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Research article

Phenology of Asian Citrus Psyllid (Hemiptera: Liviidae), With Special Reference to Biological Control by *Tamarixia radiata,* in the Residential Landscape of Southern California

Erica J. Kistner,[1,3] Ruth Amrich,[1] Martin Castillo,[1] Vincent Strode,[1] and Mark S. Hoddle[1,2]

[1]Department of Entomology, University of California, Riverside, CA 92507 (ekistner@ucr.edu; ruth.amrich@ucr.edu; martin.castillo@ucr.edu; vincent.strode@ucr.edu; mark.hoddle@ucr.edu), [2]Center for Invasive Species Research, University of California, Riverside, CA 92507, [3]Corresponding author, e-mail: ekistner@ucr.edu

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Abstract

Since its discovery in 2008, the pestiferous Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), has become widely established in residential citrus trees throughout southern California. In 2011, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a host-specific parasitoid of *D. citri*, sourced from Punjab Pakistan, was introduced into California as part of a classical biological program aimed at suppressing *D. citri* populations in urban areas. Despite these release efforts, little is known about the population dynamics of *D. citri* in urban citrus or the efficacy of *T. radiata* in controlling psyllid populations in urban-grown citrus. To address this shortcoming, the population phenology of *D. citri* was monitored biweekly for 2–3 yr on five different host plants (Rutaceae) at 11 residential sites across Riverside and Los Angeles Counties in southern California. Citrus flush growth patterns and parasitoid activity levels were also assessed. Urban *D. citri* populations were present year round at each site, with highest densities occurring over July through November. Temperature was an important indicator of overall *D. citri* densities with positive correlations across all life stages. Regularly flushing lime trees consistently supported the highest densities of psyllid eggs and nymphs, while equally vigorous flushing curry leaf plants supported the highest adult densities. While *T. radiata* activity was detected at all sites, average year-round percent parasitism was low throughout the study, averaging <5% in 2012, 2013, and 2014.

Key words: *Diaphorina citri*, urban entomology, phenology, *Tamarixia radiata*, biocontrol

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an invasive citrus pest in the United States (US) that vectors the phloem-dwelling bacterium, *Candidatus* Liberibacter asiaticus (CLas), that causes a lethal citrus disease known as huanglongbing (HLB) (Halbert and Manjunath 2004, Hall et al. 2012). CLas infection leads to foliage dieback, seed malformation, and significant yield reductions, and susceptible citrus varieties can die 5–8 yr postinfection (Bové 2006). In the US, *D. citri* has been recorded from the states of Alabama, Arizona, California, Florida, Hawaii, Louisiana, Mississippi, South Carolina, and Texas (Center for Environmental and Research Information Systems [CERIS] 2015). Subsequently, CLas has been detected in Florida (first detected in 2005), Louisiana (2008), South Carolina (2009), Georgia (2009), Texas (2012), and California (2012) (CERIS 2016). The joint invasion of *D. citri* and CLas has drastically altered commercial citrus production in the US (Hall et al. 2012). In Florida, *D. citri* and HLB were first found in 1998 and 2005, respectively, and both are widespread throughout the state (Halbert and Manjunath 2004, Bové 2006, Hall and Albrigo 2007). Some estimates suggest that >75% of Florida's citrus trees may be infected with CLas (Hall et al. 2012). The *D. citri*–CLas pathosystem has reduced the production of citrus in Florida to its lowest levels since the 1960s with over US$2 billion in estimated net losses (Hodges and Spreen 2012). Thus, this vector–plant pathogen complex is currently considered the greatest threat the US citrus industry has to manage (Hall et al. 2012).

In August 2008, *D. citri* was first discovered in San Diego County, California. This invasion is attributed to a northward range expansion from Mexico where *D. citri* and HLB are widespread (Grafton-Cardwell 2010). *D. citri* feeds on many *Citrus* (Sapindales: Rutaceae) cultivars and the related curry leaf plant, *Murraya koenigii* (L.) Sprengel (Rutaceae) (Tsai et al. 2002, Chan et al. 2011).

Citrus and to a lesser extent curry leaf plants, are widely planted in the residential landscape of southern California. Since its introduction in California, *D. citri* has become widespread in urban areas across San Diego, Imperial, Riverside, Los Angeles, Orange, and San Bernardino Counties. Smaller populations have been detected in Ventura, Tulare, Kern, Fresno, San Joaquin, Santa Clara, Santa Barbara, and San Lius Obispo Counties, and captures have been confirmed as far north as San Jose (Civerolo 2015). *D. citri* has likely established permanent populations in the San Joaquin Valley where 85% of California's US$3 billion citrus industry is located (Grafton-Cardwell et al. 2015). In 2012, CLas was detected in a single residential tree in Los Angeles County (Kumagai et al. 2013) and additional cases within 25 km of the original Clas detection site have subsequently been reported in California's urban landscape (CDFA 2015). Extensive management efforts similar to ongoing programs in Florida (Halbert and Manjanath 2004, Hall et al. 2012) are currently underway in California in an attempt to mitigate the threat this vector–pathogen complex poses to California's iconic citrus crop.

