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An assessment of interspecific competition between two introduced parasitoids of *Diaphorina citri* (Hemiptera: Liviidae) on caged citrus plants

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Abstract Two parasitoids attacking nymphs of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) are being released in California, USA in a classical biological control program. To evaluate the effect of multiple parasitoid species on *D. citri* mortality, we conducted mesocosm experiments under controlled conditions using a complete block design with 6 treatments (*D. citri* nymphs exposed to: no parasitoids; *D. aligarhensis* or *T. radiata* alone; *D. aligarhensis* or *T. radiata* released first (by 48 h); and both species released simultaneously). Parasitism of *D. citri* nymphs by *T. radiata* exceeded 60% and was unchanged when *D. aligarhensis* were present. Parasitism by *D. aligarhensis* was greatest when *T. radiata* was absent (∼28%) and was reduced in all treatments with *T. radiata* present (<3%). *D. citri* mortality and parasitoid-related mortality of *D. citri* was consistent across parasitoid treatments. Laboratory results suggest that competition between *D. aligarhensis* and *T. radiata* is asymmetric and favors *T. radiata*. It may be difficult for *D. aligarhensis* to contribute significantly to *D. citri* biological control where *T. radiata* is present. However, results reported here suggest that competition between *T. radiata* and *D. aligarhensis* is not likely to reduce parasitism by *T. radiata* or reduce parasitoid-induced mortality of *D. citri*.

Key words Asian citrus psyllid; classical biological control; competition; *Diaphorencyrtus aligarhensis*; *Tamarixia radiata*

Introduction

Diaphorina citri Kuwayama (Hemiptera: Liviidae), the Asian citrus psyllid, is the insect vector of *Candidatus*

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Liberibacter asiaticus (*C*Las), the bacterial agent responsible for huanglongbing (HLB), a deadly and incurable disease of citrus (Bové, 2006). *D. citri* was initially found in California in 2008 and spread quickly throughout urban areas of southern California by utilizing abundant backyard citrus (Hoddle, 2012). Citrus trees infected with *C*Las and exhibiting symptoms of HLB were subsequently found in Los Angeles County in 2012 (Kumagai *et al.*, 2016). The *D. citri*-*C*Las pathosystem has been particularly destructive in Florida, USA (Salifu *et al.*, 2012) and is a serious threat to the long-term viability of the citrus industry in California, which is worth approximately $2 billion USD annually (United States Department of Agriculture, 2015).

A classical biological control program is targeting *D. citri* in California (Hoddle *et al.*, 2016; Vankosky & Hoddle, 2016). The objective of this program is to reduce the density of urban *D. citri* populations, which should subsequently slow the rate at which *D. citri* acquire *C*Las from infected trees and spread it to uninfected trees (Bové, 2006). The urban-based classical biological control program is an attempt to reduce the rate of spread of *D. citri-C*Las from residential zones into commercial citrus production areas in California (Hoddle, 2012). The biological control program utilizes two host specific primary parasitoids of *D. citri*: an ectoparasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and an endoparasitoid, *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) (Hymenoptera: Encyrtidae) (Hoddle, 2012; Hoddle & Pandey, 2014; Bistline-East *et al.*, 2015; Vankosky & Hoddle, 2016). Biparental populations of both parasitoids were obtained from Punjab, Pakistan (Hoddle, 2012). Punjab, Pakistan is within the native range of *D. citri* and was chosen for foreign exploration as the climates in Pakistan and southern California have a good match (∼70%), which should ease climatic barriers that could affect the likelihood of establishment (Hoddle, 2012).

It is uncertain how *D. aligarhensis* and *T. radiata* will interact in California. Their long-term impact on *D. citri* populations is also unknown. Both parasitoid species have been introduced in Florida, USA, a major citrus production area where *D. citri-C*Las have been established since 2005 (Hall *et al.*, 2012). In Florida, observed rates of *T. radiata* parasitism are variable, and depend on how parasitism rates are assessed. For example, Qureshi *et al.* (2009) reported mean *T. radiata* parasitism rates below 20% in spring months with higher rates observed in autumn (39% and 56% in September and November, respectively), using tap samples at 28 orchards. In contrast, Michaud (2004) reported 0.2%–1.3% *D. citri* mortality due to *T. radiata* parasitism in central Florida when *D. citri* cohorts were observed in field conditions. Attempts to establish uniparental populations of *D. aligarhensis*, obtained from China (Taiwan) and Vietnam, were unsuccessful in Florida (Rohrig *et al.*, 2012).

