



## **Can the Choice Behavior and Fitness of *Tamarixia radiata* (Hymenoptera: Eulophidae) be Affected by the Citrus (Sapindales: Rutaceae) Variety Used to Rear the Asian Citrus Psyllid (Hemiptera: Liviidae)?**

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# Can the choice behavior and fitness of *Tamarixia radiata* (Hymenoptera: Eulophidae) be affected by the citrus (Sapindales: Rutaceae) variety used to rear the Asian citrus psyllid (Hemiptera: Liviidae)?

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## Abstract

Several studies have shown that the host plant influences the bioecological parameters of both pest insects and their natural enemies; however, few studies have evaluated the effect of varieties of the same plant species. This study evaluated the host-choice behavior (by a free-choice test) of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) on nymphs of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) reared on 4 sweet orange (Sapindales: Rutaceae) varieties (Hamlin, Pêra, Natal, and Valencia), and its development (biology) in nymphs reared on these same varieties, on a mandarin (cv. Ponkan), and on orange jasmine. The host-choice behavior was not affected by the host on which *D. citri* was reared, showing that *T. radiata* has a degree of plasticity in interpreting the cues for host location. In the free-choice test, the hosts did not affect the percentage of parasitism. The emergence parameter, regardless of the host used, showed viability (egg to adult) above 80% for all varieties, with the highest level for Pêra sweet orange (90.06%). The parasitoid's development period in nymphs reared on Ponkan (12.4 d) was statistically similar to the period in nymphs reared on orange jasmine (11.80 d) but statistically longer than in nymphs reared on other citrus varieties. No significant difference in the sex ratio was observed. *Tamarixia radiata* as a biological control agent of *D. citri* can be an additional component in integrated pest management in citrus groves. It can be used regardless of the citrus variety, because its behavior and biology are similar for the main commercial varieties of citrus grown in São Paulo.

Key Words: biological control; insect-plant interaction; free-choice test; parasitoid; HLB

## Resumen

Distintos estudios han mostrado la influencia de la planta hospedera en los parámetros bioecológicos, no solamente en insectos plaga, sino también en sus enemigos naturales. Sin embargo, pocos han evaluado el efecto de diferentes variedades de la misma especie de planta. Este estudio evaluó el comportamiento de elección del hospedero (por la prueba de libre elección) de *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) en ninfas de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) criadas en cuatro variedades de naranja dulce (Hamlin, Pêra, Natal y Valencia) y su desarrollo (biología) en ninfas criadas en estas mismas variedades además de mandarina (cv. Ponkan) y naranja jazmín. El comportamiento de elección no fue afectado por el huésped donde *D. citri* fue criada, mostrando que *T. radiata* posee plasticidad en la interpretación de señales para la localización del huésped. En cuanto a la prueba de libre elección, los diferentes huéspedes no afectaron el parasitismo en el ensayo biológico. En el parámetro de emergencia, independientemente del huésped utilizado, todos presentaron viabilidad (huevo-adulto) mayor que 80%, con el mayor valor obtenido para la naranja dulce Pêra (90.06%). Un tiempo de desarrollo más largo fue observado en la mandarina Ponkan (12.4 días) que no difirió estadísticamente de la naranja jazmín (11.80 días), pero fue diferente de las otras variedades de cítricos. No fue observada diferencia significativa en la proporción sexual. El uso de *T. radiata* como agente de control biológico de *D. citri* puede ser un componente adicional en el manejo integrado de plagas en plantaciones de cítricos, porque su uso es posible independientemente de la variedad utilizada, ya que su comportamiento y biología son similares en las principales variedades de cítricos utilizadas en São Paulo.

Palabras Clave: control biológico; interacciones insecto-planta; prueba de libre elección; parasitoides; HLB

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*Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is the most important parasitoid of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), the vector of the bacterium associated with the citrus disease huanglongbing (HLB) (Chen & Stansly 2014). According to Parra et al. (2010), *T. radiata* can be used as a biological control agent of *D. citri* as an additional component in integrated pest management in citrus groves. This parasitoid has successfully been used in several parts of the world (Etienne et al. 2001), and Gómez-Torres (2009) and Diniz (2013) obtained promising results in Brazil.

