×10^{-1}	0.0606×10^{-1}	0.65	0.4212
	P_3	0.0065×10^{-2}	0.0061×10^{-2}	1.14	0.2856
Adult	Quadratic regression				
	P_0	3.7388	1.042	12.87	0.0003
	P_1	-0.0550	0.0596	0.85	0.3564
	P_2	-0.0002	0.0081×10^{-1}	0.04	0.8460
	Cubic regression				
	P_0	-1.8964	1.8775	1.02	0.3125
	P_1	0.6302	0.2219	8.07	0.0045
	P_2	-0.0234	0.0751×10^{-1}	9.72	0.0018
	P_3	0.0024×10^{-1}	0.0077×10^{-2}	9.53	0.0020

TABLE 2. Comparison of statistical results of 'constant prey' and 'depleted prey' models for Type II and III functional response in the *N. barkeri*-*T. confusus* system.

Prey stage	Functional response	Model	R^2	F*	P
Egg	Type II	constant, eq.3	0.9583	1948.70	< 0.0001
		depleted, eq.5	0.9236	1057.90	< 0.0001
Quiescent larva	Type II	constant, eq.3	0.9120	1273.43	< 0.0001
		depleted, eq.5	0.7489	439.21	< 0.0001
Adult	Type III	constant, eq.4	0.9490	1184.61	< 0.0001
		depleted, eq.6	0.8347	564.65	< 0.0001

 * df = 2,25 for egg and quiescent larva and 2,23 for adult.

TABLE 3. Estimated attack rate (a, prey h^{-1}), handling time (T_h, h) and constant b and c for the functional response of *N. barkeri* to *T. confusus* density of different life stages *.

Stage	Parameter	Estimate	SE	83.4% CL	
				lower	upper
Egg	a	0.0477	0.0035	0.0427	0.0527
	T_h	0.2198	0.0394	0.1635	0.2761
Larva	a	0.0395	0.0005	0.0389	0.0402
	T_h	-	-	-	-
Quiescent larva	a	0.0510	0.0052	0.0437	0.0584
	T_h	0.4670	0.0551	0.3882	0.5458
Adult	b	0.0060	0.0024	0.0026	0.0094
	c	0.0268	0.0419	-0.0333	0.0869
	T_h	0.5772	0.0929	0.4441	0.7102

*All estimated parameters are significant at $P < 0.05$ level (95% CL > 0), except the constant c for adult stage.

As shown in Table 2, both 'constant prey' and 'depleted prey' models significantly fitted the data. However, the 'constant prey' models (eqs. 3–4) generalized greater regression coefficients (R^2) and F values and thus fitted the data better than did 'depleted prey' models. Furthermore, we found that the 'constant prey' models produced a lower searching efficiency but longer handling time (Table 3) than did 'depleted prey' models ($a = 0.1153 \pm 0.0268$ for egg, and 0.0654 ± 0.0139 for quiescent larva; $T_h = 0.2972 \pm 0.0926$ for egg, 0.3473 ± 0.1224 for quiescent larva, and 0.4070 ± 0.0907 for adult), except the handling time for egg which was shorter when data were fitted by 'constant prey' models (see Table 3) than that by 'depleted prey' models. Therefore, the 'depleted prey' models (eqs. 5–6) might have overestimated the predation of *N. barkeri*. We finally used the 'constant prey' models (eqs. 3–4) to analyse the Type II and III functional response of *N. barkeri*.

Further analyses confirmed that *N. barkeri* exhibited different types of functional response in response to *T. confusus* density of different life stages (Figure 1). The models selected significantly fitted the data: (1) Type I response on larvae, i.e., the attack rate did not change over various prey density (Figure 1d); (2) Type II response on eggs and quiescent larvae, i.e., the predation rate significantly decreased with increasing density of eggs (Figure 2b) and quiescent larvae (Figure 2f); and (3) Type III response on adults, i.e., the predation rate significantly increased when adult

density increased from 10 to approximate 20, after which significantly decreased (Figure 1h).

The handling time (T_h) on quiescent larvae and adults was significantly longer than that on eggs (83.4% CL did not overlap) (Table 3). The attack rate was not significantly different between prey life stages of egg, larva and quiescent larva (83.4% CL overlapped) (Table 3).

