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Reduced parasitism of *Diaphorina citri* (Hemiptera: Liviidae) by *Tamarixia radiata* (Hymenoptera: Eulophidae) at increasing parasitoid:host ratios

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The biological control agent *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) is being mass-produced for field release in several countries, including Brazil (Parra et al. 2016), Costa Rica, Mexico, and the United States (Flores & Ciomperlik 2017; Kerr et al. 2017), to control *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), a vector of *Candidatus Liberibacter asiaticus*, the putative bacterial pathogen that causes huanglongbing disease in citrus. Hypothetically, a higher number of the parasitoids relative to the psyllid host would increase parasitism, super-parasitism, and overall mortality of *D. citri* nymphs. However, this may not be the case because intraspecific competition can increase at higher parasitoid population levels (Visser et al. 1999; Harvey et al. 2013). Parasitoids compete for hosts under these conditions, prolonging the time required to locate and parasitize an acceptable host. As the relative number of parasitoids increases, more encounters with conspecifics occur and searching behavior is disrupted, leading to a decrease in searching efficiency and the rate of parasitism (Beddington 1975). Consistent with other parasitoid species (Ueno & Tanaka 1994), *T. radiata* females can discriminate between parasitized and unparasitized host nymphs due to marking by the females (Chen et al. 2016a). This differentiation includes nymphs parasitized by the same female or conspecific females, and super-parasitism is avoided by this capability. Single *T. radiata* females parasitized fewer third, fourth, and fifth instar *D. citri* nymphs in proportion to an increase in the overall number of nymphs (Sule et al. 2014), but no comparison was conducted in regard to multiple *T. radiata* females, as would be found in a mass-rearing system and in the field. The objective of this study was to compare the levels of parasitism and super-parasitism of *D. citri* fourth and fifth instar nymphs exposed to an increasing number of *T. radiata* females.

To compare the effects of varying release ratios of *T. radiata* to *D. citri*, while maintaining a 2:1 sex ratio (female:male) for *T. radiata*, treatments of 9 (6♀:3♂), 18 (12♀:6♂), or 45 (30♀:15♂) *T. radiata* were released into cages after adequate time was given for progeny, of a standardized ovipositing cohort of 60 adult *D. citri*, to develop based on the following methods. The study was conducted using 3 equivalent potted orange jasmine plants, *Murraya paniculata* (L.) Jack (Rutaceae), that were about 1 m tall, and had an ample

number of young shoots with flush. The *D. citri* females oviposit exclusively on flush, rather than mature leaves or stems. To prepare each plant, 4 comparable shoots were selected and the remaining shoots were removed. Plants then were rinsed thoroughly with tap water, shaken dry, and placed into individual 24.5 W × 24.5 L × 63.0 cm H BugDorm insect rearing cages (BioQuip Products Inc., Rancho Dominguez, California, USA). The cages were held at 26 ± 1 °C, 50% RH, and 14:10 h (L:D) photoperiod in an environmental chamber (model number I36VLC8, Percival Scientific, Perry, Iowa, USA).

The *D. citri* adults were obtained from a stock colony collected in Florida, and maintained for more than 10 yr on orange jasmine plants at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Biological Control Laboratory in Gainesville, Florida, USA (Kerr et al. 2017). An open 50 mL plastic vial containing 60 psyllid adults aspirated from the colony was placed upright on the potting media, and leaned against the stem of the orange jasmine plant in each experimental cage. The psyllids were given 3 d to ascend the plants and oviposit on the flush before being removed from the cage. The *D. citri* eggs and nymphs on the flush were held for 10 d to obtain the late fourth and fifth instar nymphs that are preferred by *T. radiata* for parasitism (Sule et al. 2014).

The *T. radiata* colony was established in 1998 at Florida Department of Agriculture and Consumer Services, Division of Plant Industry with specimens imported from Taiwan and southern Vietnam (Skelley & Hoy 2004). Adult parasitoids < 24 h old were aspirated at a 2:1 sex ratio (female:male) into 3 individual 50 mL glass vials. The vials were provisioned with a 0.6 cm × 5 cm strip of paper towel saturated with honey and blotted. Individual cohorts of 9 (6♀:3♂), 18 (12♀:6♂), or 45 (30♀:15♂) *T. radiata* were held in the capped vials for a 24 h mating period, and subsequently released into separate cages containing the *D. citri* nymphs that had been held 10 d to develop on the flush. The parasitoids were given 4 d to oviposit on the nymphs, after which they were removed from the cages.

For each of the three treatments 9 (6♀:3♂), 18 (12♀:6♂), or 45 (30♀:15♂) *T. radiata*, the 4 shoots with the *D. citri* nymphs and supporting flush were removed from each cage before adult parasitoid emergence at 10 to 12 d post-oviposition (Chien et al. 1991; Gomez-Torres et al. 2012). The nymphs on the flush were measured under

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a dissecting microscope (Leica S8 APO Stereomicroscope, Leica AG, Wetzlar, Germany), and classified to instar based on head capsule width: 0.40 to 0.49 mm and > 0.50 mm denoting fourth and fifth instar, respectively. The relatively few earlier instar nymphs were discarded. Each nymph was inverted to count the number of *T. radiata* eggs or larvae present on them to establish if it was unparasitized, parasitized, or super-parasitized (Chen et al. 2016a). The mean number of unparasitized, parasitized and super-parasitized nymphs for each treatment was divided by the total number of nymphs to determine the proportion in each category (5 replicates). Levels of parasitism were compared separately for fourth and fifth instar *D. citri* nymphs. Data were analyzed at a significance level of 0.05 with a 1-way ANOVA; when differences occurred, means were differentiated using Tukey's honestly significant difference (HSD) test.

