agronomy

Article

Post-Release Evaluation of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Hymenoptera: Eulophidae) for Biological Control of *Diaphorina citri* (Hemiptera: Liviidae) in Urban California, USA

Ivan Milosavljević [1,2,*], Meghan A. Vankosky [1,3], David J. W. Morgan [4], Christina D. Hoddle [1],
Rachael E. Massie [4] and Mark S. Hoddle [1,2]

[1] Department of Entomology, University of California, 900 University Ave, Riverside, CA 92521, USA; meghan.vankosky@agr.gc.ca (M.A.V.); christina.hoddle@ucr.edu (C.D.H.); mark.hoddle@ucr.edu (M.S.H.)
[2] Center for Invasive Species Research, University of California, Riverside, CA 92521, USA
[3] Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, 107 Science Place, Saskatoon, SK S7N 0X2, Canada
[4] California Department of Food and Agriculture, 4500 Glenwood Drive, Riverside, CA 92501, USA; david.morgan@cdfa.gov (D.J.W.M.); remassie@gmail.com (R.E.M.)
* Correspondence: ivanm@ucr.edu

Abstract: *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) was first released in California for biological control of *Diaphorina citri* (Hemiptera: Liviidae) in December 2014. The establishment and parasitism rates of *D. aligarhensis*, along with those of another introduced species, *Tamarixia radiata* (Hymenoptera: Eulophidae), first released in 2011, were assessed at 15 *D. aligarhensis* release and 24 no-release control sites over the period 2016–2018. Study sites with citrus trees that were infested with *D. citri* eggs, nymphs, and adults, were located in residential areas in southern California that spanned three different climatic zones: coastal, intermediate, and desert interior sites. Parasitism rates of *D. aligarhensis* were low, averaging 0.62% compared to 21.2% for *T. radiata* which had spread naturally and established widely through the study area approximately one year earlier. Recoveries of *D. aligarhensis* at release sites were made eight times in 2016 and 2017. Conversely, *T. radiata* was recovered consistently at 34 of the 39 sites surveyed. Analyses indicated that parasitism of *D. citri* nymphs by *T. radiata* exhibited delayed density-dependence with a 12-month lag associated with reductions of *D. citri* densities by 50%. Irrespective of the climatic zone, the highest frequency of parasitized *D. citri* nymphs for *T. radiata* was recorded during peak periods of citrus flush growth from March through June and October through November each year. The findings reported here suggest that it is unlikely *D. aligarhensis* has established in California and that competition from *T. radiata* may, in part, have contributed to establishment failure. Consequently, biological control efforts targeting *D. citri* in California should focus on *T. radiata*.

Keywords: Asian citrus psyllid; barriers to establishment; biological control; citrus greening; *Diaphorina aegyptiaca*; post-release evaluations; huanglongbing

check for updates

Citation: Milosavljević, I.; Vankosky, M.A.; Morgan, D.J.W.; Hoddle, C.D.; Massie, R.E.; Hoddle, M.S. Post-Release Evaluation of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Hymenoptera: Eulophidae) for Biological Control of *Diaphorina citri* (Hemiptera: Liviidae) in Urban California, USA. *Agronomy* **2022**, *12*, 583. https://doi.org/10.3390/agronomy12030583

Academic Editors: Wayne B. Hunter, Liliana M. Cano, Jawwad A. Qureshi and Ivo Tosevski

Received: 13 January 2022
Accepted: 24 February 2022
Published: 26 February 2022

1. Introduction

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was discovered infesting urban citrus in San Diego County California, USA, in 2008 [1]. The invasion of this pest in California is significant because *D. citri* transmits a bacterial pathogen, *Candidatus* Liberibacter asiaticus (CLas) [2], which causes a lethal citrus disease, huanglongbing [3]. The first tree with CLas was found in Los Angeles County, California, in 2012 and infestations are largely restricted to urban areas in southern California [4]. Backyard citrus trees in southern California are ubiquitous and can harbor *D. citri*, which facilitates the transmission and acquisition of CLas [5]. Should CLas spread from urban citrus into commercial production zones, especially the San Joaquin Valley, the major citrus producing region in the state, it would potentially jeopardize ~262,000 fruit-bearing acres [6], which supports a total of citrus-related revenue worth an estimated USD 7.1 billion per year [7].

A biological control program against *D. citri* in California was initiated in 2010 [8,9]. This program focused on two nymphal parasitoids, *Diaphorencyrtus aligarhensis* Shafee, Alam, and Argarwal (Hymenoptera: Encyrtidae) and *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), both of which were imported from Punjab, Pakistan, an area with a good climatic match to southern California [8]. Imported parasitoid species were subjected to host range and host specificity testing and approved for release [10,11]. In urban areas, biological control of *D. citri* is viewed more favorably by homeowners than large-scale treatments of backyard trees with insecticides [12], while the reduction of vector densities is critical for reducing the rate of spread of CLas by *D. citri* [13,14]. Consequently, the goal of the biological control program with *D. aligarhensis* and *T. radiata* was to use parasitoid complementarity to reduce densities of *D. citri* with the concomitant goal of slowing rates at which CLas could spread from urban areas into commercial citrus production zones.

Complementarity biological control of *D. citri* by *T. radiata* and *D. aligarhensis* aimed to reestablish in California the two species parasitoid guild from part of the presumptive native range of the target pest (from Punjab, Pakistan) [8] and to exploit parasitoid species preferences for different host stages. At the time this classical biological control program was initiated, it was reported that *D. aligarhensis*, an arrhenotokous endoparasitoid, favorably parasitized second and third instar *D. citri* nymphs [15], while *T. radiata*, an arrhenotokous ectoparasitoid, favorably parasitized fourth and fifth instar *D. citri* [16]. Subsequently, laboratory studies with both species of parasitoid sourced from Punjab, Pakistan, indicated that *D. aligarhensis* and *T. radiata* exhibited a shared life stage preference for fourth instar nymphs, a finding not previously documented [17]. Both parasitoid species can kill *D. citri* via feeding on nymphs [18–20].

In California, *T. radiata* was first released against *D. citri* in December 2011 [8]. Since this time, >23 million *T. radiata* have been released at >19,000 sites [21], with evidence of establishment at >90% of release sites in southern California, including recoveries in areas >10 km from release sites indicating extensive natural dispersal [22]. Impact studies have documented reductions >75% in *D. citri* densities in some areas, due in part to parasitism by *T. radiata*, which was recorded at >60% during periods of peak parasitoid activity [23]. The release of *D. aligarhensis* in California began in December 2014 [11]. Subsequently, >700,000 *D. aligarhensis* have been released at >300 sites in southern California since 2014 [21]. In an attempt to reduce any negative interspecific interactions that could affect the establishment of incipient populations [24], initial releases of *D. aligarhensis* were made in areas infested with *D. citri* that had not received releases of *T. radiata*.

This decision to release *D. aligarhensis* in areas with no or little competition from *T. radiata* was further enforced by reports that this parasitoid failed to establish in Florida, despite multiple releases of two thelytokous populations sourced from Taiwan (released 2000–2002) and China (released 2007–2009) [25]. *Tamarixia radiata* was first released in Florida in 1999 [26] and had reportedly established by 2001 [27] but interspecific interference could have been a factor in the lack of establishment for *D. aligarhensis* [25,28]. Other factors affecting outcomes of interspecific competition between *D. aligarhensis* and *T. radiata* and subsequent establishment likelihoods could be preferences for performance differences across heterogeneous climates and varieties of citrus cultivars, which may be more favorable for one species but not the other [29]. Range partitioning by different species of biocontrol agents attacking a shared citrus pest has been formerly recorded in California [30]. At the time this study was conducted, it was unknown whether *D. aligarhensis* had established in California, if establishment rates and efficacy of *D. aligarhensis* would vary across diverse climatic regions in southern California or different citrus cultivars, or how interactions with *T. radiata* would affect the collective impact of both parasitoid species on suppression *D. citri* populations.

