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EFFECTIVENESS OF TWO PREDATORY MITE SPECIES (ACARI: PHYTOSEIIDAE) IN CONTROLLING *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE)

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the insect vector of huanglongbing (HLB), the most devastating citrus disease in the world. The objective of this study was to evaluate the efficacy of two species of commercially available predatory mites, *Neoseiulus cucumeris* (Oudemans) and *N. barkeri* (Hughes) (Acari: Phytoseiidae), against *D. citri*. The predatory mites were evaluated by (1) determining their prey stage preference under no-choice conditions by providing eggs, 1st and 2nd instar nymphs; (2) determining the functional and numerical responses of the predators to varying densities of eggs (from 2 to 32/leaf disk); and (3) evaluating the control effect of *N. cucumeris* on *D. citri* on caged trees of *Citrus reticulata* Blanco in the field, using 1 to 8 sachets (900 ± 100 adult mites per sachet) per tree. The results indicated that *N. cucumeris* consumed eggs of *D. citri* only, whereas *N. barkeri* consumed eggs and 1st instar nymphs. Both predators displayed a Type III functional response (sigmoid). A single female adult of *N. cucumeris* consumed significantly more *D. citri* eggs than *N. barkeri*. *N. cucumeris* could lay eggs when feeding exclusively on *D. citri* eggs. The field experiment showed that control effect on *D. citri* was significantly correlated with the number of *N. cucumeris* released. Two or more sachets released per tree could achieve significant control at an initial density of 30 adult *D. citri* (male: female = 1:1) per tree. Improvement of field control efficacy of *N. cucumeris* on *D. citri* is discussed.

Key Words: huanglongbing, *Neoseiulus cucumeris, Neoseiulus barkeri,* functional and numerical response, biological control

RESUMEN

El psillido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es el insecto vector de huanglongbing (HLB), la enfermedad más devastadora de los cítricos en el mundo. El objetivo de este estudio fue evaluar la eficacia de dos especies de ácaros depredadores disponibles comercialmente, *Neoseiulus cucumeris* (Oudemans) y *N. barkeri* (Hughes) (Acari: Phytoseiidae), contra *D. citri*. Los ácaros depredadores fueron evaluados mediante (1) la determinación de su preferencia por estadios de desarrollo de la presa en condiciones de no selección, al proveer huevos y ninfas de 1^er^ y 2^do^ instar; (2) al determinar las respuestas funcionales y numéricas de los depredadores a densidades variables de huevos (de 2 a 32); y (3) al evaluar el control ejercido por *N. cucumeris* sobre *D. citri* en árboles de *Citrus reticulata* Blanco en jaulas bajo condiciones de campo, liberando 1 a 8 bolsas con ácaros por árbol (900 ± 100 ácaros adultos por bolsa). Los resultados indicaron que *N. cucumeris* consume solamente huevos de *D. citri*, mientras que *N. barkeri* consume huevos y ninfas de 1^er^ instar. Los dos depredadores mostraron una respuesta funcional Tipo III (sigmoide). Las hembras adultas de *N. cucumeris* mostraron un mayor consumo de huevos de *D. citri* que *N. barkeri*. *N. cucumeris* puede ovipositar cuando se alimenta exclusivamente de huevos de *D. citri*. El experimento en campo mostró que el nivel de control esta significativamente correlacionado con el número de *N. cucumeris* liberados. La liberación de dos o más bolsas de ácaros por árbol pueden alcanzar un control significativo cuando la densidad inicial de *D. citri* es de 30 adultos (macho: hembra = 1: 1) por árbol. El incremento del control de *N. cucumeris* sobre *D. citri* en campo es discutido.

