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# Laboratory Evaluations of the Foraging Success of Tamarixia radiata (Hymenoptera: Eulophidae) on Flowers and Extrafloral Nectaries: Potential use of Nectar Plants for Conservation Biological Control of Asian Citrus Psyllid (Hemiptera: Liviidae)

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# Laboratory evaluations of the foraging success of *Tamarixia radiata* (Hymenoptera: Eulophidae) on flowers and extrafloral nectaries: potential use of nectar plants for conservation biological control of Asian citrus psyllid (Hemiptera: Liviidae)

*Joseph M. Patt[1],\* and Eric Rohrig[2]*

## Abstract

*Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) is a specialist parasitoid of late-instar nymphs of Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), a vector of the causal agent of huanglongbing disease of citrus (Sapindales: Rutaceae). *Tamarixia radiata* is mass reared; however, parasitism levels following inundative releases have remained relatively low. One possible explanation for the low parasitism levels is the lack of sugar resources available for adult wasps in targeted release landscapes, such as abandoned commercial citrus groves and residential areas. Establishing nectar plants can be an effective means of increasing nutritional resources in targeted sites for biocontrol agents. Some eulophids forage effectively only on fully exposed nectaries, i.e., those unobstructed by other floral parts. Therefore, care must be taken to select plants that possess nectary architecture compatible with parasitoid morphology and foraging ability. A series of laboratory studies were undertaken as a first step to determine the potential for *T. radiata* to obtain sugar from natural sources in target landscapes. Following contact with a sugar spot on filter paper, the wasps engaged in stereotypical zigzagging movements, demonstrating that contact with sugar induced arrestment and induction of localized searching behavior. *Tamarixia radiata* fed on sugars found in nectar (sucrose, glucose, fructose) and honeydew (melizitose, raffinose), indicating that it should feed well on both nectar and honeydew resources. At the highest concentration tested (1 M), it preferred sucrose and melizitose, whereas at the lowest concentration tested (0.1 M), it preferred sucrose and glucose. There was no preference among sugars when tested at the 0.5 M concentration. Choice tests with wasps previously exposed to scented sucrose solution showed that the wasps were stimulated and attracted by nectar odor and could learn to associate a particular odor with the presence of nectar. Observations conducted with starved wasps on freshly cut sprigs of nectar plants showed that foraging success was highest on extrafloral nectaries and flowers with exposed nectaries. The wasps readily located the extrafloral nectaries on snap bean and cowpea, and fed on them for the majority of the observation periods. Likewise, they quickly located the fully exposed nectaries in the inflorescences of euphorbiaceous plants, such as *Euphorbia heterophylla* L. (Malpighiales: Euphorbiaceae). Foraging success declined in flowers with nectaries that were only partially exposed; the wasps' movements were deterred by floral parts or trichomes that obstructed the nectary. The wasps were unable to obtain nectar from composites and other plants with hidden nectaries. Overall, the results of these laboratory evaluations showed that *T. radiata* responds to sugars and chemical cues associated with nectaries and is capable of foraging on extrafloral nectaries and flowers with exposed nectaries.

Key Words: *Diaphorina citri*; citrus greening; huanglongbing; associative learning

## Resumen

*Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae) es un parasitoide especializado en los últimos instares de ninfas de del sílido asiático de los cítricos, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), un vector del agente causal de la enfermedad huanglongbing de los cítricos (Sapindales: Rutaceae). *Tamarixia radiata* se ha criado en masa; sin embargo, el nivel de parasitismo después de altas liberaciones se ha mantenido relativamente bajo. Una posible explicación para el nivel de parasitismo bajo es la falta de recursos de azúcar disponibles para las avispas adultas en los lugares del enfoque donde fueron liberados, tales como las plantaciones de cítricos comerciales abandonadas y las áreas residenciales. El establecimiento de plantas de néctar puede ser un medio eficaz de incrementar los recursos nutricionales en sitios específicos para agentes de control biológico. Algunos eulófidos forrajen efectivamente sólo en nectarios completamente expuestos, como aquellos que no están obstruidos por otras partes florales. Por lo tanto, se debe tener cuidado de seleccionar plantas que poseen la arquitectura nectaria compatible con la morfología del parasitoide y su capacidad de forrajeo. Se realizó una serie de estudios de laboratorio como primer paso para determinar el potencial de *T. radiata* para obtener azúcar de fuentes naturales en los lugares de enfoque. Después del contacto con una mancha de azúcar en papel de filtro, las avispas empezaron a moverse en movimientos estereotípicos en forma zigzag demostrando que el contacto con el azúcar indujo el arresto y la inducción de un comportamiento de búsqueda localizado. *Tamarixia radiata* se alimenta de azúcares que se encuentran en el néctar (sacarosa, glucosa, fructosa) y mielcilla (melizitosa, rafinosa), lo que indica que debe alimentarse bien con recursos de nectar y mielcilla. A la concentración más alta probada (1 M), prefería la sacarosa y la melizitosa, mientras que a la concentración más baja probada (0,1 M) prefería la sacarosa y la glucosa. No hubo preferencia entre los azúcares cuando se probaron a la concentración de 0,5 M. Las

