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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

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HIGHLIGHTS

- Highest *D. citri* densities were found in intermediate and coastal regions.
- Parasitism rates averaged 25% with lag density-dependent parasitism being detected.
- Parasitism by *T. radiata* was>50% greater when *L. humile* was absent.
- *Diaphorina citri* densities declined by over 75% across all study sites over four years.
- Reduced *D. citri* densities may have slowed spread of *Candidatus* Liberibacter asiaticus in California.

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ABSTRACT

The effects of climate and ants on population regulation of an invasive citrus pest, *Diaphorina citri* (Hemiptera: Liviidae), by its parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae), in southern California were examined over a four-year period. Densities of *D. citri* eggs, nymphs, and adults, and Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae), a mutualist which defends psyllid nymphs from natural enemies, citrus flush growth patterns, and parasitism rates of *T. radiata*, were monitored every four weeks on grapefruit, lemon, lime, orange, and tangerine trees at 28 urban sites across three climate types (coastal, intermediate, and desert). Significant spatial and temporal effects on *D. citri* abundance and parasitism rates were observed over this four-year period. Highest *D. citri* densities and parasitism rates were found in the intermediate and coastal regions during peaks of flushing cycles of citrus plants over March-June and September-November each year. Over the course of this study, population densities of *D. citri* declined by over 75%. Parasitism by *T. radiata* was identified as a significant mortality factor often exceeding 60% during periods of peak parasitoid activity. Analyses indicated that parasitism resulted in delayed density-dependent mortality and subsequent reductions in *D. citri* densities lagged by ~ 1 yr. Trends in *D. citri* densities and parasitism rates over time were similar on grapefruit, lemons, limes, oranges, and tangerines. Presence of *L. humile* in citrus resulted in a 3-fold increase in *D. citri* densities and control of this pest ant is needed to maximize biological control of *D. citri*.

1. Introduction

Since its discovery in California, USA, in 2008 in backyard citrus in San Diego County, the population growth and spread of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), an invasive citrus pest, was rapid in urban areas of southern California (Bayles et al., 2017; Milosavljević et al., 2017a; Thomas et al., 2017). By 2014, *D. citri* had expanded its range into the Central Valley, where 77% of California's citrus is grown (USDA NASS, 2019), and as far north as Sacramento, 850 km from the original area of invasion in San Diego County (CDFA, 2020). The invasion of *D. citri* in California is problematic because it transmits a phloem-limited bacterium, *Candidatus* Liberibacter

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asiaticus (*C*Las), which causes a debilitating citrus disease, huanglongbing (Gottwald 2010), that eventually renders citrus production unprofitable and ultimately results in tree death (Durborow, 2012; Babcock, 2018).

*C*Las was first found in California in 2012, and subsequently there have been over 2,100 cases of confirmed *C*Las-infected trees in southern California (David Morgan, CDFA, pers. comm., Nov. 2020), mostly in urban areas. Urban citrus is largely unmanaged and has potential to harbor large *D. citri* populations, which increases the likelihood of *C*Las acquisition and transmission to uninfected trees (Bayles et al. 2017). No *C*Las detections have been made in commercial citrus in the Central Valley (Milne et al. 2020). Should the *C*Las spread from backyard trees in southern California to nearby commercial citrus groves, the livelihood of California's citrus production could impact >4,000 farmers and over 120,000 ha of citrus production in the state (Babcock 2018). Commercially grown citrus contributes ~$7.1 billion to the economy of California and employs >22,000 individuals.

In an effort to slow the spread of *C*Las, a classical biological control program was initiated in 2010 (Hoddle 2012). The aim was to introduce and establish natural enemies from the presumptive native range of *D. citri*, the Indian subcontinent, in California (Grafton-Cardwell et al. 2013). The goal of this program was to reduce densities of *D. citri* in urban areas that could potentially vector *C*Las into commercial production areas (Milosavljević and Hoddle 2019). A key component of *D. citri* management is vector density reduction as this is correlated with reduced rates of *C*Las spread and huanglongbing expression (Grafton-Cardwell et al. 2013). The biological control program in California has exclusively focused on the introduction and establishment of two host-specific parasitoids, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam, and Agarwal) (Hymenoptera: Encyrtidae), sourced from Punjab, Pakistan (Hoddle et al., 2014; Bistline-East et al., 2015; Milosavljević et al., 2017a, 2017b, 2019). To date, >20 million parasitoids (*T. radiata* and *D. aligarhensis* combined; Suppl. Table 1) have been mass-produced and released at over 1,500 sites in southern California by the California Department of Food and Agriculture (CDFA, 2020). Of these two parasitoids, *T. radiata* established readily and spread rapidly. Conversely, *D. aligarhensis* may not have established in California although there have been no systematic surveys to confirm this possibility. *Tamarixia radiata* has been associated with population declines of *D. citri* in California. However, the impacts of *T. radiata* have been variable across locations and time (Kistner et al., 2016a; Milosavljević et al., 2018).

One important abiotic factor that could influence differences in *T. radiata* parasitism rates across the distribution range of *D. citri* in southern California is climate, especially temperature, which affects establishment probabilities and population dynamics of both species (Garcia et al., 2019; Taylor et al., 2019; Wang et al., 2019; Martini et al., 2020). In southern California, citrus is grown across diverse climates, ranging from cool humid coastal areas through intermediate zones to substantially hotter arid interior desert regions. Investigations of the effects of abiotic (e.g., temperature impacts on tree, pest, and natural enemy phenology [Kistner et al., 2016a; Milosavljević et al., 2018; Gómez-Marco et al., 2019]) and biotic factors (e.g., ant mutualists that protect *D. citri* from natural enemies [Tena et al., 2013; McCalla et al., 2020]) on *D. citri* population regulation by *T. radiata* have been largely limited to intermediate zones and results indicated that pest population densities and parasitism rates had high temporal variability (Kistner et al., 2016a; Milosavljević et al., 2018).

With respect to temperature, this abiotic factor has as significant effect on *D. citri* and *T. radiata* phenology, development and mortality rates (Hall et al. 2011, 2013; Kistner et al., 2016a; Gómez-Marco et al., 2019; McCalla et al., 2019; Martini et al., 2020; Milosavljević et al., 2018, 2020a,b; Ramos Aguila et al. 2020), and phenology of citrus growth (Ribeiro et al., 2012; Olesen et al., 2013; Chang et al., 2014). The effects of temperature across a broad swath of citrus production areas in southern California (i.e., coastal, intermediate, and desert interior) on the interactions between *D. citri*, *T. radiata*, and citrus phenology has not been assessed, and could, in part, explain observed variability in *D. citri* densities and *T. radiata* efficacy across areas with different climate profiles. By means of a cross geographical comparison, DeBach (1958) first ascribed varying degrees of control by introduced natural enemies of citrus pest scale, *Aonidiella aurantii* (Mask.) (Hemiptera: Diaspididae) in southern California, to temperature related factors. Therefore, assessment of the effects of climate, especially temperature, on parasitism of *D. citri* by *T. radiata* would benefit from analysis of observations over a large spatiotemporal range.

In addition to climate and temperature effects, biotic influences, especially interactions involving the invasive Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), which interferes with the suppression of *D. citri* by *T. radiata* (Milosavljević et al., 2017a; McCalla et al., 2020) needs assessment. *Linepithema humile* infests > 90% of urban citrus and ants defend > 55% of *D. citri* colonies from natural enemies (Tena et al. 2013). *Linepithema humile* is rewarded for protection of phloem feeding insects with honeydew, a carbohydrate rich food, which is highly sort after by ants. This food-for-protection mutualism reduces *T. radiata* parasitism rates of *D. citri* by ~ 80% (Tena et al. 2013). However, these observations on the interference of *L. humile* on the biological control of *D. citri* infesting backyard citrus are based on short observation periods and small sample sizes from primarily a single climatic zone (i.e., intermediate). To better understand the impacts ants have on *D. citri*-*T. radiata* interactions, assessment of the effects of *L. humile* on parasitism of *D. citri* by *T. radiata* would benefit from observations over a wide spatiotemporal range.

Consequently, this four-year study which encompassed 28 study sites examined: (1) how *D. citri* densities and *T. radiata* parasitism rates varied by temperature and time of year along a cline that extended from coastal areas through intermediate regions to interior desert zones, and (2) how *L. humile* presence affected abundance of ant-tended *D. citri* colonies and associated parasitism rates by *T. radiata*.

2. Materials and methods

2.1. Study sites

Diaphorina citri populations and *T. radiata* parasitism rates were monitored on citrus (Sapindales: Rutaceae) at 28 residential sites located in seven counties (Imperial, Los Angeles, Orange, Riverside, San Bernardino, San Diego, and Ventura) in southern California from 1 January 2015 to 31 December 2018 (Table 1). Study plants consisted of 20 orange (*Citrus sinensis*), 19 lemon (*Citrus limon*), eight grapefruit (*Citrus* × *paradisi*), five lime (*Citrus* × *aurantiifolia*), and three tangerine (*Citrus reticulata*) trees, for a total of 55 study trees older than 5 yr of age (Table 1). The study sites and surveyed trees were selected at random from the CDFA's *T. radiata* release database, chosen to be as evenly distributed as possible across climatic regions studied (Table 1). The *D. citri* parasitoids, *T. radiata* and *D. aligarhensis*, were not released at these 28 sites as part of the classical biological control program targeting *D. citri*. Each study site was located approximately 1–5 km from the nearest parasitoid release locations (CDFA, 2020; Table 1; Suppl. Table 1). Participating homeowners did no report using insecticide sprays for pest control during the course of the study.