2. Materials and Methods

2.1. Parasitoid Release and Monitoring

2.1.1. Study Sites

Fifteen residential sites, five coastal (United States Department of Agriculture [USDA] Hardiness Zone 10b), five intermediate (USDA Hardiness Zone 10a), and five desert (USDA Hardiness Zones 9a/9b) sites [31] for the release and monitoring of *D. aligarhensis* were established across five southern California counties: San Diego, Orange, Riverside, Los Angeles, and San Bernardino (Table 1; [31]). Temperature and humidity data for each of the 15 study sites were obtained from the closest meteorological station (Table 1; [32]). Each site had one lemon (*Citrus limon* L.) and one orange (*C. sinensis* L.) tree for a total of 30 study trees older than 5 years. No insecticides were applied to study trees for the duration of this project (Table 1). The release and monitoring of *D. aligarhensis* activity across study sites took place between 10 August 2015 and 29 March 2017, and 1 January 2016 and 31 December 2017, respectively (Table 1). No releases of *T. radiata* were made at these 15 sites during this time and each *D. aligarhensis* study site was located ~1–10 km from the nearest *T. radiata* parasitoid release locations ([21]; Tables 1 and S1). The sites were not examined for the presence of *T. radiata* prior to the release of *D. aligarhensis* in 2015.

2.1.2. Parasitoid Sources and Culture

Individuals of *D. aligarhensis* used for releases were collected from colonies maintained at the University of California Riverside Insectary and Quarantine Facility (UCR-IQF) and the California Department of Food and Agriculture Mt. Rubidoux Station Facility. At UCR-IQF, parasitoids were reared on second through fourth-instar *D. citri* nymphs cultured on potted 1- to 2-yr old "Volkamer" lemon plants (*C. volkameriana* V.Ten. & Pasq. [11]. All plants were grown from seed in temperature- and humidity-controlled greenhouses at UCR Ag. Operations, at 27 ± 1 °C, 50 ± 20% RH, under natural light, before they were moved to rearing cages at UCR-IQF [11]. At UCR-IQF, plants were pruned regularly to produce new leaves required for oviposition by *D. citri* females and subsequent development of nymphs [11,18]. Five *D. aligarhensis* isocage breeding lines representing different collection localities and dates from foreign exploration trips to Punjab, Pakistan, were maintained to preserve the genetic diversity of mass-produced parasitoids [33–35]. Colonies of *D. aligarhensis* were maintained in one of the five Bugdorms (model 2120F [MegaView Science Co., Ltd., Taichung, Taiwan]) labeled by locality and date of collection. Rearing cages were individually kept inside a larger Bugdorm (model 2400F). This double-cage containment was implemented to reduce the likelihood of accidental escape. All colonies were maintained at 27 °C, 40% RH, and a long-day photoperiod of 14 h [11]. Parasitoids were given water to drink and honey as an edible carbohydrate source. Newly emerged female and male parasitoids were collected daily by aspiration into individual 200 µL centrifuge tubes (model 89004-308, VWR, Radnor, PA, USA). Parasitoids were maintained at 12 ± 2 °C with droplets of 50% honey-water solution for 1–7 d prior to field release.

At the CDFA Mt. Rubidoux station rearing facility, *D. aligarhensis* colonies were maintained on mature curry leaf plants (*Bergera koenigi* L., formerly *Murraya koenigi*) infested with mixed stages of *D. citri* [21]. Parasitoid colonies were maintained in organdy-screened cages (BugDorm-2 400F, insect rearing tent; length 75 cm × width 75 cm × height 115 cm, 150 × 150 mesh size) held inside glasshouses at 27 ± 2 °C, 50 ± 6% relative humidity (RH), with a 14:10 (L:D) h photoperiod [21,36]. These colonies were initiated and maintained, in part, with *D. aligarhensis* from the original five UCR-IQF isocage lines sourced from Pakistan (see above). Adult parasitoids were aspirated daily into 150 mL plastic vials (Thermo Fisher Scientific, Waltham, MA, USA) from rearing cages. Tissue paper moistened with water was placed inside the vial to provide water, while honey, a carbohydrate resource, was streaked on vial lids. Parasitoids were maintained at 12 ± 2 °C for 1–7 d prior to their field release [21].

Table 1. Site characteristics and parasitoid release frequencies and numbers for selected *D. aligarhensis* release sites in southern California, USA.

Climate Type	County	Site Name	Latitude (deg.)	Longitude (deg.)	Elev. (m)	Surveyed Tree(s)	CIMIS Station	Date of First Parasitoid Release	Most Recent Parasitoid Release	No. *D. aligarhensis* Release Events in 2015–2017 (2016–2017 Releases)	No. *D. aligarhensis* Released in 2015–2017 (2016–2017 Releases)
Coastal	Los Angeles	Alameda	N33°59′	W118°14′	51	Lemon, Orange	174	31 August 15	7 November 16	39 (31)	6388 (4711)
	Orange	Anaheim	N33°51′	W117°44′	316	Lemon, Orange	75	17 August 15	1 March 17	49 (32)	7006 (5602)
		Fullerton	N33°53′	W117°53′	76	Lemon, Orange	75	17 August 15	15 March 17	37 (31)	6419 (4072)
		Irvine	N33°43′	W117°47′	33	Lemon, Orange	75	8 September 15	15 March 17	46 (31)	5615 (4904)
	San Diego	Fallbrook	N33°23′	W117°10′	197	Lemon, Orange	62	21 September 15	8 February 17	42 (35)	4389 (3406)
Intermediate	Los Angeles	Claremont	N34°05′	W117°42′	341	Lemon, Orange	78	31 August 15	27 March 17	26 (24)	2616 (2256)
		Pomona	N34°03′	W117°44′	255	Lemon, Orange	78	31 August 15	27 March 17	39 (31)	5595 (3512)
	Riverside	Citrus Hill	N33°51′	W117°19′	542	Lemon, Orange	44	21 September 15	8 February 17	30 (28)	3102 (2858)
		Woodcrest	N33°54′	W117°20′	479	Lemon, Orange	44	10 August 15	8 February 17	30 (23)	4449 (2929)
	San Diego	Ramona	N33°01′	W116°49′	443	Lemon, Orange	153	24 August 15	22 March 17	49 (28)	6524 (4961)
Desert	Riverside	Hemet	N33°44′	W116°49′	645	Lemon, Orange	239	8 September 15	20 March 17	20 (18)	2603 (1952)
	San Bernardino	Redlands	N34°02′	W117°13′	403	Lemon, Orange	251	5 October 15	20 March 17	29 (21)	3058 (2029)
		Warner *	N34°04′	W117°10′	440	Lemon, Orange	251	5 October 15	29 March 17	17 (15)	1981 (1151)
		Crafton	N34°03′	W117°07′	600	Lemon, Orange	251	5 October 15	29 March 17	34 (27)	3684 (2671)
	San Diego	Valley Ctr. *	N33°15′	W117°03′	348	Lemon, Orange	207	20 July 15	8 February 17	36 (29)	5542 (3191)

* Indicates study sites where *T. radiata* activity was not detected in the 2018 surveys. No next-year recoveries were made for *D. aligarhensis* in 2018.

2.1.3. Field Release and Monitoring

Releases of *D. aligarhensis* were conducted between 10 August 2015 and 29 March 2017 (see Section 2.1.1; Table 1). At each study site, parasitoid releases were contingent upon the presence of *D. citri* life stages susceptible to *D. aligarhensis* attack and available flush on study trees susceptible to *D. citri* infestation. The number of *D. aligarhensis* liberated on any release date varied, ranging from 14 to 1860 females per release date and site. This variation in numbers of parasitoids released resulted from varying levels of insectary production during the course of this study. Parasitoids contained within plastic vials with honey streaks were released onto *D. citri*-infested citrus between 8:00 a.m. and 1:00 p.m.