*Diaphorina citri* has a wide range of host plants, especially members of the family Rutaceae (Sapindales) (Aubert 1987; Halbert & Man-

junath 2004). Some studies have found that the development of *D. citri* varies on different host species (Tsai & Liu 2000; Nehru et al. 2004; Nava et al. 2007; Tsagkarakis & Rogers 2010; Teck et al. 2011; Westbrook et al. 2011) or on different varieties of the same plant species (Alves et al. 2014). Several studies have shown that not only herbivorous insects but also their natural enemies are influenced by the first trophic level (host plant) (Price 1980; Orr & Boethel 1985; Kester & Barbosa 1991; Moraes et al. 2000; Hunter 2003; Kagata & Ohgushi 2006; Malinga et al. 2014).

Tritrophic interactions, which involve plants, herbivores, and natural enemies, are basically determined by 2 factors: 1) the direct effect

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of the plant on the behavior or biology of the natural enemy (e.g., the presence or absence of trichomes); and 2) the influence of the plant on the herbivore, also affecting its biology and behavior, which in turn indirectly affects the natural enemy (Bottrell & Barbosa 1998; Legrand & Barbosa 2003; Ode 2006; Bukovinszky et al. 2008). Among the indirect effects on the natural enemy, a major factor is the quality of the host plant, which often affects the size or nutritional quality of the herbivore (Legrand & Barbosa 2003; Harvey 2005; Ode 2006; Bukovinszky et al. 2008). Qualitative and quantitative differences in an herbivore's diet can affect the performance of the organisms in a food chain (bottom-up effect), mediating interactions involving plants, herbivores, and parasitoids, and even hyperparasitoids (Harvey et al. 2003).

Variations in the quality, composition of secondary defense compounds, volatiles released after herbivory, or even morphological characteristics can differ significantly among plant species as well as among varieties of the same species (Kauffman & Flanders 1985; Hare & Luck 1991; Ode 2006; Ballhorn et al. 2008), and this could change the control efficiency of a natural enemy (Harvey 2005). Also, according to Bukovinszky et al. (2009), variations in the nutritional quality of the plant may affect the immune response of herbivores to parasitism. These differences increase the complexity of the foraging dynamics and the ability of parasitoids to interpret these signals. However, in some cases, natural enemies show wide behavioral plasticity, especially with respect to learning based on previous experience (Moraes et al. 2000).

Detailed studies on the interactions among *T. radiata*, *D. citri*, and citrus varieties are important in order to increase the efficiency of strategies for using this natural enemy as a component of integrated pest management in citrus groves. This study evaluated the host-choice behavior and biology of *T. radiata* on *D. citri* reared on several plant species and citrus varieties.

## Materials and Methods

### INSECT REARING AND PLANT MAINTENANCE

The insects used in this study were obtained from the maintenance rearing colony of the Laboratory of Insect Biology, Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture, University of São Paulo, Brazil.

To rear the psyllids and subsequently their parasitoid *T. radiata*, we used orange jasmine plants (*Murraya paniculata* [L.] Jacq.) because this species has been reported to be the preferred host of *D. citri* (Halbert & Manjunath 2004; Nava et al. 2007; Teck et al. 2011). The plants were maintained in a climate-controlled chamber at a temperature of  $25 \pm 2$  °C, RH of  $60 \pm 10\%$ , and a photoperiod of 14:10 h L:D.

The varieties used in the experiments were the sweet orange (*Citrus sinensis* [L.] Osbeck) varieties Hamlin, Natal, Pêra, and Valencia and the mandarin (*Citrus reticulata* Blanco) variety Ponkan. All the varieties were cultivated on rootstock of Rangpur lime, *Citrus limonia* Osbeck. The plants were kept in a greenhouse and irrigated daily. The plants were fertilized every 2 wk with a solution of Peters foliar fertilizer (NPK 20-20-20).

### HOST-CHOICE BEHAVIOR OF *T. RADIATA* FOR *D. CITRI* NYMPHS REARED ON FOUR CITRUS VARIETIES (FREE-CHOICE TEST)

We used cages (40 × 120 × 40 cm) covered with voile fabric. Each cage contained 1 plant of each sweet orange variety (Hamlin, Natal, Pêra, and Valencia), arranged in a randomized design in the corners of the cage (equidistant from each other). Each plant contained 50 nymphs (5th instar) of *D. citri*, previously reared on the the same variety.