The 28 study sites were classified into ten coastal (USDA Hardiness Zone 10b; Sunset Climate Zone 24), nine intermediate (USDA Hardiness Zone 10a; Sunset Climate Zones 20–22), and nine desert (USDA Hardiness Zones 9a/9b; Sunset Climate Zones 18–19) sites, based on the United States Department of Agriculture (USDA) plant hardiness zone classification system (Table 1; [Daly et al. 2012]). There were negligible differences in day length between sites (latitude between the southernmost and northernmost sites was about 1°). Air temperatures and relative humidity for each study site and sampling date were obtained from the closest weather station. Each study site was located approximately 10 km from the nearest meteorological station (Table 1; CIMIS, 2020).

Table 1
Site information, climate type, and parasitoid release information for selected *D. citri* and *T. radiata* phenology survey sites located in urban areas of southern California, USA.

Region	County	Site name	Latitude (degrees)	Longitude (degrees)	Elevation (m)	Surveyed tree(s)	CIMISstation #	CDFA's grid # for nearest parasitoid releases	Date of firstparasitoid release	Most recentparasitoid release	# parasitoid release events in grid (2015–2018)	# *T. radiata*released in grid (2015–2018)	# *D. aligarhensis* released in grid (2015–2018)
Coastal	Los Angeles	Downey	N33°55′	W118°07′	28	Grapefruit, Lime	174	MC562	16-May-14	16-Nov-16	11 (9)	7300 (5900)	0 (0)
		Los Angeles	N34°04′	W118°22′	51	Lemon, Orange	99	LO552	10-Apr-14	22-Sep-16	10 (8)	4900 (3900)	0 (0)
		Malibu	N34°02′	W118°43′	98	Lemon, Orange	99	KU548	13-May-14	22-Sep-16	11 (9)	3600 (3000)	0 (0)
	Orange	Anaheim	N33°50′	W117°56′	41	Lemon, Orange	174	MN566	23-Jun-17	10-Feb-21	63 (38)	39,010 (23310)	0 (0)
		San Clemente	N33°25′	W117°37′	36	Lemon, Lime*	75	NG596	29-Jan-14	9-Feb-18	15 (11)	2100 (1000)	3635 (3635)
	San Diego	Escondido	N33°09′	W117°07′	222	Lemon, Tangerine	173	OJ614	27-May-14	7-Nov-16	8 (6)	5300 (3900)	0 (0)
		Mira Mesa	N32°55′	W117°08′	134	Tangerine	150	OJ630	28-May-14	14-Dec-16	14 (12)	9300 (7900)	0 (0)
		San Diego*	N32°42′	W117°14′	22	Lemon*, Orange*	184	OD644	11-Apr-14	27-Dec-16	13 (11)	6400 (5400)	0 (0)
	Ventura	Newbury Park	N34°11′	W118°55′	225	Lemon, Orange	99	KI540	17-Apr-14	18-Feb-21	30 (12)	16,700 (3600)	1710 (1710)
		Ventura*	N34°15′	W119°16′	20	Lemon*, Lemon*	198	JO540	23-Apr-14	9-Feb-21	47 (30)	34,256 (24356)	0 (0)
Intermediate	Los Angeles	Arcadia	N34°07′	W118°02′	113	Orange, Orange	159	MG548	20-Sep-16	4-May-17	9 (9)	4850 (4850)	0 (0)
		Pomona	N34°04′	W117°46′	264	Lime, Orange	78	MW554	18-Apr-14	15-Sep-17	16 (16)	10,078 (10078)	1000 (1000)
	Orange	Trabuco Canyon	N33°41′	W117°38′	407	Lemon, Lemon*	71	NE578	05-Jun-14	25-Apr-16	6 (5)	1900 (1700)	0 (0)
	Riverside	Riverside	N33°55′	W117°27′	233	Grapefruit, Orange	44	NO563	29-Sep-16	13-Jan-21	67 (39)	48,236 (30436)	2547 (2547)
		Wildomar	N33°36′	W117°13′	442	Tangerine	62	OC582	19-Jul-13	20-Mar-15	7 (1)	1750 (0)	2100 (2100)
	San Bernardino	Fontana	N34°06′	W117°25′	375	Grapefruit, Lime	192	NP550	24-Apr-14	21-Oct-16	16 (14)	10,900 (9500)	0 (0)
		San Bernardino	N34°13′	W117°24′	651	Grapefruit, Lemon	192	NR540	11-Feb-14	12-Jan-18	17 (14)	4120 (3320)	1790 (1790)
	San Diego	Ramona	N33°01′	W116°55′	453	Lemon, Orange	153	OV625	29-Oct-14	4-Nov-15	4 (3)	1200 (800)	0 (0)
	Ventura	Simi Valley	N34°15′	W118°47′	260	Lemon, Orange*	217	KQ540	17-Apr-14	18-Feb-21	30 (13)	17,200 (6100)	1895 (1895)
Desert	Imperial	Brawley*	N32°59′	W115°32′	–33	Lemon*, Orange*	181	RY635	28-Mar-17	8-Feb-21	21 (8)	13,721 (6321)	0 (0)
		El Centro*	N32°47′	W115°35′	−9	Grapefruit*, Orange*	87	RV635	6-May-16	8-Feb-21	23 (11)	14,655 (7828)	525 (525)
		Salton City	N33°07′	W115°48′	−64	Lemon*, Orange	181	QZ602	12-Jun-14	10-Feb-16	4 (3)	1000 (800)	0 (0)
	Riverside	Beaumont	N33°56′	W116°59′	796	Grapefruit, Orange	251	OP588	29-Aug-14	1-Nov-16	13 (11)	4800 (3600)	0 (0)
		Blythe*	N33°38′	W114°34′	82	Grapefruit*, Orange*	135	TZ574	15-Oct-14	2-May-16	9 (7)	2400 (1800)	0 (0)
		Thermal	N33°34′	W116°10′	−21	Lime, Orange*	136	QL584	12-Jun-14	7-Dec-20	31 (21)	19,188 (12988)	0 (0)
	San Bernardino	Redlands	N34°05′	W117°10′	430	Lemon, Orange	251	OE550	1-Dec-14	3-Jan-20	23 (21)	3000 (2200)	2557 (2557)
	San Diego	Borrego Springs	N33°15′	W116°22′	173	Lemon, Orange	207	QB601	17-Apr-15	15-May-15	2 (2)	6000 (6000)	5207 (5207)
		Potrero	N32°38′	W116°38′	753	Grapefruit, Lemon, Orange	68	PN648	21-Oct-14	26-Jul-16	5 (4)	1700 (1300)	0 (0)

*Indicates study sites and trees that were excluded from statistical analyses as no D. citri were observed throughout the study period (i.e., from 2015 through 2018)

2.2. Data collection

All 55 study trees were monitored for infestations of *D. citri* and *L. humile* every four weeks throughout the 4-yr evaluation period, for a total of 52 sampling events per site per tree. The presence or absence of *L. humile* trailing on the trunk was recorded for each experimental tree by location for each sampling period. The vast majority of residential citrus in southern California sustain heavy, persistent *L. humile* infestations (Tena et al., 2013; McCalla et al., 2020). Consequently, *L. humile* presence/absence is likely to be sufficient for indicating ant attendance of hemipteran pests infesting trees. Tree canopies were divided into four quadrants (north, east, west, and south) to account for variation in *D. citri* densities and flush shoot growth with cardinal direction (Kistner et al. 2016a). Within each selected quadrant, densities of *D. citri* adults, eggs, small nymphs (instars 1–3), and large nymphs (instars 4–5), presence or absence of flush, and the number of nymphs parasitized by *T. radiata* were recorded (Milosavljević et al. 2018).

The total number of adult *D. citri* was evaluated for each sampling date and cardinal direction of study tree by counting adult psyllids during 30 sec visual inspections (for a total of 2 min per tree per sampling event). As adult female *D. citri* exclusively lay eggs on citrus flush growth (Sétamou and Bartels, 2015; Cifuentes-Arenas et al., 2018; Milosavljević et al., 2018; Carvalho et al., 2020), the density of flush growth per tree and sampling date was assessed by randomly selecting eight branches within arm's reach, from about waist to slightly above head height, while walking around each study tree (Kistner et al. 2016a). The percentage of branches bearing flush growth was calculated for each sampling date and study tree as the total number of branches with flush growth divided by the total number of flush and non-flush growth branches × 100 (Khan et al. 2014).

