Effect of *Zelus longipes* (Hemiptera: Reduviidae) on *Diaphorina citri* (Hemiptera: Liviidae) and Its Parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) under Controlled Conditions

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EFFECT OF ZELUS LONGIPES (HEMIPTERA: REDUVIIDAE) ON DIAPHORINA CITRI (HEMIPTERA: LIVIIDAE) AND ITS PARASITOID TAMARIXIA RADIATA (HYMENOPTERA: EULOPHIDAE) UNDER CONTROLLED CONDITIONS

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ABSTRACT

Studies were conducted under controlled laboratory conditions to evaluate the effect of the predator, Zelus longipes (Hemiptera: Reduviidae) on mortality of Diaphorina citri (Hemiptera: Liviidae) as well as its effect on the specialized parasitoid, Tamarixia radiata (Hymenoptera: Eulophidae). All tested life stages of Z. longipes consumed significantly more D. citri adults than nymphs. Zelus longipes nymphs were only effective as predators of D. citri adults at a predator density of 8 individuals (or specimens) per arena. The mortality of D. citri nymphs caused by Z. longipes adult females was higher at the densities of 2 or more predators per arena. All densities of male and female adult Z. longipes resulted in mortality of nearly all the T. radiata adult parasitoids offered. The importance of intraguild predation and possible interference with T. radiata are discussed.

Key Words: citrus psyllid, intraguild predation, functional response, assassin bug, Murraya

The ubiquity and importance of intra-guild predation has been considered in numerous systems involving predators, parasitoids and pathogens (Lucas 2005). Predators and parasitoids, serving as intra-guild prey, may be attacked by other generalist predators, pathogens and parasitoids (Lucas 2005). For instance, Rosenheim et al. (1993) concluded that in aphidophagous systems, the addition of generalist predators such as Zelus renardi Kolenati (Hemiptera: Reduviidae) and Nabis spp. (Hemiptera: Nabidae) generated sufficient mortality of the predator Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) to negatively affect population regulation of the in-
tended prey, *Aphis gossypii* (Glover) (Hemiptera: Aphididae).

The psyllidophagous system of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) in Florida’s cultivated Rutaceae (i.e., *Citrus, Murraya*) may also demonstrate the phenomenon of intra-guild predation (Michaud 2004). Intra-guild predation on immature stages of the introduced parasitoid *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) in Florida citrus groves resulted in > 95% mortality of the parasitoid, thereby reducing the efficacy of this biological control agent against *D. citri*.

A component of this psyllidophagous system is the assassin bug, *Zelus longipes* (L.) (Hemiptera: Reduviidae: Harpactorinae), a generalist predator (Kalsi & Seal 2011) of *D. citri* (Peña et al. 2008). The abundance of *Z. longipes* on Rutaceous plants has been verified in the field. For example, *Z. longipes* was reported preying upon *D. citri* on citrus in central and central-east Florida (Michaud 2002; Hall et al. 2008) and in *Murraya exotica* L. (formerly *M. paniculata*) in south Florida (Peña et al. 2008). *Zelus longipes* was observed eating adults of *T. radiata* on an 80-m-long *M. exotica* hedge during observation periods from 10:00 till 11:00 and from 13:00 till 14:00 each day over a period of 4 consecutive weeks (Peña et al. 2008). Numbers of *Z. longipes* recorded did not differ during morning and afternoon hours. The mean density of *Z. longipes* throughout the study ranged from 7.8 to 24.3 per 30 min of observations while the number of *D. citri* adults /flush fluctuated between 0.15 and 4.95. Peña (unpubl. 2009) recorded 0 to 12 *D. citri* adults, 0 to 52 eggs, and 0 to 62 nymphs/flush in a 20-m-long *M. exotica* hedge. During the same study, he recorded from 1 to 66 *Z. longipes* during a 2-min walk conducted 3 times a day around a 10-m-long portion of the same *M. exotica* hedge. Additionally, *Z. longipes* activity was surveyed every other day during 4 weeks for 1 h at 8:00, 11:00 and 16:00 during the spring of 2011 (AYRM, unpubl. data). A total of 1677 individuals were observed, resting or preying on *D. citri*, but also preying on other natural enemies such as the parasitoid *T. radiata* and the predator *Hippodamia convergens* Guérin-Méneville (Coccinellidae). During surveys on *M. exotica* adult activity and density in the spring and summer of 2012, *Zelus longipes* was recorded as the most frequent predator, and was observed feeding on *D. citri* and also on coccinellids, tachinid flies, and small wasps, such as *T. radiata* (BN, unpubl. data).