The mean number of unparasitized fourth and fifth instar *D. citri* nymphs remained similar across all treatments. However, the number of unparasitized fourth instar nymphs significantly increased when increasing the number of parasitoids released from 18 (12 ♀:6 ♂) to 45 (30 ♀:15 ♂) *T. radiata* (Table 1). The mean number of parasitized fourth and fifth instar nymphs decreased as the number of parasitoids relative to hosts increased. The highest level of parasitism of *D. citri* fourth and fifth instar nymphs resulted when 9 (6 ♀:3 ♂) *T. radiata* were released. The level of parasitism decreased almost proportionally, with 2.65, 2.34, and 3.40 × more parasitized fifth than fourth instar nymphs for 9 (6 ♀:3 ♂), 18 (12 ♀:6 ♂), and 45 (30 ♀:15 ♂) *T. radiata*, respectively. Super-parasitism of fourth instar nymphs took place only with 45 (30 ♀:15 ♂) *T. radiata*, and of fifth instar nymphs with 18 (12 ♀:6 ♂) and 45 (30 ♀:15 ♂) *T. radiata*. The percentage of parasitized *D. citri* nymphs for 9 (6 ♀:3 ♂), 18 (12 ♀:6 ♂), and 45 (30 ♀:15 ♂) *T. radiata* was 65%, 54%, and 13% for the fourth instar, and 92%, 89%, and 56% for fifth instar.

The relative number of *T. radiata* released into cages containing fourth and fifth instar *D. citri* nymphs, produced based on a standardized 60 *D. citri* adults, was increased to determine the amount of parasitism, super-parasitism, and its impact on mass rearing operations. The level of parasitism of the host nymphs decreased as the number of parasitoids relative to nymphs increased, probably resulting from host-feeding and competition for nymphs suitable for oviposition (Chien et al. 1991; Chen et al. 2016b). More fifth instar nymphs were parasitized irrespective of the number of parasitoids released, indicating that instar was preferred by *T. radiata* females.

Our study expands on previous findings, and indicates that increasing the number of *T. radiata* beyond optimal parasitoid:host ratios likely causes extreme competition among females for hosts. This competition could have resulted in super-parasitism, although it was infrequent. *Tamarixia radiata* is known to exhibit higher lev-

els of super-parasitism when hosts are relatively scarce (Chen et al. 2016a). For mass rearing *T. radiata*, 1 female parasitoid per 45 nymphs was suitable; about 36% of the nymphs were parasitized, and 57% killed by host feeding (Skelley & Hoy 2004). A maximum of 60% parasitism has been achieved using 1 female for 80 nymphs (Chen et al. 2017). Experimentally, however, 73% parasitism has been accomplished using 1 *T. radiata* female per nymph (Chen et al. 2016b). We observed a maximum of 92% parasitism of fifth instar nymphs at the lowest number of females and, as nymphs became less abundant relative to *T. radiata* females, the level of parasitism decreased along with an increase in the occurrence of super-parasitism.

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Summary

Tamarixia radiata females parasitized fewer host *Diaphorina citri* fourth and fifth instar nymphs as the number of females relative to hosts increased. Releasing additional females above an optimum number increased host feeding and competition for nymphs suitable for oviposition. Super-parasitism by *T. radiata*, while rare, likely indicates overpopulation, and specifically the loss of production in a mass-rearing system.

Key Words: Asian citrus psyllid; parasitoid rearing; biological control; super-parasitism

Sumario

El parasitismo del los cuarto y quinto instares de ninfas de *Diaphorina citri* por *Tamarixia radiata* disminuyó a medida que aumentaba la proporción de parasitoide:hospedero. La liberación de hembras adicionales aumentó la alimentación sobre los hospederos y la competencia por ninfas adecuadas para la oviposición. El súper-parasitismo por *T. radiata*, aunque es raro, probablemente indica una sobrepoblación y específicamente la pérdida de producción en un sistema de cría en masa.

Palabras Clave: Psílido asiático de los cítricos, cría de parasitoides, control biológico, súper-parasitismo

Table 1. Mean (± SD) number of unparasitized, parasitized, and super-parasitized fourth and fifth instar *Diaphorina citri* nymphs, and % parasitized nymphs, after 4 d exposure to a 2:1 sex ratio (female:male) of *Tamarixia radiata*^a.

<i>Tamarixia radiata</i> treatment (f:m)	Unparasitized nymphs (no.)	Parasitized nymphs (no.)	Super-parasitized nymphs (no.)	Parasitized nymphs (%)
Fourth instar nymphs				
9 (6:3)	8.6 ± 7.5 ab	16.1 ± 15.4 a	0	65
18 (12:6)	6.3 ± 3.4 b	7.5 ± 5.3 b	0	54
45 (30:15)	13.9 ± 11.3 a	2.0 ± 2.6 b	0.7 ± 1.2	13
Fifth instar nymphs				
9 (6:3)	3.5 ± 6.5	42.7 ± 36.0 a	0	92
18 (12:6)	2.2 ± 2.9	17.5 ± 11.0 b	0.1 ± 0.3	89
45 (30:15)	5.3 ± 8.2	6.8 ± 11.3 b	0.1 ± 0.6	56

^aMeans in a column followed by different lowercase letters are significantly different ($P \leq 0.05$; ANOVA and Tukey's HSD test). Unparasitized nymphs (df = 59; $P = 0.0149$ for fourth instar, and df = 59; $P = 0.2982$ for fifth instar), parasitized nymphs (df = 59; $P < 0.0001$ for fourth instar, and df = 59; $P < 0.0001$ for fifth instar).

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