In addition to timed adult counts and measures of flush availability, two flush shoots suitable for oviposition and the development of immature *D. citri* were randomly excised from within each designated tree quadrant to yield eight flush samples per study tree. If fewer flush shoots were present (the number of flush samples per study tree ranged from zero to eight), all the immature shoots were collected. Flush was removed at the node. Samples were placed individually into labeled plastic bags, placed in an ice-packed cooler in the field and transported under California Department of Food and Agriculture permit 2870 to the California Department of Food and Agriculture Mt. Rubidoux station facility. Flush samples were stored at -5°C in a freezer for up to one week before being processed.

Flush samples were examined under a dissecting microscope to estimate the number of individuals per colony as well as their life stage (eggs, small nymphs, and large nymphs) and parasitism status. The length of each flush sample, measured in cm from the apex of the flush to the base of the growth, from which counts were made was recorded so that egg and nymph counts could be standardized by flush length (Kistner et al. 2016a). Parasitism rate was calculated by sampling date and study tree as the total number of parasitized large nymphs per colony divided by the total number of parasitized and non-parasitized large nymphs per colony. These large nymphs are most preferred by T. radiata for parasitism (Hoddle and Pandey, 2014; McCalla et al., 2019). To ensure estimates of parasitism were accurate, all *D. citri* fourth–fifth instars not appearing visually parasitized (as indicated by a darkened mummification appearance or the presence of parasitoid exit holes) were examined on ventral sides for the presence of parasitoid eggs, larvae, and pupae (Kistner et al., 2016a; Milosavljević et al., 2018).

2.3. Data analysis

All statistical analyses were conducted using SAS 9.4 (SAS Institute, 2013). A linear mixed-effects model was used to test for a relationship between the observed daily temperature and climate type (i.e., coastal, intermediate, desert) over time (PROC MIXED in SAS). Fixed effects in the model included climate type, year, the four-weekly sampling period (one through thirteen), and all two-way interactions. The response variable was the observed mean daily temperature of each study site for each sampling period. Site (i.e., CIMIS station) was included as random effect in the model. Repeated measurements were recordings of mean daily temperature at each site for each sampling period. The model was fit using a normal distribution based on the variance of the response variable. This approach controls for the possibility that climate and temporal effects on patterns of variation observed for *D. citri* populations and parasitism by *T. radiata* are correlated with temperature differences (see Schoeller and Redak 2020 for more details).

Psyllid densities were analyzed using repeated measures generalized linear mixed model (GLMMs) and PROC GLIMMIX in SAS, followed by selection of the best-fit models (SAS Institute, 2013). To assist with the interpretation of potential temporal effects, sampling periods were grouped into three seasons (cool [10–15 °C, sampling periods 1–3 and 13], moderate [15.01–20 °C, sampling periods 4–6 and 11–12], warm [20.01°C or higher, sampling periods 7–10]; see Fig. 2) based on the mean daily temperature averages of study sites for each sampling period (Schoeller and Redak 2020). Fixed effects in the initial models included climate type (coastal, intermediate, desert), season (cool, moderate, warm), host plant (orange, lemon, grapefruit, lime, tangerine), percentage of shoot samples with flush, presence or absence of ants, and all two-way interactions between variables. In addition to variables listed above, percent parasitism measured at study sites during the sampling period prior to that being analyzed was included as a covariate in all models with *D. citri* abundance to account for potential delayed impacts of *T. radiata* activity on psyllid populations (Schoeller and Redak 2020). The model also included year as a fixed blocking variable to allow for long-term trends between years. Surveyed trees nested within sites were treated as replicates for each sampling date and were nested within climate type. Site was included as a random effect in the model to allow for differences between sites. Separate models were run for each of the four response variables (i.e., *D. citri* eggs, small nymphs, large nymphs, and adults). Study trees with no *D. citri* present throughout the study period were excluded from these analyses (Table 1). The model for abundance of *D. citri* adults was fit assuming a negative binomial error distribution based on the nature of the count data (Sileshi, 2006; Ver Hoef and Boveng, 2007; O'Hara and Kotze, 2010). Models for egg and nymph abundance were fit with a Poisson distribution with a log link function (for densities of immature *D. citri* per cm flush) (Schoeller and Redak 2020).

The effects of climate type, season, year, host plant, percentage of shoot samples with flush, and presence or absence of ants on parasitism by *T. radiata* were tested using repeated measures GLMMs and PROC GLIMMIX in SAS. Model parameters were similar to those described above. Additionally, population density of fourth–fifth instars *D. citri* nymphs observed at study sites during the sampling period prior to that being analyzed was included as a covariate in the model with *T. radiata* parasitism to control for the possibility that measures of *T. radiata* parasitism of fourth–fifth instars *D. citri* nymphs (i.e., preferred life stages for parasitism [Vankosky and Hoddle, 2017a, 2017b]) lagged with respect to observed psyllid densities (Hunter and Price 1998). The model was run separately for each of the three lagged intervals (i.e., 0, 1, and 2 sampling periods prior to that being analyzed), and the best-fit model (i.e., 1 sampling period prior to that being analyzed) was selected based on Akaike's information criterion (AIC) scores. The model used for parasitism rates was fit to data using a binomial distribution (Warton and Hui 2011).

The original, full models included all variables of interest. Nonsignificant variables ($P > 0.15$) were omitted (Whittingham et al., 2006; Milosavljević et al., 2016) and AIC analyses were performed to determine whether more parsimonious models performed as well as the full models. Effective degrees of freedom for fit models were estimated using Kenward–Rogers method (option ddfm = kr in SAS) (Kenward and Roger 1997). Pairwise comparisons for significant main effects were adjusted for family-wise Type I errors using the Tukey–Kramer method (Lee and Lee 2018). Significance for all tests was set at $\alpha < 0.05$.

3. Results

3.1. Effect of climate zone and sampling period on mean daily temperatures

Mean daily temperatures differed between sampling periods ($F = 2262.39$; df = 12, 2568; $P < 0.001$) and climate zones ($F = 17.99$; df = 2, 42.6; $P < 0.001$), and their interaction was significant ($F = 85.49$; df = 24, 2568; $P < 0.001$). Mean temperatures were relatively consistent across the four years ($P > 0.19$ for main and interaction effects of year; Suppl. Fig. 1). Average monthly temperatures ranged 11.5–29.8℃ for the desert climate, 13–23.5℃ for the intermediate climate, and 13–21.5℃ for the coastal climate during the study period. Average daily temperatures across the entire study period were 20.02 ± 0.63 ℃ (±SE throughout text) for the desert climate, 18.05 ± 0.45℃ for the intermediate climate, and 17.29 ± 0.43℃ for the coastal climate. Differences in mean temperatures between climates were greatest during warm seasons (i.e., June-September; Suppl. Fig. 1). The interior desert climate zone had cooler cool seasons (i.e., December–January) and higher temperatures during warm seasons (June–September) than the intermediate and coastal climates (Suppl. Fig. 1). The intermediate climate zone had temperatures that were higher during warm seasons (June–September) than the coastal climate but were similar during cool and moderate periods (i.e., January–May and October–December; Suppl. Fig. 1).

3.2. Effects of climate, time, ants, and parasitism on D. Citri densities

The average densities of each of the four *D. citri* development stages (i.e., eggs, small and large nymphs, and adults) over 1 January 2015 to 31 December 2018 are shown in Figs. 1–6. The density of *D. citri* eggs differed with climate type, year, and season (Table 2A), but not with their interactions ($P > 0.31$ for all interactions among climates, years, and seasons). Parasitism rates of *T. radiata* from the previous sampling period were significant covariates (estimate = -0.0719 ± 0.012) for observed *D. citri* egg densities in the subsequent sampling period (Table 2A; 3A–C) indicating a delayed negative effect on egg densities existed. *Diaphorina citri* egg densities were higher in intermediate and coastal climates than in desert regions ($P < 0.03$; Fig. 2A–C) but did not differ significantly between intermediate and coastal climate zones ($t = 1.41$; df = 328; $P = 0.36$) (Table 2A; Fig. 1A–C). *Diaphorina citri* egg densities were highest in 2015 and lowest in 2018 ($t = 2.03$; df = 1923; $P = 0.03$; Fig. 2A–C) regardless of climate zone in which monitoring was conducted. From 2015 through 2018, egg densities declined by over 92% (Fig. 2A–C). Irrespective of climate zone, analyses of seasonal trends showed that there was a significant difference in egg densities between cool (December–February) and moderate (March–May and October–November) ($t = -2.23$; df = 342; $P = 0.02$) and warm (June–September) and moderate (March–May and October–November) ($t = -2.97$; df = 318; $P = 0.04$) seasons, but not between cool (December–February) and warm (June–September) seasons ($t = 1.56$; df = 359; $P = 0.31$) (Fig. 2A–C). Mean egg densities exhibited increases in