Although HLB has not yet gained a foothold in California's commercial groves, there is a serious threat of CLas transmission by *D. citri* migrating between unmanaged residential landscapes into commercial citrus production areas (Boina et al. 2009). Adult *D. citri* have strong dispersal capabilities, thereby enabling *D. citri* incursion and potential establishment in both agricultural and urban habitats (Chong et al. 2010, Godfrey et al. 2013, Lewis-Rosenblum et al. 2015). Since ~36% of southern Californian gardens have citrus (Hoddle and Pandey 2014), management of urban *D. citri* populations is less straightforward compared to those in commercial orchards where insecticide applications to reduce vector densities are the standard *D. citri* control strategy (Grafton-Cardwell 2014, Richards et al. 2014). Chemical spray programs in urban areas in California have been abandoned due to a combination of high costs and some public resistance to pesticide applications to privately owned trees (Hoddle and Pandey 2014). Consequently, in the absence of insecticide treatments urban citrus provides a large refuge for *D. citri* which in turn likely increases the risk of CLas acquisition and spread that subsequently leads to HLB epidemics (Bassanezi et al. 2013).

A major component of California's emerging *D. citri* management program is classical biological control with host-specific parasitoids (Hoddle and Hoddle 2013, Hoddle and Pandey 2014, Bistline-East et al. 2015, Kistner and Hoddle 2015a). In December 2011, releases of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a host-specific parasitoid of *D. citri* sourced from Punjab Pakistan commenced. *T. radiata* is an ectoparasitoid that preferentially parasitizes fourth- and fifth-instar nymphs (Gómez-Torres et al. 2012). In addition to parasitism, female *T. radiata* can kill *D. citri* nymphs through host feeding (Chien et al. 1995). Worldwide, *T. radiata* is the most commonly employed parasitoid used for classical biological control of *D. citri* in many major citrus-growing regions (Halbert and Manjunath 2004, Hall et al. 2012). In California, the goal of this classical biological control program is to suppress urban *D. citri* population growth and spread where insecticide use targeting *D. citri* is infrequent with the intent of reducing the frequency of CLas acquisition and spread by this pest (Hoddle and Hoddle 2013, Hoddle and Pandey 2014, Kistner and Hoddle 2015a).

While *T. radiata* has provided significant suppression of *D. citri* on Réunion Island (Ètienne and Aubert 1980) and Puerto Rico (Pluke et al. 2005), extensive research indicates that this parasitoid has provided low levels of control in Florida (Tsai et al. 2002, Michaud 2004, Hall et al. 2008, 2012, Qureshi et al. 2009, Hall and Rohrig 2015). In Florida, *D. citri* mortality from parasitism is low (<5%; Michaud 2004, Qureshi and Stansly 2009), even in organic orchards and urban sites not treated with insecticides (Hall et al. 2008, 2012). There are several possible explanations for the poor performance of *T. radiata* in Florida. First, the extensive use of chemical control in Florida commercial citrus groves reduces *T. radiata* numbers (Hall and Nguyen 2010, Ferrer et al. 2011). Second, the low genetic diversity of *T. radiata* halotypes released in Florida may compromise parasitoid fitness (Barr et al. 2009, Hall et al. 2012). Third, intraguild predation by coccinellids (Michaud 2004) and ant mutualisms have been observed to reduce *T. radiata* parasitism rates (Navarrete et al. 2013). Finally, cultural practices such as frequent hedging of ornamental jasmine plants, *Murraya paniculata* (L.) Jack, a preferred *D. citri* host in urban areas, reduce urban *T. radiata* populations by removing flush with parasitized nymphs, resulting in death of the immature parasitoids while promoting *D. citri* outbreaks when hedged plants flush again (Chong et al. 2010, Hall and Rohrig 2015).

In an attempt to ensure *T. radiata* establishment and maximize efficacy in California, parasitoids for use in a classical biological control program were sourced from Punjab Pakistan, an area with ~70% climate match with major citrus production areas in California (Hoddle 2012). To preserve the genetic diversity of quarantine reared parasitoids, 17 different populations of *T. radiata* collected from Pakistan were maintained as isocage lines (Roush and Hopper 1995, Hoddle and Hoddle 2013). An HLB risk assessment model (Gottwald et al. 2014) is used by the California Department of Food and Agriculture (CDFA) to target releases of *T. radiata* in predicted high risk *D. citri*-HLB urban areas with the aim of reducing vector densities in these areas where CLas maybe present but undetected. As of July 2015, CDFA has released ~2,000,000 parasitoids at >4,000 sites across southern California. In southern California, parasitoid activity has been detected at over 100 different release sites indicating that *T. radiata* has established multiple stable populations in urban areas, some organic orchards, and it has spread unassisted to sites where it was not released (Hoddle and Hoddle 2013, Kistner and Hoddle 2015a). Despite the large ongoing release program and recoveries of *T. radiata* in southern California, the efficacy of *T. radiata* in controlling urban *D. citri* population growth in California remains largely unknown.

To address this shortcoming, urban *D. citri* populations were monitored across southern California for up to three years to evaluate the efficacy of this classical biological control program in California. Here, we report on the phenology of *D. citri* and *T. radiata* on five different types of host plants across 11 sites in Riverside and Los Angeles Counties. The results of these surveys are reported here, and these data provide important measures of *D. citri* and *T. radiata* activity at a very early stage of the biological control program.