The optimal number of natural enemy species to establish for successful classical biological control of a given target pest has generated considerable interest from biological control practitioners, ecologists, and population modelers (Hardin, 1960; DeBach & Sundby, 1963; Haigh & Maynard Smith, 1972; Quezada & DeBach, 1973; Ehler & Hall, 1982; Myers *et al.*, 1989; Briggs, 1993; Rosenheim *et al.*, 1995; Ferguson & Stiling, 1996; Denoth *et al.*, 2002). Establishment of more than one natural enemy species may result in competitive

exclusion, where one species is outcompeted and eliminated from part of the pest's range (Hardin, 1960; DeBach & Sundby, 1963). Alternatively, establishment of multiple natural enemy species may result in reduced pest mortality relative to a program that utilizes only one species that is presumed to be the most efficacious (Ehler & Hall, 1982; Briggs, 1993; Rosenheim *et al.*, 1995; Ferguson & Stiling, 1996). This situation could arise if one parasitoid has a competitive advantage in terms of development, for example, but is the less effective parasitoid (Briggs, 1993). In contrast, the efficacy of classical biological control may be increased through the use of more than one natural enemy species that have minimal competitive interactions. This outcome would likely be facilitated by niche partitioning between the two species, possibly driven by differences in preferred habitat type, climate, or host developmental stage (Haigh & Maynard Smith, 1972; Quezada & DeBach, 1973; Myers *et al.*, 1989; Denoth *et al.*, 2002).

Our current knowledge of *D. aligarhensis* and *T. radiata* is insufficient to predict if both parasitoid species will contribute to *D. citri* population suppression or if disruptive competition will result in lower levels of *D. citri* control in California. Establishment of both parasitoid species is possible as populations of *D. aligarhensis* and *T. radiata* collected for release in southern California coexist sympatrically in their native range of Punjab, Pakistan (Hoddle, 2012; Khan *et al.*, 2014) and in California, recoveries of both parasitoid species have been made concurrently at survey sites (*M. Vankosky*, personal observation). Sympatric coexistence may be possible because the two parasitoid species exhibit preferences for different host stages. *T. radiata* preferentially targets fourth and fifth instar *D. citri* nymphs (Hall *et al.*, 2012; Sule *et al.*, 2014), while *D. aligarhensis* prefers second and third instars (Rohrig, 2010; Rohrig *et al.*, 2011). However, Briggs (1993) cautioned that stage specific preferences by different natural enemy species for different host stages might reduce their overall efficacy. Coestablishment of *D. aligarhensis* and *T. radiata* may not be possible if *T. radiata* establishes in an area first (Rohrig *et al.*, 2012), as female *T. radiata* kill more hosts and because *T. radiata* larvae are superior intrinsic competitors (Rohrig, 2010).

An assessment of the competitive interactions of *D. aligarhensis* and *T. radiata* is required to predict if both parasitoid species will establish sympatrically in California, reduce *D. citri* densities, and exert long-term control of *D. citri*. As a first step in investigating potential competitive outcomes, we conducted laboratory studies using mesocosms comprised of caged citrus plants infested with *D. citri* nymphs that were exposed to *D.*

aligarhensis and *T. radiata* under 6 different treatment combinations. These experiments allowed us to evaluate the effects of competition between the two parasitoid species on their respective reproductive success and on rates of *D. citri* mortality attributable to parasitism and host feeding.