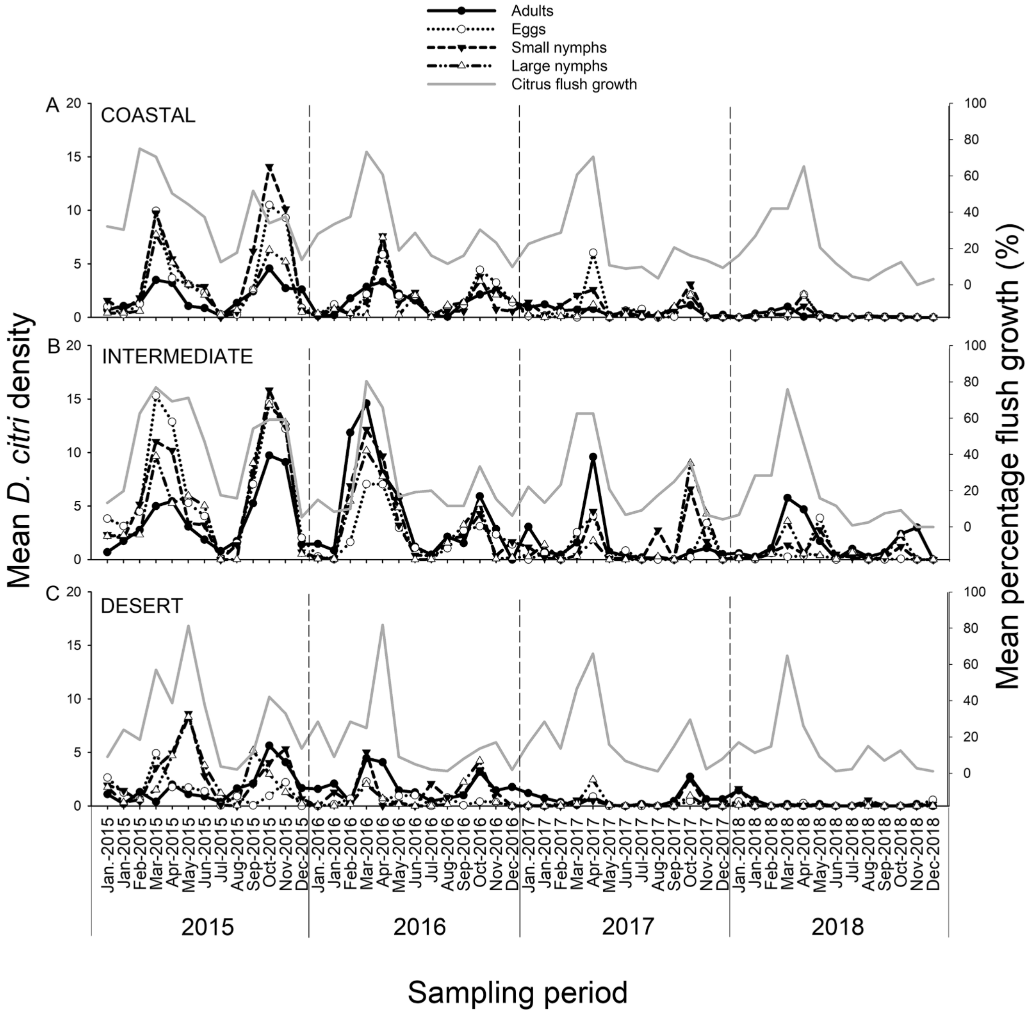

Fig. 1. Relationship between *D. citri* population densities and citrus flushing patterns over time at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean number of *D. citri* eggs, 1st–3rd instars (i.e., small nymphs), 4th–5th instars (i.e., large nymphs) per cm of flush shoot sampled, and mean *D. citri* adult counts (from timed observations) for each climate type (pooled across study sites and host plants) and mean percentage flush growth (pooled across study sites and host plants) are shown. More detail is shown in Suppl. Figs. 3–6.

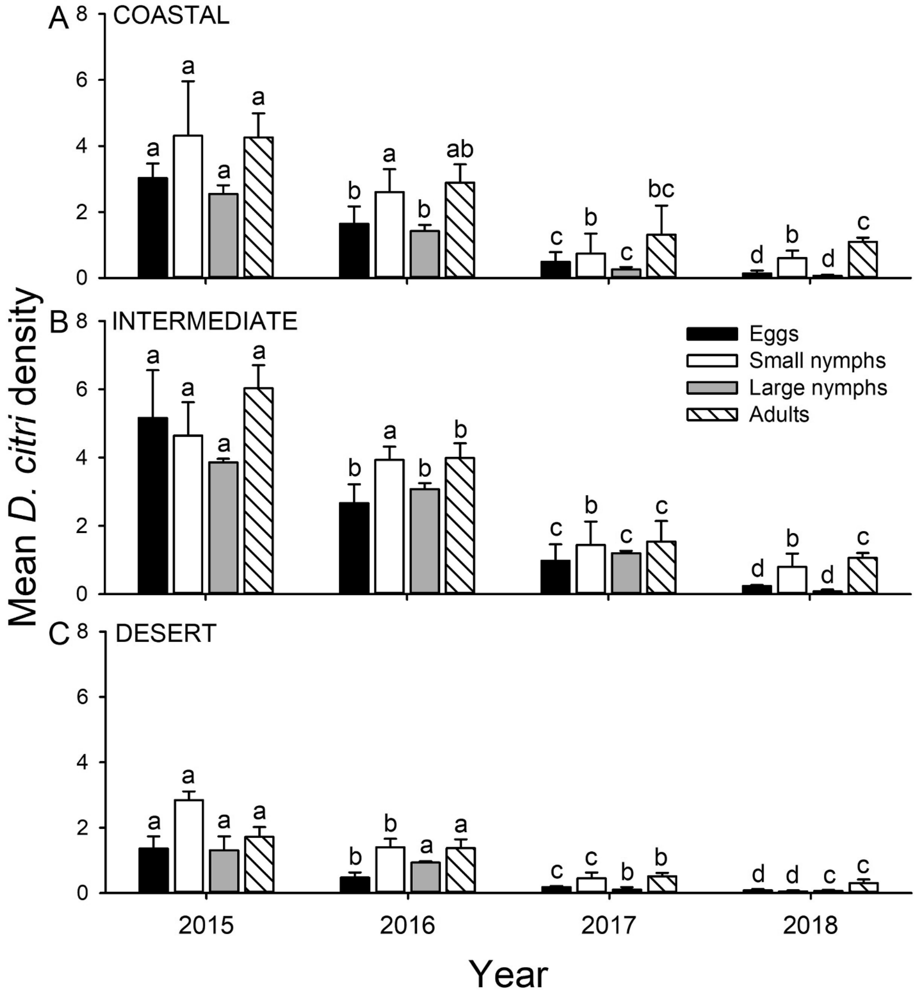

Fig. 2. Mean (+SE) annual *D. citri* population densities across sampling periods and host plants at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean number of *D. citri* eggs, 1st–3rd instars (i.e., small nymphs), 4th–5th instars (i.e., large nymphs) per cm of flush shoot sampled, and mean *D. citri* adult counts (from timed observations) for each climate type (pooled across all study sites, host plants, and sampling periods each year) are shown. Letters at the top of each bar indicate differences among groups by Tukey-Kramer adjustments for main effects of year at $\alpha < 0.05$.

March–May and October–November each year regardless of climate zone (Fig. 1A–C). The presence and density of *D. citri* eggs were consistently mediated by citrus flushing patterns (estimate = 0.1603 ± 0.064; Table 2A; Fig. 1A–C), and overall egg densities were similar on the five citrus hosts (i.e., grapefruit, lemons, limes, oranges, and tangerines) over time ($F = 1.21$; df = 4, 1204; $P = 0.34$). The density of *D. citri* eggs on flush growth was positively affected by the presence of *L. humile*, with a 3-fold increase in eggs being observed when ants were present (Table 2A; Fig. 7A). None of the interactions with host plants and ants had significant effects on *D. citri* egg densities ($P > 0.43$ for all interactions).

