Entomologia Experimentalis et Applicata

DOI: 10.1111/eea.12550

Effect of host deprivation on the foraging behavior of the Asian citrus psyllid parasitoid *Tamarixia radiata*: observations from the laboratory and the field

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Accepted: 21 October 2016

Key words: citrus, *Diaphorina citri*, classical biological control, host feeding, Hymenoptera, Eulophidae, Hemiptera, Liviidae

Abstract

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a significant citrus pest and the parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) has been released in various citrus-producing areas in classical biological control programs targeting *D. citri*. We investigated the effect of host deprivation on the foraging behavior and patch utilization by *T. radiata*. In the laboratory, females deprived of hosts for 3 days tended to leave patches of 12 nymphs without parasitizing hosts during the ca. 30 min they spent in the patch before leaving. Moreover, half of these females failed to host feed, and those that did host feed, on average, needed more than 15 min to complete feeding. Conversely, non-host-deprived females parasitized on average three nymphs before leaving patches without host feeding during the ca. 39 min they spent in the patch. These laboratory observations were compared to mass-reared female *T. radiata* that were released onto colonies of *D. citri* nymphs infesting citrus in the field. Release vials were provisioned with honey and these females had no opportunities to host feed over the 1- to 2-day containment period prior to release. When introduced onto *D. citri* colonies, 68% *T. radiata* females abandoned *D. citri* patches prior to probing hosts, in part, because Argentine ants, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), tending colonies disturbed searching parasitoids. These results from laboratory and field studies are discussed in the context of classical biological control, with the aim of understanding how to manipulate host availability and ant activity so establishment rates and impact of *T. radiata* can be improved.

Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an important citrus pest because it has the potential to spread the bacterium *Candidatus* Liberibacter asiaticus (*C*Las), one of the Liberibacter species causing the lethal and incurable citrus disease known as huanglongbing (HLB) or citrus greening disease (Bové, 2006). *Diaphorina citri* is native to the Indian subcontinent and has been inadvertently spread to the Middle East, South and Central America, and the Caribbean (Grafton-Cardwell et al., 2013). In the USA, *D. citri* has established in all major citrus production areas: Florida in 1998 (Halbert & Manjunath, 2004; Qureshi et al., 2009), Texas in 2001 (French et al., 2001), and California in 2008 (Hoddle & Hoddle, 2013).

Adult *D. citri* lay eggs exclusively on citrus flush growth, and at emergence, nymphs move to new and tender expanding shoots where they settle, form colonies, and subsequent feeding may cause distortion and death of young leaves (Hall et al., 2008). *Diaphorina citri* nymphs excrete solid honeydew which is harvested by ants. In this mutualistic relationship, ants protect immature *D. citri* from natural enemies (i.e., predators and parasitoids) and in return ants are rewarded with honeydew (Navarrete

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et al., 2013; Tena et al., 2013). *Diaphorina citri* nymphs are generally acceptable and suitable prey for a wide range of generalist predators, especially coccinellids, lacewing larvae, and syrphid fly larvae (Michaud, 2004; Qureshi & Stansly, 2009; Kistner et al., 2016). Ants tending *D. citri* colonies also disrupt foraging by generalist predators (Kistner et al., 2016).

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae) is a solitary ectoparasitoid of *D. citri* (Chu & Chien, 1991). It is arrhenotokous, synovigenic, and host feeds on *D. citri* nymphs. Females host feed on all the nymphal instars and prefer older nymphs (fourth and fifth instars) for oviposition (Chu & Chien, 1991). *Tamarixia radiata* is native to the Indian subcontinent and it has been used as a classical biological control agent to suppress *D. citri* on Reunion Island (Aubert & Quilici, 1986), Taiwan (Chien et al., 1989), and the USA [i.e., California (Hoddle & Hoddle, 2013) and Florida (Hoy et al., 2001)]. In Florida, Tsai et al. (2002) and Michaud (2004) reported that parasitism by *T. radiata* reached only 1–2%. However, a subsequent study in Florida by Qureshi et al. (2009) found parasitism rates by *T. radiata* reach 20–56%, depending on time of year and location. In southern California, *T. radiata* sourced from Punjab (Pakistan) has been widely established in backyard citrus (Hoddle & Hoddle, 2013; Hoddle et al., 2016) and life table analyses indicate that marginal rates of parasitism of *D. citri* by *T. radiata* average 21% in fall (November–September) (Kistner et al., 2016).

