

Functional Response of *Tamarixia radiata* (Hymenoptera: Eulophidae) to Densities of Its Host, *Diaphorina citri* (Hemiptera: Psylloidea)

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Abstract

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) spread rapidly in Florida following first release in 1999 for control of the Asian citrus psyllid *Diaphorina citri* (Kuwayama) (Hemiptera: Psylloidea). However, rapid spread of huanglongbing (HLB) has created a need for more effective biological control through augmentation and thus the necessity to better understand the potential of this parasitoid. Therefore, the functional response of fecundity to host density was evaluated to provide a possible indicator of success and useful information for mass rearing of *T. radiata*. The experimental unit consisted of a single 3-d-old female in a centrifuge tube with a young *Murraya paniculata* (L.) Jack (Rutaceae) shoot infested with a range of 1–60 fourth-instar *D. citri* nymphs. Shoots were changed daily for 5 d and the nymphs containing one or more parasitoid eggs noted. The number of hosts parasitized daily increased from 0.7 to 11.2 on average as host density increased from 1 to 40, above which it remained relatively constant. Percent parasitism was as high as 73.3% at density 1, but decreased at greater host densities. A random parasite equation was selected as the best-fit model, from which we obtained an attack coefficient of 12.7cm²/h, and handling time of 0.873 hour per host. We proposed the term “adjusted searching efficiency” to express the attack coefficient as a function of searching area. Similarities and differences with previous studies are discussed, as well as the need to reconcile parameters with actual behavior of *T. radiata*.

Key words: Asian citrus psyllid, attack rate, biological control

Tamarixia radiata (Waterston) is an arrhenotokous ectoparasitoid of *Diaphorina citri* (Kuwayama), vector of citrus greening disease or huanglongbing (HLB). HLB was discovered in Florida in August 2005 (Halbert and Manjunath 2004), and by December 2007, it had spread through 30 counties located south of Marion County. HLB is one of the most destructive diseases of citrus in the world (Halbert and Manjunath 2004, Bove 2006). HLB is estimated to have cost Florida's economy US\$4.4 billion and resulted in 8,257 jobs lost since 2006–2011 (Hodges and Spreen 2012). *Tamarixia radiata* has been credited with controlling *D. citri* in Reunion Island (Aubert and Quilici 1983) and providing good levels of suppression in India (Husain and Nath 1927), Puerto Rico (Pluke et al. 2008), and Guadeloupe (Etienne et al. 2001), even though it is not clear how much *D. citri* levels were reduced in these locations (Hall et al. 2013). *Tamarixia radiata* was first imported to Florida from Taiwan and Vietnam in 1998 and released in 1999–2001 (Hoy et al. 2001), and it has become established in citrus orchards according to a survey in 2006–2007 (Qureshi et al. 2009, Qureshi and Stansly 2009). However, success of *T. radiata* as an augmentive biocontrol agent in the field has not been reported.

The success of parasitoids as a biological control agent is thought to be related to the behaviors the parasitoids present in response to increasing host density, known as functional response (Huffaker et al. 1971, Hassell 2000, Fernandez-Arhex and Corley 2003, Jones et al. 2003). Functional response is important to the process of predation and parasitism (Hassell 2000), and critical in selecting biocontrol candidates by testing the capacity of parasitoids and predators to search for hosts at low host densities (Burnett 1958, Takahashi 1968, van Lenteren and Bakker 1978, Walde and Murdoch 1988, vanAlebeek et al. 1996).

Functional response of predators to prey was first described by (Holling) 1959b who characterized three types based on a linear (type I), decelerating (type II), or sigmoid (type III) response between increasing host (prey) density and number consumed. Both type II and type III functional response reach an upper asymptotic limit. Holling (1965) considered type II functional response to be typical for invertebrate predators and parasitoids and type III for vertebrate predators, which tend to concentrate their efforts on abundant prey. Hassell et al. (1977) challenged this generalization by showing that

type III functional response may be found more commonly in invertebrate predators and insect parasitoids than previously thought. This result had implications for biological control because type III functional responses, in contrast to type II, are considered stabilizing thanks to increasing searching efficiency with increased host density.