Similar to eggs, the densities of small and large *D. citri* nymphs differed across years, climate zones and seasons (Table 2B,C; Fig. 1A–C). None of the interactions among years, climate zones and seasons were significant ($P > 0.19$ for all interactions between these three variables). Mean *T. radiata* parasitism rates of fourth-fifth instar *D. citri* nymphs from the previous sampling period was a significant covariate for observed densities of small and large *D. citri* nymphs in the subsequent sampling period (small nymphs: estimate = -0.0791 ± 0.014; and large nymphs: estimate = -0.0705 ± 0.011; Table 2B,C) indicating a delayed negative effect on nymph densities existed (Figs. 4 & 5). The highest densities of small and large *D. citri* nymphs were found in, but did not differ significantly between, coastal and intermediate climate zones (small nymphs: $t = -1.12$; df = 335; $P = 0.47$; and large nymphs: $t = -1.09$; df = 321; $P = 0.57$) (Fig. 1A–C). The densities of small and large *D. citri* nymphs were highest in 2015 and lowest in 2018 (small nymphs: $t = 2.88$; df = 1772; $P = 0.02$; and large nymphs: $t = 2.09$; df = 1774; $P = 0.04$) (Fig. 2A–C) regardless of climate zone in which monitoring was conducted. Over this four-year period, populations of small and large nymphs declined by over 81% and 94%, respectively (Fig. 2A–C). Additionally, populations of small and large nymphs were higher during the moderate (March–May and October–November) than during the cool (December–February) (small nymphs: $t = 2.21$; df = 375; $P = 0.03$; and large nymphs: $t = 2.34$; df = 357; $P = 0.02$) and warm (June–September) (small nymphs: $t = 2.69$; df = 330; $P = 0.01$; and large nymphs: $t = 2.08$; df = 352; $P = 0.04$) seasons each year (Fig. 1A–C). Mean first–third and fourth–fifth instar densities of *D. citri* did not differ between cool (December–February) and warm (June–September) seasons (small nymphs: $t = 0.72$; df = 368; $P = 0.73$; and large nymphs: $t = 1.32$; df = 375; $P = 0.35$) (Fig. 1A–C). Populations of small and large *D. citri* nymphs typically had two peaks per year, around approximately April–May, and to October–November regardless of climate zone (Table 2B,C; Fig. 1A–C). Mean first–third and fourth–fifth instar densities of *D. citri* were positively correlated with citrus flushing patterns (small nymphs: estimate = 0.1904 ± 0.076; and large nymphs: estimate = 0.1579 ± 0.063; Table 2B,C; Fig. 1A–C). Trends in nymph densities over time were similar on grapefruit, lemons, limes, oranges, and tangerines (small nymphs: $F = 0.91$; df = 4, 1229; $P = 0.43$; and large nymphs: $F = 1.35$; df = 4, 1290; $P = 0.22$). Populations of small and large *D. citri* nymphs were positively affected by the presence of *L. humile* (Table 2B,C). When ants were present on sample trees densities of first–third and fourth–fifth instars increased by 3-fold in comparison to periods lacking ant activity (Fig. 7A). None of the interactions with host plants and ants had a significant effect on the observed densities of small and large *D. citri* nymphs ($P > 0.69$ for all interactions).

The densities of *D. citri* adults varied substantially across climate

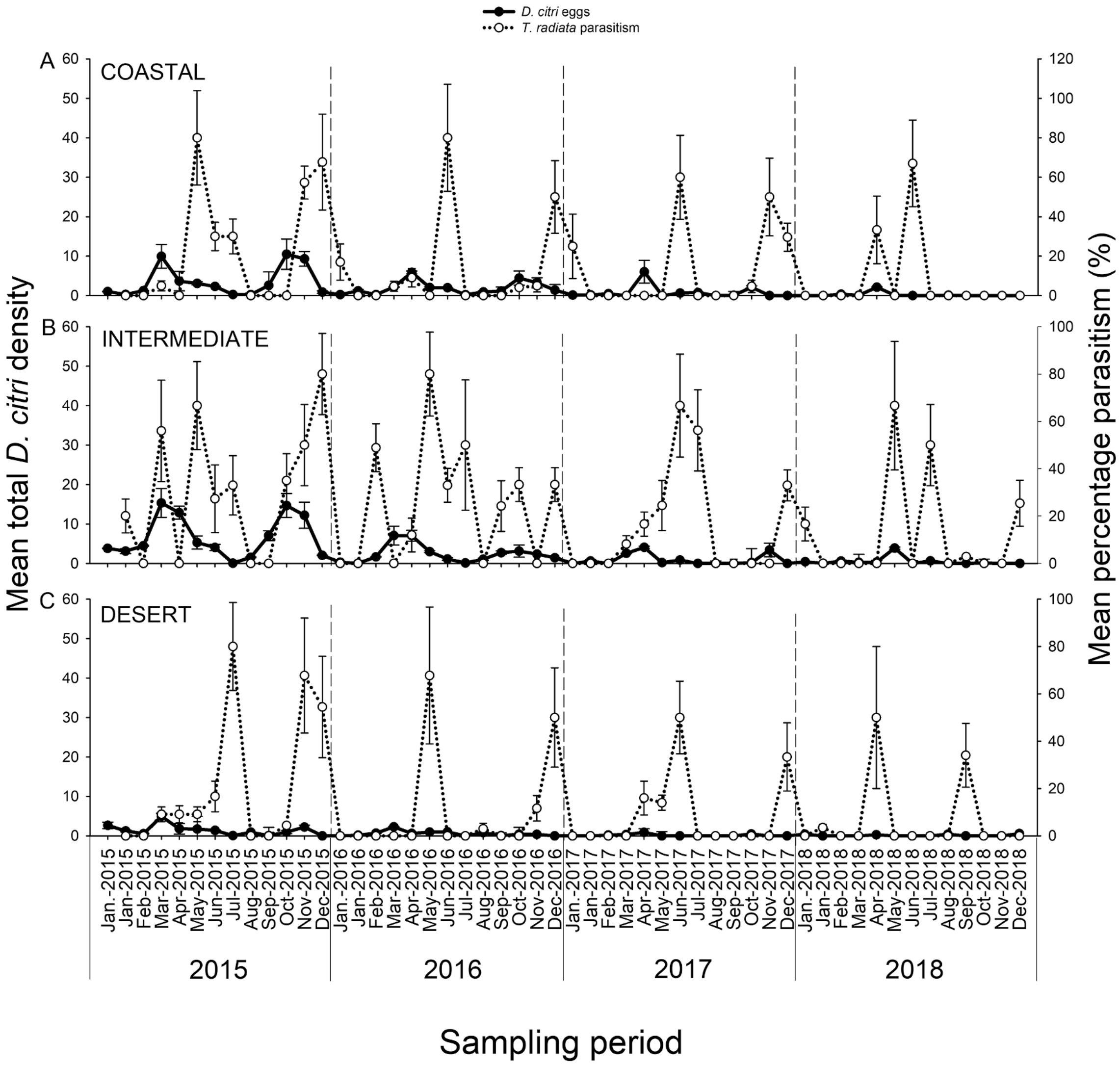

Fig. 3. Relationship between *D. citri* eggs and mean *T. radiata* parasitism rates over time at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean (±SE) number of *D. citri* eggs per cm of flush shoot sampled for each climate type (pooled across study sites and host plants) and mean (±SE) total *T. radiata* parasitism from the previous sampling period (pooled across study sites and host plants) are shown.

zones, years, and seasons (Table 2D; Fig. 1A–C). None of the interactions among climates, years, and seasons were significant ($P > 0.16$ for all interactions). *Tamarixia radiata* parasitism rates of fourth–fifth instar *D. citri* nymphs from the previous sampling period was a significant covariate (estimate = -0.0574 ± 0.012) for observed adult densities of *D. citri* in the subsequent sampling period (Table 2D; Fig. 6A–C) indicating a delayed negative effect of parasitism on subsequent densities of adult *D. citri*. Significantly more adult *D. citri* were found in intermediate climates than in coastal ($t = 2.51$; df = 325; $P = 0.03$) and desert regions ($t = 3.39$; df = 331; $P = 0.01$) (Fig. 1A–C). Mean adult densities of *D. citri* did not differ between coastal and desert climates ($t = 0.6$; df = 314; $P = 0.82$). Population levels of *D. citri* adults were highest in 2015 and lowest in 2018 ($t = 6.12$; df = 1751; $P < 0.001$; Fig. 2A–C) regardless of climate zone in which monitoring was conducted (Fig. 2A–C). From 2015 through 2018, adult densities declined by over 75% (Fig. 2A–C). Similar to eggs and nymphs, population densities of *D. citri* adults were higher during moderate (March–May and October–November) seasons than during cool (December–February) ($t = 2.1$; df = 379; $P = 0.03$) and warm (June–September) ($t = 2.51$; df = 360; $P = 0.01$) seasons (Fig. 1A–C). Mean adult densities of *D. citri* did not differ between cool (December–February) and warm (June–September) seasons ($t = 1.21$; df = 352; $P = 0.45$) (Fig. 1A–C). Mean adult densities experienced two major peaks in April–May, and October–November each year regardless of the climate zone (Fig. 1A–C). Significant positive correlations were found between mean adult densities and citrus flushing patterns over time (estimate = 0.1723 ± 0.069; Table 2D; Fig. 1A–C). The presence and density of *D. citri* adults were not affected by citrus hosts ($F = 0.8$; df = 4, 1395; $P = 0.53$) or *L. humile* presence ($F = 0.92$; df = 1, 1969; $P = 0.34$; Fig. 7A), or any interactions with these variables ($P > 0.57$ for all interactions).