The establishment of parasitoids in new areas as part of a classical biological control program depends, in part, on the physiological condition of the released individuals and subsequent post-release behavioral decisions when foraging for hosts (Mills & Wajnberg, 2008). Consequently, establishment rates of classical biological control agents may be enhanced via pre-release conditioning (e.g., provision of carbohydrate sources) that attempts to maximize the fitness of parasitoids destined for field release (Hougardy et al., 2005; Hougardy & Mills, 2006; Mills & Wajnberg, 2008). During this pre-release holding period, parasitoids harvested from mass-rearing cages are typically held in small release containers, where mating can occur and carbohydrate sources (e.g., honey) are provided. During this holding period in release vials, host deprivation occurs, and for parasitoids this results in an inability to host feed and oviposit. The absence of hosts may reduce egg maturation rates in synovigenic species like *T. radiata*, especially if protein from host feeding is needed to mature eggs. Conversely, allowing access to hosts in the pre-release period could result in the expenditure of eggs which could reduce motivation for host finding when released into the field (Mills & Wajnberg, 2008).

Here, we report on the effects of host deprivation on the foraging behavior and patch utilization by *T. radiata* under controlled laboratory conditions when a solitary female, either host deprived or previously given access to hosts, encounters a patch of *D. citri* nymphs. We then tested whether laboratory observations predicted parasitoid behavior in the field following releases of host-deprived *T. radiata* onto citrus trees infested with *D. citri* nymphs. The implication of observations made in the laboratory and field with host-deprived *T. radiata* released for classical biological control of *D. citri* is discussed.

Materials and methods

Insect rearing

Diaphorina citri used as hosts in exposure trials were sourced from colonies maintained at the University of California Riverside Insectary and Quarantine Facility (UCR IQF). *Diaphorina citri* colonies were established from field-collected material in Tustin, California, and moved to UCR IQF under California Department of Food and Agriculture (CDFA) permit no. 2870. All *D. citri* colonies were confirmed to be *C*Las-free through PCR analysis of 30 individual psyllids and reared on *Citrus volkameriana* V. Ten. & Pasq. (Rutaceae). Potted *C. volkameriana* were pruned regularly to promote flush growth necessary to stimulate ACP oviposition (Hall et al., 2008), as well as to maintain plants at sizes suitable for rearing cages. *Diaphorina citri* rearing was conducted at 29 ± 1 °C, 40 ± 5% r.h., and L14:D10 photoperiod.

Tamarixia radiata colonies were established using specimens collected from several localities from Pakistan over a 2.5-year period and moved under permit (USDA-APHIS permit number: P526P-09-02585) to UCR IQF (Hoddle & Hoddle, 2013). Parasitoids were separated according to the locality and date of collection and this resulted in the establishment of 17 isocage lines of *T. radiata* on *D. citri* at UCR (Hoddle & Hoddle, 2013). Parasitoids from each line were added to a panmictic mating cage to facilitate outcrossing and subsequent increased genetic variation in offsprings (Roush & Hopper, 1995). Progeny resulting from panmictic mating cages were used for field releases. Parasitoids were reared at 25 ± 2 °C, 60 ± 10% r.h., and L14:D10 photoperiod on curry leaf plants [*Murraya koenigii* (L.) Sprengel (Rutaceae)] infested with *D. citri*, and adults were provided access to a 50% honey solution (vol/vol). Additional details on plant preparation and maintenance, *D. citri* and *T. radiata* rearing are provided by Hoddle & Pandey (2014), Bistline-East et al. (2015), and Bistline-East & Hoddle (2016).

Experimental arenas for behavioral observations

Citrus volkameriana seedlings grown in 114-ml Ray Leach Cone-tainers (SC7 Stubby, 3.8 cm in diameter; Stuewe and Sons, Portland, OR, USA) were used for experiments. Excess leaves on transplanted seedlings were removed leaving only the two youngest leaves at the plant's apex. The soil surface was covered with a white piece of circular foam netting to reduce fungus gnat emergence and to more easily observe insects that fell or walked onto the soil (i.e., *T. radiata* adults and *D. citri* nymphs). Clear plastic 148-ml vials (Thornton Plastics, Salt Lake City, UT, USA), with three 12-mm-diameter holes (two on opposite sides, one on the bottom) covered with ultra-fine organza mesh, were inverted to fit on the vial lid that was firmly secured around the cone-tainer – see Hoddle & Pandey (2014) for a detailed figure of the experimental arena. Inverted ventilated vials enclosed test plants infested with *D. citri* nymphs and *T. radiata* females.