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pruebas de elección con avispas previamente expuestas a la solución de sacarosa con olor mostraron que las avispas fueron estimuladas y atraídas por el olor del néctar y pudieron aprender a asociar un olor particular con la presencia de néctar. Las observaciones realizadas con avispas hambrientas en ramitas recién cortadas de plantas de néctar mostraron que el éxito en el forrajeo fue mayor en nectarios extraflorales y flores con nectarios expuestos. Las avispas localizaron fácilmente los nectarios extraflorales en el frijol y el caupí, y se alimentaron de ellos durante la mayoría de los períodos de observación. Asimismo, localizaron rápidamente los nectarios totalmente expuestos en las inflorescencias de plantas euforbiáceas, tales como *Euphorbia heterophylla* L. (Malpighiales: Euphorbiaceae). El éxito del forrajeo disminuyó en flores con nectarios que sólo fueron parcialmente expuestos; los movimientos de las avispas fueron disminuidos por partes florales o tricomas que obstruyeron el nectario. Las avispas no pudieron obtener néctar de compuestos y otras plantas con nectarios ocultos. En general, los resultados de estas evaluaciones de laboratorio mostraron que *T. radiata* responde a azúcares y señales químicas asociadas con nectarios y es capaz de buscar forraje en nectarios extraflorales y flores con nectarios expuestos.

Palabras Clave: *Diaphorina citri*; enverdecimiento de los citricos; huanglongbing; aprendizaje asociativo

The Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), vectors the causal agent of huanglongbing or citrus greening, a disease that is destroying citrus groves at an alarming rate (Grafton-Cardwell et al. 2013; Hall et al. 2013). Asian citrus psyllid readily moves between commercial citrus groves and nearby residential areas and abandoned groves, underscoring the need to suppress *D. citri* at the landscape level to reduce the threat of huanglongbing (Halbert & Manjunath 2004; Boina et al. 2009; Tiwari et al. 2010; Lewis-Rosenblum et al. 2015). Immatures of *D. citri* are highly vulnerable to predation by generalist predaceous arthropods such as lady beetles, lacewings, and hunting spiders (Michaud 2004; Qureshi & Stansly 2009; Chong 2010). Late-instars of *D. citri* also are vulnerable to attack by *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae), an ectoparasite native to southern Asia (Chen & Stansly 2014a). In an attempt to suppress *D. citri* populations through inundative releases, *T. radiata* mass rearing programs have been established in Florida, Texas, and California. Abandoned citrus orchards and residential areas near commercial citrus groves are the primary target landscapes for *D. citri* suppression using releases of *T. radiata*; the frequency of insecticide applications used to suppress *D. citri* in commercial citrus groves renders them inhospitable to arthropod biological control agents (Qureshi et al. 2009, 2014; Hall & Nguyen 2010; Beloti et al. 2015).

Parasitism rates of Asian citrus psyllid by *T. radiata* have reached 70% at some localities in Florida (Hall & Rohrig 2015), but most reported parasitism levels in Florida and California have been much lower (Qureshi & Stansly 2009; Qureshi et al. 2009; Kistner et al. 2016). Females of *T. radiata* puncture nymphs of *D. citri*, killing them in the process, and feed on the nymphs' hemolymph, which provides protein and fat needed by female wasps to produce additional eggs (Chen & Stansly 2014b). Nymphal mortality from hemolymph feeding may be relatively high and may help explain why psyllid abundance has decreased in release areas with low parasitism levels (Kistner et al. 2016).

Several explanations (Qureshi & Stansly 2009; Hall & Nguyen 2010; Tena et al. 2013; Hall & Rohrig, 2015; Kistner et al. 2016) have been put forth to explain the low incidence of *D. citri* parasitism observed following release of *T. radiata*: 1) low genetic diversity of the released wasp haplotypes combined with poor performance by those haplotypes in their release environments; 2) intraguild predation by other natural enemies of *D. citri*; 3) interference from ants tending *D. citri* nymphs; 4) frequent trimming of shoots containing suitable or parasitized nymphs; 5) mortality inflicted by insecticide applications; and 6) adverse weather and climatic conditions.

Another limiting factor in wasp establishment may be a lack of non-host nutritional resources within the release site. The adults of many parasitoid species feed on nectar from flowers and extrafloral nectar glands, and on honeydew excreted by phloem-feeding insects (Leius 1960; Proctor & Yeo 1972 Jervis et al. 1993; Wäckers et al. 2005; Russell 2015). Sugar from these sources provides them with the energy needed to search for oviposition hosts and mates. In addition to hemolymph feeding, *T. radiata* also benefits from sugar feeding. For example, providing caged *T. radiata* wasps with honey improved their survivorship (Chen & Stansly 2014a), and mass rearing programs sup-

ply the wasps with diluted honey. In nature, *T. radiata* is known to feed on *D. citri* honeydew (Tena et al. 2013; Chen & Stansly 2014b), but it has not been observed feeding on floral or extrafloral nectar.