Clearly there are abundant anecdotal observations to indicate that *Z. longipes* is an abundant predator with the potential to reduce *D. citri* population suppression through intra-guild predation. To supplement those observations, here we present studies conducted under controlled conditions to evaluate the possible effect of *Z. longipes* on *D. citri* and on the specialized parasitoid, *T. radiata*.

**Materials and Methods**

Plant Material and Experimental Arenas

*Murraya exotica* (formerly known as *M. paniculata*), which is grown as an ornamental hedge in Florida, and is a host of *D. citri* was used in all experiments. *Murraya exotica* plants were grown in 3.8-L plastic pots with potting media (pine bark, 60% + Canadian sphagnum peat and vermiculite). The plants were placed inside 91.4 × 61 cm screen cages (mesh size 1 mm²) and kept inside a greenhouse to avoid unwanted arthropod infestations. Experimental arenas were constructed by cutting *M. exotica* flushes free of any damage, leaving a 5-cm stem to hold the cutting firmly upright when formed into bouquets with exposed leaves. Bouquets were formed by inserting the stems into a 26-mL vial (polystyrene, measuring 26 × 51 mm with snap-on caps, Fisherbrand® cat. # 03-338-3c) filled with 20 mL water and a small hole in the cap. The bouquets were placed inside wide-mouth mason jars (Ball®, 946 cm² volume) with a fine screen (0.25 mm²) mesh lid and a piece of wet cotton inside to provide moisture. All experiments were conducted in an insectary at the Tropical Research and Education Center (TREC), in Homestead, Florida under 25 ± 2 °C, 12:12 h L:D and 65 ± 2% RH, unless stated otherwise.

Insect Colonies

A 30 m long × 1 m tall unsprayed *M. exotica* hedge located at TREC that had a long term infestation with *D. citri* was the source of insects. Adult *D. citri* were collected with an aspirator and nymphs were collected by cutting infested flushes.

*Zelus longipes* adults of both sexes were collected from the *M. exotica* hedge with a paintbrush and placed inside plexiglass cages (30 × 30 × 30 cm) with 200 cm² of corrugated cardboard that served as oviposition substrate. Honey was supplied on 2 pieces of paper (5 × 5 cm) affixed to the cage walls. Water was provided in a clear plastic container, with a cotton roll inserted through the container lid to serve as a wick. After 2 days of oviposition the corrugated cardboard with the eggs was transferred to a new cage. *Zelus longipes* first instars emerged approximately15 days later. These nymphs were fed daily with live *Drosophila* spp. (Diptera: Drosophilidae) adults (1-3 flies per individual) until the 4th instar. Fourth instar *Z. longipes* were fed daily with adults of *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) (1-3 flies per individual), which were supplied by the Division of Plant Industry, Biocontrol Rearing Facility, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. *Drosophila* spp. was reared using ripe banana fruits kept in

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1-L plastic containers and flies were transferred to the Z. longipes cages using an aspirator. In this rearing system, Z. longipes adults were obtained 45 to 60 days after the emergence of the 1st instars. All predators were deprived of food for 24 h prior to initiating the experiments.