Twelve fourth-instar *D. citri* nymphs, a highly preferred life stage for attack by *T. radiata* (Skelley & Hoy, 2004; Gómez-Torres et al., 2012), were hand-transferred from colonies to experimental plants using a fine paint brush. All nymph transfers were completed 24 h before the test day and left to settle, feed, and acclimate. A single *T. radiata* female was then introduced into experimental arenas.

Effect of host deprivation on patch use and behavior time partitioning by *Tamarixia radiata*

To study the effect of temporary host deprivation on patch use and time partitioning across observed behaviors, newly emerged *T. radiata* females were collected daily from colonies between 15:00 and 17:00 hours. Collected females were assumed to have mated during this emergence period as copulation was frequently observed during collecting periods. Females were isolated individually in 0.8 × 3.0 cm glass vials which were provisioned with a drop of honey and closed with a cotton plug. Females were then subjected to one of two treatments: (1) host deprivation for 2 days (i.e., no *D. citri* nymphs were available for host feeding, and honey, a carbohydrate source, was the only food available), or (2) provision of one second-instar *D. citri* per day for 2 days (i.e., this was the non-host-deprived treatment). Second-instar nymphs were provided because *T. radiata* host feeds but does not lay eggs on this instar (Chu & Chien, 1991). Vials with females allocated to these two experimental treatments were kept in an environmental chamber at 25 ± 2 °C, 65 ± 10% r.h., and L14:D10 photoperiod until they were used in bioassays, which was 3 days after parasitoid emergence.

Female *T. radiata* were individually introduced into experimental arenas with citrus seedlings inoculated with 12 fourth-instar *D. citri* nymphs. Foraging parasitoids were observed visually under a compound microscope at 1.5–5× magnification and arenas were illuminated with cold light. Data recording started when the parasitoid came into contact with the plant and observations were made continuously until the parasitoid abandoned the *D. citri*-infested plant for more than 5 min or rested without walking on the plant for more than 10 min. The frequency and time in seconds spent by parasitoids searching, resting, probing, ovipositing, and host feeding were recorded. The observations were replicated 22× and 16× for non-host-deprived and host-deprived females, respectively.

After the observation period, the parasitoid was removed and the nymphs were examined to confirm oviposition. *Tamarixia radiata* lays eggs under the body of *D. citri* (Chu & Chien, 1991) and are easily observed under a microscope when nymphs are lifted off the plant substrate and inverted. We considered that *T. radiata* had failed to host feed when, after probing the dorsal surface of the nymph, hemolymph did not ooze from the wound, but females tried to feed repeatedly on probed hosts even though no hemolymph was observed. Observation of hemolymph exuding from puncture wounds and subsequent feeding on these liquids was recorded as successful host feeding.

Tamarixia radiata behavior in the field: first host use and patch leaving

To record the behavior of *T. radiata* released in the field, 34 female parasitoids that were offspring resulting from the panmictic mating laboratory culture (1–5 days old) were released and observed on 34 *D. citri* colonies (patch) infesting citrus flush. The number of third- to fifth-instar nymphs in colonies ranged from 7 to 31. Parasitoids were transported to field sites as mixed-sex groups of 10–20 individuals in 2 × 5 cm high glass vials provisioned with a drop of honey and sealed with a ventilated plastic cap. Field-released females did have access to *D. citri* nymphs for host feeding. Prior to parasitoid release, ants tending *D. citri* colonies on selected flush were manually removed from experimental patches (24 out of the 34 observed patches contained ants). The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), was the only ant species observed tending *D. citri* colonies. Parasitoids were released by holding the lip of the open vial on the margin of the *D. citri* colony. Once one female parasitoid walked onto the *D. citri* colony, the vial was removed and the following events were recorded: frequency and time spent probing hosts with the ovipositor, ovipositing, host feeding, patch abandonment prior to ovipositor probing of nymphs, and contact with ants that recolonized patches after initial manual removal. Females that left patches within 30 s of making contact with the flush infested with *D. citri* nymphs, or did not make contact with nymphs in the experimental colony, were removed from analyses. Observations of field-released female *T. radiata* were performed across 11 urban gardens on a total of 34 citrus trees infested with *D. citri* in southern California from 26 October to 9 November 2012 (Table 1).