Holling (1959a) derived the so-called disc equation to describe the type II functional response:

$$\text{Enc} = \frac{a'NT_t}{1 + a'NT_h} \quad (1)$$

where Enc = number of encounters; N = prey density; T_t = total experimental time available; T_h = handling time per prey; and “a” was termed the “instantaneous rate of discovery” also known as the “attack coefficient” (Rogers 1972). For type II functional response, \hat{a} and T_h are constant (Holling 1959a). Rogers (1972) defined \hat{a} for parasitoids as rate of search or the area covered per unit of the time, and is therefore limited by the available searching area. He suggested a refinement of the disk equation by assuming a random search:

$$N_p = N \left[1 - \exp \left(- \frac{a'T_t}{1 + a'T_hN} \right) \right] \quad (2)$$

where N_p = the number of hosts parasitized. This model assumes that, unlike the predator–prey model, parasitized hosts are not removed, and therefore hosts remain to be subsequently re-encountered.

Equations for type III sigmoid functional response were proposed by Hassell et al. (1977) and Hassell (1978). They observed that, for type III functional response, the attack coefficient (\hat{a}) increases with increasing host densities.

$$\hat{a} = \frac{bN}{1+cN} \quad (\text{from Hassell 1978 equation 3.9}). \quad (3)$$

where b and c are constants. In this case the equation of type III functional response for parasitoid is:

$$N_a = N \left[1 - \exp \left(- \frac{bTN}{1 + cN + bT_hN^2} \right) \right] \quad (4)$$

(from Hassell 1978 equation 3.11).

It has been reported that the fecundity of *T. radiata* is highly correlated with host density (Chien et al. 1991a, 1995). Chien et al. (1995) stated that the relationship between both daily and lifetime fecundity and host density showed similar parabolic responses, whereby female fecundity ascends with host density to a peak of 40 hosts per day, then decreases as host density increases up to 80 hosts per female wasp. However, these experiments were conducted at 100% relative humidity (RH), which could increase honeydew deposits that might interfere with search at high host densities. Furthermore, the late fifth-instar nymphs used may not be suitable hosts, especially when they are about to emerge as adults.

Chen (2013) also found fecundity of *T. radiata* to increase in response to host densities from 10 to 40, fourth-instar nymphs on shoots of *Murraya paniculata* L. in a growth chamber at $24 \pm 4^\circ\text{C}$, 60–80% RH, with no increase in fecundity at higher densities up to 40 per wasp. Data fitted to the Holling’s disk equation gave estimates of “ \hat{a} ” (attack coefficient) = 0.0184 ± 0.0015 per hour and T_h (handling time) = 0.045 ± 0.008 hour per host. Sule et al. (2013) also evaluated functional response of *T. radiata* under similar conditions and also saw a leveling off of fecundity at fourth instar densities above 40 per day, but reported a quite different searching efficiency of 39.99 h^{-1} as calculated using the disk equation. Given

the importance that these results are purported to have in predicting success of biological control (Barlow and Goldson 1993), we repeated these studies under favorable conditions of relative humidity and host availability, included lower host densities than Chen (2013) and compared results using both Holling’s (1959) equation (1) and Rogers’s (1972) equation (4) to make the calculations. Adjusted searching efficiency (ASE) to express attack coefficient as a function of searching area is proposed as a useful term to reconcile disparities in results among different studies.