Materials and methods

Experimental design

Citrus volkameriana V. Ten. & Pasq. (Sapindales: Rutaceae) plants were pruned to less than 200 mm tall to promote flush (i.e., new growth) and transferred from greenhouses located at University of California, Riverside (UCR) Agricultural Operations to the UCR Insectary and Quarantine Facility (IQF). Flush growth is required for oviposition by female *D. citri* and for successful development of *D. citri* nymphs (Hall *et al.*, 2012). Individual flushing *C. volkameriana* plants were isolated in small cages that consisted of 2 transparent "U"-shaped plastic risers (SW Plastics, F2191, Riverside, CA, USA) joined to produce a 30 cm × 15 cm × 15 cm rectangular structure with 2 solid plastic walls; the third wall and a sleeve for cage access were constructed from no-see-um mesh (Skeeta, Bradenton, FL, USA), as described by Bistline-East *et al.* (2015). Each caged *C. volkameriana* plant was inoculated with adult *D. citri* and females were allowed to lay eggs for approximately 10 d until they were removed using an aspirator. *D. citri*-inoculated cages were held in a climate controlled rearing room (29°C, 40% RH, 14:10 L:D photoperiod) until second and third instar *D. citri* nymphs were observed, approximately 15 d after cages were set up. At this time, cages were moved to an adjacent rearing room (23 ± 5°C, 40% RH, 14:10 L:D photoperiod) and prepared for parasitoid inoculation. All stages of *D. citri* nymphs, including eggs, were counted with the aid of an OptiVISOR (Donegan Optical Company, Inc., Lenexa, KS, USA) and the initial starting number of *D. citri* in each mesocosm was recorded. Initial *D. citri* nymph populations in mesocosms ranged from 200 to 690. In cages with more than 700 *D. citri* eggs and nymphs, excess nymphs were removed so that mortality due to competition between *D. citri* infesting the flush would be minimized. When mesocosms were inoculated with parasitoids, *D. citri* cohorts, consisted of, on average, 14% ± 2% eggs; 45% ± 3% first and second instar nymphs; 35% ± 3% third instar nymphs; and 6% ± 1% fourth and fifth instar nymphs.

Once nymphs and eggs were counted, each cage was labeled with the start date, initial number of *D. citri* infesting the plant, and the parasitoid treatment being evaluated. Each mesocosm was randomly assigned to 1 of 6 parasitoid treatments: (1) control in which *D. citri* nymphs developed in the absence of parasitoids; (2) *D. citri* nymphs exposed to *D. aligarhensis* only, (3) *D. citri* nymphs exposed to *T. radiata* only, (4) *D. citri* nymphs exposed to *D. aligarhensis* first followed by *T. radiata* 48 h later, (5) *D. citri* nymphs exposed to *T. radiata* first followed by *D. aligarhensis* 48 h later, and (6) *D. citri* nymphs exposed to *D. aligarhensis* and *T. radiata* released simultaneously. Parasitoids were introduced into mesocosms by opening microcentrifuge tubes containing mated pairs of parasitoids, less than 5 d old, inside the mesocosm and gently tapping the tube until the parasitoids emerged.

Diaphorencyrtus aligarhensis used for this experiment were sourced from colonies maintained in IQF that were originally started with material obtained from Punjab, Pakistan (experiments conducted under USDA-APHIS release permit P526P-11-00103) and are biparental (Hoddle, 2012). *T. radiata* were originally obtained from Punjab, Pakistan (experiments conducted under USDA-APHIS release permit P526P-09-02585) (Hoddle, 2012). A colony of this biparental population was maintained at the California Department of Food and Agriculture (CDFA) rearing facility at Mt. Rubidoux, Riverside, California, USA, and parasitoids from this colony were used for experiments.

The experiment was conducted between 17 March and 19 June 2016 and utilized 15 fully replicated blocks (6 mesocosms per block), initiated on a weekly basis when plant and insect material was available at the required growth stages. *C. volkameriana* were watered as required and honey droplets, supplied to the walls of the mesocosms as a carbohydrate source for the parasitoids, were replenished as needed for the duration of the experiments.

Mesocosms were inspected on alternating days for adult *D. citri*, *D. aligarhensis*, and *T. radiata* that emerged from the initial *D. citri* cohort, beginning 12 d after experimental blocks were initiated. All adult insects emerging from mesocosms were collected and counted. Collections continued until there was no insect emergence in the cages for 7 consecutive days. At that time, mesocosms were dismantled and inspected one final time for any insects that escaped earlier collections and died inside the cages. As mesocosms were dismantled, the *C. volkameriana* plants were inspected for mummies without exit holes (i.e., parasitoids that died during larval or pupal development). Parasitoid mummies without exit holes were separated

from the plant surface and inspected with an OptiVI-SOR or microscope to determine if *D. citri* mummies had been parasitized by *D. aligarhensis* or *T. radiata*. These mortality data were added to the appropriate parasitoid tallies.