Materials and Methods

Study Sites

Eleven urban sites for monitoring *D. citri* densities, host plant flushing patterns, and parasitoid activity were selected based on University of California Riverside (UCR) and CDFA *T. radiata* release data. Five sites in Los Angeles County were set up in 2012 and surveys ran for three consecutive years at these sites (Table 1). In 2013, six additional sites, three sites in Riverside County and three in Los Angeles County, were added. Surveys at these latter six sites

Table 1. Site information for selected *D. citri* and *T. radiata* phenology survey sites in urban areas of southern California

Site street	Start date	End date	Location	County	Tree(s)	*Tamarixia* Status
Penn Mar	25 Jan. 2012	25 Jan. 2015	34°03′88.48″ N; 118°04′45.63″ W; elevation 71 m	Los Angeles	1 lime	Invaded[a,b]
Maplefield	25 Jan. 2012	25 Jan. 2015	34°04′42.79″ N; 118°02′14.02″ W; elevation 44 m	Los Angeles	1 lemon	632 released[c] over 4 events
Poinsettia	25 Jan. 2012	25 Jan. 2015	34°05′36.45″ N; 118°01′07.12″ W; elevation 87 m	Los Angeles	1 lemon	Invaded[d]
Strozier	25 Jan. 2012	25 Jan. 2015	34°04′71.31″ N; 118°05′02.71″ W; elevation 73 m	Los Angeles	1 lemon	Invaded[e]
Badillo	25 Jan. 2012	25 Jan. 2015	34°08′74.50″ N; 117°94′57.97″ W; 193 m	Los Angeles	1 lemon	Invaded[f]
Rodeway Inn	16 Jan. 2013	25 Jan. 2015	34°12′18.75″ N; 117°90′70.22″ W; 166 m	Los Angeles	2 curry leaf plants	903 released over 9 events
Asuza	16 Jan. 2013	25 Jan. 2015	34°12′88.25″ N; 117°90′74.30″ W; 159 m	Los Angeles	2 orange, 2 lemon	1,859 released over 11 events
Lochmoor	16 Jan. 2013	25 Jan. 2015	33°95′37.44″ N; 117°31′50.77″ W; 420 m	Riverside	2 lemon, 2 orange, 2 grapefruit	2,089 released over 9 events
Jurupa	16 Jan. 2013	25 Jan. 2015	33°99′29.75″ N; 117°50′61.37″ W; 214 m	Riverside	2 lemon, 2 lime, 1 orange, 1 grapefruit	791 released over 6 events
Indiana	16 Jan. 2013	25 Jan. 2015	33°88′36.40″ N; 117°49′73.92″ W; 293 m	Riverside	6 oranges	Invaded[g]
Maywood	16 Jan. 2013	25 Jan. 2015	33°98′38.25″ N; 118°17′89.74″ W; 43 m	Los Angeles	1 lemon	Invaded[b]

[a] Invaded indicates that *T. radiata* colonized the site without deliberate human assistance.
[b] 0.2 km away from nearest release site.
[c] Released indicates that *T. radiata* was deliberately released at site as part of the biocontrol program.
[d] 1.4 km from nearest release site.
[e] 0.8 km from nearest release site.
[f] 2.4 km from nearest release site.
[g] 1.4 km from nearest release site.
[h] 0.75 km from nearest release site.

were conducted for two consecutive years (Table 1). *T. radiata* was purposely released as part of the classical biocontrol program at five of the 11 survey sites. Additional *T. radiata* release information is provided in Supp. Table 1 (online only). GPS coordinates were obtained for each designated survey tree using a handheld Garmin GPS unit (GPSMP 64, Garmin Ltd., Lenexa, KS). The phenology study consisted of 11 orange, 11 lemon, three lime, three grapefruit, and two curry leaf plants (*Murraya koenigii*, [Rutaceae]), a close relative of citrus for a total of 30 study trees (Table 1). Survey trees across all study sites were healthy, mature (>5 yr of age), and easily accessible. Each survey tree was divided into four designated quadrants (north, east, west, and south), and trees were monitored biweekly for densities of adult and immature (i.e., eggs, small nymphs [instars 1–3], and large nymphs [instars 4–5]) *D. citri*, immature leaf flush patterns, and *T. radiata* activity.

Surveys for Adult *D. citri* and Calculation of Insect Days

Timed 2 min *D. citri* adult counts were made in each of the four quadrants of each experimental tree for a total of 8 min per tree. The mean biweekly number of adult *D. citri* per tree was converted to cumulative insect days (i.e., adult-days). This technique combines time and the number of insects observed as a way of estimating the area under the population curve for each tree and represents the insect burden on host plants that can be compared to determine site effects (Ruppel 1983, Khan et al. 2014). The adult-days for each sampling period was estimated as

$$\text{adult-days} = (X_{i+1} - X_i)\left[(Y_i + Y_{i+1})/2\right]$$

where X_i and X_{i+1} are adjacent sampling periods and Y_i and Y_{i+1} are the corresponding insect counts. The cumulative adult days was then computed by sequentially summing the individual biweekly insect days over the course of the survey for each individual tree surveyed. Cumulative insect-days per tree can then be averaged to provide an estimate of the mean insect load across study trees. This method enabled us to compare adult *D. citri* population densities over the entire survey period within and across host plants located in different sites.

Monitoring Host Plant Flush Phenology, *D. citri* Eggs, Nymphs, and Percent Parasitism