3.3. Effects on T. Radiata parasitism rates

Parasitism of *D. citri* nymphs by *T. radiata* was found at all study sites. *D. aligarhensis* activity was not observed at these locations. Parasitism rates of *T. radiata* differed with season (Table 2E; Figs. 3–6), but did not differ significantly with climate zone ($F = 0.41$; df = 2, 251.8; $P = 0.66$) or year ($F = 1.21$; df = 3, 1765; $P = 0.31$), or interactions of season with climate zone, season with year, and climate zone with year ($P > 0.35$ for all interaction terms). The density of large nymphs from a site's previous sampling period had a significant effect on *T. radiata* parasitism rates in the subsequent sampling period (estimate = 0.3004 ± 0.044; Table 2E)

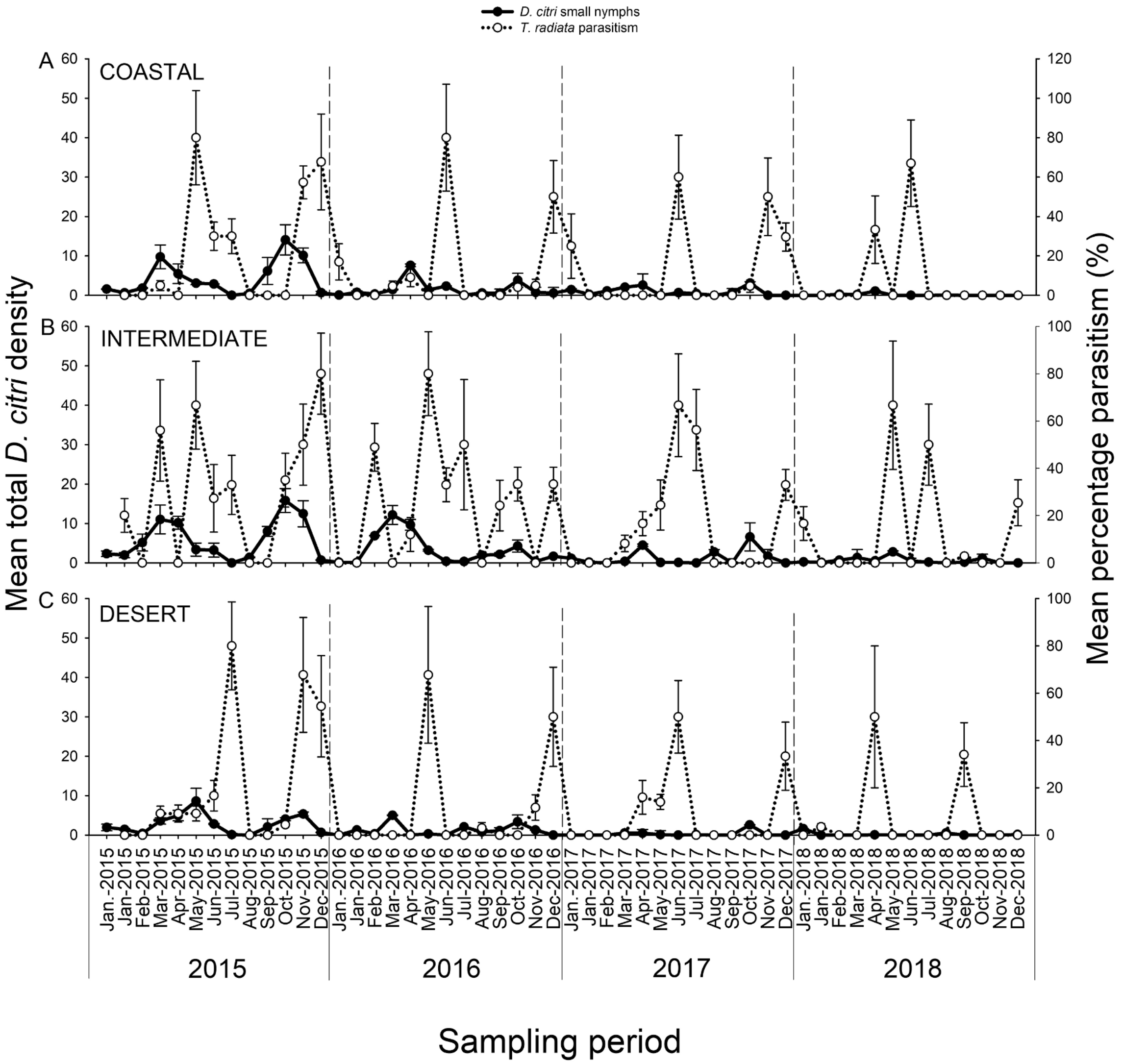

Fig. 4. Relationship between *D. citri* 1st–3rd instars (i.e., small nymphs) and mean *T. radiata* parasitism rates over time at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean (±SE) number of *D. citri* small nymphs per cm of flush shoot sampled for each climate type (pooled across study sites and host plants) and mean (±SE) total *T. radiata* parasitism from the previous sampling period (pooled across study sites and host plants) are shown.

indicating a density-dependent lag existed (Figs. 3–6). Analyses of seasonal trends showed that there was a significant difference in parasitism rates between cool (December–February) and moderate (March–May and October–November) (t = -2.05; df = 266; P = 0.04) and warm (June–September) and moderate (March–May and October–November) (t = -3.4; df = 277; P = 0.01) seasons, but not between cool (December–February) and warm (June–September) seasons (t = 1.22; df = 263; P = 0.44) (Table 2E; Figs. 3–6). *T. radiata* parasitism rates were observed to be highest over April–May and October–November each year regardless of climate zone (Figs. 3–6). Mean parasitism rates were relatively consistent across coastal (22.3 ± 3.51%), intermediate (24.3 ± 5.12%), and desert (17.8 ± 3.42%) climate zones (Figs. 3–6). Significant positive correlations were found between *T. radiata* parasitism and percentage flush growth over time (estimate = 0.7325 ± 0.129; Table 2E), and overall patterns in parasitism rates were similar on the five citrus hosts (F = 1.09; df = 4, 1151; P = 0.18). Finally, *T. radiata* reproductive success was negatively affected by *L. humile* presence. Parasitism rates were reduced by ~ 50 percent when ants were active on trees (Table 2E; Fig. 7B). None of the interactions with citrus hosts and ants had a significant effect on *T. radiata* parasitism rates (P > 0.27 for all interactions terms).

4. Discussion

Seasonal increases in *D. citri* densities coincided with citrus flushing patterns and favorable temperatures. This pattern is consistent with observations from Florida and Brazil (Hall et al., 2008; Qureshi et al., 2009; Chong et al., 2010; Beloti et al., 2013; Sétamou and Bartels, 2015; Cifuentes-Arenas et al., 2018; Laranjeira et al., 2020; Martini et al., 2020) and from previous studies in southern California (Kistner et al., 2016a; Bayles et al., 2017; Milosavljević et al., 2018). Consequently, the highest numbers of *D. citri* eggs, nymphs, and adults were found in April-May and September-November when average daily temperatures consistently fell within or close to *D. citri* 's optimal temperature range of 25–28 °C (Milosavljević et al., 2020a).

Conversely, the lowest numbers of *D. citri* recorded were in January and February. The observed temperature averages during winter periods were 13.24℃ at desert sites, 13.78℃ at coastal sites, and 14.31℃ at intermediate sites, ~2-6℃ above the estimated lower developmental threshold of 8-11℃ for *D. citri* (Milosavljević et al., 2020a). *D. citri* adults and nymphs can survive several hours at temperatures as low as −8℃ and can resist frost inducing temperatures for several weeks (Hall et al. 2011). These adverse low temperatures are well below southern