Augmentation with plants that provide floral and extrafloral nectar can be an effective means of increasing sugar resources in a variety of target landscapes (Leius 1967; Patt et al. 1997a; Landis et al. 2000; Ellis et al. 2005; Gurr et al. 2005; Heimpel & Jervis 2005; Wäckers et al. 2005; Fiedler et al. 2008; Lundgren 2009; Jonsson et al. 2010; Brennan 2013, 2016). In addition to sugars, extrafloral nectar has low concentrations of amino acids (Heil 2015); therefore, plants with extrafloral nectaries should be beneficial to *T. radiata*. Whether augmentation with nectar-producing plants in target landscapes will result in positively influencing *T. radiata* populations or not will necessitate evaluation of wasp retention and parasitism levels in experimental sites with nectar plants versus control sites lacking those plants.

The first step in this process is selecting candidate nectar plants for field testing. Because of their small size and lack of specialized mouthparts, certain chalcid wasp species can efficiently forage only in flowers with nectaries that are fully exposed or only slightly obstructed by petals or other floral parts (Patt et al. 1997b). They may have difficulty moving within folded or constricted floral structures to obtain nectar, or be repulsed by patches of trichomes on the petals or other floral parts. For these reasons, care must be taken to select plant species with floral architectures that are compatible with the morphology and foraging abilities of the target species. For example, many flowering ornamental plants have been bred for showy flowers, and their nectaries are either absent or hidden by multiple whorls of petals. If residential areas and commercial landscapes are the target landscape for *T. radiata* release, then nectar plants for these sites will need to have both the requisite floral architectures and ornamental value. Because extrafloral nectaries have evolved to be accessible to predaceous and parasitic arthropods (Wäckers et al. 2005; Lundgren 2009), and they have not been subjected to artificial selection, the selection of plant species with extrafloral nectaries with respect to nectary accessibility is less problematic.

Here we describe the results of a series of laboratory studies undertaken as a first step to determine the potential for *T. radiata* to obtain sugar from nectar plants in target landscapes. These studies examined 1) whether *T. radiata* foraging movements are arrested following contact with sugar; 2) whether *T. radiata* has a preference for sugars from either honeydew or nectar; 3) the ability of *T. radiata* to obtain nectar from extrafloral nectaries and from flowers that varied in their degree of nectary accessibility; and 4) whether *T. radiata* can learn to recognize chemosensory cues associated with nectar.

## Materials and Methods

### PARASITOID SOURCE

The *T. radiata* wasps used for these studies were provided by the Florida Department of Agriculture and Consumer Services, Gainesville,

Florida. The wasps were shipped via overnight mail in vials containing water wicks and filter paper spotted with diluted honey (1:1 v/v honey-to-water ratio). Upon arrival, the wasps were transferred to nylon screen cages (30 cm L × 30 cm H × 30 cm W) supplied with water stations and filter paper spotted with diluted honey. The cages were held in an incubator with a temperature of 25 ± 1 °C and a 8:16 h L:D photoperiod. Wasps used in the tests were 7 to 14 d old. Aspirators were used to transfer the wasps from the holding cage to the experimental arenas. Only female wasps were used in the experiments.

SUGAR PREFERENCE

Observations were conducted for *T. radiata* feeding behavior on sugars commonly present in either nectar (sucrose, glucose, fructose) or honeydew (maltose, raffinose, melizitose) (Sigma-Aldrich, St. Louis, Missouri) to determine if 1) foraging movements were arrested following contact with sugar; and 2) the wasps preferred sugars from honeydew or nectaries. Prior to the observations, the wasps were starved overnight in cages furnished with water dispensers to increase their hunger levels. For each observation, individual wasps were placed on a filter paper strip (5 mm wide × 25 mm long; No. 1, Qualitative, Whatman, Maidstone, United Kingdom) spotted with a 100 μL aliquot of a single test sugar. Three concentrations (0.1, 0.5, or 1.0 M) of each sugar solution were used in the tests.

To facilitate observation of the wasp's behavior, the paper strip was mounted to a clip that, in turn, was positioned under the objective of a stereomicroscope (Model SZX9, Olympus Corporation, Tokyo, Japan). Feeding behavior was verified by observing the wasp on a computer monitor connected to a video camera (Model acA1300-60g, Basler AG, Ahrensburg, Germany) mounted on the stereomicroscope. To control for thirst, control spots consisting of distilled water were also presented to the wasps. To assist the observer in visualizing the sugar spot and wasp feeding behavior, the solutions were colored light pink by the addition of 50 μL of red food coloring (McCormick & Co., Inc., Hunt Valley, Maryland) to 10 mL of each stock sugar solution and the water control. Each wasp was released within a few mm of the sugar spot and the observation initiated when the wasp began to crawl on the strip. Wasps that flew from the strip within 30 s after placement were excluded from the study.

Each observation lasted until the wasp flew from the strip or until 300 s had elapsed. The amount of time each wasp spent feeding on the sugar spot was recorded for each observation period. The numbers of wasps tested on each sugar–concentration treatment were as follows: sucrose (0.1 M: $n = 14$; 0.5 M: $n = 21$; 1.0 M: $n = 36$); melizitose (0.1 M: $n = 6$; 0.5 M: $n = 14$; 1.0 M: $n = 19$); raffinose (0.1 M: $n = 5$; 0.5 M: $n = 13$; 1.0 M: $n = 10$); glucose (0.1 M: $n = 6$; 0.5 M: $n = 10$; 1.0 M: $n = 19$); fructose (0.1 M: $n = 3$; 0.5 M: $n = 10$; 1.0 M: $n = 19$); and maltose (0.1 M: $n = 6$; 0.5 M: $n = 8$; 1.0 M: $n = 7$).