Calculations and statistics

For all mesocosms, the total number of adult *D. citri* that emerged was recorded. The total number of parasitoids (*D. aligarhensis* or *T. radiata*) was expressed as the sum of all adults (living and dead) collected from the cages, plus the number of mummies from which no adult parasitoids emerged. The total number of insects that emerged from each mesocosm was expressed as the sum of all *D. citri*, *D. aligarhensis*, and *T. radiata*. Using the total number of insects, percent parasitism by *T. radiata* and *D. aligarhensis* was calculated for each parasitoid species using Equation (1) (Qureshi *et al.*, 2009; Sule *et al.*, 2014) and used for statistical analyses that assessed the effect of parasitoid treatment on percent parasitism by each parasitoid species.

$$\%\text{Parasitism} = 100\,[(\text{Number of parasitoids})/$$
$$(\text{Total mesocosm emergence})]. \quad (1)$$

Mortality of *D. citri*, due to all potential abiotic and biotic sources (i.e., parasitism, host feeding by female parasitoids, unknown factors, etc.) in the mesocosms was calculated using the Henderson–Tilton formula (Equation (2); Henderson & Tilton, 1955), which calculates percent mortality based on the initial and final insect population in the control relative to the treatments. Henderson–Tilton mortality was calculated for treatments 2, 3, 4, 5, and 6 in each block of the experiment using values from the control (treatment 1). In Equation (2), the abbreviation ACP (Asian citrus psyllid) is used in place of *D. citri*.

$$\%\,\text{Mortality} = 100 \times$$
$$\left[\frac{(\text{Final ACP after Treatment}) \times (\text{Initial ACP in Control})}{(\text{Initial ACP before Treatment}) \times (\text{Final ACP in Control})} \right].$$
$$(2)$$

Seven treatment mesocosms from 3 experimental blocks had negative percent mortality values. In all 3 blocks, the initial *D. citri* nymph population in the control exceeded 400, but less than half of those nymphs emerged as adults. Thus, the treatment cages in these

blocks had more adults emerge than the controls. We elected to exclude mesocosms with negative mortality percentages from the analyses to compare mortality between treatments.

The percentage of unemerged *D. citri* in each mesocosm, due to all possible mortality factors was calculated using Equation (3).

$$\%\text{Dead } D.\ citri = 100\,[(\text{Initial } D.\ citri\ \text{nymphs}-$$
$$\text{Adult } D.\ citri)\,/\,(\text{No. of initial } D.\ citri\ \text{nymphs})]. \quad (3)$$

Using these values, mortality of *D. citri* resulting from parasitoid activity (i.e., parasitism, host feeding, indirect effects of parasitoids) was estimated for the mesocosms in each block using Equation (4).

$$\% D.\ citri\ \text{mortality due to parasitoids}$$
$$= (\%\text{Unemerged } D.\ citri\ \text{in Treatment})$$
$$- (\%\text{Unemerged } D.\ citri\ \text{in Control}). \quad (4)$$

D. citri parasitism by *T. radiata* was compared between treatments in which *T. radiata* was released (treatments 3–6) with one-way analysis of variance (ANOVA) using the GLM procedure (SAS Institute, 2009), where the level of significance for the analysis was set at $\alpha = 0.05$. Parasitism of *D. citri* by *D. aligarhensis* was compared between Treatments 2–5 using the Kruskal–Wallis test (NPAR1WAY procedure; $\alpha = 0.05$), as this dataset (raw or transformed) did not meet the assumptions of parametric testing (SAS Institute, 2009). Significant Kruskal–Wallis results were further explored using paired Mann–Whitney U tests to separate treatment means (SAS Institute, 2009); results of U tests were compared to a Bonferroni-corrected level of significance ($\alpha = 0.008$).