Tamarixia radiata were collected on the M. exotica hedge and then placed with M. exotica flushed infested with D. citri 4th and 5th instars. Psyllid infested flushes were formed into large bouquets inserted in polystyrene containers (26 x 51 mm, Fisherbrand®) and then placed inside a plexiglas cage (30 x 30 x 30 cm). Then, 20 T. radiata adults were introduced to the cages for 4 days. After the oviposition period, flushes with presumably parasitized D. citri nymphs were transferred to emergence cages where parasitoids began to emerge approximately 12-15 days later. Honey and water were provided in the same way as for Z. longipes. This procedure was repeated every 10 days.

Susceptibility of Different Psyllid Life Stages to Predation by Zelus longipes

Experimental arenas (glass mason jars with volume of 946 cm$^3$) were prepared with either 2 M. exotica flushes and 20 adults of D. citri (presumably mated, 1:1 ϕ:♂) or 1 flush of M. exotica and 20 D. citri 5th instars. Sets of 1, 2, 4 or 8 1-d-old unfed Z. longipes adults (♂ and ♀ tested separately), or 1st instars of Z. longipes were introduced into the arenas. Experimental arenas with D. citri but no predators were used as a control. Twenty four h after introducing the predators, the flushes were inspected under a stereomicroscope and the number of D. citri eggs, dead D. citri and Z. longipes per flush were counted. Each predator-stage density was replicated 5 times.

Susceptibility of Tamarixia radiata Adults to Predation by Zelus longipes

Experimental arenas were prepared with 2 M. exotica flushes and 20 1-d-old T. radiata adults (1:1 ϕ:♂). Sets of 1, 2, 4 or 8 1-d-old unfed Z. longipes adults (♂ or ♀), or 1, 2, 4, 8, or 12 1st instars of Z. longipes were introduced into the arenas. Twenty four h later, the number of live and dead adults of both predator and prey was counted. The untreated control was set up in the same way but contained no Z. longipes. The response variables were the number of dead T. radiata adults and number of surviving Z. longipes. Each predator density was replicated 5 times.

Statistical Analysis

The response variables were analyzed using analysis of variance performed with PROC GLM (SAS 2001). Tukey’s test was used to determine significant differences between means ($P < 0.05$). The number of D. citri eggs was not normally distributed (PROC GPLOT, SAS 2001) so a $\sqrt{(x+1)}$ transformation was used to normalize the data.

Functional Response of Zelus longipes to Diaphorina citri Adults

Six densities of D. citri adults (presumably mated, 1:1 ϕ:♂) (4, 8, 12, 16, 20, and 24 individuals per arena) were offered to a single 1-d-old Z. longipes, presumably mated female adult. Both the prey and the predator were placed in the glass jar experimental arena with 1 flush of M. exotica. Twenty four h later, the numbers of live and dead adults of D. citri were counted. Each prey density was replicated 6 times. The untreated control for each density consisted of the prey without the predator. The type of functional response was determined using the polynomial logistic regression model (Juliano 2001) analyzed in SAS (SAS Institute 2001).

$$N_e = \frac{\exp(P_0 + P_1N_0 - P_2N_e + P_3N_e^2)}{1 + \exp(P_0 + P_1N_0 - P_2N_e + P_3N_e^2)}$$

Where $N_e$ is the number of prey eaten, $N_0$ is the initial number of prey, and $P_0, P_1, P_2$, and $P_3$ are parameters estimated in the model.

The handling time and attack constant were estimated using the NLIN procedure (Juliano 2001) with the Hassell equation as a model (Hassell 1978):

$$N_e = N_0[1 - \exp(-aTN_e) + bTN_e + aT^2N_e^2]$$

In this equation, $a$ is the attack constant, $b$ is a constant, $T_h$ is handling time and $T$ is total time available for Z. longipes to search for and attack the D. citri adults.