Prey stage preference

When supplied with prey of different life stages, *N. barkeri* significantly preferred *T. confusus* larvae over adults and significantly preferred the latter over eggs for feeding ($F_{3,72} = 18.69$, P < 0.0001); however, there was no significant difference in number of prey fed detected between adults and quiescent larvae and between quiescent larvae and eggs (Figure 2).
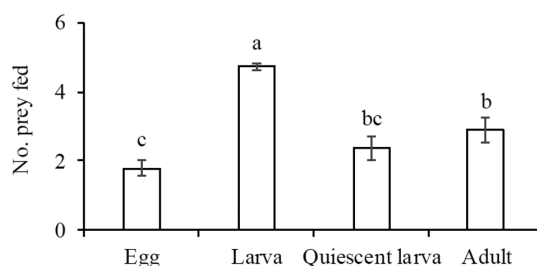

FIGURE 2. Mean (± SE) number of *T. confusus* of different life stages fed by *N. barkeri* in 24 h. Columns with the same letters are not significantly different (ANOVA: P > 0.05).

Discussion

In the present study we have identified the type of functional response of *N. barkeri* to *T. confusus* density by including all active and quiescent life stages that the predators encounter in nature. *N. barkeri* displayed a Type I response on prey larvae, a Type II response on eggs and quiescent larvae and a Type III on female adults (Tables 1 and 3; Figure 2). We show that the 'constant prey' models (eqs. 3–4; Holling 1966; Hassell 1978) were more appropriate to fit our data than did 'depleted prey' models (eqs. 5–6; Rogers 1972; Hassell 1978) (Table 2). These may attribute to that *N. barkeri* did not completely consumed the prey (sucked body fluids only) and left the fed bodies in situ, and thus most likely reencountered during subsequent prey searching; predators reencountering the prey bodies might in turn decline their searching efficiency. Therefore, we argue that for predator species like *N. barkeri*, the choice of 'constant prey' or 'depleted prey' functional models largely depends on whether the reencountering of fed bodies influences the predators' searching behaviors.

Our results suggest that *N. barkeri* is potentially a highly effective biological control agent of *T. confusus*, for three reasons. First, a Type I response was detected on *T. confusus* larvae and a Type III response on female adults. Natural enemies imposing a Type I or III functional response are more potentially manage the prey populations and can be considered as efficient biocontrol agents (Fernández-arhex & Corley 2003). Because a Type I response implies a density-independent predator attack rate (Fernández-arhex & Corley 2003), where the number of prey larvae fed by *N. barkeri* increased with larval density (Figure 1c–1d). A Type III response on female adults was defined as a sigmoid curve (Figure 1g–1h), where the response initially accelerated due to increasingly efficient of predators in finding prey (Fernández-arhex & Corley 2003) then gradually levelled off probably because of the satiation and prolonged handling time (Berryman 1999;

Jeschke 2007; Li *et al.* 2018). In turn, direct density dependence is detected when prey density was low (≈ 20 female adults in this study). Thus, a Type III response cannot eliminate a prey population but can potentially stabilize predator-prey interactions (Hassell 1978; Collins *et al.* 1981; Berryman 1999), maintaining the population of *T. confusus* female adults at a low density.

Second, as reported in many pest species attacked by *N. barkeri* [for example, the two-spotted spider mite *Tetranychus urticae* Koch (Fan & Petitt 1994a; Jafari *et al.* 2012b), citrus red mite *Eotetranychus kankitus* Ehara (Li *et al.* 2017), broad mite *Polyphagotarsonemus latus* (Banks) (Fan & Petitt 1994b), or western flower thrips *Frankliniella occidentalis* Pergande (Wu *et al.* 2015)], a Type II response of *N. barkeri* was also detected on *T. confusus* eggs and quiescent larvae in the present study due to an inverse density-dependent predation. The Type II response is usually more common than the Type I and III responses (Fernández-arhex & Corley 2003). However, a Type II response does not imply a potential failure to control *T. confusus* using *N. barkeri*, rather biological control success using *N. barkeri* with a Type II functional response has been widely reported, for example the *Thrips tabaci* (L.) (Hansen 1988; Wu *et al.* 2014), *Potyphagotarsonemus latus* (Banks) (Fan & Petitt 1994b) and *Eotetranychus kankitus* Ehara (Li *et al.* 2017), although the mechanisms behind the successes are not well known. Koehler (1999) indicated that predators with Type II response are particularly efficient at low prey densities. Hence, it is assumed that augmentative release of *N. barkeri* early in the season when the prey density is low may be able to prevent pest outbreak later in the season.