Palabras Clave: huanglongbing, *Neoseiulus cucumeris, Neoseiulus barkeri,* respuestas funcional y numérica, control biológico

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of huanglongbing (HLB) or citrus greening, which is a destructive citrus disease associated with the phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (Bové 2006). Currently, HLB is seriously impacting citrus production in nearly 40 countries, including the 3 countries that produced the most citrus, i.e., China (Fan et al. 2009), Brazil (Lopes et al. 2007) and the United States (Qureshi & Stansly 2009). Control of *D. citri* is a key step in HLB management (Chao et al. 1979). Integrated control based on natural enemies, bio-rational materials, chemicals, and cultural practices could be the most acceptable pest management tactics (Yang et al. 2006; Juan-Blasco et al. 2012). Natural enemies of *D. citri* include predators such as ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), spiders (Aranae), and hoverflies (Diptera: Syrphidae), parasitoids such as *Diaphorencyrtis aligarhensis* (Shafee, Alam & Agaral) (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), and certain entomopathogens (Aubert & Quilici 1984; Hoy & Nguyen 2001; Michaud 2002, 2004; Pluke et al. 2005; Yang et al. 2006; Qureshi & Stansly 2008, 2009). However, any single tactic use alone is unlikely to provide sufficient suppression of *D. citri* populations, so that more proactive and augmentative approaches to biological control should be developed (Juan-Blasco et al. 2012).

Both *D. citri* populations and the incidence of HLB were significantly lower in many citrus groves in Zhaoqing City, Guangdong Province, China, where *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) was released for *Panonychus citri* (McGregor) (Acari: Tetranychidae) control than in nearby groves where only synthetic pesticides were heavily used (Zhang et al. 2010; Ouyang et al. 2011). It was speculated that the predatory mites or other natural enemies might have kept *D. citri* population under control, which led to the low HLB incidence. Our observations in laboratory confirmed that *N. cucumeris* consumed *D. citri* eggs (Fig. 1).

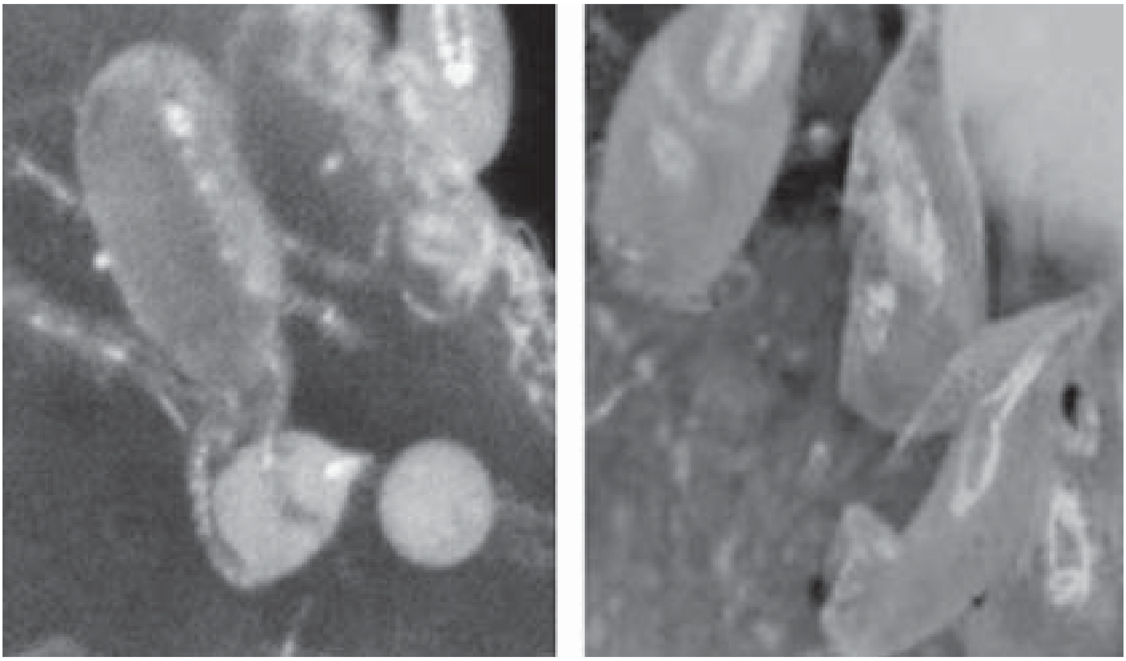

Fig. 1. Eggs of *Diaphorina citri* consumed by *Neoseiulis cucumeris* as seen by stereo microscope (Nikon®, SMZ1000) in the laboratory. The photo on the left shows the feeding of a *N. cucumeris* mite on *D. citri* eggs. The photo on the right shows the remains of *D. citri* eggs that have been consumed by *N. cucumeris.*

Juan-Blasco et al. (2012) found that the predatory mite, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), fed on both eggs and nymphs of *D. citri*, and showed promise for biological control against *D. citri* (Juan-Blasco et al. 2012); and they speculated that certain phytoseiid mites may serve as additional biological control agents of the psyllid.