D. citri population growth is limited by the availability of flush growth on host trees (Hall et al. 2008). Therefore, leaf growth on each tree, in each quadrant, was estimated biweekly using a standardized area of 172 cm^2 which was delineated using a metal hoop (37 cm radius). The hoop was randomly set along the tree's outer foliage at shoulder height and only branches that terminated inside the hoop were inspected. Leaf growth at the end of each branch was assessed for the presence or absence of flush growth. Flush growth consisted of three age-sequential stages, all of which are characterized by soft, light green tissue (Chan et al. 2011). In contrast, nonflush growth consists of stiff, dark green, mature leaves. These data were used to determine percentage flush growth (i.e., number of branches with flush growth/[(number of flush growth branches + nonflush growth branches) x 100]) per tree at each sampling site and date (Khan et al. 2014). After flush counts were completed, two branches with flush from each quadrant were sampled from outside the hoop for a total of 8 branch samples per tree. The branch samples were returned to the UCR Insectary & Quarantine facility for processing in compliance with CDFA permit number 2870. In quarantine, branch samples were refrigerated and processed over a 1–2-d period. Branch processing involved measuring the length of flush and counting the number of *D. citri* eggs, first–third instars (small nymphs), and fourth–fifth instars (big nymphs) for each tree by sampling date and location (Kistner and Hoddle 2015a). Mean biweekly numbers of *D. citri* eggs, first–third instars, and fourth–fifth instars per centimeter of branch were then converted to cumulative insect days (i.e., egg and nymph-days) per tree, which provided an estimate of the total egg or nymph population load on trees per site over the entire survey period (Ruppel 1983). These calculations were identical to those discussed above to calculate adult-days. Parasitism of *D. citri* nymphs was determined by lifting each nymph off the host stratum and using a microscope to check the ventral side for the presence of *T. radiata* eggs (found on the anterior edge of the third pair of coxa), larvae, or pupae. Any parasitoids found were recorded by sampling date and site, removed, and preserved in 95% ethanol in labeled vials for future genetic analyses. These data were used to determine actual percentage parasitism of nymphs (i.e., total number of parasitized nymphs/[(total number of nonparasitized large nymphs + total number of parasitized large nymphs) × 100]). Mean actual percentage parasitism was calculated for each tree by sampling date and location.

Meteorological Data

Mean biweekly maximum, minimum, and average temperatures; relative humidity; and rainfall data were obtained from seven nearby weather stations. Weather stations used were: Rosemead Gardens (34° 03′ N, 118° 02′ W) for Penn Mar, Maplefield, Poinsettia, and Strozier; South Gate Park for Maywood (33° 94′ N, 118° 18′ W); Baldwin Park-Denovo Dental (34° 10′ N, 117° 97′ W) for Badillo; Rosedale Community #1 Station (34° 14′ N, 117° 90′ W) for Asuza and Rodeway Inn; Arlington Heights (33° 89′ N, 117° 47′ W) for Indiana; Canyon Crest Golf (33° 95′ N, 117° 33′ W) for Lochmoor; and Cloverdale Estates (33° 97′ N, 117° 56′ W) for Jurupa. All weather stations were within 1–8 km of each study site (The Weather Underground 2015). Climate data were used to prepare average biweekly summaries for temperature, humidity, and rainfall for sites over the duration of the study (Suppl. Fig. 1 [online only]).

Statistical Analysis

Before analyses were conducted, data were assessed for normality using the Shapiro–Wilk's test. Cumulative *D. citri* days across all life stages (eggs, first–third instars, fourth–fifth instars, and adults) were log transformed while actual percent parasitism and percent flush growth were arcsine root-transformed (Zar 1999). Sample periods with no *D. citri* fourth–fifth instars present were excluded from percentage parasitism analyses because *T. radiata* exclusively parasitizes these large nymphs (Gómez-Torres et al. 2012). Since only five Los Angeles County sites, each containing one tree, were surveyed in 2012, one-way repeated measures ANOVAs were run for actual percent parasitism, and percent flush growth to compare within year phenology data differences between sites. Phenology data collected from 2013–2015 were analyzed separately because six additional sites were added in 2013 for a grand total of 11 sites monitored from 2013–2015. To avoid temporal and spatial pseudoreplication, we employed linear mixed models with site, host plant (oranges, lemons, limes, grapefruit, and curry leaf plants), and year (2013 and 2014), as fixed variables, with repeated measures (i.e., sample date) taken on trees nested within sites as random variables (Crawley 2013). Models examining differences in cumulative *D. citri* days only included tree as a random variable since this value is the sum of all insect density measurements per tree in 2013 and 2014. Linear mixed models were run for each cumulative *D. citri* day per life stage, actual percent parasitism, and percent flush growth to compare differences in phenology data between host plants within and across sites. In the percent parasitism models, we also included *T. radiata* release status, "invaded" (self-introduced from neighboring release sites) versus "released" (i.e., deliberately released at a study site as part of the biological control program) as a fixed variable. This was necessary, as *T. radiata* was only deliberately released at five out of the 11 study sites as part of the biological control program and this parasitoid invaded the other six sites without human assistance. Significant differences between site and host plant means when detected were separated with Tukey's HSD at the 0.05 level of significance. Repeated measure ANOVAs and linear mixed models were run using the lme4 package in R 3.2.0 (Bates et al. 2014). For both the 2012 and the 2013–2015 datasets, Pearson correlation analyses were performed using the stats package in R.3.2.0 (R Development Core Team 2015) to assess the relationship between *D. citri* life stage densities, percent parasitism, percent flush growth, and environmental conditions (temperature, relative humidity, and rainfall). All statistical analyses were conducted in R 3.2.0 (R Development Core Team 2015).

Results

D. citri Phenology on Different Host Plants

Biweekly mean egg densities on host plants experienced two small population spikes in January and April 2012 and two larger spikes in September and November 2012 (Fig. 1A). Mean biweekly 2012 egg densities were positively correlated with average temperature ($r = 0.14$; $P = 0.01$) and percentage flush growth ($r = 0.13$; $P = 0.01$), but were not correlated with rainfall or relative humidity ($P > 0.05$). In 2013, mean biweekly egg densities experienced population spikes in March through June, August, and October through December (Fig. 1A). In 2014, egg densities exhibited small spikes in January, April, and May (Fig. 1A). Mean biweekly egg densities in 2013 and 2014 were positively correlated with average temperature ($r = 0.21$; $P < 0.01$) and percent flush growth ($r = 0.17$; $P = 0.01$), but were not correlated with rainfall or relative humidity ($P > 0.05$).