Materials and Methods

Colonies

Rearing methods generally followed Skelley and Hoy (2004). *Murraya paniculata* was grown from seed and transplanted into 3.92-liter Air-Pots (TerraHydro, Carmichael, CA) filled with 40% Canadian sphagnum peat plus bark, vermiculite, perlite, dolomitic limestone, and a wetting agent (Fafard 4P Professional Growing Mix, Sun Gro Horticulture, Agawam, MA). Plants were grown in an unheated naturally ventilated hoop style trussed greenhouse covered with insect screen mesh plus an additional polyethylene roof and raisable sides used during cool weather. Each selected plant had at least eight shoots of new growth, with each new shoot about 3 cm in length and the terminal bud not fully open. Plants were sprayed as needed four or more days before use with 1% M-pede soap (Dow AgroSciences LLC) to control unwanted psyllids and other pests.

Diaphorina citri were reared in BugDorm-2400 Insect Rearing Tents (75 by 75 by 115 cm³, MegaView Science Co. Ltd., Taichung, Taiwan) inside an air-conditioned glass greenhouse maintained at 22–32°C, 50–80% RH (HOBO RH/ Temp/ Light/ External Data Logger- H08-004-02, Onset Computer Corp. Onset Computer Corporation, Bourne, MA). Nine flushing *M. paniculata* plants were placed into each cage, and ~1,500 *D. citri* were released and held for 72 h to oviposit. Plants were then moved to a similar clean cage for about 10 d until eggs hatched and nymphs developed to the fourth instar.

Six *M. paniculata* plants infested with fourth nymphal instar psyllids were transferred to a wooden frame cage with polyacrylic sides inside a 4- by 4-m air-conditioned polycarbonate greenhouse maintained at $26 \pm 1^\circ\text{C}$, 55–85% RH. One hundred and twenty females and sixty male *T. radiata* were released inside for 7 d, and progeny were collected from day 8 until no more were found (around day 22).

Functional Response

Experimental arenas were prepared from conical polypropylene 50-ml centrifuge tubes (28 by 115 mm², Fisher Scientific, Pittsburg, PA), into which were placed a single 7-cm young shoot of *M. paniculata* infested with 1, 3, 6, 10, 20, 30, 40, 50, or 60 fourth-instar psyllid nymphs. Healthy young shoots with an excess of nymphs were chosen, and small paintbrush was used to remove extra nymphs to achieve the desired densities. This method was preferred to adding nymphs which then tended to crawl off the shoots.

Six days after *T. radiata* were released into the *D. citri* colony, small (2 cm) portions of shoots containing one parasitized nymph with the wasp close to pupal stage were excised and placed individually into a glass tube 75 mm long by 12 mm in diameter (Fisher Scientific, Hampton, NH). Tubes were checked frequently, and wasps removed immediately upon emergence (Chen and Stansly 2014) to a Petri dish with fourth-instar *D. citri* nymphs, where they were kept for 72 h with host nymphs changed every 24 h.

One randomly chosen 3-d-old parasitoid pair was released into each centrifuge tube sealed with Parafilm (Bemis NA, Neenah, WI) ventilated by small holes made with a number 1 insect pin. The total searching area included the interior surface of the centrifuge tube (103 cm^2) plus the two sides of the leaves, giving a total of $\sim 147\text{ cm}^2$. Arenas were placed in a Percival model I-36LL-1 incubator with two fluorescent lights on each of two shelves and set for $25 \pm 1^\circ\text{C}$, 55–85% RH, and a photoperiod of 14:10 (L:D) h. Shoots were replaced every 24 h for 5 d. Exposed nymphs were then inverted under a stereoscopic microscope to check for parasitoid eggs. Six replications were completed for each host density.

Scotophase Activity

Single young shoots of *M. paniculata* infested with 10 fourth-instar *D. citri* nymphs were placed in 50-ml centrifuge tubes as above. One randomly chosen 3-d-old *T. radiata* pair was released into each centrifuge tube and maintained in incubators as above from 8:00 p.m. to 6:00 a.m. at $25 \pm 1^\circ\text{C}$, 55–85% RH without light. *Tamarixia radiata* pairs in the control groups were held the same conditions except in light for the same time period. Nymphs were then checked for parasitization. There were 10 replications.