Statistical analysis

The ANOVA test was used to determine whether significant differences existed for laboratory data between the mean time spent by host-deprived and non-host-deprived parasitoids to lay an egg and to probe a host, as well as the total time spent in the patch. A Kruskal–Wallis test was used to compare the percentage of time spent (i.e., time partitioning) in each behavior by host-deprived and non-host-deprived parasitoids. A linear model assuming normal error variance was used to analyze the number of oviposition events as a function of the residence time in the patch. We examined all possible regression models with linear, power, log, exponential, and polynomial functions, and selected the model with the highest coefficient of determination within those tested that satisfied the underlying model assumptions.

Generalized linear models were used to compare the mean number of hosts parasitized and probed per patch, as well as the proportion of *T. radiata* females that parasitized at least one *D. citri* nymph per patch and the proportion of females that left the patch prior to contacting a host. A Poisson error variance was assumed for the number of hosts parasitized and probed per patch, and a binomial error variance was assumed for the probability that *T. radiata* females parasitized at least one *D. citri* nymph in the patch. The assumed error structures were then assessed with a heterogeneity factor equal to the residual deviance divided by the residual degrees of freedom. If over- or underdispersion was detected, the significance of the explanatory variables was reevaluated with an F-test after rescaling the statistical model with a Pearson's χ^2 value divided by the residual degrees of freedom (Crawley, 2007). The statistical software package 'R' v.3.1.2 (http://www.R-project.org) was used for all analyses which were conducted at the 0.05 level of significance.

Table 1 Sampling dates and field sites, number of *Diaphorina citri* colonies sampled per site, and citrus varieties onto which *Tamarixia radiata* was released

Sampling date	Locality	Zip code	Citrus variety	No. *D. citri* colonies/tree
26/10/2012	Fullerton	92833	Valencia	3
		92835	Navel	1
		92833	Navel	4
	Pico Rivera	90660	–	2
			Navel	2
			Navel	3
02/11/2012	Calimesa	92320	Lime	2
	Riverside	92509	Valencia	4
07/11/2012	Riverside	92509	Valencia	3
			Valencia	3
			Valencia	1
09/11/2012	Pico Rivera	90660	Navel	2
	Pomona	91767	Lemon	2
			Valencia	2
Total				34

–, unknown.

Results

Duration of host use events in the laboratory

Tamarixia radiata females parasitized a total of 81 *D. citri* nymphs (65 were parasitized by non-deprived and 16 were parasitized by host-deprived females), probed and rejected 219 (184 by non-deprived, 35 by host-deprived females), host fed on 12 (only one nymph was fed on by non-deprived, 11 nymphs were fed on by host-deprived females), and failed to host feed on seven nymphs despite ovipositor probing (all by host-deprived females). The time females needed to oviposit ranged from 50 to 550 s. Mean oviposition times were not significantly different but was marginally longer for host-deprived than for non-host-deprived females: 241.1 ± 60.7 s ($n = 20$) vs. 151.6 ± 18.6 s ($n = 4$) ($F_{1,22} = 3.36$, $P = 0.08$). The time females spent probing and rejecting nymphs ranged from 5 to 650 s and it was independent of previous host deprivation—mean probing time for host-deprived vs. non-host-deprived females: 51.7 ± 9.2 s ($n = 22$) vs. 46.8 ± 6.0 s ($n = 10$) ($F_{1,27} = 0.22$, $P = 0.65$). Host feeding (i.e., host probing and subsequent consumption of hemolymph) took on average 1 103.2 ± 93.3 s ($n = 11$ host-deprived females and 1 non-host-deprived female combined). Six host-deprived females failed to feed on seven nymphs that they probed and these nymphs were rejected by females. These females spent an average of 1 077.1 ± 257.8 s ($n = 6$) (ranging from 440 to 2 205 s) on failed host-feeding events.

