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Article

Functional response of *Amblyseius andersoni* and *Neoseiulus neoreticuloides* (Acari: Phytoseiidae) to adults of the wolfberry gall mite *Aceria pallida* (Acari: Eriophyoidae)

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Abstract

The gall mite, *Aceria pallida* Keifer (Acari: Eriophyoidae) is an economically important pest of wolfberry *Lycium barbarum* L. and can cause significant losses to plant production. Two species of phytoseiid predatory mites, *Amblyseius andersoni* Chant and *Neoseiulus neoreticuloides* Liang & Hu were found on *L. barbarum* in Bayan Nur city, Inner Mongolia, China. We assessed the potential of these two phytoseiid species as biological control agents against *A. pallida*, using functional response experiments with seven prey densities (5, 10, 20, 40, 60, 80 and 100 adults of *A. pallida*) on a wolfberry leaf under 25ºC ± 1ºC, 60% ± 5% RH and a 16:8 h (L:D) photoperiod. Overall, the predation of both species increased with increase in prey density. The consumption of *A. andersoni* female was significantly greater than that of conspecific males and *N. neoreticuloides* female at high prey densities. Both phytoseiid species displayed a type II functional response to *A. pallida*. Female *A. andersoni* had a higher attack rate (5.961) and a shorter handling time (0.014 d) than male *A. andersoni* (1.619; 0.019 d) and female *N. neoreticuloides* (0.719; 0.023 d). The maximum attack rate (T/T_h) was estimated to be 71.43 and 52.63 for female and male *A. andersoni*, respectively, while it was 43.48 for female *N. neoreticuloides*. Both female and male *A. andersoni* consistently consumed significantly more prey than *N. neoreticuloides* across all densities of *A. pallida* adults.

Keyword: biological control; eriophyoid mite; functional response; predatory mites; type II

Introduction

Wolfberry, *Lycium barbarum* L. is used as a medicinal plant and food in China. The gall mite, *Aceria pallida* Keifer (Eriophyoidae) is an economically important phytophagous mite associated with wolfberry (Rong & Wang 1983; Wu *et al*. 2017; Liu *et al*. 2019b), in the major wolfberry production areas of China (Kuang 1983; Rong & Wang 1983; Zhang *et al.* 2000; Wu *et al*. 2017). Like most eriophyoid mites (Keifer *et al*. 1982; Westphal & Manson 1996), *A. pallida* caused the gall formation on the plant and other abnormalities resulting in loss of plant growth (Wu *et al*. 2017). *Aceria pallida* is mainly controlled using acaricide applications (Xu *et al*. 2014). However, *A. pallida* completes most of its development in galls, and this protects it from acaricide treatments (Xu & Duan 2005). Biological control, using predatory mites that mainly feed on tetranychid mites and eriophyoid mites (McMurtry & Croft 1997), can be a viable alternative to chemical control and can be used to control *A. pallida*.

Two species of predatory mites, *Amblyseius andersoni* Chant and *Neoseiulus neoreticuloides* Liang & Hu were found on wild Chinese wolfberry, *L. barbarum*, in Inner Mongolia (Liu *et al*. 2019a). *Amblyseius andersoni* is a type III generalist predator that feeds on a variety of prey and non-

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prey foods (Zhang & Sanderson 1993; Koveos and Broufas 2000; Duso *et al*. 2011; Lorenzon *et al*. 2012; Li *et al*. 2019; McMurtry *et al*. 2013), and it is used in the biological control of spider mites (Tetranychidae) in orchards (Markó *et al*. 2012; Szabó & Pénzes 2013). *Neoseiulus neoreticuloides* was initially described on elms in China (Liang & Hu 1988), but no further studies have been conducted on this species. However, several species of the genus *Neoseiulus* are known for their potential as biological control agents of eriophyoid mites (McMurtry & Croft 1997). One example is *Neoseiulus baraki* Athias-Henriot that controls *Aceria guerreronis* Keifer (Lima *et al*. 2015).

Thus, it is worthy of interest and useful to be considered in the general scenario of control of *A. pallida*, one of the most serious pests on wolfberry, by exploring effective predatory mites that were found to co-occur with this eriophyid species. This study evaluated the predatory potential of two phytoseiid species—*A. andersoni* and *N. neoreticuloides*—on the gall mite *A. pallida*, basing functional response experiments.

Functional response (FS) experiments are used to evaluate the effectiveness of a predator for controlling a particular prey species (Xiao & Fadamiro 2010). It describes the relationship between consumption rate of an individual predator and different prey density (Farazmand *et al*. 2012), and can be classified into three types (Holling 1959): Type I FS (the number of prey consumed increases linearly to a maximum, then remains constant as prey density increases), Type II FS (the prey consumption increases as prey density increases but at a decelerating rate towards an asymptote), and Type III FS (the number of prey eaten approaches an asymptote as a sigmoid function).