Statistical Analysis

Functional Response

A two-factor factorial analysis was used to determine the significant effects of age and density. Fecundity and superparasitism as proportions were subjected to analysis of variance (ANOVA) with mean separation determined by Fisher's LSD ($P < 0.05$). Values were first subjected to a square-root transformation to satisfy the equal-variance assumption of ANOVA (JMP software, SAS Institute Inc. 2013).

Model Selection

We considered the parasitization outcome to be binary, and so the number of hosts parasitized should follow a binomial distribution with the probability of being parasitized given by the corresponding expressions in the appropriate functional response equations. To choose among these, we fitted our data using maximum likelihood to expressions (2) and (4). The best-fitted model was chosen as the one with smallest Bayesian information criterion (BIC) based on the likelihood function (Schwarz 1978). Attack coefficient and handling time were estimated based on the appropriate model equation. Attack coefficient is defined as a function of the area a parasitoid covers in a unit of time (Rogers 1972); therefore, we propose using searching efficiency (ASE) in this paper, which was calculated as: $\text{ASE} = \text{attack coefficient} \times \text{searching area available}$ (147 cm^2). Data were also fitted to equation (1) in order to compare estimates of the unknown parameters with Sule et al. (2014).

Results

Functional Response

Fecundity did not vary significantly within host densities over the 5-day study period ($F = 1.5118$, $df = 4$, $P = 0.1991$). In contrast, the effect of density on fecundity was highly significant ($F = 150.6340$, $df = 8$, $P < 0.0001$) as it was for average fecundity among densities over all days ($F = 330.51$; $df_{(\text{model, error})} = 8, 45$; $P < 0.0001$). The effect of density on fecundity was still significant even after the low densities (1, 3, and 6) were eliminated ($F = 75.3679$; $df_{(\text{model, error})} = 5, 30$; $P < 0.0001$). Fecundity increased with host density from 1–40 hosts to a maximum of 11 eggs per day, with no

significant differences among host densities of 40, 50, and 60 (Table 1, Fig. 1).

Percent parasitism was highest at 73.3% at the lowest (1 host) density and least at 18.6% at the highest (60 hosts) density ($F = 87.5$; $df = 8, 45$; $P < 0.0001$; Table 1; Fig. 2A). Percent superparasitism (the number of superparasitized hosts over parasitized hosts) varied significantly among densities ($F = 4.68$; $df = 8, 45$; $P < 0.0001$) from 16.2% at 6 hosts per female to 1 or less at 30 hosts per female or greater with no differences among the last four densities (Table 1, Fig. 2B).

Parasitization During Scotophase

No oviposition during scotophase was observed over the 10 replications, suggesting that parasitization does not take place in darkness. The mean (\pm SE) oviposition in the control groups was 3.4 ± 0.03 . Consequently, the 10-h dark period was eliminated when fitting to functional response; that is, the total experimental period (T) relevant for functional response analysis was assumed to be 14 h.

Model Selection

The calculated BIC values for the Rogers (1972) type II equation (2) and Hassell (1978) type III equation (4) were -978.1 and -972.5 , respectively, so the type II equation was chosen as the best-fit model. The fitted parameters using type II functional response equation (2) were $\hat{a} = 0.0864\text{ h}^{-1}$ ($\text{SE} = 0.0082$), and $T_h = 0.873\text{ h}$ per host ($\text{SE} = 0.044$). Given a total searching area of 147 cm^2 , ASE was estimated at $12.70 \pm 1.21\text{ cm}^2/\text{h}$ ($0.21 \pm 0.02\text{ cm}^2/\text{min}$).

Estimates obtained by fitting our data to Holling's disc equation (1), which unlike Rogers's (1972) equation, takes unit parameters into consideration were $\hat{a} = 7.45\text{ cm}^2/\text{h}$ ($\text{SE} = 0.53$), and $T_h = 0.85\text{ h}$ per host ($\text{SE} = 0.04$).