Phytoseiid mites are among the most important predatory mites. They are widely used for biological control of mite and small insect pests of fruit, green vegetable, and other crops worldwide (Kostiainen & Hoy 1996; Opit et al. 2004; Arthurs et al. 2009; Juan-Blasco et al. 2012). *Neoseiulus cucumeris* and *N. barkeri* (Hughes) (Acari: Phytoseiidae) are both commercially available in China. *Neoseiulus cucumeris* was first introduced to China from England in 1997 (Zhang et al. 2002). *Neoseiulus barkeri*, a native predatory mite of China, occurs worldwide (De Moraes et al. 2004; Wu et al. 2009). Both species are mass-produced and widely used for controlling the citrus red mite *P. citri* in China (Zhang et al. 2002; Ouyang et al. 2007; Wei et al. 2013). These predators are also widely applied for controlling *Thrips tabaci* Lindman, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) and other small vegetable greenhouse pests in many countries (van Houten et al. 1995; Kostiainen & Hoy 1996; Shipp & Wang 2003; Hoy 2011).

Few studies have been conducted in using *N. cucumeris* and *N. barkeri* for *D. citri* control, although we observed that *N. cucumeris* could feed on *D. citri*. The goal of this study was to evaluate the potential of *N. cucumeris* and *N. barkeri* as biological control agents for control of *D. citri*. The objectives of this study were to, (1) determine prey preferences of the 2 predator species under no-choice conditions by providing eggs, 1st and 2nd instar nymphs, (2) estimate the functional and numerical responses of the predators to varying egg densities; and (3) evaluate the predation capacity of *N. cucumeris* in the field.

MATERIALS AND METHODS

Rearing of Predatory Mites and *Diaphorina citri*

The colonies of *N. cucumeris* and *N. barkeri* used in the laboratory experiments were initially obtained from the breeding laboratory of the Guangdong Academy of Agricultural Sciences. The populations had been reared on the flour mite, *Acarus siro* L. (Acari: Acaridae), on bran in our laboratory for 6 months before the experiments. Eggs and nymphs of *D. citri* were obtained from a population reared on *Murraya paniculata* L. (Sapindales: Rutaceae) in a greenhouse at the Guangdong Entomological Institute. The initial population had been collected from the campus of Sun Yat-sen University in 2009.

Prey Stage Preference

One female adult of *N. cucumeris* or *N. barkeri*, which was 8-day-old, was separated from the colony and transferred onto an arena with a fine-bristle brush. The arena was similar to that described by Overmeer (1981), consisting of a 7.0 cm diam white nanometer sponge placed in the center of a 9.0 cm diam, 1.2 cm deep Petri dish (Overmeer 1981). One 6.0 cm diam filter paper disc was placed on the sponge, and a smaller plastic cover placed on the filter paper. The foam mat was kept water-saturated using tap water to prevent mite escape. After the predatory mite had been starved for 24 h, 10 individuals of each developmental stage (eggs, 1st or 2nd instar nymphs of *D. citri*) on *M. paniculata* flushes were transferred onto the arena. A small, water-saturated sponge was used to keep the flushes fresh in order to prevent the *D. citri* individuals from dehydrating or starving. The Petri dish was covered to maintain humidity. The Petri dishes in the control treatment each had the same numbers of eggs, 1st or 2nd instar nymphs, but without a predatory mite. All Petri dishes were kept in a climate chamber for 24 h at 25 ± 2 °C, 75 ± 5% RH and 16:8 h L:D. After 24 h, the number of *D. citri* eggs and nymphs consumed were determined. Each treatment contained 30 replicates.