Mean biweekly first–third instar densities on lime experienced major population spikes in January and November as well as minor peaks in May, July, and August through September 2012; densities on lemon exhibited smaller population spikes with the largest occurring in September 2012 (Fig. 1B). Mean biweekly first–third instar densities in 2012 were positively correlated with average temperature ($r = 0.38$; $P < 0.001$), but were not correlated with percent flush growth, rainfall, or relative humidity ($P > 0.05$). Mean biweekly first–third instar densities persisted at low levels year round on all host plants when flush was present with major population spikes occurring in both spring and fall of 2013 and 2014 (Fig. 1B). Lime hosted the highest first–third instar densities in late August–November of 2013 (Fig. 1B). Mean biweekly first–third instar densities from 2013–2015 were positively correlated with average temperature ($r = 0.32$; $P = 0.01$) and percentage flush growth ($r = 0.23$; $P = 0.01$), but were not correlated with rainfall or relative humidity ($P > 0.05$).

D. citri fourth–fifth instars reached their highest densities beginning in September before declining in November, but overall densities remained low throughout 2012 (Fig. 1C). Mean biweekly fourth–fifth instar densities in 2012 were positively correlated with average temperature ($r = 0.44$; $P < 0.001$) and negatively correlated with rainfall ($r = -0.37$; $P < 0.001$). No significant correlations were found between 2012 mean biweekly fourth–fifth instar densities and percentage flush growth, relative humidity, or parasitism rates ($P > 0.05$).

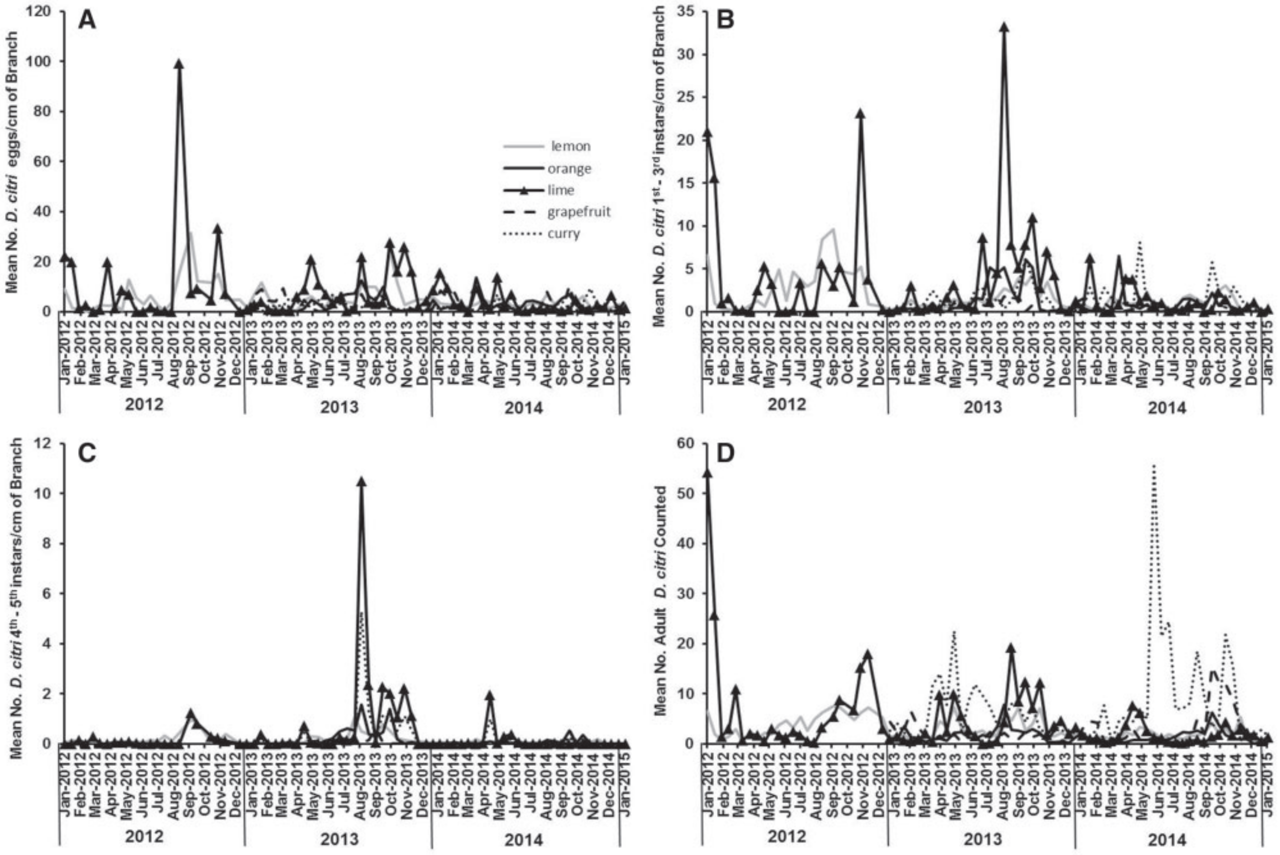

Fig. 1. Relationship between host plants and biweekly *D. citri* population densities. (**A**) Mean number of *D. citri* eggs per centimeter of branch sample. (**B**) Mean number of *D. citri* first–third instars per centimeter of branch sample. (**C**) Mean number of *D. citri* fourth–fifth instars per centimeter of branch sample. (**D**) Mean number of *D. citri* adults per tree from 2-min visual counts. *D. citri* numbers for each host plant were averaged across Los Angeles and Riverside County study sites. *D. citri* population data in 2012 were collected solely from lemon and lime trees within Los Angeles County.