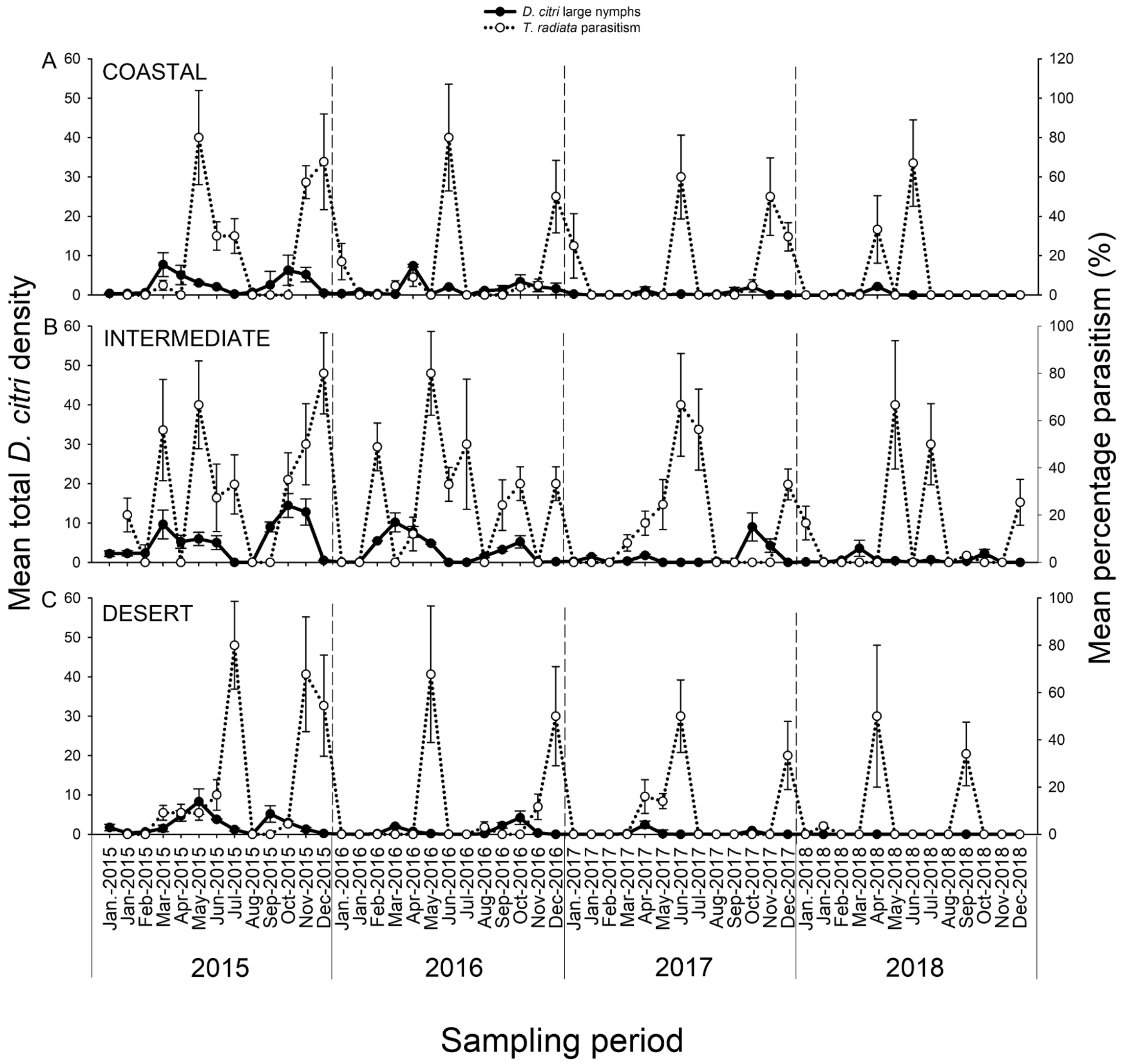

Fig. 5. Relationship between *D. citri* 4th–5th instars (i.e., large nymphs) and mean *T. radiata* parasitism rates over time at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean (±SE) number of *D. citri* large nymphs per cm of flush shoot sampled for each climate type (pooled across study sites and host plants) and mean (±SE) total *T. radiata* parasitism from the previous sampling period (pooled across study sites and host plants) are shown.

California's typical winter temperatures. Consequently, during winter periods *D. citri* populations may have declined naturally due to the lack of citrus flush suitable for nymph development rather than unfavorable low temperatures (Suppl. Fig. 2).

The observed population trends and densities of *D. citri* eggs, nymphs, and adults were similar among grapefruit, lemons, limes, oranges, and tangerines across climate zones over time. These observations confirm previous findings from southern California and Arizona (Kistner et al., 2016a; Milosavljević et al., 2018; Gómez-Marco et al., 2019). However, Kistner et al. (2016a) reported that vigorously flushing limes and curry leaf plants (*Murraya koenigii* [L.] Sprengel [Rutaceae]) consistently supported higher densities of *D. citri* when compared to oranges, lemons, and grapefruit plants. Collectively, these studies indicated that flush availability is heterogeneous throughout the year and across different areas, perhaps reflecting variability in horticultural practices (e.g., pruning, fertilization, and irrigation) among residencies. Flush heterogeneity may be important for sustaining *D. citri* and *T. radiata* populations in urban southern California.

Urban citrus growing in desert areas consistently hosted lower year-round *D. citri* densities when compared to intermediate and coastal sites. High extreme temperatures were observed at desert sites during summer seasons. Correspondingly, temperatures averaged 28.5°C at desert sites from June through September over the 4-yr study period, with daytime average highs exceeding 40°C for > 6 h when compared to coastal and intermediate sites that had daily averages/highs of 21.03/27.4°C and 23.05/32.9°C, respectively, during summer periods. Brief exposures (<4h) to these excessively high summer temperatures (>40°C) can induce reduced fecundity, locomotor deficits, and rapid mortality in *D. citri* (Hall et al., 2011; Martini et al., 2018; Milosavljević et al., 2020a). Detrimental impacts of excessive heat may be further amplified by adverse temperature-induced effects on host plants upon which *D. citri* are developing (Gómez-Marco et al. 2019). Thus, the combined stresses of heat on *D. citri* fitness in conjunction with reduced host quality (e.g., duration and quantity of flush availability) in desert areas may have collectively reduced *D. citri* populations.

Analyses of parasitism rates detected delayed density-dependent regulation of *D. citri* populations by *T. radiata*. The observed seasonal peaks in *D. citri* population densities in the spring and fall each year coincided with the highest levels of *T. radiata* parasitism, which often exceeded 60% during periods of peak parasitoid activity, before *D. citri* levels declined. Consequently, the numbers of surviving large *D. citri* nymphs, *T. radiata*'s preferred life stage for oviposition (Hall et al., 2013;

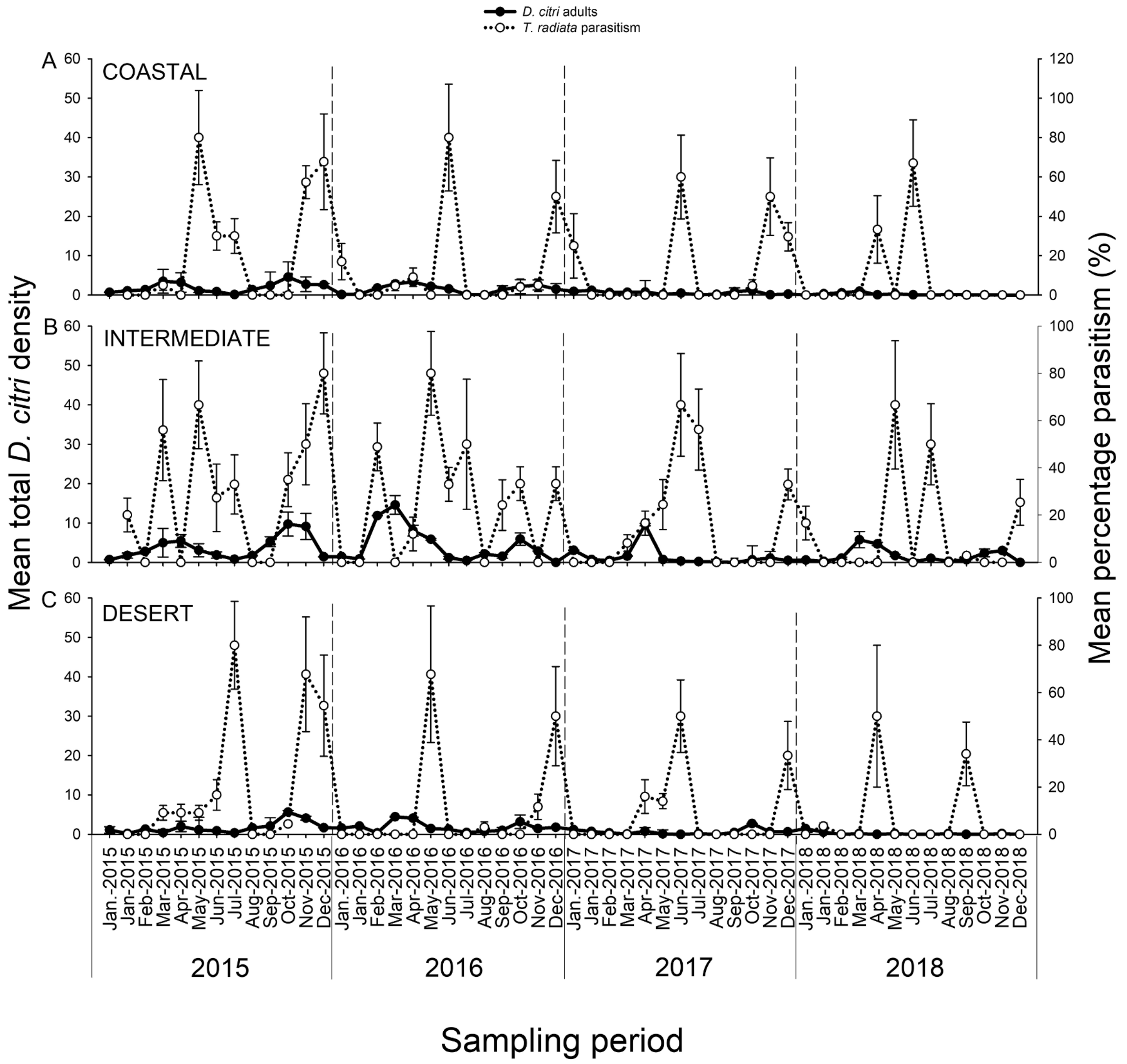

Fig. 6. Relationship between *D. citri* adults and mean *T. radiata* parasitism rates over time at study sites belonging to either the (A) coastal, (B) intermediate, or (C) desert climate zones in southern California from January 2015 through December 2018. Mean (±SE) number of *D. citri* adult counts (from timed observations) for each climate type (pooled across study sites and host plants) and mean (±SE) total *T. radiata* parasitism from the previous sampling period (pooled across study sites and host plants) are shown.