Functional and Numerical Responses

A mated female adult of *N. cucumeris* or *N. barkeri*, which was 8-day-old, was separated from the colony and transferred onto a leaf disc of *M. paniculata* on an arena as described above. After 24 h starvation, eggs of *D. citri* at densities of 2, 4, 8, 16, or 32 were transferred onto the leaf discs with a fine-bristle brush. Each treatment contained 30 replicates. The control treatment had the same densities of *D. citri*, except with no predatory mites on it. Every 24 h, the consumed eggs of *D. citri* were examined under a stereo-microscope (Nikon®, SMZ1000), all eggs of *D. citri* were replaced with fresh ones, and any newly laid eggs by predatory mites were removed from the examination arenas and counted to determine the numerical response. The test lasted 5 days. The experiments of *N. cucumeris* and *N. barkeri* were conducted simultaneously.

Field Experiment

The experimental population of *N. cucumeris* was obtained from Fujian Yanxuan Bio-Preventing and Controlling Technology Co., Ltd. The number of living adults of *N. cucumeris* per sachet (14 cm × 10 cm paper envelope) was 900 ± 100 determined by preliminary sample examination.

The experiment was conducted from Sep 2011 to Jan 2012 in Sihui City, Guangdong Province. The minimum, maximum, and mean daily temperature averaged 21-27 °C, 29-35 °C, and 24-32 °C in Sep; 19-23 °C, 24-31 °C, and 20-27 °C in Oct; 14-24 °C, 17-30 °C, and 19-26 °C in Nov; 4-19 °C, 13-25 °C, and 8-18 °C in Dec; 6-16 °C, 11-23 °C, and 8-13 °C in Jan, respectively. Relative humidity averaged 70-90% during the study period. The data were obtained from the local weather station.

A 4-yr-old *Citrus reticulata* Blanco grove was used for the experiment. The canopy of the tree was about 1.3 m high × 1.0 m diam. Each tree was covered with a nylon mesh cage (1.8 m high × 2.0 m diam) with a mesh size of 125 µm (Fig. 2) to prevent the predatory mites and psyllids from escaping. All arthropods inside the cages were eliminated by spraying 25 g/L deltamethrin emulsion 2000 × (Decis®, Bayer CropScience China Co., Ltd.) on 10 Sep using a knapsack pressure sprayer (5319-5L, Qiaojuan gardens facility Co. Ltd., Shanghai, China) maintained at 2.0×10^2 KPa during spraying. To ensure no arthropods were left inside the cages, a further application of 0.05% aqueous emulsion of horticulture mineral oil (Sunspray® Ultra Fine, Sunoco Inc, USA) was conducted on 20 Sep using the same sprayer. Additionally, manual removal of any observable remaining arthropods was conducted as necessary. To promote new flushes, fertilization and pruning were conducted on 15 Sep. Thirty adults of *D. citri* in a reproductive state (male: female = 1: 1) collected from a nearby grove were released into each cage when new flushes were beginning to occur on 30 Sep. *Neoseiulus cucumeris* at different densities were released into the cages on the same day. A total of 4 treatments (i.e,. 1, 2, 4 and 8 sachets per cage, respectively) were used in the experiment. The sachets were attached at the base of branches dispersedly. There was no *N. cucumeris* release in the control group. Each treatment included 3 replicates, 3 trees per replicate. A randomized complete design was used for allocating the blocks.

Fig. 2. Mesh cages used in the field experiment. Each cage contained one small (1.3 m tall) 4-yr-old *Citrus reticulate* tree. Thirty *Diaphorina citri* adults (1:1 sex ratio) together with various numbers of sachets of *Neoseiulus cucumeris* were released into each cage and changes in the *D. citri* population density over the subsequent 3 months were determined.

The number of adult psyllids on the entire tree in each cage was counted by field inspection on 7 Nov 2011, 7 Dec 2011, and 7 Jan 2012, respectively. It was observed that there were predatory mites, eggs and nymphs of *D. citri* in the new flushes, but their sizes were too small to survey on the entire tree directly. In order not to excessively disrupt the experiment, the numbers were not surveyed in this experiment.

Data Analysis

All data were analyzed using SPSS analysis software (13.0 for Windows; SPSS Inc, Chicago, USA). Data for prey stage preference were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at 5% level of significance.