Finally, when supplied with prey of all active and quiescent stages, *N. barkeri* strongly preferred the active larvae and adults for feeding. As only active prey feed and act as pathogen vectors in the orchards, *N. barkeri* preferring larvae and adults may rapidly minimise damages caused by pest feeding and pathogen infestation. Likewise, as only females could found next generation, *N. barkeri* focusing on *T. confusus* female adults is likely to destroy the reproductive units of a prey population quickly and reduce the possibility of population increase of future generations. Therefore, compared to other tarsonemid mites who less likely attack the older duetonymphs or completely ignore female adults, for example, the two-spotted spider mite (e.g., Burnett 1971; Takafuji & Chant 1976; Moghadasi *et al.* 2013; Naeem *et al.* 2017), *N. barkeri* may quickly suppress the prey populations indicating its high potential in biological control of *T. confusus*.

The mechanisms regulating the prey stage preference of phytoseiid mites are still not very clear. The optimal foraging theory assumes that natural selection generally favors animal foraging behaviors that maximize the energetic gains while minimize the costs involved in prey searching, processing and handling, inducing a trade-off between benefit and costs (Krebs 1972; Begon *et al.* 1996; Blackwood *et al.* 2001). According to this theoretical assumption, *N. barkeri* should prefer the quiescent larvae, because prey have grown in body size when develop to mature larval stage which contains more resource, and the quiescent larvae (as well as eggs) are immobile and unable to defend or escape. In contrast, our results show that *N. barkeri* preferred the active larvae and adults over the eggs and quiescent larvae (Figure 2). To our best knowledge, the only study involving the quiescent forms in preference tests is performed by Clements and Harmsen (1990). They reported that when competes with a stigmaeid predator mite [*Zetzellia mali* (Ewing)] for a given life stage of European red mite *Panonychus ulmi* (Koch), the phytoseiid *Typhlodromus caudiglans* (Schuster) prefers the active forms over the eggs and quiescent forms.

The phytoseiid mites lack eyes but could locate their prey by various means (Gerson *et al.* 2003). Early studies (Putman 1962; Mori & Chant 1966; Mcmurtry *et al.* 1970) reported that phytoseiids detect their prey by direct contact. In acarines various hairs on their bodies function as mechanoreceptors, sensing movement and airborne vibrations from both their prey and the

surrounding air (Gillespie & Spagna 2009). Krantz (1970) further stated that the legs of acarines have many tactile setae, and the first and second pairs of legs are provided with elongate setae, trichobothria, which probably are chemo-receptors. Moreover, the first pair of legs of Phytoseiidae are longer than other mite families, may serve primarily to locate and identify the potential prey encountered (Takafuji & Chant 1976; Gillespie & Spagna 2009). The results of significantly more larvae and adults fed (Figure 2) assume that *N. barkeri* located the active mites more easily and more frequently and accepted more for feeding. These may due to two reasons as suggested by Sabelis (1990) and Blackwood *et al*. (2001): (1) active mites are slightly larger than quiescent ones (i.e., larvae > eggs, and adults > quiescent larvae) particularly after feeding occurs; and (2) larvae and adults are active, increasing probability of prey encountered by predators and possibly enticing predators to investigate with their front legs. *N. barkeri* significantly preferring *T. confusus* larvae over adults for feeding may attribute to the better defence of adults, while the non-significant preference between prey adults and quiescent larvae is the result of behavioural interaction between prey searching of the predator and defence of the prey adults. These specific prey searching and predatory behaviors of acarines may be the causes of varied functional responses of *N. barkeri* on different life stages of *T. confusus*.

In conclusion, using the *N. barkeri-T. confusus* system, we show that the 'constant prey' functional response models were better to fit our data than the 'depleted prey' models as the prey residues left were reencountered by the predators during the subsequent prey searching. We demonstrated that *N. barkeri* has potential to be a highly effective biological agent of *T. confusus*. Based on its functional response to *T. confusus* density and performance of prey stage preference, we propose two mechanisms behind the potential success of *N. barkeri* in biological control of *T. confusus*. First, the Type I and III response of *N. barkeri* to *T. confusus* larva and adult density and preference of these two life stages for feeding imply that *N. barkeri* is capable to quickly decline the possibility of current fruit damage due to prey feeding or pathogen infection and to potentially prevent the prey population build-up later in the season. Second, although a Type II functional response of *N. barkeri* detected on eggs and quiescent larvae, the positive relationship between the number of prey fed and prey density implies that augmentative release of *N. barkeri* early in the season when the prey density is low may also suppress the pest population. The variance of functional response type of *N. barkeri* on different life stages of *T. confusus* may attribute to the predators' prey stage preference behaviors. Our study provides insights into the biological control ecology of *N. barkeri*.

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