Avoidance of Parasitized Hosts by Female Wasps of Tamarixia radiata (Hymenoptera: Eulophidae), Parasitoid of Diaphorina citri (Hemiptera: Liviidae), Vector of Citrus Greening Disease

Authors: Chen, Xulin, Bordini, Gabriela Pavan, and Stansly, Philip A.

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Avoidance of parasitized hosts by female wasps of *Tamarixia radiata* (Hymenoptera: Eulophidae), parasitoid of *Diaphorina citri* (Hemiptera: Liviidae), vector of citrus greening disease

Xulin Chen[1,*], *Gabriela Pavan Bordini*[2], *and Philip A. Stansly*[1]

Many insects are known to avoid already infested or parasitized insect hosts to reduce competition among offspring, often by responding to chemical signals deposited previously by an ovipositing female (Prokopy 1981; Nufio & Papaj 2001; Stelinski et al. 2007). These signals permit females to distinguish utilized and unutilized hosts by contact chemoreception (Seeley 1989). Such marking pheromones have been found in Coleoptera, Diptera, Hymenoptera, Lepidoptera, and Neuroptera, and more than 20 families of phytophagous insects (Prokopy 1981). For hymenopteran parasitoids, 150 to 200 species have demonstrated the ability in nearly every family to discriminate between parasitized and non-parasitized insect hosts (van Lenteren 1981).

It has also been reported that parasitic female Hymenoptera are less likely to self-superparasitize than superparasitize another conspecific (Vandijken et al. 1992; Danyk & Mackauer 1993; Vanbaaren et al. 1994). The rationale would be that, whereas superparasitizing another conspecific might eliminate a potential competitor, superparasitizing herself would only increase competition among sibs (Vanalphen & Visser 1990). Three possible mechanisms have been proposed to explain how parasitoids might guard against self- and conspecific-superparasitism. First, there could be a 2-component marking system with 1 short-lived component that guards against self-superparasitism and a 2nd, longer-lived component guarding against conspecific-superparsitism (Field & Keller 1999). Second, there may be perceivable differences among individuals in 1 or more marking pheromone components (Nufio & Papaj 2001). Third, individuals may learn to discriminate between and remember recently parasitized hosts (Ueno & Tanaka 1996). However, Vanalphen et al. (1987) reported that inexperienced parasitoids are capable of discriminating parasitized from non-parasitized hosts, indicating that host discrimination does not have to be gained through learning, even though superparasitism may be influenced by experience (Vanalphen & Visser 1990).

Previous studies reported that female *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) wasps can discriminate between parasitized and non-parasitized hosts to avoid superparasitism (Chien et al. 1991a). Husain & Nath (1923) observed superparasitism in the field during Dec and Jan when hosts were scarce, but not at times when hosts were abundant. Chien et al. (1991a,b) observed superparasitism rates of up to 5.6% when host density was low and active space was limited. Chen (2013) found superparasitism to decrease with increasing host density over a range from 10 to 60 per parasitoid, with the highest rate of 37.9% at the lowest host density. Whether the generally low rate of superparasitism at higher host densities was due to host marking remained unknown. The objective of this study was to determine whether a *T. radiata* female wasp can avoid superparasitism by identifying hosts parasitized by herself or by a conspecific.

Tamarixia radiata colonies were maintained at the Southwest Florida Research and Education Center in Immokalee, Florida, generally following methods of Skelley & Hoy (2004) using *Murraya paniculata* (L.) Jacq. (Sapindales: Rutaceae) as host plant. Nine newly trimmed plants with healthy flushes were held in a BugDorm™ 2400 insect rearing tent (75 × 75 × 115 cm) (MegaView Science Co. Ltd., Taichung, Taiwan) inside an air-conditioned glass greenhouse controlled during spring 2014 to approximately 27 °C, 65% RH (HOBO® RH/Temp/Light/External Data Logger H08-004-02, Onset Computer Corp., Bourne, Massachusetts). About 1,500 *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) adult psyllids were introduced into the cage for 72 h to oviposit. Adults were then removed, and plants were held in the same cage for about 10 d until nymphs hatched and developed to 4th instars. Six of these plants infested with 4th instar psyllid nymphs were transferred into a wooden framed cage (60 × 80 × 90 cm) with polyacrylic sides maintained in an air-conditioned polycarbonate greenhouse at 26 ± 1 °C, 55 to 85% RH, and a 14:10 h L:D photoperiod. Two hundred female and 60 male *T. radiata* adults were then released into the cage and removed after 7 d. The *T. radiata* progeny were collected from day 8 until no more could be found.

Six days after *T. radiata* adults were released into the colony, small (2 cm) portions of shoots containing 1 parasitized nymph with the wasp close to the pupal stage were excised and placed individually into a glass tube 75 mm long × 12 mm in diameter (Fisher Scientific, Hampton, New Hampshire). Tubes were checked frequently, and wasps were removed immediately upon emergence (Chen & Stansly 2014).

Possible behavioral effects were investigated using a marking technique for readily distinguishing among nymphs identified as parasitized or not parasitized. Ten 4th instar *D. citri* nymphs were randomly placed on a young *M. paniculata* shoot with 6 leaflets in a Petri dish. A Pigma® marker (Micro® Archival) was used to randomly mark 5 hosts with a small dot on the thorax. One 24-h-old *T. radiata* female wasp was released into this arena (*N* = 20) to evaluate possible response to the mark. After 24 h, all hosts were inverted under a stereoscopic microscope to check for *T. radiata* eggs indicating parasitization.