The type of functional response of each predatory mite was determined using a curve estimation regression analysis of the proportion consumed as a function of prey density. The equation $N_e/N_o = a + bN_o + cN_o^2 + dN_o^3 + e$ was used to determine the type of functional response where N_e is the proportion of prey consumed, N_o is the number of prey offered, a is the intercept, b, c, and d are the linear, quadratic, and cubic coefficients, respectively. Type I responses were described by an intercept and a constant positive slope b. For Type II responses, the linear coefficient b was < 0. The proportion of consumed prey consumed declined linearly with the initial number of prey offered. Type III responses were characterized by a linear coefficient $b > 0$ and a quadratic coefficient $c < 0$ (Trexler et al. 1988; Juliano 2001; Xiao & Fadamiro 2010).

In the second step of the analysis, a nonlinear regression was used to estimate the parameters that fit the data to a mechanistic model. The model incorporating prey depletion for a type III functional response was expressed as follows:

$$N_e = N_o\{1 - \exp[(d + bN_o)(T_h N_e - T)/(1 + cN_o)]\}$$

where N_o is the initial density, N_e is the number of prey attacked, b, c, and d are the constants related to the attack coefficient, T_h is the handling time per prey, T is the total time available (24 h), which accounts for Type III functional responses with prey depletion (Rogers 1972; Juliano 2001).

The above equation was reduced to the following expression (Juliano 2001):

$$N_e = N_o\{1 - \exp[bN_o(T_hN_e - T)]\}.$$

The parameters of functional response b (attack coefficient) and T_h (handling time) were estimated using nonlinear regression. To determine the difference of functional response between *N. cucumeris* and *N. barkeri*, consumptions under different densities of prey were analyzed by comparing means by the Paired-Samples t-test ($\alpha = 0.05$).

The numerical response was calculated using regression analysis with a hyperbolic model (Carrillo & Peña 2012). The hyperbolic model is described by the equation: $y = ax/(b + x)$ where y is the daily oviposition by *N. cucumeris* at various prey densities x, a is the maximum daily oviposition (i.e. plateau), and b is the prey density needed to achieve a half-maximal estimated response.

The population densities of adult *D. citri* under different treatments were analyzed by Tukey's HSD test at 5% level of significance. The correlation between population densities of adult *D. citri* and the number of *N. cucumeris* released was analyzed by Pearson's correlation.

Results

Prey Stage Preference

Under no-choice conditions, one *N. cucumeris* female adult consumed 1.70 ± 0.34 *D. citri* eggs, one *N. barkeri* female adult consumed 0.20 ± 0.11 eggs, and there was no prey mortality in the control treatment. There were significant differences between these 3 treatments ($F = 19.92$; df = 2, 87; $P < 0.05$) when eggs of *D. citri* were provided as the prey. *Neoseiulus cucumeris* did not consume any 1st instar nymphs, whereas *N. barkeri* consumed 0.80 ± 0.41 1st instar nymphs, and there was no prey mortality in the control treatment. There were no significant differences between these 3 treatments ($F = 3.85$; df = 2, 87; $P > 0.05$) when 1st instar nymphs were provided as prey. The experiment indicated that neither of the 2 predator species consumed any 2nd instar nymphs.

Functional and Numerical Responses

The number of *D. citri* eggs consumed by *N. cucumeris* per day increased from 0.40 ± 0.15 at a prey density of 2 eggs per arena to 3.60 ± 1.28 at a prey density of 32 eggs per arena. The number of *D. citri* eggs consumed by *N. barkeri* per day increased from 0 at a prey density of 2 eggs per arena to 0.80 ± 0.29 at a prey density of 32 eggs per arena. The results of the nonlinear regression analysis (Table 1) indicated that both predators exhibited Type III functional response to *D. citri* eggs (Fig. 3).

The parameters were estimated according to the Holling III functional response model (Table 2). The functional curves of the 2 predator species were significantly different when *D. citri* eggs were provided ($t = 3.70$; df = 4; $P < 0.05$). *Neoseiulis cucumeris* generally consumed more eggs of *D. citri* than *N. barkeri*.