To obtain the exact number of nymphs on each plant, 1 pair of *D. citri* adults was confined on each plant for a period of 24 h for oviposition. After this period, the pair was removed and the development of the offspring was followed until they reached the 5th instar. The excess nymphs (over 50) on the plants were removed with a soft brush (24 h before the plant and nymphs were offered to the parasitoids). In the center of each cage, 3 pairs of 2-d-old *T. radiata* were released and allowed to parasitize the nymphs for 48 h under natural conditions. The percentage of parasitism was the parameter used to determine the host-choice behavior of *T. radiata*.

After this period, the parasitoids were removed and the plants together with the parasitized nymphs were maintained in climate-controlled chambers at a temperature of  $25 \pm 2$  °C, RH of  $60 \pm 10\%$ , and a photoperiod of 14:10 h L:D. The plants were left for 7 d, the average period required to differentiate the parasitized from the non-parasitized nymphs. The percentage of parasitism in each variety was obtained by counting the parasitized nymphs, which are opaque and dark brown ("mummified") (Etienne et al. 2001). The experimental design was completely randomized, with 12 replications in total.

### BIOLOGY OF *T. RADIATA* IN *D. CITRI* ON VARIOUS HOSTS

For this study, the sweet orange varieties Hamlin, Pêra, Natal, and Valencia, the mandarin variety Ponkan, and orange jasmine were used. Each plant contained fifty 5th instar nymphs of *D. citri* reared previously on the same host. The nymphs were offered to a pair of *T. radiata* adults (2 d old) for a period of 24 h in mesh sleeve cages maintained in climate-controlled chambers at a temperature of  $25 \pm 2$  °C, RH of  $60 \pm 10\%$ , and a photoperiod of 14:10 h L:D. After this period, the parasitoids were removed and the plants maintained under the same conditions.

To obtain the exact number of psyllid nymphs, the same method as in the previous experiment was used. The following parameters were evaluated: parasitism, development time (egg to adult), adult emergence (viability), and sex ratio.

### STATISTICAL ANALYSES

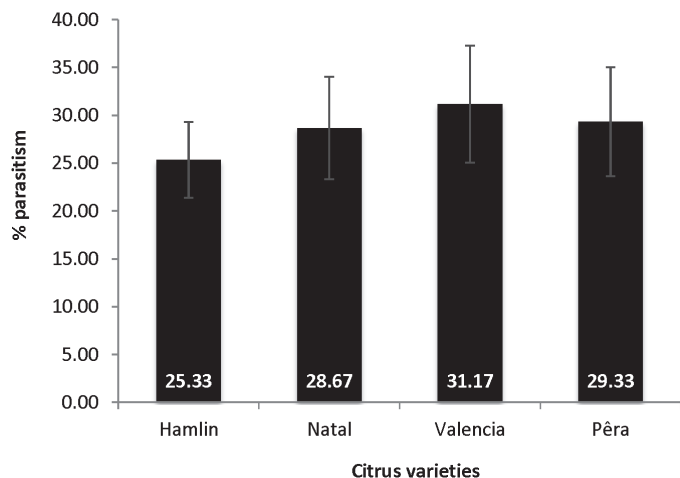
Data for viability and development time were analyzed for normality, homoscedasticity, and the presence of outliers by optimal Box-Cox transformation; then, the data were subjected to analysis of variance (ANOVA), and the means were compared with the Tukey test ( $P \leq 0.05$ ). The nonparametric chi-squared ( $\chi^2$ ) test was used to evaluate the sex ratio.

The data for parasitism were analyzed using generalized linear models (GLM) (Nelder & Wedderburn 1972) through a quasi-Poisson distribution; the *F* value was calculated by ANOVA of the model. The quality of fit was determined using a half-normal graph of probabilities with simulation envelope (Demétrio & Hinde 1997; Hinde & Demétrio 1998). All analyses were conducted with the aid of SAS<sup>®</sup> version 9.1 (SAS Institute 2003).

## Results

### HOST-CHOICE BEHAVIOR OF *T. RADIATA* FOR *D. CITRI* NYMPHS REARED ON FOUR CITRUS VARIETIES (FREE-CHOICE TEST)

The citrus varieties used to rear *D. citri* did not affect the choice behavior of the parasitoids in the free-choice test. The mean parasitism ranged from 25.33% for Hamlin to 31.17% for Valencia ( $F = 0.213$ ;  $df = 3,47$ ;  $P = 0.886$ ) (Fig. 1).



**Fig. 1.** Mean percentage ( $\pm$  SE) of parasitism of *Tamarixia radiata* on nymphs of *Diaphorina citri* reared on 4 citrus varieties. Means do not differ by Tukey's test ( $P > 0.05$ ).