One-way ANOVA ($\alpha = 0.05$) was used to determine if parasitoid treatment affected *D. citri* mortality, as calculated using the Henderson–Tilton formula (Henderson & Tilton, 1955), and if parasitoid-related mortality differed between treatments.

Results

In Block 9 of the experiment, the *C. volkameriana* host plant died in the control cage, resulting in no adult *D. citri* emergence (100% mortality). Therefore, Block 9 was excluded from analyses assessing overall mortality and mortality due to parasitism because mortality in the treatment

Table 1 Initial number of *Diaphorina citri*, adult *D. citri* emergence, and the number of unemerged (dead) *D. citri* given for the 6 experimental treatments across 15 experimental blocks (mean ± SE; DA = *Diaphorencyrtus aligarhensis*; TR = *Tamarixia radiata*).

Treatment	Initial *D. citri* population	*D. citri* adults (alive)	Dead *D. citri*
Control	387 ± 23	159 ± 23	227 ± 33
DA only	387 ± 65	64 ± 70	312 ± 49
TR only	473 ± 104	70 ± 65	311 ± 157
DA first	457 ± 22	37 ± 11	327 ± 37
TR first	484 ± 68	30 ± 53	359 ± 86
DA + TR	564 ± 19	86 ± 32	356 ± 38

cages could not be calculated relative to the control. The control cage of Block 12 was contaminated with adult *T. radiata* when *D. citri* nymphs were susceptible to parasitism, thus Block 12 was also excluded from mortality analyses. All data from the treatment cages in Blocks 9 and 12 were included in analyses to determine the effect of treatment on parasitism, as parasitoid emergence was observed in all treatment cages for both blocks. Table 1 summarizes the mean (±SE) number of initial *D. citri*, the number of *D. citri* adults that emerged, and the number of dead *D. citri*. Mean values were calculated using all replicates from each block included in the statistical analyses; these values were not compared directly because the mean initial number of *D. citri* ranged considerably between treatments (e.g., 387 ± 23 in the control and 564 ± 19 in mesocosms with both parasitoids released simultaneously; Table 1). In the control treatment, where *D. citri* were not exposed to parasitoids, mean *D. citri* mortality was 58.7%

The rate at which *D. citri* nymphs were parasitized by *T. radiata* was not affected by the composition of the parasitoid species within the treatment cages, or by the order of parasitoid introduction ($F_{3,56} = 0.78$, $P = 0.5121$; Fig. 1). Parasitism of *D. citri* by *D. aligarhensis* was affected by parasitoid treatment ($\chi^2 = 19.42$, df = 3, $P = 0.0020$). The greatest rate of parasitism by *D. aligarhensis* was observed in cages without *T. radiata* (Fig. 2). The lowest rate of parasitism by *D. aligarhensis* was observed in cages where *D. aligarhensis* were introduced 48 h after *T. radiata* (Fig. 2).

Mortality of *D. citri*, as calculated using the Henderson–Tilton equation, did not differ between mesocosm treatments ($F_{4,53} = 0.65$; $P = 0.6302$). Mean mortality of *D. citri* was less than 70% in cages with *D. aligarhensis* only, *T. radiata* only, and in cages where the parasitoid species were introduced simultaneously (Fig. 3A). Mean *D. citri* mortality was greater than 70% in cages where both parasitoids were present, but were introduced 48 h apart (Fig. 3A).
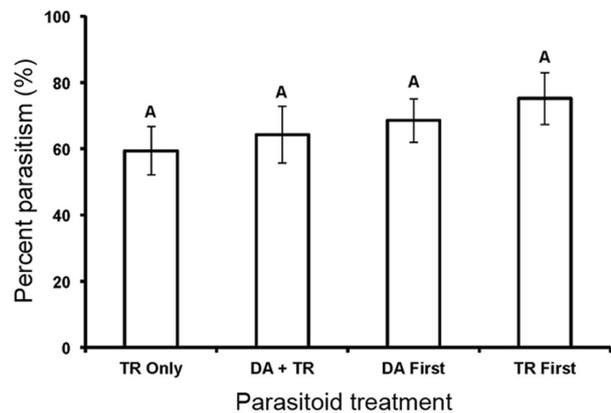

Fig. 1 Mean (±SE) rates of parasitism by *Tamarixia radiata* (TR) observed in mesocosms where only *T. radiata* was released (TR Only), where *Diaphorencyrtus aligarhensis* (DA) and *T. radiata* were introduced simultaneously (DA + TR), where *D. aligarhensis* was released 48 h before *T. radiata* (DA First), and where *T. radiata* was introduced 48 h before *D. aligarhensis* (TR First). Means with the same letters are not significantly different ($P > 0.05$).