Prey Preference of Zelus longipes

Prey preferences of 1st instar nymphs, adult females or males of Z. longipes, were determined by choice tests. The prey species offered were T. radiata, D. citri, and A. suspensa adults. In the first choice test, one flush of M. exotica containing 15 adults of D. citri and 15 adults of T. radiata was introduced into the experimental arena followed by the introduction of a 1st instar, an adult male, or an adult female Z. longipes. The arena was sealed thereafter. Twenty four h later, the numbers of live and dead adults of D. citri and T. radiata were counted. Each experimental unit was replicated 15 times. The β index proposed by Manly et al. (1972) was used to quantify prey preference.
\[
\alpha = \frac{\ln(Ne)}{N} \quad \text{and} \quad \beta = \frac{1}{(1 + a)}
\]

Where \( N \) and \( N' \) are the numbers of each provided prey type and \( Ne \) and \( Ne' \) are the numbers of each prey type killed. The preference \( \beta \) index assigns values from 0 to 1, where 0.5 represents no preference. Mean \( \beta \) values were considered significant when 95% confidence intervals based on the \( t \)-distribution did not overlap with \( \beta = 0.5 \).

The same methodology was used for a separate experiment in which adult \( Z. \) longipes (male and female) had to choose between 15 adults of \( A. \) suspensa and 15 adults of \( D. \) citri.

**RESULTS**

Susceptibility of Different Psyllid Stages to Predation by \( Zelus \) longipes

All tested stages of \( Z. \) longipes consumed significantly more \( D. \) citri adults than nymphs (\( F = 77.05; \) df = 1, 149; \( P < 0.001 \)). In addition, adult female and male \( Z. \) longipes showed significantly higher predation rates on \( D. \) citri than did 1st instar \( Z. \) longipes (\( F = 11.68; \) df = 2, 147; \( P < 0.001 \)).

\( Zelus \) longipes adults at densities of 2 to 8 predators per arena caused significantly higher mortality (females: \( F = 117.58; \) df = 4, 20; \( P < 0.001 \); males: \( F = 28.77; \) df = 4, 20; \( P < 0.001 \)) of \( D. \) citri adults than did a single male or female (Table 1). However, the average number of prey consumed per adult predator decreased significantly when the number of predators in the arenas increased (females: \( F = 83.62; \) df = 4, 20; \( P < 0.001 \); males: \( F = 8.65; \) df = 4, 20; \( P < 0.001 \)) (Table 1).

\( Zelus \) longipes nymphs were only effective as predators of \( D. \) citri adults at a predator density of 8 per arena (\( F = 14.04; \) df = 4, 20; \( P < 0.001 \)). The number of prey consumed per \( Z. \) longipes nymph was higher when a single predator was released in the arena compared with 2, 4 and 8 predators per arena, which caused low mortality similar to that observed in the predator-free control (Table 1).

The mortality levels of \( D. \) citri nymphs caused by \( Z. \) longipes adult females were density dependent. Mortality was significantly higher at a predator density of 8 females/arena, followed by 4 or 2 females/arena, and 1 female/arena; the latter showed mortality rates similar to that observed in the predator-free control (Table 1).

Four to 8 \( Z. \) longipes males per arena caused the highest number of dead \( D. \) citri nymphs compared with the results obtained at lower predator densities or in the untreated control (\( F = 13.17; \) df

| Table 1. Mean Numbers (± SEM) of \( Diaphorina \) citri Adults and Nymphs Found Dead in Treatments with Different Densities of \( Zelus \) longipes Adult Females, Adult Males or 1st Instars. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( Zelus \) Stage | Predator density | \( D. \) citri prey | \( D. \) citri adults | \( D. \) citri nymphs |
|                 | dead | killed/predator | dead | killed/predator |
| Adult female    | 0     | 20            | 0.2 ± 0.2 c | 0.0 ± 0.0 d |
|                 | 1     | 20            | 14.8 ± 1.4 b | 14.8 ± 1.4 a |
|                 | 2     | 20            | 18.6 ± 0.7 a | 9.3 ± 0.3 b |
|                 | 4     | 20            | 18.0 ± 0.5 a | 4.5 ± 0.1 c |
|                 | 8     | 20            | 19.8 ± 0.2 a | 2.5 ± 0.02 cd |

Means followed by different letters within columns separated by lines differ significantly (Tukey's test, \( P < 0.005 \)).