Materials and methods

Predator source and rearing

Colonies of *A. andersoni* and *N. neoreticuloides* were initiated from specimens collected in September 2017 on a Chinese wolfberry plant at Bayan Nur city (40º28′N–108°11′E), Inner Mongolia, China. Both predator species were maintained with all stages of the fruit mite, *Carpoglyphus lactis* L. on refined sugar and yeast. The rearing unit consisted of a piece of plastic film spread on a water-saturated foam, which sat in a 30×20×10 cm plastic box half-filled with water to prevent mites from escaping. All rearing units were maintained under laboratory conditions (25ºC ± 1ºC, 60% ± 5% RH and a 16:8 h (L:D) photoperiod) since they were collected in September 2017.

Prey culture

The colony of *A. pallida* was obtained from Chinese wolfberry at the same location of predatory mite collection. Wolfberry branches with mite galls were collected and stored at 4°C. The mites within the galls were directly used in the following experiments. *Aceria pallida* are very small (the adult is 200–240 μm) (Kuang 1983), and the juvenile stage is difficult to distinguish under stereoscope. Thus, only adults of *A. pallida*, which are distinguishable by yellow-orange colour, were used in the experiments. These were collected from galls with diameters of 2–4 mm as described in Wu *et al*. (2017).

Experimental arenas

The experimental arena used was modified from Munger cells (Nguyen *et al*. 2013). From top to bottom, the cells were successively made up of a transparent acrylic board (top board; 20×35 mm, 2-mm thick), another transparent acrylic board (middle board; 20×35 mm, 2-mm thick) with a 10-mm diameter hole in the centre, a wolfberry leaf (20× 35 mm), a piece of wet filter paper (20× 35 mm) and another transparent acrylic board (bottom board; 20×35 mm, 2-mm thick) with a 10-mm diameter hole in the centre. The top board, middle board, wolfberry leaf and bottom board were

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tightly clamped together with two metal clips to form an enclosed cell. The filter paper enabled the leaf to remain fresh during the experiment. The cells were placed on a PVC tray covered with wet gauze.

Functional response

The predatory mites were shifted from the stock colony to a similar rearing unit and fed with all stages *A. pallida* for three successive generations (30 days) before being used in the experiments. Three-day-old males and gravid females of *A. andersoni*, and three-day-old gravid females of *N. neoreticuloides* were randomly selected from the cultures and individually transferred into the experimental cells. The reason for the omission of male *N. neoreticuloides* was that it had lower consumption for *A. pallida* adults and the data couldn't fit equation models to discriminate the type of functional response (unpublished data). The predatory mites were starved for 24 h before being used in the experiments. Female mites who had oviposited during starvation were placed singly in the new cell. Adults of *A. pallida* were offered as prey to male and female adults. Each cell was randomly subjected to one of the seven densities of *A. palida* (5, 10, 20, 40, 60, 80 and 100 adults), which were transferred from the galls into the cells with a fine camel-hair brush. Then females/males of *A. andersoni* or *N. neoreticuloides* were allowed to feed for 24 h, and the number of prey eaten was recorded. Each density treatment was replicated 10 times.

Statistical analysis

To discriminate between type II and type III functional responses, a polynomial logistic regression was performed between the proportion of prey consumed (N_e/N_0) and initial prey density (N_0) (Juliano 2001; Timms *et al*. 2008), using SigmaPlot 12.5 software (SigmaPlot 2013).

$$N_e/N_0 = a + bN_0 + cN_0^2 + dN_0^3 + e, \qquad (1)$$

where N_e = number of prey consumed, N_0 = initial number of prey, a = intercept, b = linear, c = quadratic and d = cubic coefficient. From the equations obtained for the proportions of prey consumed, the linear coefficients (b) and the quadratic coefficient (c) were observed, thus allowing determination of the type of functional response. If $b < 0$, the functional response is type II, if $b > 0$ and $c < 0$, the functional response is type III. The handling time and attack rate were estimated using non-linear least squares regressions from PROC NLIN of SAS (SAS Institute 2002). Since prey items were not replaced during the experimental period, the random predator equation of Rogers was appropriate to describe the type II functional response parameters (2) (Rogers 1972; Mendes *et al*. 2018):

$$N_a = N_0\{1-\exp[\alpha(T_h N_a - T)]\}, \qquad (2)$$

where α = attack rate, T_h = handling time and T = experimental time. The values of α and T_h were compared between two predators using the 95% confidence intervals. Two-way ANOVA followed by Tukey's multiple comparisons test was applied to compare the consumption of predators among prey densities within and between predator species.