In 2013, mean biweekly fourth–fifth instar densities experienced a minor spike in May and two major spikes in August and October through December (Fig. 1C). In contrast, only one minor population spike occurred in 2014 during May (Fig. 1C). Mean biweekly fourth–fifth instar densities from 2013–2015 were positively correlated with average temperature ($r = 0.25$; $P < 0.01$) and negatively correlated with rainfall ($r = -0.12$; $P < 0.05$). No significant correlations were found between 2013–2015 biweekly fourth–fifth instar densities and percentage flush growth, relative humidity, or parasitism rates ($P > 0.05$).

Mean biweekly adult densities on lime were highest in January 2012 followed by a slight increase in March 2012 and a more gradual population build up beginning in September 2012 before declining again in December 2012 (Fig. 1D). Adult density fluctuations on lemon were less pronounced than lime with densities increasing in June, and reaching an asymptote in September that held until the end of 2012 (Fig. 1D). Mean biweekly adult densities in 2012 were positively correlated with average temperature ($r = 0.22$; $P = 0.02$), but were not correlated with percentage flush growth, rainfall, or relative humidity ($P > 0.05$). In 2013, mean biweekly adult densities on all host plants experienced one population spike from April to May followed by a second peak in August through November (Fig. 1D). In 2014, adult densities on curry leaf plants increased rapidly in June followed by two smaller peaks in September and November (Fig. 1D). Adult populations on grapefruit spiked in October 2014 while adult densities on lemon, orange and lime exhibited minor peaks in the spring and fall of 2014 (Fig. 1D). Mean biweekly adult densities from 2013–2015 were positively correlated with average temperature ($r = 0.14$; $P < 0.001$) and percent flush growth ($r = 0.11$; $P = 0.02$), but were not correlated with rainfall or relative humidity ($P > 0.05$).

Comparison of Cumulative *D. citri* Days on Different Hosts Plants Within and Across Sites

D. citri Egg-Days

From 2013 to 2015, cumulative *D. citri* egg-days differed across the five host plants ($F = 24.85$; df = 4, 13; $P < 0.001$; Fig. 2) and the 11 study sites ($F = 10.64$; df = 10, 13; $P < 0.001$). Cumulative egg-days did not differ between years ($F = 1.18$; df = 1, 13; $P = 0.28$). The year by host plant ($F = 2.76$; df = 4, 13; $P = 0.04$), year by site ($F = 1.94$; df = 10, 13; $P = 0.04$; Suppl. Fig. 2A [online only]), and site by host plant interactions ($F = 16.11$; df = 4, 13; $P < 0.001$; Suppl. Fig. 3A [online only]) were all significant. Mean cumulative egg-days were highest for lime compared to all other host plants (Tukey HSD < 0.01; Fig. 2).

D. citri First–Third Instar-Days

From 2013 to 2015, cumulative *D. citri* first–third instar-days differed across the five host plants ($F = 36.21$; df = 3, 13; $P < 0.001$; Fig. 2) and the 11 study sites ($F = 39.31$; df = 10, 13; $P < 0.001$). Cumulative first–third instar-days did not differ between years ($F = 0.31$; df = 1, 13; $P = 0.58$). The year by host plant ($F = 3.72$; df = 4, 13; $P = 0.01$), year by site ($F = 4.08$; df = 10, 13; $P < 0.001$; Supp. Fig. 2B [online only]), and site by host plant interactions were all significant

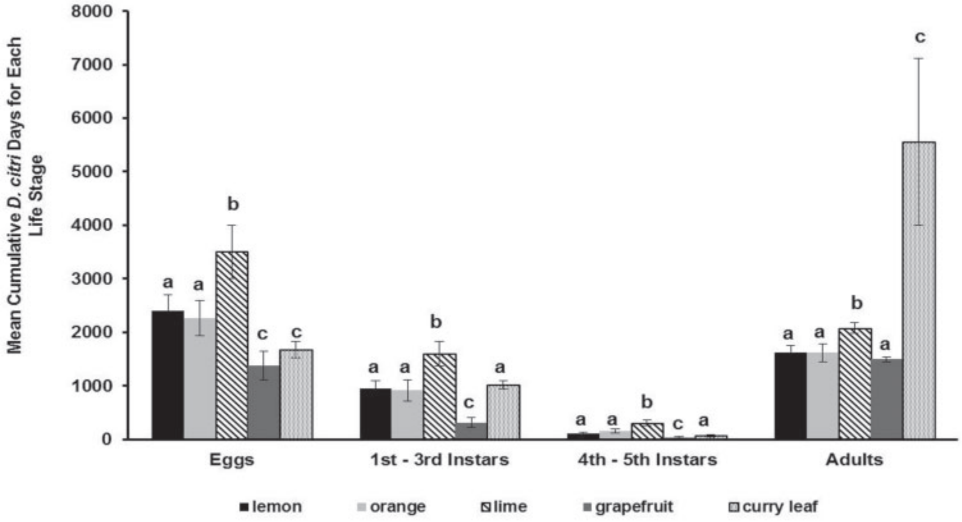

Fig. 2. Mean (± SE; untransformed) cumulative *D. citri* egg, first–third instar, fourth–fifth instar, and adult days from 2013–2015 on oranges, lemons, limes, grapefruit, and curry leaf plants. Different letters within a life stage indicate significant differences between host plants (Tukey HSD test, $P > 0.05$). *D. citri* days for each host plant were averaged across eight Los Angeles and three Riverside County study sites.