Discussion

Chien et al. (1995) reported peak fecundity of about 25 eggs per female per day at a host density of 40 but a decrease at higher host densities up to 80. Sule et al. (2014) reported a maximum of 21.2 nymphs (fourth instar) parasitized per female during a single 24-h exposure to 80 nymphs beyond which incidence of parasitism leveled off. We found number of hosts parasitized, percent parasitism, and percent superparasitism were all significantly influenced by host density over a 3–8 d old range. The number parasitized increased to

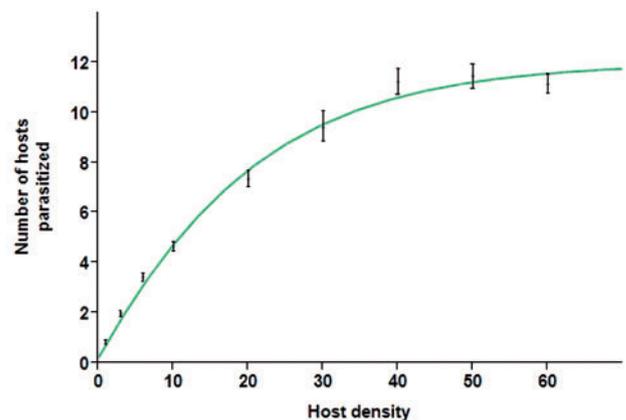


Fig. 1. Mean (SE error bars) number of parasitized hosts per day by one 3-d-old *T. radiata* female at nine host densities. A smoothed curve based on non-linear least squares has been superimposed (black and white).

Table 1. Mean \pm SE number of parasitized hosts, percent parasitism, and percent superparasitism at nine different host densities from 1 to 60 fourth-instar nymphs per arena per day

Host density	Parasitized hosts	Percent parasitism (%)	Percent superparasitism (%)
1	0.73 \pm 0.08 G	73.33 \pm 8.21 G	10.00 \pm 5.57 A
3	1.97 \pm 0.13 F	65.56 \pm 4.37 F	14.44 \pm 4.56 A
6	3.43 \pm 0.19 E	57.22 \pm 3.26 E	16.22 \pm 4.27 A
10	4.63 \pm 0.25 D	46.33 \pm 2.56 D	13.17 \pm 2.52 A
20	6.86 \pm 0.40 C	34.41 \pm 1.98 C	9.25 \pm 2.65 AB
30	9.40 \pm 0.50 B	31.33 \pm 1.67 B	1.03 \pm 0.72 BC
40	11.20 \pm 0.46 A	28.00 \pm 1.16 A	0.35 \pm 0.25 C
50	11.43 \pm 0.37 A	22.90 \pm 0.75 A	0.43 \pm 0.43 C
60	11.13 \pm 0.42 A	18.55 \pm 0.70 A	0.27 \pm 0.27 C

Means in the same column followed by the same letter are not significantly different (Fisher's LSD, $P < 0.05$).