EVALUATION OF WASP FORAGING ABILITY ON POTENTIAL NECTAR PLANT SPECIES

A series of observations were conducted to determine whether *T. radiata* could successfully forage for nectar from extrafloral nectaries and from the floral nectaries of plants that varied with respect to their floral architecture and horticultural, agronomic, and conservation utility. The plant species used in the observations were selected to encompass representatives exhibiting a variety of the following traits: 1) degree of nectary accessibility, i.e., fully exposed, partially exposed, partially hidden, and hidden (Fig. 1); 2) geographic origin, i.e., native species or exotic ornamental; and 3) utility, i.e., garden ornamental, agricultural groundcover, hardy in ruderal situations (Table 1). All of the candidate plant species selected for this study bloomed or produced extrafloral nectaries for 3 wk or more, because shorter nectar

production times would discount their utility as nectar sources in target landscapes.

Test plants of each species were grown in pots in an outdoor research garden in Fort Pierce, Florida. Prior to the observations, inflorescences or sprigs with extrafloral nectaries were cut with a razor blade and placed in tap water; the submerged ends were cut again to prevent wilting and inserted into a 10 mL vial filled with tap water. The plant specimens were brought to the laboratory immediately and then positioned on the stage of a stereomicroscope for observation. Wasps were starved overnight, placed on flowers or extrafloral nectaries, and their foraging behavior observed until they left the sprig or 300 s had elapsed. The wasps were placed on the sprig a few mm below the flowers or extrafloral nectaries and allowed to move freely about the sprig, and were observed using the equipment described for the sugar preference experiment. Observations were facilitated by the use of a computer screen attached to a video camera mounted on the stereomicroscope. Observations were conducted between 10 AM and 3 PM. For each wasp, the durations of the following behaviors were scored: crawling, sitting, grooming, and feeding on nectar. The numbers of wasps tested on each plant species are shown in Table 1.

CONDITIONED RESPONSE TO NECTAR ODOR

A choice test was performed to determine whether *T. radiata* could learn to recognize aroma cues associated with nectar. We followed the method used by Patt et al. (1999) to examine similar behavior in 2 other eulophid species. The choice test arena consisted of 16 individual cups (3 mm high × 4.0 mm wide) attached to the backside of a glass Petri dish (9.0 cm in diameter). The cups were arranged into an inner (3.0 cm diameter) and outer ring (3.5 cm in diameter) of 8 cups each, with each cup situated 5 mm apart from its nearest neighbor. Each cup contained a 10 μL aliquot of artificial nectar; the control nectar consisted of 1.0 M sucrose solution and the scented nectar consisted of a 1:1 (v/v) mixture of 1.0 M sucrose solution and banana flavor extract (McCormick & Co., Inc., Hunt Valley, Maryland) (after Lewis & Takasu 1990). The control and scented nectars were placed in alternating cups.

Wasps were starved overnight as described for the sugar preference experiment. At the beginning of the assay, a single wasp was placed on a filter paper strip spotted either with 1.0 M sucrose solution or a 1:1 (v/v) mixture of 1.0 M sucrose solution and banana flavor extract, and was allowed to feed for 60 s. The nectar solutions were colored pink as described for the sugar preference experiment. Wasps that failed to feed for the entire 60 s pre-test conditioning period were discarded. Following the pre-test conditioning period, the wasp was gently transferred with an artist's paintbrush (size 0) to the center of the test arena and then permitted to walk freely and choose among the individual cups.

Discovering the nectar required the wasp to crawl up the side of the cup and enter it. Each wasp was observed for 300 s or until it either discovered a nectary or left the test arena. For each experiment, the number of wasps that discovered each type of nectary was recorded, whereas wasps that left the arena or failed to enter a nectary cup were recorded as non-respondents. Wasps that left the arena within 30 s following placement were discounted from the test. Each wasp was tested only once. Forty-eight wasps were pre-test exposed to the control sucrose solution and 55 wasps were pre-test exposed to the sucrose and banana flavor extract solution.