($F = 25.17$; df = 4, 13; $P < 0.001$; Suppl. Fig. 3B [online only]). Mean cumulative first–third instar-days were highest on limes (Tukey HSD < 0.01) and lowest on grapefruit (Tukey HSD < 0.01; Fig. 2).

D. citri Fourth–Fifth Instar-Days

From 2013 to 2015, cumulative *D. citri* fourth–fifth instar-days differed across the five host plants ($F = 7.49$; df = 4, 13; $P < 0.001$; Fig. 2) and the 11 study sites ($F = 9.91$; df = 10, 13; $P < 0.001$). In 2014, cumulative fourth–fifth instar-days declined by 28% ($F = 19.48$; df = 1, 13; $P < 0.001$; Fig. 1C). The host plant by year ($F = 4.79$; df = 4, 13; $P = 0.002$), site by year ($F = 4.61$; df = 10, 13; $P < 0.00$; Suppl. Fig. 2C [online only]), site by host plant ($F = 15.00$; df = 4, 13; $P < 0.001$; Suppl. Fig. 3C [online only]), and site by host plant by year ($F = 3.98$; df = 4, 13; $P = 0.007$) interactions were also significant. Like earlier *D. citri* life stages, mean cumulative fourth–fifth instar-days were highest on limes (Tukey HSD < 0.01) and lowest on grapefruit (Tukey HSD < 0.01; Fig. 2).

D. citri Adult-Days

From 2013 to 2015, cumulative *D. citri* adult-days differed across the five host plants ($F = 77.88$; df = 4, 13; $P < 0.001$; Fig. 2) and the 11 study sites ($F = 26.39$; df = 10, 13; $P < 0.001$). Cumulative adult-days did not differ between years ($F = 0.31$; df = 1, 13; $P = 0.10$), but there were significant year by site ($F = 4.08$; df = 10, 13; $P < 0.001$; Suppl. Fig. 2D [online only]) and site by host plant ($F = 15.00$; df = 4, 13; $P < 0.001$; Suppl. Fig. 3D [online only]) interactions. Mean cumulative adult days were 125% higher on curry leaf plants compared to all other host plants (Tukey HSD < 0.01; Fig. 2).

Citrus Flush Patterns

In 2012, percent flush growth varied significantly across the five Los Angeles County sites ($F = 7.64$; df = 4, 88; $P < 0.001$), which was due, in part, to differences in host plant flushing patterns. Limes produced high levels of flush from January to March and exhibited smaller peaks in flush production in April, May, October, and November 2012; no flush was produced from June through September 2012 (Fig. 3A). Lemons consistently produced flush year round and peak lemon flushing events coincided with limes (Fig. 3A). Percent flush growth in 2012 was negatively correlated with average temperature ($r = -0.43$; $P < 0.001$), and was not correlated with rainfall or relative humidity ($P > 0.05$).

From 2013 to 2015, percent flush growth varied across the 11 study sites ($F = 9.11$; df = 10, 1533; $P < 0.001$) and the five host plants ($F = 2.66$; df = 4, 1533; $P = 0.03$), but not between years ($F = 0.28$; df = 1, 1533; $P = 0.60$). There was a significant year by host plant interaction ($F = 3.40$; df = 1, 1533; $P = 0.02$), suggesting that host plants responded differently to climatic variation between years. The site by host plant ($F = 1.53$; df = 4, 1533; $P = 0.20$), site by year ($F = 1.04$; df = 10, 1533; $P = 0.40$), and site by year by host plant ($F = 1.39$; df = 4, 1533; $P = 0.24$) interaction terms were not significant. Differences in flushing patterns among sites were strongly influenced by host plant and climate. All host plants exhibited a major flush event beginning in January and ending in March in 2013 and 2014 (Fig. 3A). Limes and curry leaf plants had the highest percent flush growth compared to the other host plants (Tukey HSD < 0.05). While lemon flushed more frequently than grapefruit or orange, these three host plants did not differ in overall percent flush growth since orange and grapefruit exhibited greater flush growth during major flush events (Tukey HSD > 0.05; Fig. 3A). Percent flush growth from 2013–2015 was negatively correlated with average temperature ($r = -0.28$; $P < 0.001$), but was not correlated with rainfall or relative humidity ($P > 0.05$).

Parasitism by *T. radiata*

In 2012, mean parasitism rates were low, averaging ~ 0.6% (range, 0–7.6%) and did not differ across the five Los Angeles County sites ($F = 0.35$; df = 4, 76; $P = 0.84$; Suppl. Fig. 4). Parasitoid activity on lemons peaked in November 2012 while parasitoid activity on limes peaked in December 2012 (Fig. 3B). No correlations were found between percent parasitism and any climatic factors, percent flush growth, or *D. citri* densities ($P > 0.05$) in 2012.

From 2013 to 2015, mean parasitism rates remained low, averaging ~ 5.0% (range, 0–18.6%) across the 11 study sites. Mean parasitism rates varied significantly across host plant ($F = 32.04$; df = 4, 606; $P < 0.001$; Fig. 3B), sites ($F = 12.68$; df = 10, 606; $P > 0.001$; Suppl. Fig. 4), and *T. radiata* release status ($F = 13.78$; df = 1, 606; $P < 0.001$), but not between years ($F = 0.18$; df = 1, 606; $P = 0.67$).