#### BIOLOGY OF *T. RADIATA* IN *D. CITRI* ON VARIOUS HOSTS

Similarly to the results of the free-choice test, the hosts on which nymphs of *D. citri* were reared did not affect the percentage of parasitism. The highest parasitism rates, which averaged 24%, were obtained on the sweet orange varieties Hamlin and Natal; the lowest parasitism rate was observed on Ponkan (mandarin), with a mean of 18%. For the emergence parameter, regardless of the host used, the viability (egg to adult) of above 80% did not differ among the hosts tested, with the highest level obtained for the Pêra sweet orange (90.06%). There was no significant difference in sex ratio as a function of the host (Table 1). However, the development time was longest on Ponkan (12.04 d), which did not differ statistically from the time on orange jasmine (11.80 d), but did differ from the time on the other citrus varieties (Table 1).

## Discussion

#### CHOICE BEHAVIOR OF *T. RADIATA* FOR *D. CITRI* NYMPHS REARED ON FOUR CITRUS VARIETIES (FREE-CHOICE TEST)

The reproductive success of a parasitoid is defined by its ability to find sexual partners and hosts (Dicke & Grostal 2001). Therefore, knowledge of the factors that influence the host search is important. The host-choice behavior of female adults of *T. radiata* is directly as-

sociated with the release of volatile compounds from the plants that is induced by nymphs of *D. citri* (Mann et al. 2010). According to these authors, the female parasitoids are more strongly attracted to citrus plants infested with nymphs (rather than to plants or nymphs alone), and are attracted by the presence of the greening pathogen (Martini et al. 2014). Variations in several factors, for example volatiles, may show different profiles in varieties of the same plant species (Ballhorn et al. 2008), making foraging and particularly the control exerted by a natural enemy even more complex (Harvey 2005).

The results obtained in this study demonstrated that *T. radiata* has a high plasticity and is able to recognize the induced volatiles released by plants attacked by *D. citri* nymphs, independently of the variety on which the nymphs were reared. The volatile compounds are perceived by different types of antennal sensilla found in *T. radiata*. The number of each type of sensillum differs in males and females, and the most abundant type of sensilla in females is associated with host choice (Onagbola et al. 2009).

In a recent study, Khan et al. (2014) observed that the parasitism level of *T. radiata* was similar between 2 host plant species (on average 26%, for both species), which were the Kinnow tangerine variety and sweet orange. According to Wang & Messing (2004), for parasitoids, the success of the offspring development is strongly influenced by the female's choice of the most suitable host, especially for idiobiont ectoparasitoids such as *T. radiata*.

Results differing from those of the present study were reported by Paiva & Parra (2012) in field conditions, with respect to the 4 citrus varieties most commonly planted in São Paulo. They observed the highest parasitism on Hamlin (35.6%), followed by Pêra (19.2%), Valencia (8.6%), and Natal (7.3%). These differences may be related to the initial populations of *D. citri* in various areas of the state, or to the large number of volatile compounds in the environment.

#### BIOLOGY OF *T. RADIATA* IN *D. CITRI* ON VARIOUS HOSTS

Artificial selection for new varieties may have changed the chemistry of crop plants, and some cases may alter the performance not only of the herbivores but also of their parasitoids (Gols & Harvey 2009). Therefore, varieties of the same species may also affect parasitoids, either directly or indirectly, in plant-herbivore-parasitoid interactions (Soglia et al. 2006).

On different host plant species or varieties, the performance of a host and its parasitoid can be positive (Harvey et al. 2003; Sznajder & Harvey 2003) or negative (Bottrell & Barbosa 1998; Legrand & Barbosa 2003; Ode 2006), although the adverse effects of food-plant characteristics are usually less pronounced on the parasitoid performance than on the herbivore (Gols & Harvey 2009). This may explain the present

**Table 1.** Mean ( $\pm$  SD) of parasitism rate, survival, development time, and sex ratio of *Tamarixia radiata* reared on *Diaphorina citri* on various host plants (temperature:  $25 \pm 2$  °C; RH:  $60 \pm 10$ %; photoperiod: 14:10 h L:D).