STATISTICAL ANALYSES

For the sugar preference test, feeding times within the same sugar solution concentration were compared among the different sugars with the Kruskal–Wallis test (ANOVA) as the data did not meet parametric assumptions. Significant ANOVA was followed by pairwise mul-
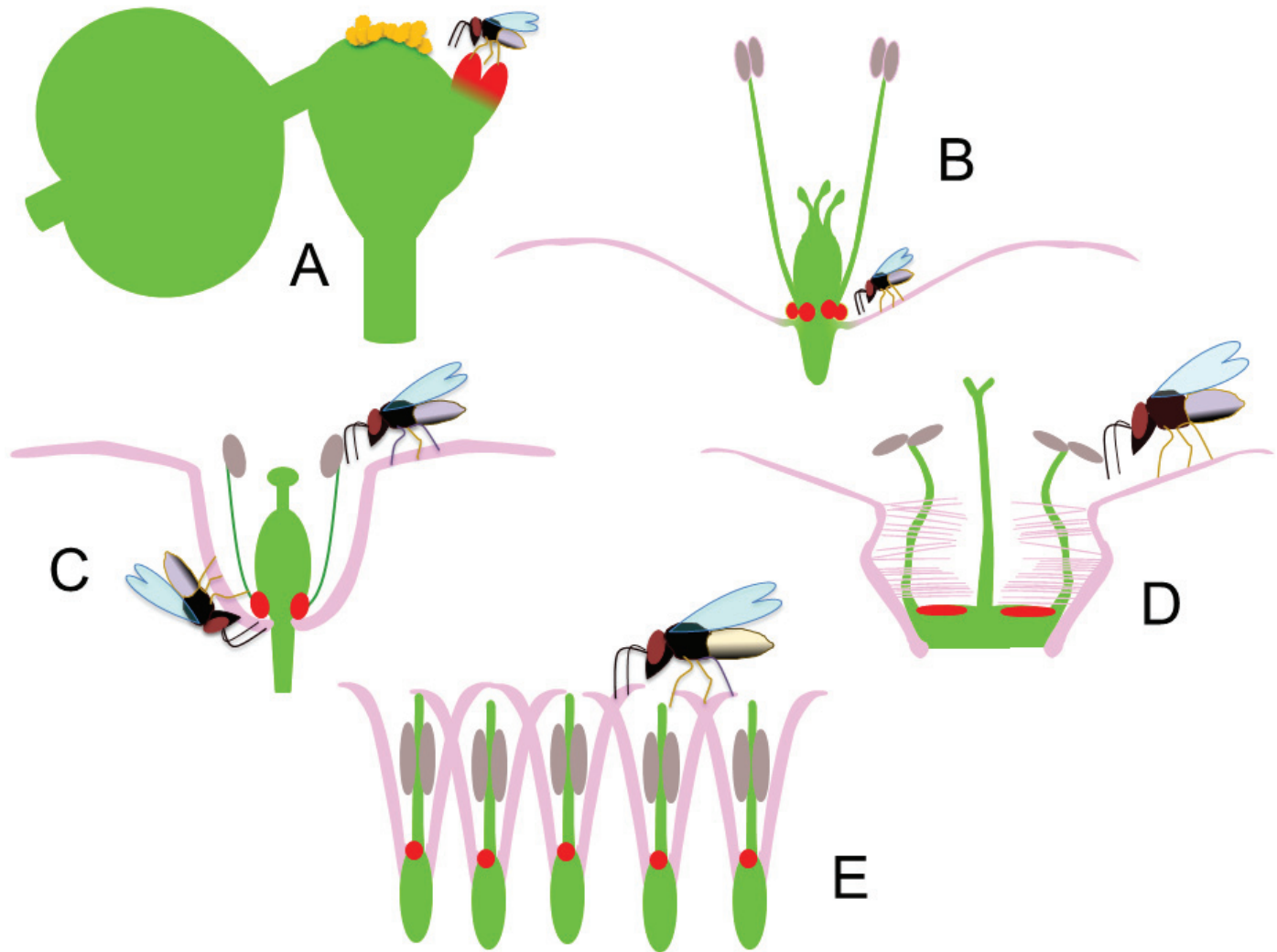
**Fig. 1.** Diagrammatic representation of nectary architectures presented to *Tamarixia radiata* in foraging evaluations. Location of nectaries shown in red. A. Cyathium of euphorbiaceous species with exposed nectaries. B. Partially exposed nectaries as found in buckwheat. C. Partially hidden nectaries as found in alyssum. D. Partially exposed nectaries covered with trichomes as found in marjoram. E. Hidden nectaries as found in composites. Drawings are only indicative of size and spatial relationships and are not to scale.

tiple comparisons using the Dunn method (SigmaPlot® software, version 11.2.0, Systat Software, Inc. San Jose, California). This procedure was also used to compare the mean percentage of observation time spent feeding on the nectaries of the various plant species tested. In the test of conditioned responses to nectar odor, a log–likelihood test (*G*-test) with the Yate correction was used to compare each cup selection and pre-test exposure treatment combination (Zar 1999) using a 1:1 observed-to-expected ratio. A significance level of $P \leq 0.05$ was used in all statistical tests performed.

## Results

### SUGAR PREFERENCE

When foraging *T. radiata* wasps contacted the sugar spot on the filter paper strip, they ceased movement and fed, showing that sugar feeding resulted in arrestment of the foraging movements. When presented with a water spot, the wasps drank briefly (mean $\pm SE$ = 9.1 ± 1.5 s, $n$ = 32); conversely, they fed extensively when presented with a spot of 1.0 M sucrose solution (263 ± 9.1 s, $n$ = 30). Following contact with

the sugar spot, the wasps engaged in stereotypical zigzagging localized searching movements before returning to the spot to feed further. The wasps fed on sugars from both nectar and honeydew; only maltose was not fed upon (Fig. 2). At the highest concentration, a preference was shown for sucrose and melizitose ($H$ = 36.52; df = 4; $P \leq 0.001$), and for fructose and glucose at the 0.1 M concentration ($H$ = 21.61; df = 5; $P \leq 0.001$). No preference was shown among the sugars tested at the 0.5 M concentration. These results indicate that, in nature, *T. radiata* will feed on sugars from nectar as well as from honeydew.