Post-release monitoring was conducted at all study sites every two weeks from 1 January 2016 through 31 December 2017 (see Section 2.1.1; Table 1). Experimental trees were divided into quadrants (north, east, west, and south) to control for differences in psyllid densities related to cardinal direction [23]. The numbers of larval and adult *D. citri*, and the number of nymphs parasitized by either *D. aligarhensis* or *T. radiata*, were recorded per quadrant per tree sampled [23,37,38]. Citrus flush density was assessed on each sampling date by randomly selecting eight branches around the study tree, within arm's reach and ~1.5–2 m above the ground [23,37,39]. The total number of *D. citri* individuals per colony and parasitism status were estimated by excising two randomly selected flush growths from each cardinal quadrant for a total of eight samples. If fewer than eight flush shoots were present, all were collected. Collected flush samples were placed singly into sealable bags in an insulated cooler with ice in the field and transported back to the laboratory for processing in compliance with CDFA permit 2870. Samples were refrigerated at 4 °C for 1–2 days before being processed. The length (cm) of each collected flush sample was recorded so that numbers of larval and adult psyllids could be estimated by the length of the sampled flush [23,39]. All psyllid mummies with parasitoid exit holes were counted, identified by parasitoid species, and removed: *T. radiata* emerges from the anterior thoracic region [20], whereas *D. aligarhensis* emerges from the posterior abdominal region [15,19] of mummified *D. citri*.

Dead nymphs lacking exit holes were removed from flush and inspected under a dissecting microscope to determine causes of mortality and the frequency of successful parasitism. Fourth and fifth instar nymphs were inspected on ventral sides for the presence of larval *T. radiata* [17,40,41]. Second through fourth instar nymphs with no obvious presence of *T. radiata* eggs or larvae were dissected for the presence of larval *D. aligarhensis* [19]. Under a dissecting microscope, each nymph was placed in a drop of saline solution on a depression slide and dissected using stainless steel insect pins (size 000) attached to wooden satay stick handles. Eggs, larvae, and pupae found within *D. citri* nymphs were classified as *D. aligarhensis*, added to the appropriate parasitoid tallies, and preserved in labeled vials in 95% ethanol for possible future genetic analyses. The total rates of parasitism were calculated across the two parasitoid species for each tree by study site and sampling date. Species-specific parasitism rates were calculated after excluding host instars that were unsuitable for the development of immature parasitoids.

2.2. *Parasitoid Establishment*

Establishment was monitored at 39 residential sites at which no releases of *T. radiata* were made (15 previously received *D. aligarhensis* and 24 served as controls) in February–March 2018 and September–October 2018 (Tables 1 and S1). No insecticide applications were made at these study sites. The control sites were >1 km apart from the closest *D. aligarhensis* and *T. radiata* release sites. The methods described above were used to assess parasitoid establishment through recovery of *D. citri* mummies with parasitoid exit holes and dissections of *D. citri* nymphs to detect *D. aligarhensis* eggs, larvae, or pupae. Surveys of selected sites were timed to coincide with the presence of citrus flush infested with *D. citri* life stages that *D. aligarhensis* could attack. If citrus flush and *D. citri* nymphs were not present at sites, repeat visits were made until optimal conditions for parasitoid activity occurred. If parasitoids were not detected after two consecutive surveys at sites where conditions were suitable for their activity, the site was classified as "not established".

2.3. Data Analysis

All statistical results were generated using SAS (version 9.4; [42]). The relationship between observed daily temperatures and climate type was estimated with a Gaussian linear mixed-effects model and PROC MIXED procedure in SAS. The observed mean daily temperatures of each study site were aggregated into mean monthly values that were considered repeated measurements. The fixed effects included in the model were year, climate type, and sampling month. All two-way interactions of the fixed models were included in the initial models. The site main effect and all its interactions with climate type, year, and sampling month were considered random. This analytical approach controls for potential temporal effects of climate on observed psyllid populations and parasitism rates related with temperature differences [23].

Psyllid densities were analyzed with hierarchical generalized linear mixed models (GLMMs) with a log link function and Poisson error structure. Parameters for the GLMMs were estimated using the PROC GLIMMIX in SAS [42]. Biweekly sampling periods of study sites were classified into sampling seasons, namely, cool (10–15 °C; sampling periods 1–4, 23, and 24), moderate (15.01–20 °C; sampling periods 5–10 and 19–22), and warm (20.01–25 °C; sampling periods 11–18), which facilitated the interpretation of potential temporal effects (see [23]). The initial full model included the main effects of climate type (coastal, intermediate, desert), host plant (orange, lemon), season (cool, moderate, warm), and their interactions as fixed effects. For the covariate percentage of shoot samples with flush, year was included as a blocking variable. Random effects included site and host tree identity to account for the non-independence of observations taken from within each site and repeated measures, respectively. This analytical approach accounts for the pseudo-replication inherent in hierarchical experimental designs [23]. The models also included measurements of the percent parasitism from the previous sampling period as a covariate to control for possible time-lagged impacts of parasitism on recorded psyllid densities [23].

GLMMs with the same model formula and binomial error structure [43] were used to assess the effects of climate type, season, year, and host plants on parasitism by each of the two parasitoid species over time. The model also included psyllid densities from the prior sampling period as a covariate. This analytical approach controls for possible delayed density-dependent mortality from parasitoids. Only the densities of nymphal instars vulnerable to attack by each species of parasitoid (second through fourth stage nymphs for *D. aligarhensis* parasitism and fourth and fifth stage nymphs for *T. radiata* parasitism) were utilized in species-specific models. Climate effects were assessed using 3 lag periods of 0, 1, and 2 sampling months prior to that being analyzed. Best fit time lag (i.e., one month prior to the sampling period being analyzed) was selected based on Akaike's and Bayesian information criteria (AIC and BIC, respectively) [23].

The full regression models included all the variables. Insignificant variables were removed using a critical p-value of 0.15, while the AIC and BIC analyses were performed until no further improvements to fits were achieved [23,44,45]. The Kenward–Roger adjustment (option ddfm = kr in SAS) was used to calculate the denominator degrees of freedom for the fixed effects [23,42]. The Tukey–Kramer HSD test was used to see which group mean pairs were significantly different at $\alpha < 0.05$.

3. Results

3.1. Effects of Climate Type and Sampling Month on Mean Daily Temperatures

Daily mean temperatures varied with climate zones ($F = 4.17$; df = 2, 11; $p = 0.04$) and sampling months ($F = 612.12$; df = 11, 1418; $p < 0.001$), while climate × sampling month interaction was also significant ($F = 8.35$; df = 22, 1418; $p < 0.001$). Mean temperatures were similar in 2016 and 2017 ($p > 0.28$ for main and interaction effects of year; Figure 1). Average monthly temperatures ranged from 9.3–24.9 °C for the coastal climate, from 10.6–29.1 °C for the intermediate climate, and from 11.2–32.8 °C for the desert climate throughout the study. Daily temperatures averaged 17.65 ± 0.18 °C (±SE throughout text) at coastal sites, 19.18 ± 0.2 °C at intermediate sites, and 19.89 ± 0.24 °C at desert sites. Major differences in temperatures between climate zones were recorded during warm seasons (climate × month effect; Figure 1). The coastal climate zone had lower temperatures during warm months than the intermediate and desert climates but temperatures were insignificantly different during cool and moderate months (Figure 1). Temperatures for the intermediate climate closely matched the desert climate during warm months (Figure 1).