Host	<i>n</i>	Parasitism (%)	Viability: egg to adult (%)	Development time: egg to adult (d)	Sex ratio <sup>a</sup>
Hamlin	17	24.94 $\pm$ 2.59 a	85.04 $\pm$ 3.04 a	11.52 $\pm$ 0.11 b	0.69 $\pm$ 0.03
Pêra	16	19.38 $\pm$ 2.45 a	90.06 $\pm$ 2.10 a	11.37 $\pm$ 0.11 b	0.66 $\pm$ 0.05
Natal	16	24.25 $\pm$ 1.40 a	85.81 $\pm$ 2.58 a	11.41 $\pm$ 0.09 b	0.74 $\pm$ 0.04
Valencia	16	21.87 $\pm$ 2.36 a	88.58 $\pm$ 2.75 a	11.42 $\pm$ 0.14 b	0.68 $\pm$ 0.04
Ponkan	17	18.12 $\pm$ 1.25 a	80.75 $\pm$ 3.53 a	12.04 $\pm$ 0.16 a	0.77 $\pm$ 0.03
Orange jasmine	16	22.75 $\pm$ 2.29 a	85.47 $\pm$ 2.47 a	11.80 $\pm$ 0.12 ab	0.68 $\pm$ 0.03
<i>F</i>		1.76	0.91	4.69	
<i>df</i>		5, 92	5, 92	5, 92	
<i>P</i>		0.13	0.49	$\leq 0.05$	

Means followed by the same letter in a column do not differ by Tukey's test ( $P > 0.05$ ).  
<sup>a</sup>non-significant by  $\chi^2$  test.



results obtained, that different citrus hosts had no effect on parasitism, viability, or sex ratio.

The effects on biological parameters of the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) on the California red scale (*Aonidiella aurantii* [Maskell]; Hemiptera: Diaspididae) reared on several cultivars of citrus were very noticeable in the study of Hare & Luck (1991). Among the hosts used, tangerine leaves (*Citrus unshiu* Marcovicz) were the least suitable for both parasitoid and pest development (Hare & Luck 1991).

The effect of the host plant on the biology of parasitoids was also observed for other eulophids. Kauffman & Flanders (1985) found reduced egg-to-adult viability of the parasitoid *Pediobius foveolatus* (Crawford) (Hymenoptera: Eulophidae) on larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), on certain soybean cultivars. In addition to the egg-to-adult viability, the hosts also affected the insect development time. Similar results were obtained recently for the same parasitoid, *P. foveolatus*, on larvae of *Henosepilachna vigintioctopunctata* F. (Coleoptera: Coccinellidae) on several eggplant and tomato varieties (Wang et al. 2013). These effects may be due mainly to differences in nutritional requirements and the presence of secondary plant metabolites, which may affect some aspects of the biology of the host and its parasitoid (e.g., parasitism and viability) (Ballhorn et al. 2008; Gols & Harvey 2009; Bukovinszky et al. 2009), altering the control efficiency of the parasitoid (Harvey 2005).

According to Gols & Harvey (2009), adverse effects on the natural enemies may not be directly related only to the quality of the host plant. The effects of trophic interactions on plants are strongly associated with the specificity of the parasitoid (van der Meijden 1996; Sznajder & Harvey 2003; Harvey 2005). In specific parasitoids such as *T. radiata*, the biological parameters are little affected by the host herbivore's diet, be it different plant species or different varieties that may contain secondary compounds (Sznajder & Harvey 2003) or be nutritionally inadequate.

Unlike *D. citri*, whose development is affected by citrus species (Tsai & Liu 2000; Nava et al. 2007; Tsagkarakis & Rogers 2010; Westbrook et al. 2011) or varieties (Alves et al. 2014), its parasitoid *T. radiata* is not affected by the host plant, according to the present study. These results may indicate that these parasitoids are able to select those hosts, among the several nymphs (50) offered in this study, that are most suitable for the development of their offspring. This selection occurs because, in insect parasitoids, the fitness of the offspring is affected by the host chosen by the female, particularly in idiobiont ectoparasitoids.

In summary, the results obtained in this study show that only the parasitoid development time was influenced by the host on which *D. citri* was reared. These results are promising because they establish that *T. radiata* can be used to control the Asian citrus psyllid regardless of the variety of citrus, as its host-choice behavior and biology are similar on the main commercial varieties of citrus grown in São Paulo State. *Tamarixia radiata* can be an additional component of integrated pest management in citrus groves. Focusing the releases of the parasitoid on organic citrus areas, on urban areas with large populations of an alternate psyllid host (*M. paniculata*), or on abandoned groves can increase the population of the parasitoid for subsequent dispersal to commercial citrus areas (Parra et al. 2010).

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