\( Zelus \) longipes nymphs were only effective as predators of \( D. \) citri adults at a predator density of 8 per arena (\( F = 14.04; \) df = 4, 20; \( P < 0.001 \)). The number of prey consumed per \( Z. \) longipes nymph was higher when a single predator was released in the arena compared with 2, 4 and 8 predators per arena, which caused low mortality similar to that observed in the predator-free control (Table 1).

The mortality levels of \( D. \) citri nymphs caused by \( Z. \) longipes adult females were density dependent. Mortality was significantly higher at a predator density of 8 females/arena, followed by 4 or 2 females/arena, and 1 female/arena; the latter showed mortality rates similar to that observed in the predator-free control (Table 1).

Four to 8 \( Z. \) longipes males per arena caused the highest number of dead \( D. \) citri nymphs compared with the results obtained at lower predator densities or in the untreated control (\( F = 13.17; \) df

Means followed by different letters within columns separated by lines differ significantly (Tukey's test, \( P < 0.005 \)).
The average number of \textit{D. citri} nymphs consumed per \textit{Z. longipes} male was significantly higher than the control when 2 predators were released per arena \((F = 4.13; \text{df} = 4, 20; P < 0.01)\) (Table 1).

Compared to the control, only the treatment with 8 \textit{Z. longipes} nymphs could reduce significantly the number of \textit{D. citri} nymphs \((F = 3.82; \text{df} = 4, 20; P < 0.01)\). The number of prey consumed per \textit{Z. longipes} nymph was density dependent. The average number of \textit{D. citri} nymphs consumed per \textit{Z. longipes} nymph was significantly higher when 1 predator was released per arena and it decreased as the number of predators increased \((F = 18.83; \text{df} = 4, 20; P < 0.01)\) (Table 1).

**Susceptibility of \textit{Tamarixia radiata} Adults to Predation by \textit{Zelus longipes}**

All densities of male \((F = 93.98; \text{df} = 4, 20; P < 0.001)\) and female \((F = 318.71; \text{df} = 4, 20; P < 0.001)\) \textit{Z. longipes} adults resulted in approximately 90\% mortality of the parasitoids offered compared to the untreated control (< 1\%). Mortality of the adult parasitoids increased with increasing densities of \textit{Z. longipes} nymphs. Densities as low as 2 nymphs per arena significantly increased \textit{T. radiata} mortality relative to natural mortality (control) \((F = 28.77; \text{df} = 4, 20; P < 0.001)\) (Fig. 1).

**Functional Response of \textit{Zelus longipes} to \textit{Diaphorina citri} Adults**

The regression analysis resulted in the following coefficient estimates: intercept \(a = -1.1742 \pm 1.57\) (SE), \(r^2 = 0.56, P = 0.4545\); linear \(b = 0.8682 \pm 0.4106\) (SE), \(r^2 = 0.447, P = 0.003\); quadratic \(c = -0.0621 \pm 0.0304\) (SE), \(r^2 = 3.45, P = 0.0041\) and cubic \(d = 0.00125 \pm 0.00067\) (SE), \(r^2 = 3.45, P = 0.0041\). The linear coefficient \(b\) was > 0 indicating that adult female \textit{Z. longipes} showed a Type III functional response (Juliano 2001). The model produced the following values for the parameters of the Hassel equation: The handling time (Th) was 1.15 ± 0.08 h and the attack rate constant \(a\) was 0.017 ± 0.004 (Fig. 2).

**Prey Preference of \textit{Z. longipes}**

First instar \textit{Z. longipes} nymphs expressed a significant preference for \textit{T. radiata} adults (95\% confidence intervals did not include \(\beta = 0.5\)) over \textit{D. citri} adults. Neither male nor female adults of \textit{Z. longipes} showed a preference for one prey type over the other (Fig. 4). In the second experiment \textit{Z. longipes} females showed a significant preference for \textit{A. suspensa} adults (95\% confidence intervals did not include \(\beta = 0.5\)) over \textit{D. citri} adults, but males did not show any preference (Fig. 3).