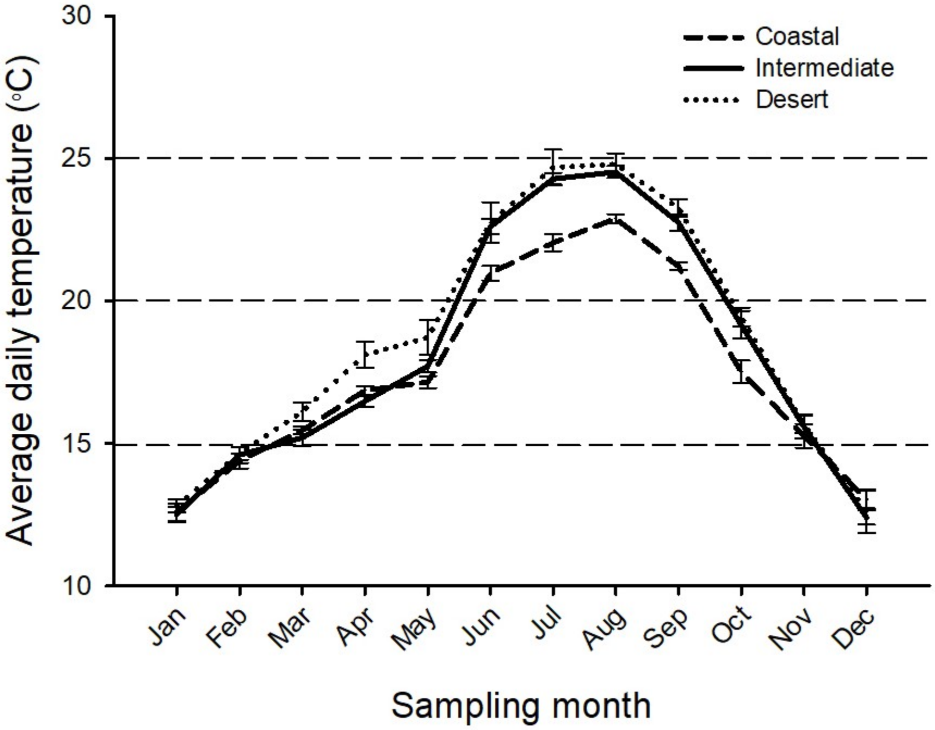

Figure 1. Mean (±SE) temperatures of sampling periods at study sites belonging to either the coastal (CIMIS stations: 62, 75, 174), intermediate (CIMIS stations: 44, 78, 153) or desert (CIMIS stations: 207, 239, 251) climate zones in southern California from January 2016 through December 2017 study period. Sampling months were grouped into three seasons: cool (10–15 °C), moderate (15.01–20 °C), or warm (>20.01 °C).

3.2. Effects of Climate Type, Season, and Parasitism on D. citri Densities

Psyllid densities differed with season but not with climate zone (Table 2A). Psyllid densities were negatively correlated with parasitism rates from the previous sampling period (Table 2A, Figure 2), indicating the existence of time-delayed negative impacts on psyllid densities. Psyllid densities differed between cool and warm months and moderate and warm months, but not between cool and moderate months (Table 2A). Psyllid densities experienced two peaks in February through May and September through November each year irrespective of climate type (Figure 2). Mean psyllid densities were positively correlated with citrus flushing patterns (Table 2). Psyllid population trends were similar on lemons and oranges over time (Table 2). Psyllid densities were 50% higher in 2016 than in 2017 (Table 2A, Figure 2) irrespective of climate zone. None of the interactions between tested variables were significant ($p > 0.15$).

Table 2. Results of generalized linear mixed-effects models examining the main and interaction effects of climate type and host plants on recorded (A) total psyllid densities and (B) parasitism rates of *T. radiata* at field sites from January 2016 through December 2017. The selected regression model for psyllid abundance also included a covariate of total parasitism rates from the prior sampling period (A). The selected regression model for parasitism rates of *T. radiata* also included a covariate of densities of late fourth and fifth instar psyllid nymphs from the previous sampling period (B). The final minimal and most adequate regression models for each response variable are presented below. The critical level of significance was set at 0.05 *.

Parameter		**Estimate**	**SE**	**df**	*t*	*p*	**95% Conf. Interval**	
							Lower	**Upper**
(A) Psyllid densities								
Climate type	Coastal_Intermediate	0.3094	0.5231	54.33	0.59	0.82	−0.7393	1.3581
	Coastal_Desert	0.3662	0.5796	92.48	0.63	0.81	−0.7848	1.5171
	Desert_Intermediate	−0.0568	0.5742	70.74	-0.11	0.99	−1.2018	1.0882
Year	2016_2017	0.3126	0.1389	634.9	2.25	0.048 *	0.0126	0.6126
Season	Cool_Moderate	−0.2295	0.5874	74.82	-0.39	0.92	−1.3998	0.9407
	Cool_Warm	1.5459	0.6485	105.7	2.38	0.02 *	0.6201	2.3817
	Moderate_Warm	1.7755	0.5616	136.3	3.16	0.006 *	0.6648	2.8861
Crop	Lemon_Orange	0.5701	0.4282	673.9	1.33	0.19	−0.2708	1.4109
Flush abundance		6.6711	0.5043	689	13.23	<0.001 *	5.6808	7.6611
Total parasitism		−0.0326	0.0119	677.8	−2.74	0.006 *	−0.0549	−0.0092
(B) Parasitism of *T. radiata*								
Climate type	Coastal_Intermediate	0.0964	0.4858	35.08	0.19	0.97	−0.9728	1.1655
	Coastal_Desert	0.5147	0.5604	18.97	0.92	0.65	−0.7187	1.7481
	Desert_Intermediate	−0.4183	0.5509	26.24	−0.76	0.73	−1.6308	0.7941
Year	2016_2017	0.6787	0.4112	465	1.65	0.13	−1.5669	0.2098
Season	Cool_Moderate	0.7115	0.4696	55.84	1.52	0.13	−1.6344	0.2113
	Cool_Warm	4.0058	0.6947	74.18	5.77	<0.001 *	2.5750	5.4365
	Moderate_Warm	2.3531	0.5845	76.5	4.03	<0.001 *	1.1492	3.5568
Crop	Lemon_Orange	0.2844	0.3456	413.5	0.79	0.44	−0.4816	1.0505
Flush abundance		1.6528	0.5425	463.4	3.05	0.009 *	0.5355	2.7700
Large nymphs		−0.7012	0.1989	382.6	3.52	<0.001 *	0.3102	1.0922

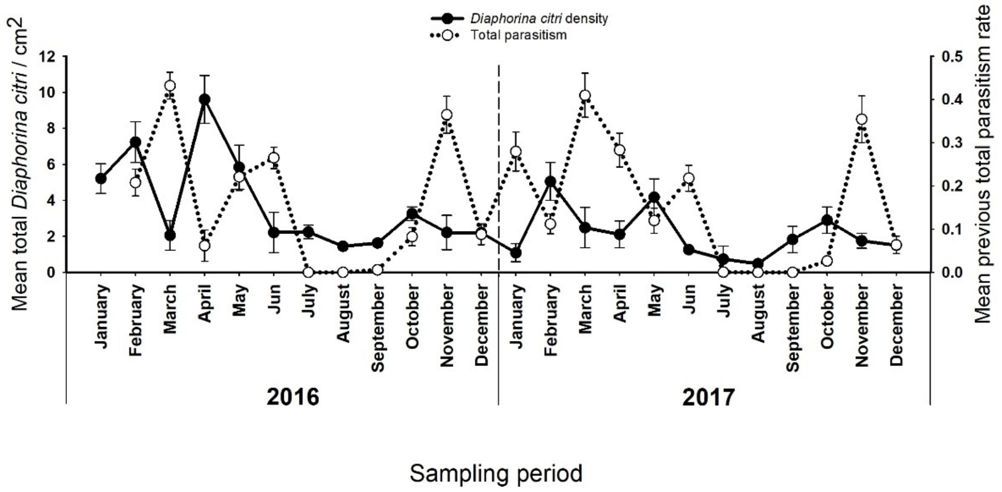

Figure 2. The relationship between *Diaphorina citri* densities and total parasitism rates across field sites from January 2016 through December 2017. Mean (±SE) total *D. citri* population densities (pooled across all psyllid life stages) and mean (±SE) total parasitism rates (pooled across *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* parasitism rates) from the previous sampling interval (pooled across climate types and host plants) are shown.