Results

Predation of A. andersoni *and* N. neoreticuloides

The predation of the two predators increased significantly with increasing prey density (female *A. andersoni*: $P < 0.0001$; male *A. andersoni*: $P < 0.0001$; female *N. neoreticuloides*: $P < 0.0001$).

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Female *A. andersoni* consumed significantly more prey than male *A. andersoni* and female *N. neoreticuloides* ($F_{12, 189}$ = 257.8, *P* <0.0001). The interaction between predator sex for *A. andersoni* and gall mite density was also significant for the number of prey eaten ($F_{6,126}$ = 232.6, *P* <0.0001). The maximum number of prey killed was 57.6, 33.5 and 23.7 for female and male *A. andersoni*, and female *N. neoreticuloides*, respectively (Table 1).

TABLE 1. Consumption (mean ± SE) of *A. pallida* adults by *A. andersoni* and *N. neoreticuloides* at different prey densities

Prey density	*A. andersoni*		*N. neoreticuloides*
	Female	Male	Female
5	4.80±0.13aG	4.80±0.13aE	4.70±0.15aF
10	9.30±0.21aF	8.50±0.22aD	8.30±0.26aD
20	18.30±0.40aD	14.70±0.47bC	11.10±0.38bC
40	35.80±0.51aC	28.80±0.81bB	15.30±0.47bB
60	52.00±0.47aB	32.90±0.50bA	22.20±0.57bA
80	57.10±0.62aA	33.40±0.60bA	23.30±0.63bA
100	57.60±0.69aA	33.60±0.58bA	23.70±0.65bA

Means followed by different lower-case letters within a row are significantly different; the means followed by different upper-case letters within a column are significantly different (Two-way ANOVA, Tukey's test, *p*<0.05).

Functional response of A. andersoni *and* N. neoreticuloides *to adult* A. pallida

Both phytoseiid species displayed a Type II functional response to densities of *A. pallida* adults (b<0) (Figure 1; Table 2). The attack rate (α) of female *A. andersoni* was 3-fold higher and the handling time (T_h) was 0.75-fold lower than that of males (Table 3). Female *A. andersoni* had an attack rate 8-fold higher and a handling time shorter than female *N. neoreticuloides* (Table 3). The maximum attack rate (T/T_h) was estimated to be 71.43 for female *A. andersoni*, 52.63 for male *A. andersoni* and 43.48 for female *N. neoreticuloides*.

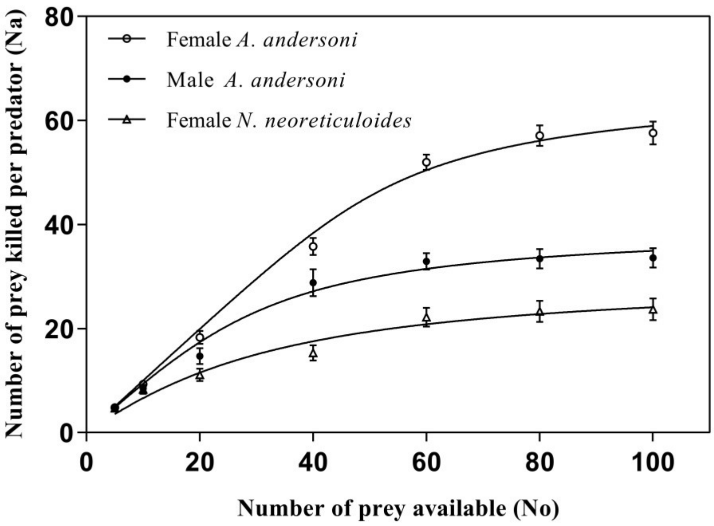

FIGURE 1. Functional response of *A. andersoni* and *N. neoreticuloides* fed on *A. pallida* adults. Points represent the observed number of prey consumed at each initial prey density.

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TABLE 2. Estimates of coefficients in a binomial logistic regression of the proportion of *A. pallida* adults consumed by *A. andersoni* and *N. neoreticuloides* as a function of initial prey density.

Predator Species	Type	Parameters	Estimate	SE	t	*P* value
Female *A. andersoni*	II	Intercept(*a*)	0.9617	0.0176	54.77	<0.0001
		Linear (*b*)	-0.0022	0.0018	-1.24	0.2190
		Quadratic(*c*)	3.29E-05	4.03E-05	0.81	0.4175
		Cubic (*d*)	-5.03E-07	2.52E-07	-1.20	0.0499
Male *A. andersoni*	II	Intercept (*a*)	0.9681	0.0279	34.66	<0.0001
		Linear (*b*)	-0.0101	0.0028	-3.61	0.0006
		Quadratic(*c*)	7.59E-05	6.42E-05	1.18	0.2410
		Cubic (*d*)	-3.91E-07	4.01E-07	-0.98	0.3330
Female *N. neoreticuloides*	II	Intercept (*a*)	1.1062	0.0252	43.86	<0.0001
		Linear (*b*)	-0.0353	0.0025	-13.98	<0.0001
		Quadratic(*c*)	0.0005	5.79E-0.5	9.30	<0.0001
		Cubic (*d*)	-2.73E-06	3.62E-0.7	7.54	<0.0001

TABLE 3. Attack rate and handling time (days) (means ± SE) for *A. andersoni* and *N. neoreticuloides* feeding on *A. pallida* adults.