Mortality associated with parasitoid activity (i.e., host feeding by female parasitoids, parasitism, and indirect effects of parasitoid presence) did not differ between cage treatments ($F_{4,60} = 1.12$, $P = 0.3538$; Fig. 3B).

Discussion

Parasitism by *T. radiata* exceeded 60% in all treatments where *T. radiata* was released and the presence of *D. aligarhensis* did not affect the rate of parasitism by *T. radiata*. Previous laboratory studies using *T. radiata* and *D. aligarhensis* from the same populations used in this study found that *T. radiata* foraging was not affected by the presence of heterospecific or conspecific competitors (Vankosky & Hoddle, 2017). In the current experiment, mated female *T. radiata* foraged for *D. citri* nymphs in a more realistic landscape than in the aforementioned
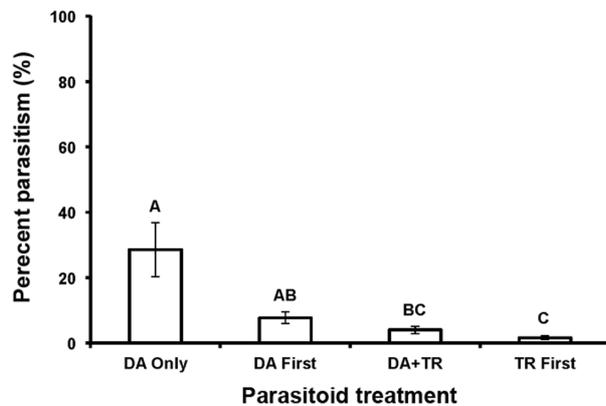
Fig. 2 Mean (±SE) rates of parasitism by *Diaphorencyrtus aligarhensis* (DA) observed in mesocosms where only *D. aligarhensis* was released (DA Only), where *D. aligarhensis* and *Tamarixia radiata* (TR) were introduced simultaneously (DA + TR), where *D. aligarhensis* was released 48 h before *T. radiata* (DA First), and where *T. radiata* was introduced 48 h before *D. aligarhensis* (TR First). Means with the same letters are not significantly different ($P > 0.05$).

laboratory study, and may have been less likely to encounter *D. aligarhensis* while searching for hosts. However, when taken together, both studies (i.e., laboratory studies [Vankosky & Hoddle, 2017] and the mesocosm experiments reported here) support the conclusion that parasitism rates of *T. radiata* are not affected by the presence of *D. aligarhensis*, irrespective of the scale of the experiment (i.e., Petri dishes vs. small plants), time available for search (i.e., 1 h vs. several days), and varying host densities at which these two parasitoids concurrently searched for hosts. This result is important as it suggests that in southern California, the recent and ongoing release of *D. aligarhensis* (Bistline-East *et al.*, 2015; Vankosky & Hoddle, 2016) will not likely have an adverse effect on *D. citri* biological control exerted by previously established populations of *T. radiata*.