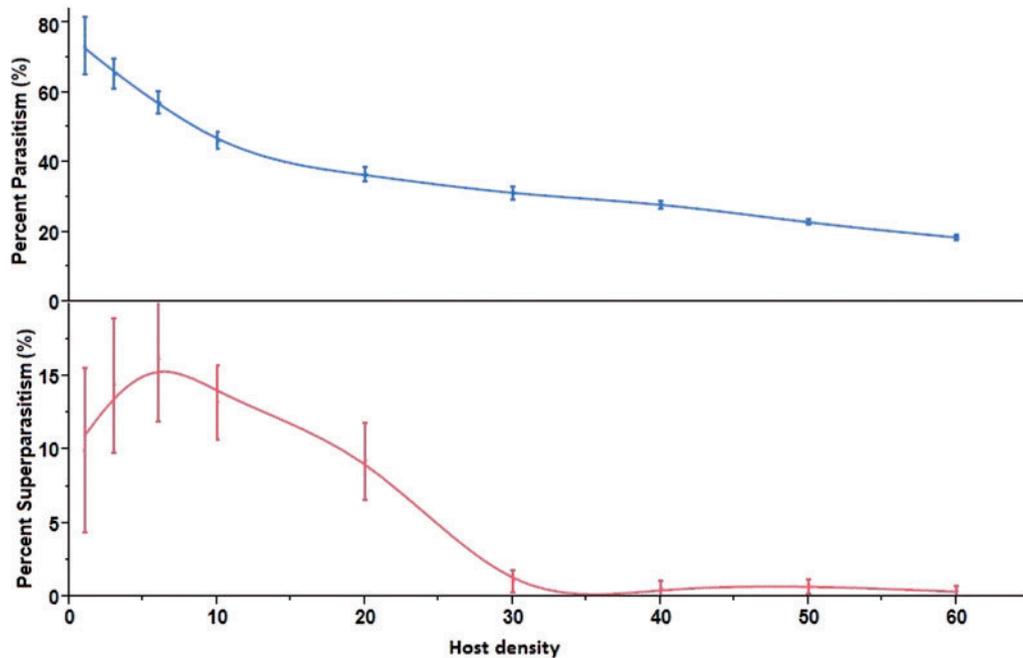


Fig. 2. A smoothed curve of mean (%) parasitism (A) and superparasitism (B) at nine host densities. Error bars at each point represent the standard error of the mean (black and white).

a maximum of 11.2/d from the lowest density through 40 hosts per female, and then remained relatively constant through 60 hosts per wasp, indicating an upper limit had been reached. Percent parasitism declined with host density, which was highest (73%) at the lowest density. Host feeding may have been the reason that a 100% percent parasitism was not reached at low host densities. Chien et al. (1995) and Sule et al. (2014) used larger areas than we did to test host density effects, which might explain the higher fecundities they observed.

Superparasitism was high at host densities ranging from 1 to 20, and negligible at densities ≥ 30 . Chien et al. (1991b) reported that *T. radiata* females were able to discriminate between parasitized and nonparasitized hosts, although superparasitism still occurred when nonparasitized hosts were available. Superparasitism is wasteful because only one egg can mature to the adult stage and any others will be killed by the first hatched (Chien et al. 1991a, Harvey et al. 2013). We found percent parasitism to be greater at a host:parasitoid ratio 1:30 than at 1:40, whereas superparasitism was not significantly different at the two host densities. On the other hand, more hosts were parasitized at a 1:40 parasitoid:host ratio. Therefore, while the 1:30 ratio may result in more efficient use of hosts, the

1:40 ratio may result in more efficient use of other resource of time or space for mass rearing.

We found a type II response by *T. radiata* to host density over 5 d. However, there are many reasons given why type III responses could be difficult to detect including exposure to the hosts in a fixed area (Hassell et al. 1977, van Lenteren and Bakker 1978) and fixed periods of search (Huffaker et al. 1971, van Lenteren and Bakker 1978, Collins et al. 1981). Furthermore, an accelerating trend leading to a sigmoid (type III) response could occur at lower densities but be masked over a large host density range. While the type III functional response is thought to have a stabilizing effect and are therefore more likely to lead to population regulation (Hassell et al. 1977), the shape of the functional response curve has yet to be linked to success of biocontrol (Fernandez-Arhex and Corley 2003).