**DISCUSSION**

Our research suggests that \textit{Z. longipes} can reduce the population of \textit{D. citri} in \textit{M. exotica}. Females were slightly more efficient predators of \textit{D. citri} adults than are males, which may be explained by the fact that \textit{Z. longipes} females are larger and perhaps need more prey than the males (Kalsi 2011). Due to their small size, 1st instar nymphs of \textit{Z. longipes} were not able to reduce psyllid numbers to the same extent as the adults; Groups of 2 or 3 \textit{Z. longipes} nymphs were often attacked by a single prey, and thus an individual nymph consumed fewer psyllids than did an individual adult \textit{Z. longipes}. Similar results were noted with \textit{Zelus exsanguis} Stål attacking \textit{Ephestia...}

Imatures and adults of Z. longipes consumed relatively fewer 5th instars of D. citri than adult D. citri. Apparently Z. longipes prefers to eat mobile prey instead of sessile prey. Visual stimuli seem to be the most important cue needed for reduviids to trigger an attack, as shown in work with Z. renardii and Rhynocoris marginatus Fab. (Hari-dass et al. 1988; Cisneros & Rosenheim 1998). In contrast, other Harpactorinae like Scipinia repax Stål and Nagusta sp., which are specialist predators of spiders, seem to rely on other sensory cues for orienting to prey (Jackson et al. 2010). Tama-rixia radiata adults were suitable prey for adults and nymphs of Z. longipes, suggesting the possibility that intra-guild predation may be disruptive to biological control of the psyllid. This may also explain low levels of parasitism at certain sites (Peña et al. 2008). Kalsi & Seal (2011) mentioned that Z. longipes can be a predator of other natural enemies like Orius spp. Zelus renardii has been reported as an important predator of parasitoids of the genus Aphytis, thus interfering with biological control of the California red scale Aonidiella aurantii (Maskell) (Heimpel et al. 1997). No cannibalistic behavior was observed among Z. longipes individuals during any experiment, likely as a result of the short duration (24 h) of trials. According to personal observations by BN of the Z. longipes colony, cannibalism will begin after 3 d of starvation. Similar behavior was observed by Evans (1976) with the pirate bug An-thocoris confusus (Reuter).

Zelus longipes female adults had a type III functional response to D. citri adults (Juliano 2001). Type III functional response is typical for generalist predators that can switch from one prey to another (Hassell 1978; Akre & Johnson 1979; van Baalen et al. 2001). Kalsi (2011) found that both genders of Z. longipes adults had a type II functional response when feeding upon flies of the genus Euxesta. The different types of func-tional responses for the same predator species may be explained by the use of different prey with different behaviors and size.

As generalist predators, reduviids are thought not to express strong prey preferences (Louis 1974). Our work confirms that Z. longipes adults do not exhibit a preference for D. citri versus T. radiata as prey items. However, Z. longipes 1st instar nymphs prefer T. radiata adults over D. citri adults, which may be due to the comparatively smaller size of the parasitoid. For instance, Cogni et al. (2002) inferred from choice experiments that Z. longipes adults prefer smaller prey. In this study, adult females of Z. longipes showed a prey preference for A. suspensa adults over D. citri adults; in this case the females chose a bigger prey, which may be explained by greater nutri-tional needs of the females or by their bigger size in comparison with males.

Our research indicated that Z. longipes adults could potentially prey upon adults of D. citri un-
nder field conditions. Considering the high populations of this reduviid seen in south Florida, this predator may contribute to *D. citri* mortality. However, it was also demonstrated in this research that the parasitoid *T. radiata* is a suitable prey for *Z. longipes*. The prey preference studies showed that 1st instar nymphs had a preference for the smaller parasitoid over the larger *D. citri*. Hence, the real effect of this predator on parasitoids and other natural enemies should be tested under field conditions, particularly in *M. exotica* systems where *Z. longipes* appears to be more frequent than on *Citrus* spp.

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