3.3. D. aligarhensis Establishment and Parasitism Rates

A total of 39,990 females and 30,371 males of *D. aligarhensis* were released from August 2015 through March 2017 at the 15 sites monitored (Table 1). Parasitism by *D. aligarhensis* was exceptionally low, averaging 0.62 ± 0.3% from 2015 through 2017 (Figure 2). Parasitism rates of *D. aligarhensis* averaged 0 ± 0%, 0.89 ± 0.2%, and 0.53 ± 0.1% during the cool, moderate, and warm seasons, respectively (Figure 2). Parasitism rates of *D. aligarhensis* averaged 0.63 ± 0.4% at coastal sites, 0.91 ± 0.6% at intermediate sites, and 0.39 ± 0.2% at desert climate sites. Because of this scarcity of recovery data for *D. aligarhensis*, no definite conclusions could be drawn about the importance of climate effects on parasitism rates. We recovered 12 *D. aligarhensis* (all larvae and eggs) from nine trees that were surveyed in 2016 and 2017. All recoveries were from just six sites and occurred within one month of release. No *D. aligarhensis* were recovered in 2018 from all 39 study sites, 15 of which previously received *D. aligarhensis* and 24 of which served as controls (Tables 1 and S1). Hence, it was concluded that *D. aligarhensis* had failed to establish and all subsequent references to parasitism refer to *T. radiata* only.

3.4. Effects on T. radiata Parasitism Rates and Recovery Success

Parasitism of *D. citri* nymphs by *T. radiata* was found at all 15 *D. aligarhensis* release sites. Overall, *T. radiata* accounted for ~97% of total parasitism observed (Figure 2). Time of year, but not climate, affected *T. radiata* parasitism rates (Table 2B). Parasitism rates were significantly correlated with the previous month's density of large *D. citri* nymphs (instars four and five are the preferred stages for parasitism) (Table 2B, Figure 3). The mean proportions of nymphs parasitized by *T. radiata* differed between cool and warm months and moderate and warm months, but not between cool and moderate months (Table 2B, Figure 3). Parasitism rates were highest in February through May and October through December each year irrespective of climate zone (Figures 2 and 3). Parasitism by *T. radiata* was relatively consistent across coastal (21.4 ± 2.4%), intermediate (21.9 ± 2.4%), and desert (17.6 ± 2.8%) climate sites (Table 2B). Parasitism rates were positively correlated with citrus flushing patterns over time (Table 2B). Average parasitism rates of *T. radiata* were not affected by citrus hosts or year (Table 2B). None of the interactions between the tested variables were significant ($p > 0.32$). In 2018, *T. radiata* was recovered at 34 of the 39 sites surveyed (i.e., 87% of the total sites surveyed) (Tables1 and S1). This included 21 control sites that were located up to 5 km away from the closest *T. radiata* release site, indicating that parasitoids had dispersed naturally from release sites. In addition, ~87% of *D. aligarhensis* release sites were colonized by *T. radiata*.

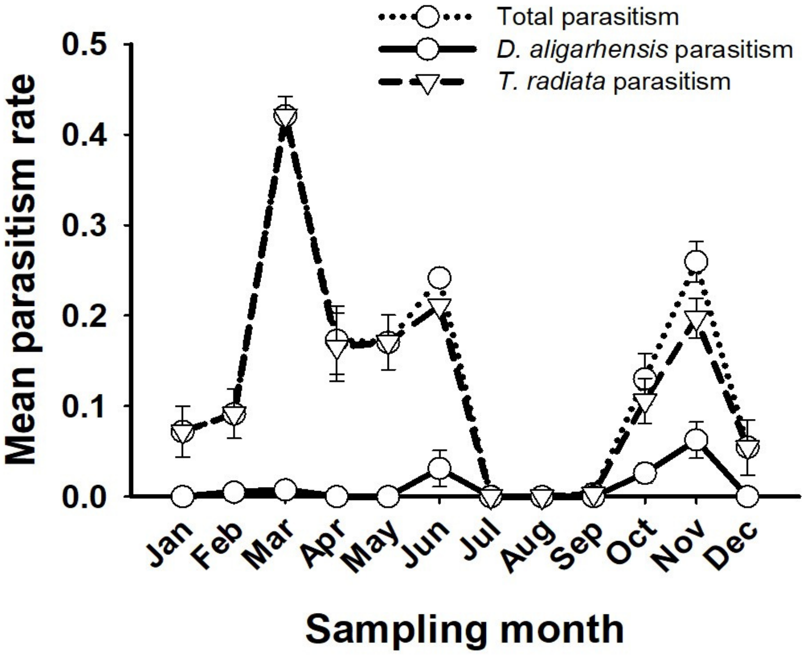

Figure 3. Mean (±SE) total and species-specific parasitism rates of *Diaphorina citri* by *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* at study sites in southern California from January 2016 through December 2017 (pooled across study years, climate types, and host plants).

4. Discussion

The results presented strongly suggest that despite releasing ~40,000 female *D. aligarhensis*, this parasitoid failed to establish at the 15 release sites used in this study and *D. aligarhensis* was not subsequently recovered from surveyed study sites after releases ceased. A total of >700,000 female and male *D. aligarhensis* released over 2014–2018 across >300 sites dispersed over all three climatic zones also appear to have failed to establish and mass rearing and releases of this species ceased in 2019 (DJW Morgan CDFA, *unpublished data*). When recoveries of *D. aligarhensis* were made at release sites, parasitoid activity was only found in the month following release, parasitism rates were low (<1%), and this parasitoid likely contributed very little to observed reductions in *D. citri* densities at study sites.

The reasons for the low levels of parasitism by *D. aligarhensis* and failure to establish are not conclusively known, but may be due, in part, to competition from *T. radiata* that had naturally invaded and established at release sites. Previous studies using *D. aligarhensis* and *T. radiata* from the same source areas (i.e., Punjab, Pakistan) used in this study indicated that competition between *D. aligarhensis* and *T. radiata* for *D. citri* nymphs is asymmetric and *T. radiata* dominates to the detriment of *D. aligarhensis* [17,40,41]. Additionally, female *D. aligarhensis* parasitize fewer hosts per unit time than *T. radiata* [18] and when multiparasitism occurs, *D. aligarhensis* larvae are inferior intrinsic competitors and are killed by *T. radiata* larvae [19]. The superior competitiveness of *T. radiata* may have prevented the establishment of *D. aligarhensis* in Florida [25,28], and possibly also in California.

In Punjab, Pakistan, where *D. aligarhensis* was sourced, this parasitoid exhibited a very strong association with *Diaphorina aegyptiaca* Puton, infesting leaves of Assyrian plum, *Cordia myxa* L. (SZ Khan, CD Hoddle, and MS Hoddle, pers. obs.). *Diaphorina aegyptiaca* on *C. myxa* was also reported as a host for *D. aligarhensis* in India where *D. aligarhensis* is also native [46]. *Cordia myxa* is a fruit-bearing tree that may be grown in close proximity to citrus orchards or interspersed through orchards. Infestations of *D. aegyptiaca* could act as reservoirs from which *D. aligarhensis* disperse and attack less preferred or less suitable hosts such as *D. citri* infesting neighboring citrus trees. From foreign exploration efforts in Pakistan, nearly half as many *D. aligarhensis* (1023) as *T. radiata* (2021) were reared at UCR-IQF from *D. citri* collected [47]. A lack of a preferred alternative host species, such as *D. aegyptiaca*, could also explain, in part, the failure of *D. aligarhensis* to establish in California and its prevalence in Pakistani citrus orchards where it coexists with *T. radiata*. Further, in comparison to *T. radiata*, *D. aligarhensis* is more likely to be attacked by hyperparasitoids of which at least 17 species are known from Southeast Asia and Pakistan [47–51], and at least 8 of these genera are present in California [50]. Although not documented, an additional impediment to establishment of *D. aligarhensis* may have been the acquisition of hyperparasitoid species already resident in California.