Effect of host deprivation on patch use and time partitioning

The mean total time spent by *T. radiata* females foraging for 12 *D. citri* nymphs in a patch was independent of host exposure treatments (i.e., non-host-deprived vs. host-deprived females: 1 817.0 ± 232.1 vs. 2 334.1 ± 369.4 s; $F_{1,36} = 1.55$, $P = 0.22$). However, time partitioning across behavioral events differed between host- and non-host-deprived females (searching: $K = 6.63$, $P = 0.01$; probing: $K = 11.92$, $P = 0.0006$; ovipositing: $K = 13.25$, $P = 0.0003$; resting and grooming: $K = 0.11$, $P = 0.74$; host feeding: $K = 18.78$, $P<0.0001$; Figure 1). Non-host-deprived females spent most of their time searching (46.1%), ovipositing (24.6%), and probing (20%), whereas little time was spent host feeding (1.6%). In comparison, host-deprived females spent the majority of time engaged in host-feeding events (successful host-feeding events comprised 35.5% of total time; failed host-feeding 12.8%; searching 31.5%; resting 9.9%; ovipositing 6.4%; and probing 4%; Figure 1).

On average, non-host-deprived females probed and parasitized more *D. citri* nymphs than host-deprived females (probed hosts: $F_{1,36} = 7.18$, $P = 0.011$; parasitized hosts: $F_{1,36} = 7.74$, $P = 0.0084$; Figure 2). Similarly, the proportion of *T. radiata* females that parasitized at least one *D. citri* nymph was higher for females without host deprivation than for females deprived of hosts: 0.91 ± 0.06 vs. 0.25 ± 0.11 ($F_{1,36} = 18.99$, $P = 0.0001$). Independent of host deprivation, *T. radiata* females did not parasitize all *D. citri* nymphs available in the patch. The maximum number of parasitized hosts during the experimental time exposure (ca. 30 min) was seven nymphs for both host- and non-host-deprived females. The cumulative number of parasitism events for non-host-deprived females increased steadily during the first 1 000 s of the experiment (Figure 3). Females laid an average of three eggs during this period, and they needed an average additional ca. 1 000 s to lay a fourth egg.

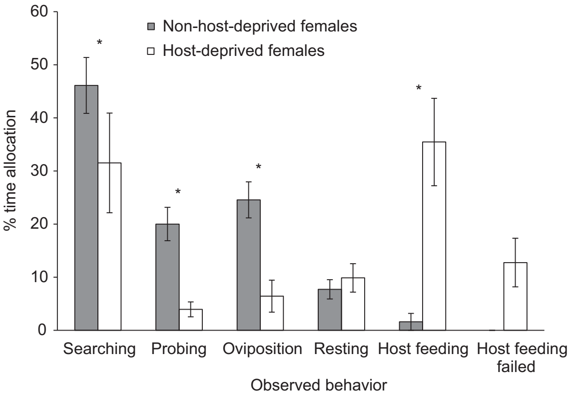

Figure 1 Time partitioning among six categories of behavior (%; mean ± SE) of non-host- vs. host-deprived *Tamarixia radiata* females searching in a patch of 12 *Diaphorina citri* nymphs. Asterisk indicates a significant difference between females within a type of behavior (Kruskal–Wallis test: $P<0.05$).

One of the 22 (5%) non-host-deprived females and 11 of the 16 (66%) host-deprived females engaged in host feeding when exposed to *D. citri* nymphs (Figure 2). Moreover, 6 of the 11 (54.5%) host-deprived females failed to host feed when they probed the host. In the three instances that females were observed to parasitize and host feed in the same patch, host feeding always occurred after parasitism.

First host use in the laboratory and field

Field observations of host-deprived *T. radiata* females released onto *D. citri* patches differed from behavior sequences observed in the laboratory (Figure 4). Oviposition events were rare in the field when *T. radiata* females encountered and probed the first nymph. Eleven of 34 (32.4%) females observed in the field probed the first nymph encountered. Five of these females engaged in host feeding (14.7%) after probing nymphs. Since we could not observe whether *D. citri* fluids oozed from the wounded nymphs in the field, we cannot confirm that *T. radiata* fed. Five probing events out of the other six events observed in the field were shorter than 50 s, which is the laboratory-derived minimum time for a probing event that ends in oviposition. Consequently, only 1 of the 34 (2.9%) females observed might have parasitized the first probed nymph. In the laboratory, *T. radiata* females laid an egg under 13 of 17 nymphs (76.5%) that were probed for more than 50 s.