### EVALUATION OF WASP FORAGING SUCCESS ON POTENTIAL NECTAR PLANT SPECIES

*Tamarixia radiata* foraging success was highest on extrafloral nectaries and on flowers with exposed nectaries (Table 1) ($H$ = 97.36; df = 12; $P \leq 0.001$). When placed near the extrafloral nectaries of snap bean (*Phaseolus vulgaris* L.; Fabaceae) and cowpea (*Vigna unguiculata* [L.] Walp.; Fabaceae), all but one of the tested wasps located the extrafloral nectaries and the rest spent most of the observation period feeding on them. Likewise, most wasps readily located the completely exposed nectaries found in the inflorescences of plants in the family Euphor-

**Table 1.** Nectary accessibility and foraging success in *T. radiata*.

| Common name, species, and family | Nectar accessibility | Plant utility | No. of wasps | Wasps feeding on flowers or nectaries (%) | Mean observation time spent nectar feeding (%)[a] |
|---|---|---|---|---|---|
| Cowpea *Vigna unguiculata* Fabaceae | extrafloral nectaries | N-fixer, ground cover, grove border, green manure | 19 | 96 | 98a |
| Snap bean *Phaseolus vulgaris* Fabaceae | extrafloral nectaries | N-fixer, ground cover, grove border, green manure | 23 | 100 | 87a |
| Crown-of-thorns *Euphorbia milii* Euphorbiaceae | exposed | ornamental | 40 | 98 | 79a |
| Wild poinsettia *Euphorbia heterophylla* Euphorbiaceae | exposed | native | 14 | 93 | 78a |
| Spotted spurge *Chamaesyce maculata* Euphorbiaceae | exposed | cosmopolitan weed | 10 | 90 | 64b |
| Grassleaved spurge *Euphorbia graminea* Euphorbiaceae | exposed | common weed | 24 | 50 | 61b |
| Buckwheat *Fagopyrum esculentum* Polygonaceae | partially exposed | ground cover, grove border, green manure | 22 | 50 | 73b |
| Alyssum *Lobularia maritima* Brassicaceae | partially hidden | ornamental | 12 | 33 | 57b |
| Cilantro *Coriandrum sativum* Apiaceae | partially hidden | home garden, grove border | 5 | 20 | 6b |
| Marjoram *Origanum majorana* Lamiaceae | shallow floral tube | home garden, ornamental | 15 | 13[b] | 61b |
| Frog fruit *Phyla nodiflora* Verbenaceae | shallow floral tube | native, ornamental, ground cover | 10 | 0 | 0 |
| Tropical milkweed *Ascelepias curassavica* Apocynaceae | deep floral tube | ornamental | 19 | 45[b] | 60b |
| Pentas *Pentas lanceolata* Rubiaceae | deep floral tube | ornamental | 23 | 13[b] | 32b |
| Spanish needles *Bidens alba* Asteraceae | composite with deep floral tube | native | 21 | 19[b] | 12b |
| Indian blanket *Gaillardia aestivalis* Asteraceae | composite with deep floral tube | native, ornamental | 5 | 0 | 0 |

[a]Numbers in the same column with different letters are different at $P \leq 0.05$ (ANOVA).
[b]Wasps did not enter the corolla tube to feed but scavenged nectar residue on petals.

biaceae, such as crown-of-thorns (*Euphorbia milii* des Moulins), wild poinsettia (*Euphorbia heterophylla* L.), grassleaved spurge (*Euphorbia graminea* Jacquin), and spotted spurge (*Chamaesyce maculata* [L.] Small) (Fig. 1A), and fed extensively on them.

The wasps' foraging success noticeably declined in flowers with nectaries that were only partially exposed; they did not enter or search within floral tubes for nectar and seemed to be deterred by reflexed floral parts or trichomes obstructing the corolla aperture. For example, only half of the wasps on buckwheat (*Fagopyrum esculentum* Moench; Polygonaceae) were able to locate the partially exposed nectaries of the flowers (Fig. 1B). On alyssum (*Lobularia maritima* [L.] Desvaux; Brassicaceae), whose flowers have partially hidden nectaries, the wasps searched between florets in the dense inflorescences, and some individuals successfully accessed the nec-taries by probing through the clefts at the base of the sepals (Fig. 1C). However, only about a third of the wasps successfully located alyssum nectaries.