McCalla et al., 2019), and the subsequent numbers of adults, eggs and small nymphs, decreased by over 75% across all study sites over four years. This suggests that egg laying by fewer surviving adults each year cannot overcome the density dependent mortality that results from parasitism. Further, it is likely that *Tamarixia*-inflicted mortality on *D. citri* populations was underestimated because mortality from host feeding (Tena et al., 2017; Gebiola et al., 2018) and unsuccessful parasitism (Abram et al. 2019) by female parasitoids and loss of parasitized *D. citri* nymphs through intraguild predation (Kistner et al. 2016a) could not be quantified from field collected samples in this study. Regardless, our results provide strong evidence that *T. radiata* is likely limiting urban *D. citri* populations across southern California.

Observed parasitism rates by *T. radiata* were similar across climate zones and years. Correspondingly, previous findings indicated that *T. radiata* is possibly more tolerant of temperature extremes when compared to *D. citri* (McCalla et al., 2019; Milosavljević et al., 2020a, 2020b). In addition, elevated temperatures not exceeding the critical thermal maximum of 37–41°C observed for *T. radiata* increases efficacy (Li et al., 2018; McCalla et al., 2019; Ramos Aguila et al., 2020). This potential proclivity for warmer temperatures may have, in part, facilitated the rapidity with which *T. radiata* established across urban southern California (Hoddle et al. 2016) and subsequent reductions of *D. citri* densities reported here. Collectively, these outcomes may provide indirect support for sourcing *T. radiata* from Punjab Pakistan, an area with ~ 70% climate match with citrus production areas in California (Hoddle and Pandey 2014).

It is also likely that generalist predators were responsible for some reduction of psyllid nymphs (Michaud, 2004; Qureshi and Stansly, 2009; Kistner et al., 2016b, 2017; Irvin et al., 2021). This study did not measure predation. Kistner et al. (2016b) however, reported that syrphid (Diptera: Syrphidae) and lacewing (Neuroptera: Chrysopidae) larvae accounted for ~ 86% of predation events and could cause up to 93% mortality of field-deployed *D. citri* cohorts. Numerous multi-predator studies have demonstrated a positive relationship between predator biodiversity and predation of insect herbivores in several different agricultural ecosystems (reviewed by Crowder and Jabbour 2014) and it is likely that natural enemy complementarity contributes to *D. citri* biological control, especially in urban citrus where these studies were conducted. Further assessment of *T. radiata*'s contribution to mortality of *D. citri* nymphs in California would therefore benefit from field experiments that assess the individual and combined effects of host feeding, intraguild predation of parasitized nymphs, and predation on

Table 2
Results of repeated measures analyses examining the effects of regional climate zone (coastal, intermediate, desert), season (cool, moderate, warm), citrus hosts (i.e., grapefruit, lemon, lime, orange, tangerine), presence or absence of flush, presence or absence of Argentine ant (*L. humile*), and their interactions on densities of (A) *D. citri* eggs, (B) small [i.e., 1st–3rd instar] nymphs, (C) large [i.e., 4th–5th instar] nymphs, (D) and adults, and (E) *T. radiata* parasitism rates recorded across field sites from 2015 through 2018. All models for *D. citri* abundance included percent parasitism measured at sites during the prior sampling period as a covariate (A-D). Percent parasitism models included *D. citri* densities from the previous sampling period as a covariate (E). Only minimal adequate models for each response variable are shown. *$P < 0.1$; **$P < 0.05$; ***$P < 0.01$.

(A) Eggs	**Num *df***	**Den *df***	***F***	***P***
Climate type	2	303.9	3.29	0.04**
Year	3	1832	2.35	0.04**
Season	2	348.6	5.24	0.01**
Flush abundance	1	1493	6.21	0.01**
Ants	1	1909	6.11	0.02**
Parasitism	1	1959	37.44	<0.01***
(B) Small nymphs	**Num *df***	**Den *df***	***F***	***P***
Climate type	2	312.8	2.95	0.04**
Year	3	1833	4.37	0.01**
Season	2	358.4	4.51	0.01**
Flush abundance	1	1535	6.33	0.01**
Ants	1	1924	8.91	0.01**
Parasitism	1	1964	31.37	<0.01***
(C) Large nymphs	**Num *df***	**Den *df***	***F***	***P***
Climate type	2	323.1	3.71	0.03**
Year	3	1822	4.91	0.03**
Season	2	370.3	3.17	0.04**
Flush abundance	1	1680	6.23	0.01**
Ants	1	1964	16.61	<0.01***
Parasitism	1	1974	38.50	<0.01***
(D) Adults	**Num *df***	**Den *df***	***F***	***P***
Climate type	2	324.3	6.37	0.01**
Year	3	1761	17.00	<0.01***
Season	2	363.3	5.78	0.03**
Flush abundance	1	1760	6.13	0.01**
Parasitism	1	1964	21.60	<0.01***
(E) Parasitism	**Num *df***	**Den *df***	***F***	***P***
Season	2	269.5	6.01	0.01**
Flush abundance	1	1610	5.51	0.01**
Ants	1	1967	5.90	0.01**
Large nymphs	1	1979	45.41	<0.01***

pest population densities.

The combined impact by *T. radiata* and generalist predators has reduced *D. citri* densities in urban citrus by approximately 70 percent since the classical biocontrol program started (Hoddle, 2012; Hoddle and Hoddle, 2020). Correspondingly, this study is the first report of density-dependent reduction of *D. citri* by *T. radiata*. Significant reductions in *D. citri* densities may have resulted in substantially reduced rates of spread of this pest and *C*Las, especially from southern California into the Central Valley, where the majority of California citrus is grown. We recommend that biological control programs against *D. citri* in the aftermath of invasion focus on establishing stable populations of *T. radiata* for sustained suppression of *D. citri* populations.

Tamarixia radiata parasitism rates of *D. citri* were > 50% higher when colonies were not tended by *L. humile*. Conversely, when ants were active on trees, the densities of *D. citri* eggs and nymphs, increased 3-fold in comparison to trees lacking ant activity. No effects of ants on adult *D. citri* densities were observed possibly because adult psyllids do not produce harvestable honeydew (Tena et al., 2013; McCalla et al., 2020). These results are consistent with other studies from California (Tena et al., 2013; Anastasio et al., 2021) indicating that *L. humile* management programs (McCalla et al., 2020; Milosavljević and Hoddle, 2021) that reduce densities of foraging ants improve the efficacy *T. radiata* as a biological control agent of *D. citri* (Schall et al. 2018). Liquid baits consisting of sugar water and ultra-low concentrations of insecticide are extremely effective at quickly reducing densities of foraging workers and killing subterranean *L. humile* colonies (Schall et al. 2018). Liquid bait can be delivered to ants inside biodegradable hydrogel beads (Tay et al., 2017; Milosavljević and Hoddle, 2021), which are deployed on the ground under trees (McCalla et al. 2020). In the absence of ants, pest densities decline markedly, and the percentage of flush infested with *D. citri* is significantly reduced (Schall et al. 2018). Subsequently, *L. humile* control in residential citrus is recommended.

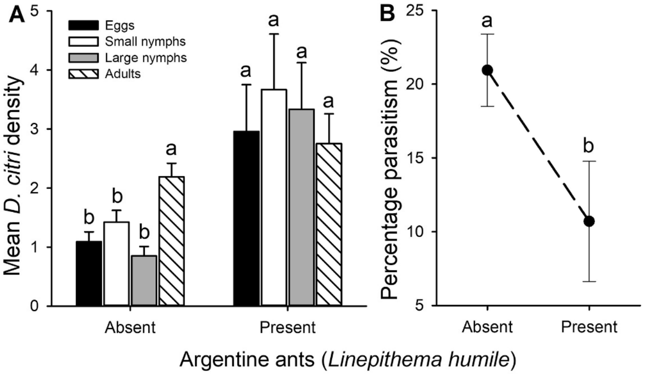

Fig. 7. Relationship between the presence and absence of *L. humile* and (A) mean (+SE) *D. citri* population densities (i.e., mean number of *D. citri* eggs, 1st–3rd instars [i.e., small nymphs], 4th–5th instars [i.e., large nymphs] per cm of flush shoot sampled, and mean *D. citri* adult counts [from timed observations]) pooled across climate types and years; and (B) mean (±SE) total *T. radiata* parasitism rates of study sites (pooled across climate zones and years). Different lowercase letters denote differences among groups by Tukey-Kramer adjustments for main effects of *L. humile* presence or absence at $\alpha < 0.05$.

CRediT authorship contribution statement

Ivan Milosavljević: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Project administration, Writing - original draft, Writing - review & editing. **David J.W. Morgan:** Conceptualization, Methodology, Data curation, Project administration, Funding acquisition, Writing - review & editing. **Rachael E. Massie:** Conceptualization, Methodology, Investigation, Data curation, Project administration, Writing - review & editing. **Mark S. Hoddle:** Conceptualization, Methodology, Investigation, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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