Parasitism by *D. aligarhensis* in these experiments did not exceed 30% and was greatest when *D. aligarhensis* foraged alone (28.5%). *D. citri* parasitism by *D. aligarhensis* was significantly reduced to 7.7% or lower when *T. radiata* was present. The reduction in *D. aligarhensis* parasitism observed here could be the result of competitive interactions between heterospecific female parasitoids (Mohamad *et al.*, 2015; Xu *et al.*, 2015). However, in small-sized and structurally simple experimental arenas, *D. aligarhensis* oviposited as often in the presence of female *T. radiata* as they did when they were alone (Vankosky & Hoddle, 2017). In those arenas, female parasitoids had 1 h to search and were restricted to a small foraging space with only 20 potential hosts to choose
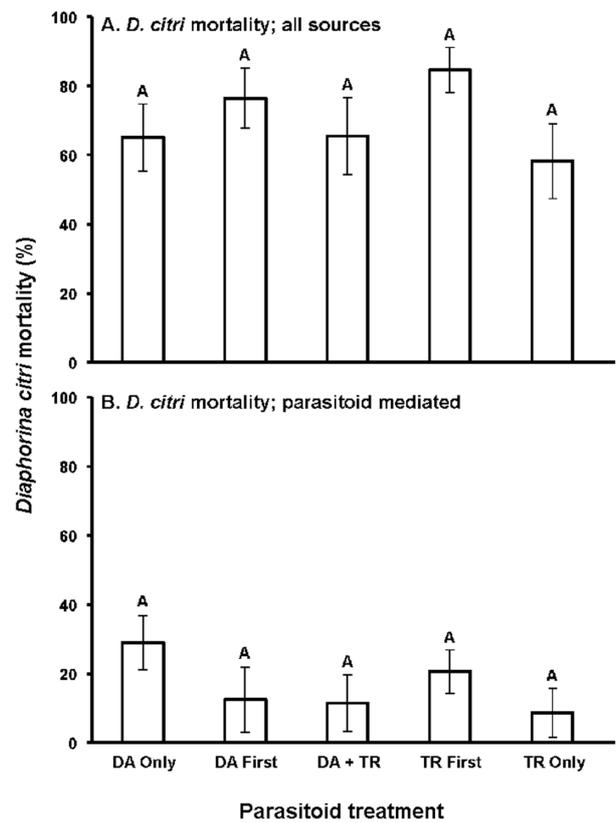

Fig. 3 Mean (±SE) mortality of *Diaphorina citri* in mesocosms attributed to: (A) all possible sources of mortality (i.e., parasitism, host feeding by female parasitoids, competition between *D. citri* nymphs, abiotic, and unknown factors), and (B) parasitoid activity (i.e., parasitism, host feeding, superparasitism, and multiparasitism). In both panels, means with the same letters are not significantly different ($P > 0.05$).

from. In the current study, female parasitoids foraged for an excess of hosts distributed on a small citrus plant contained within a small cage for several days. Therefore, we expect that direct interactions between *D. aligarhensis* and *T. radiata* females were less likely to occur in mesocosms with an excess of time than in competition studies conducted at the scale of a Petri dish with limited foraging time (Vankosky & Hoddle, 2017).

An alternative explanation to direct competition between female parasitoids is that the low rates of *D. aligarhensis* parasitism observed in the presence of *T. radiata* resulted from intrinsic larval competition in which larval *T. radiata* killed or outcompeted *D. aligarhensis* larvae when multiparasitism occurred. Specifically, Rohrig (2010) observed that *T. radiata* larvae killed *D. aligarhensis* larvae that were less than 5 d old when both species inhabited the same *D. citri* host. In the current study, *D. aligarhensis* that had a 48 h developmental advantage had

a higher rate of parasitism than when both species were introduced simultaneously or when *T. radiata* was introduced 48 h in advance of *D. aligarhensis*. In Rohrig's study (2010), *D. aligarhensis* eggs deposited after *T. radiata* oviposited never survived, which potentially explains why the rate of *D. aligarhensis* parasitism was low in mesocosms first inoculated by *T. radiata*. Repeating this study using different time intervals between *D. aligarhensis* and *T. radiata* introductions would further elucidate the impact of intrinsic larval competition between these 2 parasitoid species. It would be interesting to repeat these experiments in the presence of *D. citri* predators, as intraguild predation could contribute to parasitoid mortality causing parasitism rates to be underestimated in field conditions (Michaud, 2004).

Based on the results of the mesocosm experiments conducted here, we expect that *D. aligarhensis* parasitism in southern California will be higher in habitats where *T. radiata* is absent, but that low levels of parasitism by *D. aligarhensis* might be possible where both species occur sympatrically. Heterospecific competition between *D. aligarhensis* and widely established *T. radiata* may have been one of several factors that contributed to the failure of *D. aligarhensis* to establish in Florida (Rohrig *et al.*, 2012). Other factors, including an environmental mismatch, may have also contributed to the failure of *D. aligarhensis* to establish in Florida (Rohrig *et al.*, 2012). It may be difficult for *D. aligarhensis* to establish in California because of heterospecific competition between *D. aligarhensis* and *T. radiata*, especially in areas where *T. radiata* is established (Hoddle *et al.*, 2016).