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[1] University of Florida/IFAS, Southwest Florida Research and Education Center, Immokalee, Florida 34142, USA

[2] Universidade de São Paulo, Escola Superior de Agricultura “Luiz de Queiroz”, Ave. Pádua Dias 11, Piracicaba, São Paulo, 13418-900, Brazil

*Corresponding author; E-mail: xulin527@ufl.edu

Table 1. Mean ± SE number of non-parasitized and self- or conspecific-parasitized hosts probed and parasitized by a 24-h-old *Tamarixia radiata* female wasp during a 1 h observation time.

Experiment	Host type	Probing	Parasitization
Self-identification	Self-parasitized	1.51 ± 0.43	0.25 ± 1.53
	Non-parasitized	3.63 ± 0.53	2.63 ± 0.42
Conspecific-identification	Conspecific-parasitized	0.25 ± 0.16	0.13 ± 0.13
	Non-parasitized	2.63 ± 0.26	2.13 ± 0.22

To evaluate preference for self-parasitized versus non-parasitized hosts, one 5 cm long *M. paniculata* shoot infested with thirty 4th instar *D. citri* nymphs was placed in a Petri dish, and a single mated 24-h-old *T. radiata* female wasp was released into this arena for oviposition. After 24 h, 6 randomly chosen parasitized hosts were transferred to a new arena where a young clean *M. paniculata* shoot with 6 leaflets was attached at the bottom of a Petri dish with double-sided cellophane tape. One parasitized and one non-parasitized host were placed at a random location on each of 6 leaflets. Parasitized hosts were identified by a small dot on the thorax made with a Pigma® marker. The same female parasitoid which had parasitized the 6 hosts was released into this Petri dish and maintained under constant observation for 1 h by using a stereoscopic microscope to note the number of hosts contacted or probed. Nymphs were then inverted under the stereoscopic microscope to assess the number of parasitized or superparasitized nymphs as indicated by the presence of one or more eggs, respectively (N = 10). The same procedure was used for conspecific-discrimination except that a new 24-h-old *T. radiata* female wasp was released (N = 10).

A 3rd choice experiment was conducted to evaluate superparasitism on conspecific- versus self-parasitized hosts. The same setup was used as described before. However, as preliminary results indicated little parasitism was likely to occur on parasitized eggs during 1 h, exposure time was lengthened to 24 h. Therefore, the Petri dishes were placed in a growth chamber at 25 °C, 70 to 85% RH, and a 14:10 h L:D photoperiod (HOBO® RH/Temp/Light/External Data Logger H08-004-02, Onset Computer Corp., Bourne, Massachusetts). Nymphs were inverted under a stereoscopic microscope after 24 h to check for superparasitism (N = 20).

Statistical analyses were done using JMP software (SAS Institute 2013). Preference among the 2 host types was assessed using Student's t-test ($P < 0.05$) to compare parasitization of Pigma®-marked hosts versus clean hosts, as well as probes and parasitization between parasitized hosts and non-parasitized hosts or between conspecific-parasitized and self-parasitized hosts.

Incidence of parasitism was not significantly different between clean hosts and Pigma®-marked hosts (mean ± SE: 2.05 ± 0.98 and 2.10 ± 1.12, respectively) ($F = 0.0$; $\text{df}_{(\text{model, error})} = 1, 38$; $P = 0.8823$). This result indicated that the Pigma® mark had no effect on host choice.

A significant difference between non-parasitized and self-parasitized hosts was observed in the number of hosts probed ($F = 9.1$; df = 1, 18; $P = 0.0093$) and the number of hosts parasitized ($F = 27.8$; df = 1, 18; $P < 0.0001$) during 1 h (Table 1). Results for conspecific-identification also showed significant differences in incidence of probing ($F = 58.8$; df = 1, 18; $P < 0.0001$) and parasitism ($F = 59.7$; df = 1, 18; $P < 0.0001$) between parasitized and non-parasitized hosts (Table 1).

Similarly, a significant difference in the number of hosts parasitized was observed between the self-parasitized hosts (0.45 ± 0.14) and the conspecific-parasitized hosts (1.05 ± 0.22) over 24 h ($F = 5.3$; df = 1, 38; $P = 0.03$). Female wasps were observed to ignore the parasitized host or probe briefly, only to run off and rest. The superparasitism rate of less than or equal to 1 observed when no non-parasitized hosts were available contrasted with an oviposition rate of about 11 eggs per 24 h expected if sufficient non-parasitized hosts were available (Chen 2013; Sule et al. 2014), indicating strong avoidance of marked hosts.

Summary

Female wasps of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) avoided probing or parasitizing hosts that the same or another female wasp had already parasitized. Furthermore, they discriminated against hosts parasitized by herself versus another female wasp when these were the only choices. Nevertheless, incidence of superparasitism was low under these conditions compared with normal parasitism, underscoring the strength of the deterrent mark.

Key Words: superparasitism; host discrimination; host marking

Sumario

La avispa *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) evita sondar o parasitar huéspedes ya parasitados, que sea de ella o de otra hembra. Además, ellas discriminan contra huéspedes parasitados por sí misma frente a las de otra hembra cuando solo existan estas dos opciones. Sin embargo, la incidencia de superparasitismo fue reducido bajo estas condiciones comparados a parasitismo normal, lo que subraya la fuerza de la marca disuasiva.

Palabras Clave: superparasitismo; discriminacion del hospedero; marcaje del hospedero

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