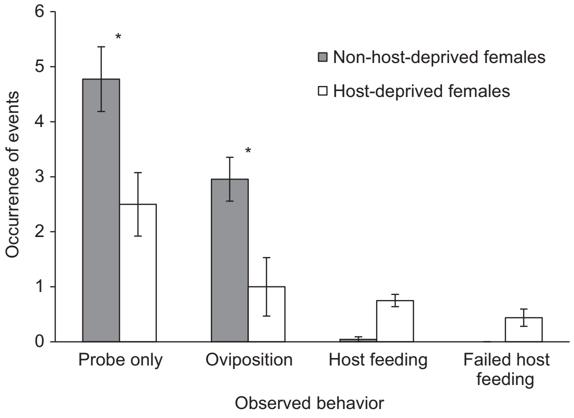

Figure 2 Mean (± SE) number of *Diaphorina citri* nymphs probed, parasitized, successfully host fed, and unsuccessfully host fed on by non-host- vs. host-deprived *Tamarixia radiata* females searching in patches of 12 nymphs. Asterisk indicates a significant difference between females within an event [GLM (semi-Poisson distribution): $P<0.05$].

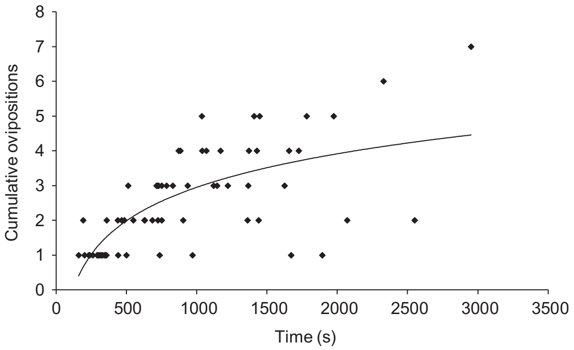

Figure 3 Number of oviposition events for *Tamarixia radiata* as a function of residence time in a patch of 12 *Diaphorina citri* nymphs (equation of the line: $\text{oviposition} = 0.048^{*}\text{time}^{0.58}$; $R^2 = 46.7$; $F_{1,63} = 55.19$, $P<0.0001$).

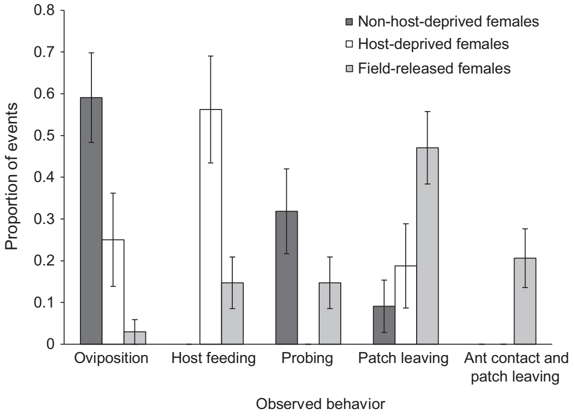

Figure 4 Mean (± SE) proportion of five behavioral events of *Tamarixia radiata* when females encountered the first host in a patch of 12 nymphs in the laboratory and colonies of 7–31 nymphs of *Diaphorina citri* in the field. Laboratory females include non-host- and host-deprived females.

A mismatch between laboratory and field observation was evident with 'patch leaving'. Most *T. radiata* females (23 out of 34: 67.7%) left the patch before probing at least one *D. citri* in the field, whereas only 5 of 38 *T. radiata* females (13.2%) left the patch in the laboratory (including both host- and non-host-deprived females) ($F_{1,71} = 23.86$, $P<0.0001$) (Figure 4). In the field, 7 of these 23 females (30%) left the patch because of contact with foraging ants.