Attack coefficient is considered to define the steepness of the curve approaching the upper fecundity asymptote and estimates the proportion of area searched during the experimental period (Gitonga et al. 2002). In our experiment, *T. radiata* was able to search 12.7 ± 1.21 cm²/h based on Rogers's (1972) equation (2) and 7.45 ± 0.53 cm²/h based on Holling's equation (1). Holling's

Table 2. Attack coefficient (\hat{a}) of *T. radiata* female attacking fourth-instar *D. citri* nymphs at increasing density using $T = 24$ h, $T_h = 0.6$ h⁻¹, and an estimated search area of 190 cm² (Sule et al. 2014)

Density (N)	Hosts parasitized (Enc)	\hat{a} (h ⁻¹)	\hat{a} (cm ² /h)
15	13.2	0.055	10.40
20	16.8	0.06	11.47
40	20.2	0.04	8.08
80	21.2	0.023	4.46
100	22.0	0.02	3.87

Table 3. Comparisons of handling time (T_h), attack coefficient (\hat{a}), and ASE among Chien et al. (1995), Sule et al. (2014), and our results

Comparisons	T_h (h)	\hat{a} (/h)	ASE (cm ² /h)
Chien et al. (1995)	0.437 (T = 24 h) 0.218 (T = 12 h) 0.072 (T = 4 h)	0.03	6.885
Sule et al. (2014)	0.6	0.06–0.02 ^a	11.47–3.87
Equation (1) in this paper	0.85	–	7.45
Equation (2) in this paper	0.873	0.0864	12.7

^aOur calculation.

Sule et al. (2014) reported ^a = 39.99

equation takes units into consideration and generates attack coefficient with units cm²/h directly. A comparable attack coefficient (cm²/h) from Rogers's (1972) equation was obtained by calculating ASE. Sule et al. (2014) also reported a type II functional response for *T. radiata* when a mature female was introduced for oviposition to densities ranging from 2 to 120 fourth-instar *D. citri* nymphs for 24 h under lab conditions. Using equation (1), they reported handling time (T_h) 0.6 h⁻¹ for fourth instars, which is close to our estimate of 0.85 h⁻¹. However, they also reported searching efficiency for fourth-instar hosts of 39.99 h⁻¹, which is over 400-fold higher than our estimate of 0.86! However, using their estimated value of $T_h = 0.6$ h⁻¹, the calculated values for \hat{a} came out much closer to ours than the published value (Table 2). Chien et al. (1995) reported an attack coefficient of 0.78/d (0.03/h), which was similar to our results. The available searching area in their experiment was ~229.5 cm², resulting in an ASE = 6.885 cm²/h, which was also very close to our result (Table 3).

Handling time is defined as the time needed by a parasitoid to pursue, subdue, oviposit, and recover (Holling 1959a). Estimates for handling time also vary among studies. Chien et al. (1995) calculated handling times for *T. radiata* that varied from 26.2 to 13.1 to 4.3 min depending on whether total time was considered as 24 h, 12 h (active time), or 4 h (peak oviposition time), respectively. Sule et al. (2014) calculated a handling time for fourth-instar hosts of 0.6 h over 24-h total time, which is not far from the 0.87 h estimate from our research calculated on the basis of 14-h total time. All estimates of handling time greatly exceed 61 s estimated by Chien et al. (1995) based on behavioral observations. One reason may be that other time-consuming activities, such as resting and grooming, are not taken into consideration, leading to overestimates of handling time by functional response equations (Chong and Oetting 2007). Thus, there is a need to reconcile these calculations with observational handling times.

Both Holling (1959a) and Rogers (1972) stated that functional response equations may explain the data but not necessarily actual

behavior. Reconciling functional response with behavioral observations is a goal yet to be realized (Fernandez-Arhex and Corley 2003). Validation of functional response with behavioral observations will provide more practical comparisons among biological control agents, and help optimize rearing conditions in the laboratory as well as releasing conditions in the field.

Results presented here provide a starting point for improving control of *D. citri* in the field. However, this experiment was conducted in a Petri dish with limited searching area in contrast to field conditions. A study now in progress is focused on determining the relationship between parasitoid and different host densities at the colony level, which will provide a closer approximation to field conditions.

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