The number of eggs laid by each *N. cucumeris* female per day (y) and the prey density (x) were highly correlated ($R^2 = 0.99$, $P = 0.003$; Fig. 4). The maximum daily oviposition (a) of *N. cucumeris* was 0.31 ± 0.01 eggs. The prey density needed to achieve half the maximum response (b) was 4.11 ± 0.40 eggs per arena.

Field Experiment

The results indicated that the number of *D. citri* adults in the control treatment was significantly higher than in the treatments with *N. cucumeris* in the 1st, 2nd and 3rd month evaluations after treatment, respectively ($F = 27.64$, df = 4, 10, $P < 0.05$; $F = 63.53$, df = 4, 10, $P < 0.05$; $F = 56.04$, df = 4, 10, $P < 0.05$) (Table 3), although the numbers of *D. citri* adults increased in all treatments. The population densities of *D. citri*

Table 1. Coefficients of the polynomial nonlinear regression of predation proportion for *Neoseiulus cucumeris* and *Neoseiulus barkeri* against *Diaphorina citri* eggs under laboratory conditions.

Species	Parameter	Estimate	SE	t	P
N. cucumeris	Intercept	0.1327	0.0079	16.8865	0.0377
	Linear (b)	0.0411	0.0028	14.8380	0.0428
	Quadratic (c)	-0.0034	0.0002	-15.3032	0.0416
	Cubic (d)	0.00007	0.000005	14.4929	0.0439
N. barkeri	Intercept	-0.0342	0.0005	-75.4716	0.0084
	Linear (b)	0.0198	0.0002	123.8187	0.0051
	Quadratic (c)	-0.0014	0.00001	-105.2318	0.0060
	Cubic (d)	0.00002	0.0000003	94.5189	0.0067

Note: The regression equation is as follows: $N_e/N_o = a + bN_o + cN_o^2 + dN_o^3 + e$.

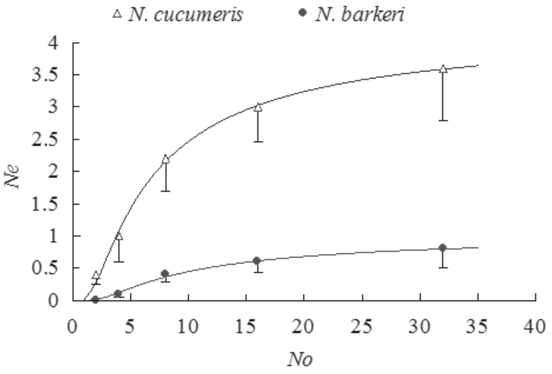

Fig. 3. Functional response of *Neoseiulis cucumeris* adult females (upper curve) and of *N. cucumeris* adult females (lower curve) to *Diaphorina citri* eggs on leaf discs. N_o = the density of *D. citri* eggs. N_e = the number of *D. citri* consumed per *N. cucumeris* female or *N. barkeri* female per day.

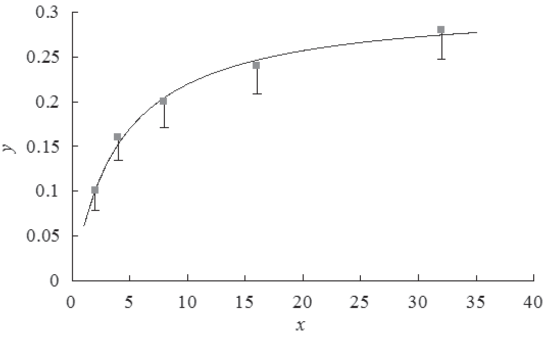

Fig. 4. Numerical response of *Neoseiulis cucumeris* adult females to *Diaphorina citri* eggs on leaf discs, where. x = the density of *D. citri* eggs per arena, and y = the number of eggs laid by a *N. cucumeris* adult female per day.

adults in each surveyed month were significantly correlated with the number of predatory mites released (Table 4).