Although marjoram (*Origanum majorana* L.; Lamiaceae), penny-royal (*Piloblephis rigida* (Bartram ex Benth.) Raf.; Lamiaceae), and frog fruit (*Phyla nodiflora* [L.] Greene; Verbenaceae) have open, shallow flowers with partially hidden nectaries, their petals and corolla tubes were covered with trichomes, which repulsed the wasps and prevent-ed them from reaching the nectary (Fig. 1D). The wasps did not at-tempt to crawl into the narrow corollas of the 2 common composite species tested: Spanish needles, *Bidens alba* (L.) (Asteraceae), and In-dian blanket, *Gaillardia aestivalis* (Walter) Rock (Asteraceae) (Fig. 1E). Contact with the pollen of these composites seemed to irritate them and resulted in extensive grooming.
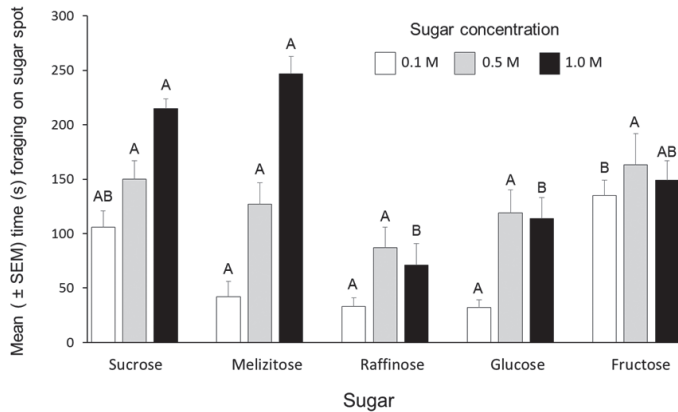
**Fig. 2.** Mean (± SE) feeding time of *Tamarixia radiata* when presented with different concentrations of sugars commonly occurring in nectar (sucrose, fructose, glucose) and honeydew (melizitose, raffinose). Bars within the same concentration having different letters are different at $P ≤ 0.05$ (ANOVA).

The wasps did not enter the relatively deep floral tubes of 2 common garden ornamentals, tropical milkweed (*Asclepias curassavica* L.; Apocynaceae) and pentas (*Pentas lanceolata* [Forssk.] Deflers; Rubiaceae). The corolla tube of pentas contained numerous non-glandular trichomes that precluded entry by *T. radiata*, and the nectaries of milkweed were hidden within the hood-like coronal pouches. However, on both of these plants, the wasps scavenged on what appeared to be nectar deposits left on the petals and hoods by bees and butterflies.

### CONDITIONED RESPONSE TO NECTAR ODOR

The wasps that fed on the unscented sucrose solution showed no preference for cups with either the unscented or banana-scented artificial nectar, whereas the wasps that fed on the banana-scented sucrose showed a significant preference for the cups with the sucrose and banana flavor extract ($G = 9.61$; df = 1; $P ≤ 0.01$) (Fig. 3). A similar proportion of wasps from each of the pre-test conditioning treatments did not choose a cup in the test arena (19% from the unscented control and 11% from the banana-scented treatment).

## Discussion

Scoring the foraging success of *T. radiata* on different types of nectaries in the laboratory was a straightforward means to identify the nectary architectures compatible with the wasp's foraging abilities.
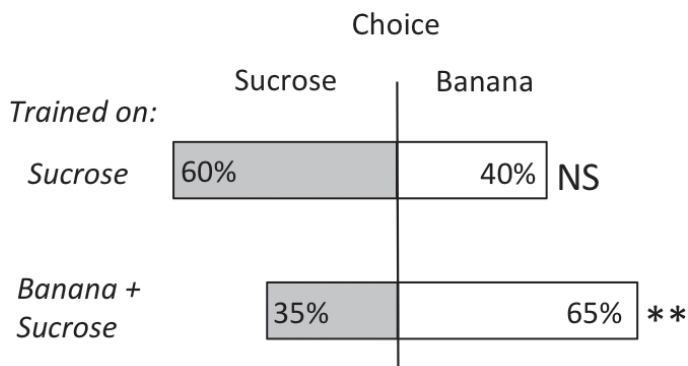


**Fig. 3.** Choice of cups with either unscented sucrose solution or with banana-scented sucrose solution made by *Tamarixia radiata* following a pre-test exposure to either 1.0 M sucrose solution or 1.0 M sucrose solution and banana flavor extract (*G*-test; ** = $P ≤ 0.01$; NS = not significant).

Specifically, *T. radiata* can forage only on plant species with flowers that have exposed nectaries, or on species with extrafloral nectaries. The value of evaluating the compatibility of nectar plants with target biocontrol agents has been shown in other situations in which conservation biological control parameters were being established (Wäckers 2004; Olson & Wäckers 2007; Sivinski et al. 2011; Géneau et al. 2012; Wäckers & van Rijn 2012; Russell 2015; Nave et al. 2016).