Predator species	Attack rate (a) (Asymptotic 95% CI)	Handling time (T_h) (Asymptotic 95% CI)	Maximum attack rate (T/Th)	P > F
Female *A. andersoni*	5.961±0.819 (4.328–7.595)	0.014±0.0004 (0.014–0.015)	71.43	<0.0001
Male *A. andersoni*	1.619±0.297 (1.026–2.212)	0.019±0.002 (0.016–0.024)	52.63	<0.0001
Female *N. neoreticuloides*	0.719±0.090 (0.539–0.899)	0.023±0.003 (0.018–0.028)	43.48	<0.0001

Discussion

This is the first study of the functional responses of the predatory mites *A. andersoni* and *N. neoreticuloides* to *A. pallida* density. *Amblyseius andersoni* consumed more prey than *N. neoreticuloides* did across all prey densities. Our results suggest that *A. andersoni* have an effective predatory potential for the control *A. pallida* on wolfberry plants in China.

The two phytoseiid species displayed a Type II functional response to *A. pallida*, similar to that of *A. andersoni* when fed on *Panonychus ulmi* Koch (Tetranychidae) (Koveos & Broufas 2000). A type II functional response is common in phytoseiid mites (Afshar & Latifi 2017; Alfaia *et al*. 2018; Barbosa *et al.* 2019). Phytoseiid predators with a type II functional response, such as *Neoseiulus californicus* McGregor, *Neoseiulus cucumeris* Oudemans, *Neoseiulus barkeri* Hughes and *Amblyseius swirskii* Athias-Henriot, were proved to be efficient for control of pest organisms, especially at low prey densities (Koehler 1999; Jafari *et al.* 2012; van Lenteren 2012; Calvo *et al*. 2015; Song *et al*. 2016; Patel and Zhang 2017; Akyazi & Liburd 2019; Bazgir *et al*. 2020). The attack rate and handling time determine the magnitude of the functional response (Pervez & Omkar 2005). Female *A. andersoni* response to *A. pallida* included a higher attack rate (5.961) and a shorter handling time (0.014 d) than other phytoseiid biocontrol agents, such as *A. swirskii* to *Eotetranychus frosti* McGregor eggs (0.1142, 0.4858h) (Bazgir *et al.* 2020), and *Neoseiulus womersleyi* Schicha to *Tetranychus urticae* Koch eggs at 25℃ (5.467, 0.056 d) (Sugawara *et al.* 2018). The maximum attack rate representing predation capacity is obtained by dividing the experiment time (1 day) on

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handling time (T/T_h) (Fathipour *et al.* 2017). As the handling time decreased, predation capacity during one day increased. Female *A. andersoni* had a higher maximum attack rate (57.6) than male *A. andersoni* (33.5), or female *N. neoreticuloides* (23.7).

Several factors can affect the functional response and predation rate, including the sex of the predator (Parajulee *et al*. 1994). Female *A. andersoni* consumed more prey and showed a higher attack rate and a shorter handling time than did conspecific males. A similar trend was observed for female and male *N. cucumeris* feeding on different stages of *Bemisia tabaci* Gennadius (Li *et al*. 2017). Functional responses can be influenced by physical characteristics of the host plant (Koveos & Broufas 2000; Ahn *et al*. 2010). Thus we conducted the experiments on leaves of wolfberry, the original habitat of predator and prey. To avoid the deviation of feeding experience (Castagnoli & Simoni 1999; Mendes *et al*. 2018), both phytoseiid species were uniformly fed with wolfberry mite galls for 30 days prior to bioassay. In addition, the type of functional response is not constant, which can be influenced by the alternative food and the stage of prey (Ganjisaffar & Perring 2015; Li *et al*. 2018; Fathipour *et al*. 2020). Due to the small size of *A. pallida* (Kuang 1983), only adult *A. pallida* were provided in the present study.

Nevertheless, it is difficult to replicate a natural environment by a simple experimental arena (O'Neil 1989). Therefore, further field studies will be necessary to determine the efficiency of each species of predator under more realistic conditions for offering a reference point by using native predatory mites as biocontrol agents against *A. pallida*.

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