All study sites had steady year-round Argentine ant (*Linepithema humile* Mayr) activity (data not shown). Argentine ants infest >90% of all residential citrus trees growing in southern California where they protect more than 55% of *D. citri* colonies from natural enemies, in exchange for a sugar-rich honeydew [52]. This mutualistic relationship can impair parasitoid oviposition performance and reduce efficacy of *T. radiata* by >80% [23,52,53]. Field observations revealed that the majority of interactions between Argentine ants and *T. radiata* on citrus branches infested with ants resulted in mortality of this parasitoid or its deterrence by tending ants [54]. Nearly all oviposition attempts by *T. radiata* were frequently interrupted by Argentine ants, allowing psyllids to escape parasitism. In the context of this study, *L. humile* likely impeded *D. aligarhensis*'s efficacy, even though putative negative impacts were not quantified.

Factors promoting the field persistence of *D. aligarhensis* on *D. citri* need determination. For example, self-introduced populations of *D. aligarhensis* into various citrus-growing regions, including Réunion Island [55], Saudi Arabia [56,57], Taiwan [58], Colombia [59], and the Philippines [60], generally provided low levels of *D. citri* control, especially in areas where *T. radiata* was already present (e.g., Réunion Island and Taiwan) or later introduced. For example, in the Philippines, where *D. aligarhensis* was widespread, releases of *T. radiata* dominated the *D. citri* parasitoid guild within 12 months of release at study sites [61]. A similar outcome was observed in Saudi Arabia where *D. aligarhensis* was recorded inflicting mortality of 60–70% on *D. citri* in limes [57]. Releases of *T. radiata* at sites with established *D. aligarhensis* populations resulted in the suppression of *D. aligarhensis* and *T. radiata* emerged as the dominant parasitoid species [56].

An important, albeit unintentional, byproduct of this work assessing the establishment and impact of *D. aligarhensis* on *D. citri* was the opportunity to simultaneously investigate the impacts of *T. radiata* on this pest in urban areas. At sites where *D. aligarhensis* was released, *T. radiata* accounted for >95% of total parasitism rates. For the 2016–2017 survey, *T. radiata* parasitism rates averaged 21%, similar to levels reported in Pakistan [39], southern California [23,37], Texas [62], and Florida [28,63], and lower than rates reported from Réunion Island [55] and Puerto Rico [64]. Secondly, with respect to *T. radiata* analyses of parasitism rates, delayed density-dependent regulation of *D. citri* populations with a lag of ~12 months was detected. Following the peaks of *D. citri* activity in the spring and fall of 2016, mean *T. radiata* parasitism increased to a peak of over 46%, before *D. citri* levels declined in 2017 to levels that were ~50% of previous recorded densities. Finally, in the 2018 surveys, *T. radiata* was recovered at 34 of 39 sites (i.e., 87%) monitored, indicating a strong propensity for natural dispersal and establishment. The highest psyllid densities and frequencies of parasitized *D. citri* nymphs coincided with citrus flushing periods. Consequently, parasitism of *D. citri* by *T. radiata* peaked in March through June and October through November each year when daily temperature averages consistently fell within an optimal temperature range of 20–28 °C for development [65]. The results of work reported here are supported by previous studies that also indicated the significant role that natural enemies such as *T. radiata* have played in suppressing *D. citri* densities in urban citrus [23,37].

In conclusion, classical biological control introductions into the USA of thelytokous (Florida) and arrhenotokous (California) populations of *D. aligarhensis* failed to establish. The reasons for this are uncertain, however, competition from *T. radiata* is suspected as a significant contributing factor. With respect to establishment success in classical biological control, an elucidation of factors adversely affecting the successful establishment of natural enemies is important. The identification and understanding of barriers (e.g., interspecific competition, hyperparasitism, intraguild predation, or poor climate matching) that impede agent establishment can inform decision-making during critical steps of program development [66–68]. In this regard, interspecific competition may be important and could deserve consideration when classical biological programs for *D. citri* are being developed. Consequently, classical biological control programs targeting *D. citri* should probably only consider the use of *T. radiata*. This suggestion is supported by results from meta-analyses that indicate considerable suppression of insect pests is often attained by a single species of natural enemy [69] and that increased levels of control are usually not achieved by releasing multiple species of natural enemies [70]. Documentation of successes and failures of natural enemy releases in the context of classical biological control, both in terms of establishment and suppression of target pest populations, is recommended so that factors contributing to observed outcomes can be better understood and used to inform the development of future projects [68].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy12030583/s1: Table S1: Site, climate type, and host plant information for selected *D. aligarhensis* control sites located in urban areas of southern California, USA.

Author Contributions: Conceptualization, I.M., M.A.V., D.J.W.M., C.D.H., R.E.M. and M.S.H.; methodology, I.M., M.A.V., D.J.W.M., C.D.H., R.E.M. and M.S.H.; formal analysis, I.M.; investigation, I.M., M.A.V., D.J.W.M., C.D.H., R.E.M. and M.S.H.; data curation, I.M., M.A.V., D.J.W.M., C.D.H. and R.E.M.; project administration, I.M., M.A.V., D.J.W.M., C.D.H., R.E.M. and M.S.H.; writing—original draft, I.M. and M.S.H.; writing—review & editing, I.M., M.A.V., D.J.W.M., C.D.H., R.E.M. and M.S.H.; funding acquisition, M.S.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported, in part, from funds provided by the California Citrus Research Board (award number 5500-191) and the United States Department of Agriculture Multi-Agency Coordination (USDA-MAC) Agreement Number #15-8130-0336-CA.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy.

Acknowledgments: We would like to thank Alejandro Muniz, Grace Radabaugh, and Robert Dempster (California Department of Food and Agriculture), and Martin Castillo, Michael Lewis, Ruth Amrich, and Nagham Melham (University of California Riverside) for their contribution to the mass rearing of released parasitoids, field work, and processing of data. We are grateful to homeowners in southern California who provided unlimited access to their properties to conduct survey work over a three-year period.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Grafton-Cardwell, E.E. How serious is the threat of Asian citrus psyllid and Huanglongbing? *Citrograph* **2010**, *1*, 8–10.
2. Bové, J.M. Huanglongbing: A destructive, newly emerging, century-old disease of citrus. *J. Plant Pathol.* **2006**, *88*, 7–37.
3. Gottwald, T.R. Current epidemiological understanding of citrus Huanglongbing. *Annu. Rev. Phytopathol.* **2010**, *48*, 119–139. [CrossRef]
4. Milne, A.E.; Gottwald, T.; Parnell, S.R.; Alonso Chavez, V.; Van den Bosch, F. What makes or breaks a campaign to stop an invading plant pathogen? *PLoS Comput. Biol.* **2020**, *16*, e1007570. [CrossRef] [PubMed]
5. Bayles, B.R.; Thomas, S.M.; Simmons, G.S.; Grafton-Cardwell, E.E.; Daugherty, M.P. Spatiotemporal dynamics of the Southern California Asian citrus psyllid (*Diaphorina citri*) invasion. *PLoS ONE* **2017**, *12*, e0173226.
6. United States Department of Agriculture National Agricultural Statistics Services (USDA NASS). Citrus Production Forecast: 2019. Available online: www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/Citrus_Forecast/index.php (accessed on 10 January 2022).
7. Babcock, B. Economic impact of California's citrus industry. *Citrograph* **2018**, *9*, 36–39.
8. Hoddle, M.S. Foreign exploration for natural enemies of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the Punjab of Pakistan for use in a classical biological control program in California USA. *Pak. Entomol.* **2012**, *34*, 1–5.
9. Milosavljević, I.; Hoddle, M.S. Advances in classical biological control to support IPM of perennial agricultural crops. In *Integrated Management of Insect Pests: Current and Future Developments in IPM*; Kogan, M., Heinrichs, E.A., Eds.; Burleigh Dodds Science Publishing: Cambridge, UK, 2019; pp. 415–450. [CrossRef]
10. Hoddle, M.S.; Pandey, R. Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina citri* (Hemiptera: Liviidae: Euphyllurinae: Diaphorinini) in California. *J. Econ. Entomol.* **2014**, *107*, 125–136. [CrossRef] [PubMed]
11. Bistline-East, A.; Pandey, R.; Keçeci, M.; Hoddle, M.S. Host range testing of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) for use in classical biological control of *Diaphorina citri* (Hemiptera: Liviidae) in California. *J. Econ. Entomol.* **2015**, *108*, 940–950. [CrossRef]
12. Milosavljević, I.; Schall, K.A.; Hoddle, C.D.; Morgan, D.J.W.; Hoddle, M.S. Biocontrol program targets Asian citrus psyllid in California's urban areas. *Calif. Agric.* **2017**, *71*, 169–177. [CrossRef]
13. Bassanezi, R.B.; Montesino, L.H.; Gimenes-Fernandes, N.; Yamamoto, P.T.; Gottwald, T.R.; Amorim, L.; Filho, A.B. Efficacy of area-wide inoculum reduction and vector control on temporal progress of huanglongbing in young sweet orange plantings. *Plant Dis.* **2013**, *97*, 789–796. [CrossRef] [PubMed]
14. Grafton-Cardwell, E.E.; Stelinski, L.L.; Stansly, P.A. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annu. Rev. Entomol.* **2013**, *58*, 413–432. [CrossRef] [PubMed]
15. Rohrig, E.; Shirk, P.D.; Hall, D.G.; Stansly, P.A. Larval development of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), an endoparasitoid of *Diaphorina citri* (Hemiptera: Psyllidae). *Ann. Entomol. Soc. Am.* **2011**, *104*, 50–58. [CrossRef]
16. Sule, H.; Muhamad, R.; Omar, D.; Hee, A.K.W. Parasitism rate, host stage preference and functional response of *Tamarixia radiata* on *Diaphorina citri*. *Int. J. Agric. Biol.* **2014**, *16*, 783–788.
17. Vankosky, M.A.; Hoddle, M.S. Two parasitoids of *Diaphorina citri* (Hemiptera: Liviidae) have shared, stage-specific preference for host nymphs that does not impact pest mortality rates. *Fla. Entomol.* **2019**, *102*, 49–58.
18. Skelley, L.H.; Hoy, M.A. A synchronous rearing method for the Asian citrus psyllid and its parasitoids in quarantine. *Biol. Control* **2004**, *29*, 14–23. [CrossRef]
19. Rohrig, E. Biology and Behavior of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) an Endoparasitoid of *Diaphorina citri* (Hemiptera: Psyllidae). Ph.D. Thesis, University of Florida, Gainesville, FL, USA, 2010.
20. Chen, X.; Stansly, P.A. Biology of *Tamarixia radiata* (Hymenoptera: Eulophidae), parasitoid of the citrus greening disease vector *Diaphorina citri* (Hemiptera: Psylloidea): A mini review. *Fla. Entomol.* **2014**, *97*, 1404–1413. [CrossRef]