DISCUSSION

The results from this study demonstrated the potential value of using *N. cucumeris* in a *D. citri* integrated pest management program. Both *N. cucumeris* and *N. barkeri* consumed *D. citri* eggs. Additionally, *N. barkeri* also consumed small numbers of 1st instar nymphs. Consumption of *D. citri* eggs by *N. cucumeris* was significantly greater than that by *N. barkeri*. We observed that *N. cucumeris* readily located the provided eggs. Immediately after crawling on the eggs, *N. cucumeris* predators began to feed on them. *Neoseiulis barkeri* mites tended to search near the edge of the arena and ignored nearby eggs. The prey stage preference of *N. cucumeris* in this study was different from previous studies in which *N. cucumeris* preferred 1st instars over eggs of *Tetranychus urticae* Koch or *F. occidentalis* (Shipp & Whitfield 1991; Croft et al. 2004). Different nutritional benefits of each prey (Popov & Kondryakov 2008; Blackwood et al. 2001), morphological traits such as long dorsal shield setae (Croft et al. 2004) and the size and activity of each predatory species (Sabelis 1985) were reported to play roles in prey stage preferences of predatory mites. Nevertheless, since both *D. citri* nymphs and adults can acquire and transmit the HLB pathogen, preference by the predator for the egg stage of *D. citri* should be considered desirable for HLB management because the elimination of *D. citri* eggs reduces the probability of disease transmission.

TABLE 2. ATTACK COEFFICIENT (*B*), AND HANDLING TIME (T_H) (95% CL) OF *NEOSEIULUS CUCUMERIS* AND *NEOSEIULUS BARKERI* AT DIFFERENT *DIAPHORINA CITRI* EGG DENSITIES ESTIMATED USING THE FUNCTIONAL RESPONSE MODEL

Species	b	T_h	R^2
N. cucumeris	0.071 ± 0.010	0.263 ± 0.003	0.931
N. barkeri	0.008 ± 0.001	1.129 ± 0.023	0.958

Note. The Type III functional response is determined by solving the following equation:

$N_e = N_o\{1 - \exp[bN_o(T_hN_e - T)]\}$.

Solomon (1949) divided the response of consumers (predators) to the density of their resources (prey) into functional and numerical responses. The former response type describes how the consumption rate of individual consumers changes with resource density, and the latter response type shows how the per capita reproductive rate changes with resource density (Solomon 1949). Functional and numerical responses have been widely used to evaluate the effectiveness of predatory insects and mites (Shipp & Whitfield 1991; De Clercq et al. 2000; Badii et al. 2004; Xiao & Fadamiro 2010; Carrillo & Peña 2012). The functional response of a predator as a function of prey density generally follows one of 3 mathematical models (Holling 1959a, 1959b, 1961). In the Type I functional response, the number of prey killed increases linearly at a constant rate as a function of prey density. In the Type II response, the number of prey killed increases up to a maximum (predator saturation). However, the proportion of dead prey killed declines with increasing prey density. In the Type III response, predation results in a sigmoid curve wherein the proportion of prey consumed is positively dependent on density over a prey density range. However, predator saturation occurs at high prey densities (Xiao & Fadamiro 2010). Predators with Type III response are capable of regulating prey populations (Holling 1965).

TABLE 3. POPULATION DENSITIES OF ADULT *DIAPHORINA CITRI* ON CAGED FLUSHING 4-YR-OLD *CITRUS RETICULATA* TREES ONTO EACH OF WHICH 30 *DIAPHORINA CITRI* ADULTS (1:1 SEX RATIO) WERE RELEASED TOGETHER WITH VARIOUS NUMBERS OF SACHETS OF *NEOSEIULUS CUCUMERIS*. EACH SACHET CONTAINED 900 ± 100 ADULT MITES.

	Density of Adult *D. citri* Population		
Number of Sachets with *N. cucumeris*	After 1 Month	After 2 Months	After 3 Months
0 (Control)	383.67 ± 31.55 a	271.67 ± 2.40 a	198.67 ± 5.67 a
1	313.00 ± 30.81 ab	213.33 ± 2.67 b	167.67 ± 4.63 a
2	229.00 ± 23.00 bc	162.33 ± 21.98 b	100.33 ± 12.86 b
4	175.33 ± 3.71 c	97.00 ± 7.21 c	86.33 ± 10.49 b
8	74.00 ± 11.06 d	52.67 ± 7.62 d	43.67 ± 4.84 c

Means followed by a different letter in the same column indicates a significant difference (Tukey's HSD, $P < 0.05$).