Discussion

In the field, our observational data indicated that *T. radiata* females tend to abandon patches of appropriately aged *D. citri* nymphs without probing. Even though we removed foraging *L. humile* from experimental patches and excluded replicates where females left the patch prior to 30 s or did not make contact with hosts, 68% of released females abandoned patches without using hosts. This result can be partially explained by the presence of *L. humile*, which returned to patches and disturbed the foraging parasitoids in 21% of observations. *Tamarixia radiata* on *D. citri* patches were sensitive to close approaches of *L. humile*, which caused flight responses. During previous field releases of *T. radiata*, we observed *L. humile* capturing foraging parasitoids and returning this prey to nests. Consequently, although *L. humile* has not impeded the establishment of *T. radiata* in urban citrus in Southern California (Hoddle & Hoddle, 2013; Hoddle et al., 2016), the presence of this ant might have retarded rates of establishment and spread. After the 1st year of releases, *T. radiata* had been recovered in the majority of release sites and it had spread unassisted up to 13 km from the nearest release areas (Hoddle & Hoddle, 2013; Hoddle et al., 2016). These results indicate that *T. radiata* has been able to find and parasitize *D. citri* patches, possibly exploiting those that were unattended by *L. humile*. Tena et al. (2013) reported that for residential citrus *L. humile* tend more than 55% of the *D. citri* colonies composed of fourth and fifth nymphal instars on infested trees, the same life stages onto which parasitoids were released for this study. The intensity of the mutualism between *L. humile* and *D. citri* depends, among other factors, on the densities of *D. citri* patches (Tena et al., 2013). The probability that a *D. citri* colony was tended by ants was almost 100% when colonies had more than 20 nymphs, whereas <40% of colonies with fewer than 10 nymphs were tended (Tena et al., 2013). Consequently, *T. radiata* might exploit low-density patches more easily than high-density patches, without being attacked by *L. humile*. Therefore, to minimize interference from *L. humile*, *T. radiata* releases should be made directly onto *D. citri* patches that are not tended by ants, or if unattended colonies are not available, parasitoids should be released directly on patches comprised of 20 or fewer nymphs as ant attendance will be less intensive. If this is not possible, then pre-emptive ant control measures (e.g., deployment of liquid ant baits) at pre-selected release sites several days in advance of parasitoid releases may be beneficial.

The discrepancies in host use between laboratory and field experiments as reported here may also be due to causes that were not quantified. For example, in one of the few studies comparing parasitoid foraging results from both laboratory and field experiments, Casas et al. (2004) found that *Aphytis melinus* DeBach, a parasitoid of armored scales infesting citrus, rejected hosts in the field at much higher rates than in the laboratory. This result occurred because 90% of encounters in the field were with dead hosts, which accumulated on twigs and fruits (Casas et al., 2004). However, encounters with dead *D. citri* nymphs by *T. radiata* females was likely not the cause of the early departure, as dead nymphs are readily identifiable and were seldom observed in experimental patches. It is possible that premature patch abandonment by *T. radiata* resulted from shadows or perceived movement of the person releasing parasitoids onto patches. Additionally, time of day, affected by light intensity and ambient temperature at time of release, may have increased the propensity of *T. radiata* to fly after release from vials. However, despite these factors that could have influenced premature patch abandonment, we excluded from analyses females that left patches within 30 s of making contact with the flush or did not make contact with *D. citri* nymphs. These field observations underscore our lack of knowledge about host searching and host location by *T. radiata* foraging for *D. citri* nymphs in citrus.

The slow host-handling for oviposition and host feeding observed in the laboratory may confer a reproductive disadvantage to *T. radiata* when ants are tending patches of *D. citri* nymphs in the field. Female *T. radiata* needed almost 3 min to lay an egg in a forth-instar nymph and >18 min to host feed on the same life stage. These long periods increase the risk to *T. radiata* of disturbance and predation by ants, with the latter being greater during oviposition when female parasitoids have reduced mobility (Weisser et al., 1994; Heimpel et al., 1997; Völkl & Kroupa, 1997; Barzman & Daane, 2001; Martinez-Ferrer et al., 2003). Although there are examples of natural enemies that are able to forage in ant-tended patches (Mackauer & Völkl, 1993), our field observations indicated that *T. radiata* is harassed by foraging *L. humile*. This ant species also disrupts parasitism of other common parasitoids of scale citrus pests such as *A. melinus* and the encyrtid *Metaphycus* spec. (Martinez-Ferrer et al., 2003; Choe & Rust, 2006). Taking into account that *T. radiata* is sensitive to disturbance by ants, the long time needed to host feed, and the fact that in some cases females fail to host feed, releases of parasitoids that have host fed could minimize the risk of negative interactions with ants tending *D. citri* patches.