21. California Department of Food and Agriculture (CDFA). CDFA ACP/HLB Regulation and Quarantine Boundaries: 2021. Available online: https://www.cdfa.ca.gov/plant/acp/regulation.html (accessed on 10 January 2022).
22. Hoddle, M.S.; Amrich, R.; Hoddle, C.D.; Kistner, E.J. Where's *Tamarixia*? *Citrograph* **2016**, *7*, 64–66.
23. Milosavljević, I.; Morgan, D.J.; Massie, R.E.; Hoddle, M.S. Density dependent mortality, climate, and Argentine ants affect population dynamics of an invasive citrus pest, *Diaphorina citri*, and its specialist parasitoid, *Tamarixia radiata*, in Southern California, USA. *Biol. Control* **2021**, *159*, 104627. [CrossRef]
24. DeBach, P.; Sundby, R. Competitive displacement between ecological homologues. *Hilgardia* **1963**, *34*, 105–166. [CrossRef]
25. Rohrig, E.A.; Hall, D.G.; Qureshi, J.A.; Stansly, P.A. Field release in Florida of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), an endoparasitoid of *Diaphorina citri* (Homoptera: Psyllidae), from mainland China. *Fla. Entomol.* **2012**, *95*, 479–481. [CrossRef]
26. Hoy, M.A.; Nguyen, R.; Jeyaprakash, A. Classical biological control of the Asian citrus psylla-release of *Tamarixia radiata*. *Citrus Ind.* **1999**, *80*, 20–22.
27. Hoy, M.A.; Nguyen, R. Classical biological control of Asian citrus psylla. *Citrus Ind.* **2001**, *81*, 48–50.
28. Qureshi, J.A.; Rogers, M.E.; Hall, D.G.; Stansly, P.A. Incidence of invasive *Diaphorina citri* (Hemiptera: Psyllidae) and its introduced parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae) in Florida citrus. *J. Econ. Entomol.* **2009**, *102*, 247–256. [CrossRef]
29. Milosavljević, I.; McCalla, K.A.; Ratkowsky, D.A.; Hoddle, M.S. Effects of constant and fluctuating temperatures on development rates and longevity of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae). *J. Econ. Entomol.* **2019**, *112*, 1062–1072. [CrossRef]
30. Quezada, J.; DeBach, P. Bioecological and population studies of the cottony-cushion scale, *Icerya purchasi* Mask., and its natural enemies, *Rodolia cardinalis* Mul. and *Cryptochaetum iceryae* Will., in southern California. *Hilgardia* **1973**, *41*, 631–688. [CrossRef]
31. Daly, C.; Widrlechner, M.P.; Halbleib, M.D.; Smith, J.I.; Gibson, W.P. Development of a new USDA plant hardiness zone map for the United States. *J. Appl. Meteorol. Climatol.* **2012**, *51*, 242–264. [CrossRef]
32. California Irrigation Management Information System (CIMIS). California Department of Water Resources' California Irrigation Management Information System: State of California, Sacramento, CA: 2021. Available online: http://www.cimis.water.ca.gov (accessed on 10 January 2022).
33. Hopper, K.R.; Roush, R.T.; Powell, W. Management of genetics of biological-control introductions. *Ann. Rev. Entomol.* **1993**, *38*, 27–51. [CrossRef]
34. Roush, R.T.; Hopper, K.R. Use of single family lines to preserve genetic variation in laboratory colonies. *Ann. Entomol. Soc. Am.* **1995**, *88*, 713–717. [CrossRef]
35. Hoddle, M.S.; Hoddle, C.D. Classical biological control of Asian citrus psyllid with *Tamarixia radiata* in urban Southern California. *Citrograph* **2013**, *4*, 52–58.
36. Ruais, D. The Effects of an Artificial Holding Diet on the Fecundity of the Ectoparasitic Wasp *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae). Ph.D. Thesis, California State Polytechnic University, Pomona, CA, USA, 2018.
37. Kistner, E.J.; Amrich, R.; Castillo, M.; Strode, V.; Hoddle, M.S. Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of Southern California. *J. Econ. Entomol.* **2016**, *109*, 1047–1057. [CrossRef] [PubMed]
38. Milosavljević, I.; Amrich, R.; Strode, V.; Hoddle, M.S. Modeling the phenology of Asian citrus psyllid (Hemiptera: Liviidae) in urban Southern California: Effects of environment, habitat, and natural enemies. *Environ. Entomol.* **2018**, *47*, 233–243. [CrossRef] [PubMed]
39. Khan, S.Z.; Arif, M.J.; Hoddle, C.D.; Hoddle, M.S. Phenology of Asian citrus psyllid (Hemiptera: Liviidae) and associated parasitoids on two species of citrus, kinnow mandarin and sweet orange, in Punjab Pakistan. *Environ. Entomol.* **2014**, *43*, 1145–1156. [CrossRef] [PubMed]
40. Vankosky, M.A.; Hoddle, M.S. The effects of conspecific and heterospecific interactions on foraging and oviposition behaviours of two parasitoids of *Diaphorina citri*. *Biocontrol Sci. Technol.* **2017**, *27*, 739–754. [CrossRef]
41. Vankosky, M.A.; Hoddle, M.S. An assessment of interspecific competition between two introduced parasitoids of *Diaphorina citri* (Hemiptera: Liviidae) on caged citrus plants. *Insect Sci.* **2019**, *26*, 119–127. [CrossRef] [PubMed]
42. Institute SAS. *SAS/STAT, Version 9.4 User's Guide*, 2nd ed.; SAS Institute: Cary, NC, USA, 2013.
43. Warton, D.I.; Hui, F.K. The arcsine is asinine: The analysis of proportions in ecology. *Ecology* **2011**, *92*, 3–10. [CrossRef] [PubMed]
44. Milosavljević, I.; Esser, A.D.; Crowder, D.W. Effects of environmental and agronomic factors on soil-dwelling pest communities in cereal crops. *Agric. Ecosyst. Environ.* **2016**, *225*, 192–198. [CrossRef]
45. Whittingham, M.J.; Stephens, P.A.; Bradbury, R.B.; Freckleton, R.P. Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.* **2006**, *75*, 1182–1189. [CrossRef] [PubMed]
46. Hayat, M. Taxonomic notes on Indian Encyrtidae (Hym.: Chalcidoidea): II. *J. Nat. Hist.* **1981**, *15*, 17–29. [CrossRef]
47. Hoddle, M.S.; Hoddle, C.D.; Triapitsyn, S.V.; Khan, S.Z.; Arif, M.J. How many primary parasitoid species attack nymphs of *Diaphorina citri* (Hemiptera: Liviidae) in Punjab, Pakistan? *Fla. Entomol.* **2014**, *97*, 1825–1828. [CrossRef]
48. Tang, Y.Q. On the parasite complex of *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in Asian-Pacific and other areas. In Proceedings of the 4th International Conference on Citrus Rehabilitation, Chiang Mai, Thailand, 4–10 February 1990; Volume 4, pp. 240–245.