Even with the wasps' limited foraging ability, a number of species could be considered as potential nectar plants, and all of them have horticultural and agronomic utility in citrus-growing regions. For example, cowpea is a nitrogen-fixing plant that can be used as a ground cover, green manure, or cattle forage. It grows well in hot climates and is fairly drought tolerant; some varieties tolerate root-knot nematode and other pests (Wang et al. 2006). Some native legumes with extrafloral nectaries, such as partridge pea (*Chamaecrista fasciculata* [Michx.] Greene; Fabaceae) and native cassias (*Senna* species; Fabaceae), grow on uplands adjacent to citrus orchards and provide extrafloral nectar for a variety of insect predators and parasitoids (Koptur et al. 2015). Passionflowers (*Passiflora* species; Passifloraceae) have extrafloral nectaries, and a number of ornamental and native species may be suitable nectar plants for *T. radiata* in gardens and border areas. Likewise, elderberry (*Sambucus* species; Adoxaceae) has large extrafloral nectaries (Mizell 2015) and is common near irrigation ditches adjacent to citrus groves. Whether ants would restrict to any appreciable degree the wasps' access to the extrafloral nectaries of any of these species needs to be examined (Tena et al. 2013).

Plants in the family Euphorbiaceae have numerous exposed floral nectaries that were readily visited by the wasp. Crown-of-thorns (*E. milii*) is a popular ornamental perennial available in a variety of colors and growth habits that are heat and drought tolerant. Two native poinsettia species, *E. heterophylla* and *E. cyathophora* (Murray) Griseb., thrive in ruderal areas and could grow in abandoned groves and along the borders of commercial groves. Weedy spurges, such as grassleaved spurge (*E. graminea*) and spotted spurge (*C. maculata*) may also provide nectar in these neglected areas. Interestingly, the 'Diamond Frost' ornamental cultivar of *E. graminea* has concave nectaries, giving them a partially hidden architecture that prevented *T. radiata* from foraging on them. So, although Diamond Frost has a much showier inflorescence than the weedy wild type, it is not a good nectar plant for *T. radiata*. Perhaps a new cultivar can be bred that would retain the showy white bracts while retaining an exposed nectary architecture. Likewise, showier cultivars of the long-day blooming *E. heterophylla* and *E. cyathophora* could be developed for use as ornamentals in gardens and other landscaped areas. Although it is used as an ornamental, the Christmas Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) is a short-day plant that blooms in the winter. In the winter months, *D. citri* reproduction is at an annual low (Hall et al. 2008), so *E. pulcherrima* would probably not have much utility as a nectar source for *T. radiata*.

Plants in the family Apiaceae were not examined, but further studies should test their potential as nectar plants. For example, dill (*Anethum graveolens* L.) has exposed nectaries that would likely be accessible to *T. radiata* (Patt et al. 1997a). However, dill may not grow well in the summer heat of citrus-producing areas and would have to be used in the early and later parts of the growing season. Two other eulophid species, *Pediobius foveolatus* (Crawford) and *Edovum puttleri* Grissell, readily forage on dill flowers. However, on another umbel species, cilantro (*Coriandrum sativum* L.), *P. foveolatus* was able to push past the flower's recurved petals to feed on the nectaries whereas *E. puttleri* could not do so (Patt et al. 1997b). In the current study, *T. radiata* also could not maneuver around recurved petals of cilantro florets to reach the nectaries. That 3 eulophid species of similar size displayed varying foraging success on the same plant species highlights the need to

evaluate their foraging abilities on a case-by-case basis for the purpose of selecting potential nectar plant species for each of them.

Our future work will test whether nectar plants can maximize the survivorship and efficacy of *T. radiata* in residential landscapes and abandoned groves. Other studies have shown that augmenting residential landscapes with nectar plants can enhance biological control of certain pest species (Ellis et al. 2005; Rebek et al. 2006). Urban landscapes are amenable to a conservation biological control strategy because ecological disturbance tends to be minimal and these landscapes lend themselves to manipulations of plantings (Raupp et al. 1992; Shrewsbury & Raupp 2000; Tooker & Hanks 2000; Ellis et al. 2005). In contrast, abandoned citrus groves are challenging in this regard because the nectar plants must grow in ruderal conditions, compete with exotic grasses, and withstand drought conditions. *Euphorbia heterophylla* and partridge pea may be suitable nectar plants capable of growing in the agronomically challenging conditions found in abandoned citrus groves. Other native species that can tolerate ruderal conditions should be examined for their suitability as nectar plants for *T. radiata*.

For nectar plant augmentation to be a successful management strategy for *T. radiata*, several key questions will need to be addressed concerning the ecological relationship between *T. radiata* and potential nectar plant species within each type of target landscape. We will need to determine whether *T. radiata* will commute between the canopies of *D. citri* host plants and nectar plants growing at ground level. If they do commute, then we will need to determine 1) if there is an optimal spatial relationship between nectar plants and *D. citri* host plants, i.e., how localized is the effect of augmentative plantings of nectar plants; and 2) whether planting densities and species composition have effects on wasp establishment success and psyllid parasitism levels.

Lastly, the choice test demonstrated that *T. radiata* is stimulated by nectar odor and could learn to recognize olfactory cues associated with nectar. This finding suggests *T. radiata* can use olfactory cues to locate nectar sources, as has been shown for other parasitoid wasp species (Lewis & Takasu 1990; Lewis et al. 1998; Patt et al. 1999; Wäckers 2004; Zhu et al. 2013; Foti et al. 2016). Tests will need to be conducted to determine whether *T. radiata* wasps exposed to nectar odors during rearing are more adept at locating nectar sources following their release than odor-naïve wasps.

## Acknowledgments

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