In the laboratory, host-deprived females tended to host feed before abandoning patches without parasitizing *D. citri* nymphs. *Tamarixia radiata* is a synovigenic parasitoid, and nutrients obtained from host feeding are generally used to mature eggs in these parasitoids (Jervis et al., 2008). Interestingly, female *T. radiata* deprived of hosts for up to 10 days can lay eggs when maintained in the laboratory at 25 °C (Chien & Chu, 1996). Importantly, the sequence of patch use was not reported by Chien & Chu (1996) and it is unknown whether host-deprived females needed to host feed prior to the 10 days host deprivation period in order to start laying the eggs after this elapsed time. We can think of several explanations for *T. radiata* females abandoning patches of *D. citri* nymphs after host feeding. Firstly, females may have no mature eggs for oviposition as some parasitoid species need lengthy periods, perhaps as much as 1 day, to mature the eggs after host feeding (Heimpel et al., 1998; Casas et al., 2000). The length of time needed for *T. radiata* to mature eggs is unknown. Alternatively, host fed females might contain mature eggs but they need to rest after host feeding before they can initiate oviposition behaviors. For example, *A. melinus* rests after host feeding (A Tena, pers. obs.). Additionally, Hougardy & Mills (2007) reported that the majority of host-deprived parasitoids used in field assays rested after being released, even though they were in good physiological condition to lay eggs.

In our laboratory assays, non-host-deprived *T. radiata* parasitized an average of three *D. citri* nymphs per patch. As with other parasitoids (Cronin & Strong, 1993; Völkl, 1994; Weisser et al., 1994), *T. radiata* underexploited host patches as females parasitized only 25% of the nymphs available in a patch comprised of 12 nymphs within ca. 30 min. This rate of deposition equates to an average of ca. 30% of the eggs laid by *T. radiata*, as 2-day-old females lay on average 12 eggs over a 24-h period (Chu & Chien, 1991). Interestingly, Chu & Chien (1991) also reported patch underexploitation when females were constrained to a single patch of 20 nymphs over a 24-h period. Several hypotheses have been suggested to explain the phenomenon of underexploitation of host patches by parasitoids (for a detailed discussion see Heimpel & Casas, 2008). For classical biological control programs, seemingly premature patch abandonment may be positive because it may facilitate the spread of newly introduced parasitoid species (Hougardy & Mills, 2006). Mills & Wajnberg (2008) further suggest that patch-choice decisions by parasitoids that determine the distribution of parasitoid foraging effort among patches are more likely to influence the impact of classical biological control agents on target pest populations than foraging decisions made within host patches.

Overall, observations of *T. radiata* released on *D. citri* patches in the field do not correspond with laboratory observations. In the field, *T. radiata* females exhibited a high tendency to leave patches on which they were released without parasitizing *D. citri* nymphs. However, laboratory and field assays provided results that can guide releases of *T. radiata* in Southern California and other citrus-producing regions where this parasitoid is being considered for introduction as part of a classical biological control program. First, *T. radiata* is sensitive to interference from ants tending *D. citri* nymphs. Consequently, if *D. citri* is tended by ants we recommend releasing parasitoids in trees without ants or implementing ant suppression programs prior to release. If this option is not feasible, *T. radiata* should be released close to small colonies (<20 *D. citri* nymphs) to minimize antagonistic ant interactions. Second, host-deprived parasitoids tend to host feed and disperse in the laboratory and field. Considering the long time *T. radiata* needs to host feed, and that some females fail to successfully host feed and need to repeat the process, the release of non-host-deprived females is recommended so oviposition events are more likely to occur upon first contacts with *D. citri* nymphs following releases onto host patches in the field.

Acknowledgements

We thank Anna Soper for providing psyllids, host plants, and parasitoids to conduct laboratory experiments. Christina Hoddle, UCR, and Grace Radabaugh, CDFA, assisted with field observations. We thank cooperating homeowners in Southern California for unlimited access to citrus in their gardens. This work was supported, in part, by funds from the Citrus Research Board to RS (5500-196) & MSH (5500-194) and the California Department of Food and Agriculture's Specialty Crop Grant SCB10007 awarded to MSH. AT was a recipient of a postdoctoral fellowship from the MCINN (Juan de la Cierva program) and his research at UCR was funded by the Marie-Curie International Research Exchange Scheme No. 269196 (Programme: FP7-PEOPLE-2010-IRSES).

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