Potential loss of efficacy in biological control programs involving multiple species of natural enemy is a significant concern (Ehler & Hall, 1982; Briggs, 1993; Rosenheim *et al.*, 1995; Ferguson & Stiling, 1996). In the current study, we observed that mortality of *D. citri*, ranged from approximately 60%–80% relative to the control treatment without parasitoids and *D. citri* mortality rates did not differ significantly across parasitoid treatments. This suggests that if only one parasitoid species utilizes a patch, if both species arrive simultaneously, or if one species arrives before the other the overall rate of *D. citri* mortality caused by *T. radiata* or *D. aligarhensis* should not be adversely affected. This analysis indicates that efforts to establish *D. aligarhensis* are not likely to interfere with current levels of biological control of *D. citri* achieved by *T. radiata* in southern California. It also suggests that parasitism is one of several sources of mortality affecting the survival of *D. citri* nymphs and other sources of mortality not measured here, such as host feeding, superparasitism or multiparasitism, and naturally occurring mortality due to competition between *D. citri* nymphs

or overcrowding likely contributed to reduced *D. citri* survivorship rates in comparison to control treatments. These sources of mortality probably affect *D. citri* populations in field conditions too, but their rates have not been quantified. In this experiment, estimates of *D. citri* mortality resulting from competition between nymphs or overcrowding on flush was likely inflated because of the limited number of oviposition sites available for female *D. citri* inside the mesocosms.

Estimating the total mortality caused by parasitoids (i.e., death of nymphs due to parasitism, host feeding, superparasitism or multiparasitism) is difficult, especially in the field and in mesocosm studies using caged plants infested with hosts. Khan *et al.* (2014) estimated parasitism rates of *D. citri* by both species of parasitoid in citrus orchards in Pakistan, but did not estimate other parasitoid-mediated sources of mortality (e.g., host feeding). Kistner *et al.* (2016) estimated mortality due to *T. radiata* parasitism under field conditions and acknowledged that other sources of parasitoid-mediated mortality, particularly host feeding, likely occurred and were probably important contributors to *D. citri* mortality. However, mortality sources and rates in addition to parasitism were not quantified in these life table studies (Kistner *et al.*, 2016). Results from this study suggest that parasitoid-mediated *D. citri* mortality constituted approximately 10%–30% of mean *D. citri* mortality, and this estimate did not differ significantly between parasitoid treatments. Consequently, this result suggests that in the presence of *T. radiata* and *D. aligarhensis*, alone or in combination, mortality of *D. citri* will be relatively constant irrespective of the time females of either species arrive at host patches. To the best of our knowledge, these results represent the first attempt to attribute *D. citri* mortality to parasitoid activity when both of its primary parasitoids are active on *D. citri* patches. Future work, conducted in the field, should endeavor to build upon the results reported here, and those reported by Kistner *et al.* (2016).

In conclusion, our results suggest that introducing *D. aligarhensis* into southern California will not significantly affect the level of *D. citri* suppression currently provided by *T. radiata* or adversely impact *T. radiata* populations. However, our results also indicate that *D. aligarhensis* may not be able to establish in habitats already occupied by *T. radiata*. Currently, *D. aligarhensis* release efforts in southern California are focused on areas where *T. radiata* has not been released (Vankosky & Hoddle, 2016). We recommend that this strategy be maintained with the goal that *D. aligarhensis* establishes populations in habitats that are either not suitable for *T. radiata* or provide an advantage to *D. aligarhensis* (e.g., due to differences in climate preferences). Hypotheses

generated from experiments reported here will be addressed with field-based research programs investigating the effects of long-term releases in southern California of mass produced *D. aligarhensis* on the establishment, spread, impact, and phenology of this parasitoid (Vankosky & Hoddle, 2016).

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Disclosure

The authors have no conflicts of interest to disclose.

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