49. Bistline-East, A.; Hoddle, M.S. *Chartocerus* sp. (Hymenoptera: Signiphoridae) and *Pachyneuron crassiculme* (Hymenoptera: Pteromalidae) are obligate hyperparasitoids of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) and possibly *Tamarixia radiata* (Hymenoptera: EulopHidae). *Fla. Entomol.* **2014**, *97*, 562–566. [CrossRef]
50. Bistline-East, A.; Hoddle, M.S. Biology of *Psyllaphycus diaphorinae* (Hymenoptera: Encyrtidae), a hyperparasitoid of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Hymenoptera: Eulophidae). *Ann. Entomol. Soc. Am.* **2016**, *109*, 22–28. [CrossRef]
51. Milosavljević, I.; McCalla, K.A.; Bistline-East, A.; Hoddle, M.S. Threshold temperatures and thermal requirements of *Psyllaphycus diaphorinae* (Hymenoptera: Encyrtidae), a hyperparasitoid of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Hymenoptera: Eulophidae). *Fla. Entomol.* **2020**, *103*, 23–31. [CrossRef]
52. Tena, A.; Hoddle, C.D.; Hoddle, M.S. Competition between honeydew producers in an ant-hemipteran interaction may enhance biological control of an invasive pest. *Bull. Entomol. Res.* **2013**, *103*, 714–723. [CrossRef] [PubMed]
53. Anastasio, O.E.; Mathis, K.A.; Rivera, M.J. Impacts of invasive ant-hemipteran interaction, edge effects and habitat complexities on the spatial distribution of ants in citrus orchards. *Agric. Ecosyst. Environ.* **2021**, *310*, 107299. [CrossRef]
54. McCalla, K.A. Implications of Argentine Ant Management for Biological Control of the Asian Citrus Psyllid and Other Ant-Tended Pests in Citrus. Ph.D. Thesis, University of California, Riverside, CA, USA, 2019.
55. Aubert, B.; Quilici, S. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea), through eulophid and encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island. In Proceedings of the Ninth Conference of the International Organization of Citrus Virologist, Puerto Iguazu, Argentina, 9–13 May 1983; International Organization of Citrus Virologists: Riverside, CA, USA, 1984; Volume 9, pp. 100–108.
56. Bové, J.M. Greening in the Arabian Peninsula: Toward new techniques for its detection and control. *FAO Plant Protect. Bull.* **1986**, *34*, 7–14.
57. Al-Ghamdi, K.M.S.; Faragalla, A. Parasitism of *Diaphoria* [sic] *citri* (Kuwayama) (Homoptera: Psyllidae) by *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) on Mexican lime trees, western Saudi Arabia. *Bio Sci. Res. Bull.* **2000**, *16*, 99–105.
58. Chiu, S.C.; Aubert, B.; Chien, C.C. Attempts to establish *Tetrastichus radiatus* Waterson (Hymenoptera, Chalcidoidea), a primary parasite of *Diaphorina citri* Kuwayama in Taiwan. In Proceedings of the Tenth Conference of the International Organization of Citrus Virologists, Valencia, Spain, 17–21 November 1986; International Organization of Citrus Virologists: Riverside, CA, USA, 1988; Volume 10, pp. 265–268.
59. Kondo, T.; Wooley, J.B.; Arciniegas, K.T.; Campos-Patiño, Y. First report in Colombia and diagnosis of *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae), a parasitoid wasp of *Diaphorina citri* (Hemiptera: Liviidae). *Caldasia* **2022**, *44*. [CrossRef]
60. Gavarra, M.R.; Mercado, B.G.; Gonzales, C.I.; Aubert, B.; Tontyaporn, S.; Buangsuwon, D. Progress report: *D. citri* trapping, identification of parasite and possible field establishment of the imported parasite, *Tamarixia radiata* in the Philippines. In Proceedings of the Fourth International Asia Pacific Conference on Citrus Rehabilitation, Chiang Mai, Thailand, 12 September 1990; pp. 246–250.
61. de Jesus, L.A.; Macasaet, A.A. Insect vector occurrence and greening disease spread in citrus orchard. *Philipp. J. Crop Sci.* **1991**, *16*, 23.
62. Chow, A.; Sétamou, M. Parasitism of *Diaphorina citri* (Hemiptera: Liviidae) by *Tamarixia radiata* (Hymenoptera: Eulophidae) on residential citrus in Texas: Importance of colony size and instar composition. *Biol. Control* **2022**, *165*, 104796. [CrossRef]
63. Hall, D.G.; Rohrig, E. Bionomics of Asian citrus psyllid (Hemiptera: Liviidae) associated with orange jasmine hedges in southeast central Florida, with special reference to biological control by *Tamarixia radiata*. *J. Econ. Entomol.* **2015**, *108*, 1198–1207. [CrossRef] [PubMed]
64. Pluke, R.W.; Qureshi, J.A.; Stansly, P.A. Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. *Fla. Entomol.* **2008**, *91*, 36–42. [CrossRef]
65. McCalla, K.A.; Keçeci, M.; Milosavljević, I.; Ratkowsky, D.A.; Hoddle, M.S. The influence of temperature variation on life history parameters and thermal performance curves of *Tamarixia radiata* (Hymenoptera: Eulophidae), a parasitoid of the Asian citrus psyllid (Hemiptera: Liviidae). *J. Econ. Entomol.* **2019**, *112*, 1560–1574. [CrossRef] [PubMed]
66. Beirne, B.P. Avoidable obstacles to colonization in classical biological control of insects. *Can. J. Zool.* **1985**, *63*, 743–747. [CrossRef]
67. Stiling, P. Why do natural enemies fail in classical biological control programs? *Am. Entomol.* **1993**, *39*, 31–37. [CrossRef]
68. Van Driesche, R.G.; Hoddle, M.S. Classical arthropod biological control: Measuring success, step by step. In *Biological Control: Measures of Success*; Gurr, G.M., Wratten, S.D., Eds.; Springer: Dordrecht, The Netherlands, 2000; pp. 39–75.
69. Myers, J.H.; Higgins, C.; Kovacs, E. How many insect species are necessary for the biological control of insects? *Environ. Entomol.* **1989**, *18*, 541–547. [CrossRef]
70. Denoth, M.; Frid, L.; Myers, J.H. Multiple agents in biological control: Improving the odds? *Biol. Control* **2002**, *24*, 20–30. [CrossRef]