Predators with type II response are particularly efficient at low prey densities (Koehler 1999).

For both predatory mites, the relationship between *D. citri* egg density and number of *D. citri* consumed indicated a Type III functional response (sigmoid) model. Sigmoid functional responses indicate generalist predators. These predators readily switch from one food species to another or concentrate their feeding in areas where certain resources are most abundant (Holling 1959a). These responses are called prey-dependent responses because the feeding rate of consumers depends only on the prey density (Holling 1959a; Murdoch 1977). Type III functional responses are commonly regarded as efficient biological control agents (Holling 1965; Pervez & Omkar 2005; Laumann et al. 2008). Type III responses are less common than Type II responses (Mills & Lacan 2004). The functional response curves indicated that *N. cucumeris* consumed significantly more *D. citri* eggs than *N. barkeri*. *Neoseiulis cucumeris* showed greater potential for controlling *D. citri* than *N. barkeri.*

The result of field experiments indicated that control outcome on *D. citri* in the 1st month was significantly correlated with the number of *N. cucumeris* released. Two sachets or more of predatory mites released per tree achieved significant psyllid population reduction, compared to the untreated control. However, the psyllid populations increased under all treatments. This might be due to the high initial density of *D. citri* as well as the limited control efficacy of *N. cucumeris* against the psyllid. We speculate that the main factor affecting population fluctuation of *D. citri* adult in the 1st month was different from that in the following 3 months. The factor operating in the 1st month was likely correlated with the flushing peak of citrus and the oviposition peak of *D. citri*. Population fluctuation of *D. citri* adults mainly depended on the offspring of the initially released *D. citri* and was significantly affected by *N. cucumeris*. During the following months, the oviposition of *D. citri* was much less and the most likely factor was the influence of lower temperature (averaged 8-18 ºC in Dec 2011 and 8-13 ºC in Jan, 2012) and fewer new flushes. These months were a part of the over-wintering period of *D. citri* and the duration of the adult stage was relatively long in Guangdong. The population fluctuation of *D. citri* adults mainly depended on natural death, and not on the eggs consumed by *N. cucumeris*.

These results showed *N. cucumeris* could suppress the population of *D. citri* to a certain degree. Considering *N. cucumeris* can be easily and cheaply mass-reared (Zhang et al. 2002), this predatory mite might be applied as a biological control agent for control of *D. citri*. A further study is needed to determine how well *N. cucumeris* could control *D. citri* populations under actual field conditions in which *D. citri* is able to freely colonize the citrus trees.

Besides *D. citri,* the citrus red mite, *P. citri,* is another important pest of citrus in several regions of the world (Huang 2009). The population peak of *P. citri* usually occurs later than citrus

TABLE 4. CORRELATION BETWEEN THE POPULATION DENSITIES OF *DIAPHORINA CITRI* ADULTS AND THE NUMBER OF *NEOSEIULUS CUCUMERIS* MITES ON 4-YR-OLD *CITRUS RETICULATA* TREES SUBJECTED TO DIFFERENT *NEOSEIULUS CUCUMERIS* DENSITIES.

	Population Densities of Adult *D. citri* Treated with Different *N. cucumeris* Densities		
Pearson's Correlation	After 1 Month	After 2 Months	After 3 Months
Number of *N. cucumeris*	–0.921*	–0.922*	–0.895*

Correlation is significant by Pearson's correlation test at the 0.01 level (2-tailed, $n = 15$).

flushing, whereas the oviposition peak of *D. citri* is synchronous with flushing (Huang 2009). Hoy (2011) reported that build-up of predatory mite *Euseius tularensis* (Acari: Phytoseiidae) population on citrus red mites allowed the predator to achieve a better control of citrus thrips population later in the season. Presence of *P. citri* or pollen in the groves should be helpful in increasing the predatory mite reproductive rate and stabilizing the predator's populations. This might result in better control of *D. citri* by the predators.

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