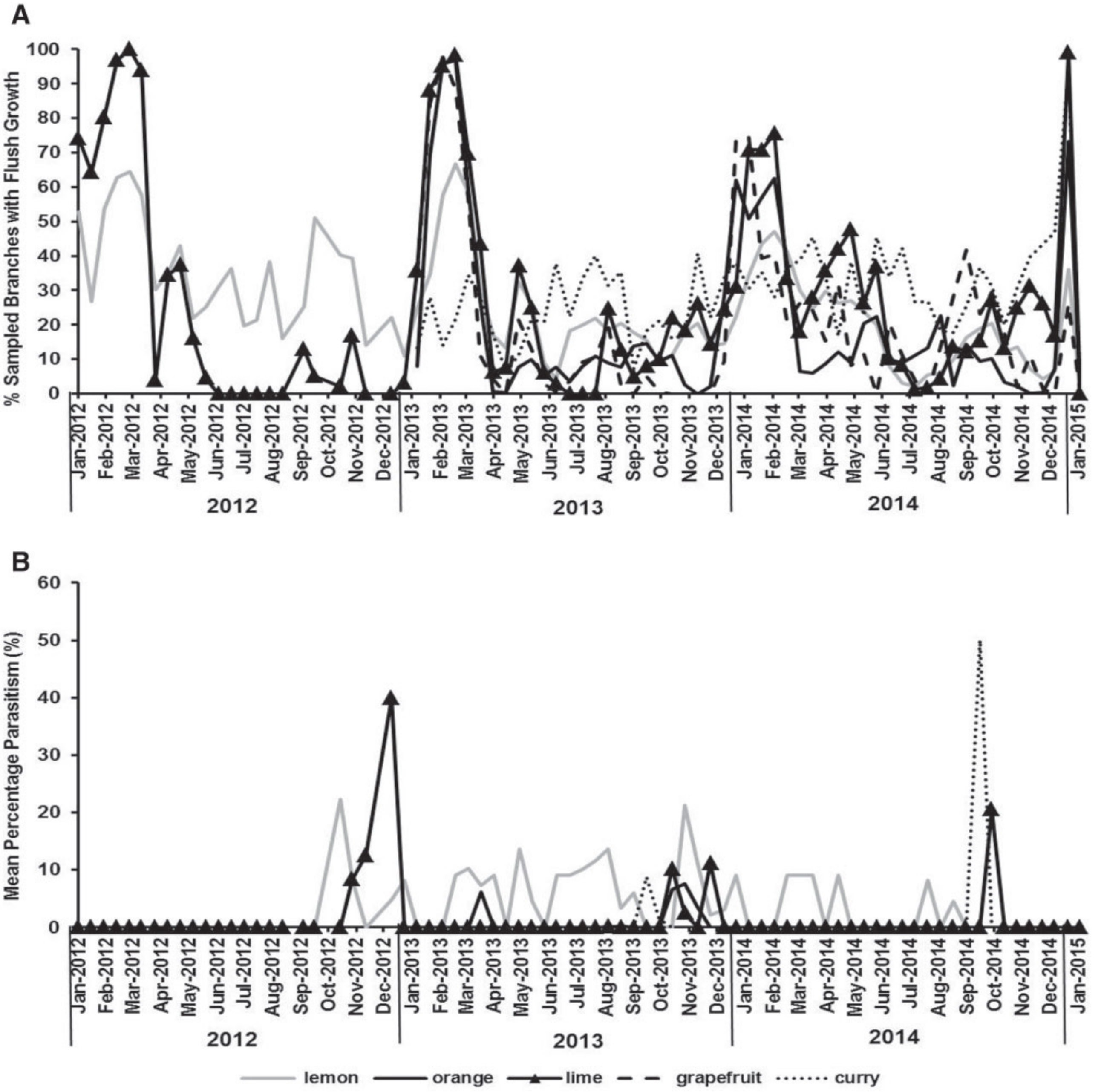

Fig. 3. (**A**) Biweekly flushing patterns for oranges, lemons, limes, grapefruit, and curry leaf plants combined across Los Angeles and Riverside County study sites. (**B**) Biweekly percentage parasitism trends for *T. radiata* attacking *D. citri* nymphs on oranges, lemons, limes, grapefruit, and curry leaf plants. Parasitism data were combined for each host plant across Los Angeles and Riverside County study sites. Flush and parasitism data from 2012 were only collected from lemon and lime trees within Los Angeles County.

The year by host plant interaction term ($F = 2.75$; df = 4, 606; $P = 0.04$) was significant. The year by *T. radiata* release status ($F = 0.38$; df = 1, 606; $P = 0.41$), site by year ($F = 0.89$; df = 4, 633; $P = 0.53$), and site by year by host plant ($F = 0.43$; df = 4, 633; $P = 0.73$) interaction terms were not significant. Major parasitoid activity on most host plants was observed in fall of 2013 and 2014 (Fig. 3B), following summer population growth of *D. citri* fourth–fifth instars (Fig. 1C). Parasitoid activity on oranges, limes, and curry leaf plants decreased substantially in winter and remained low over spring. Interestingly, lemons exhibited parasitoid activity year round (Fig. 3B), as well as the highest overall parasitism rates compared to all other host plants (Tukey HSD < 0.01). Overall parasitism rates were significantly higher at Lochmoor in Riverside County, averaging 18.6%, compared to all other sites (Tukey HSD < 0.001). Parasitism rates also varied within host plants across sites as indicated by the significant site by host plant interaction term ($F = 24.86$; df = 10, 606; $P < 0.001$). For instance, lemons at Lochmoor, a designated *T. radiata* release site in Riverside County, exhibited a 40-fold greater mean parasitism rate compared to all other sites by lemon trees (Tukey HSD < 0.05; Fig. 4). In contrast, no parasitoid activity was detected on grapefruit trees at Lochmoor and Jurupa, oranges at Azusa, or lemons at Badillo and Maplefield (Fig. 4). No correlations were found between 2013–2015 parasitism rates and any climatic factors, percent flush growth, or *D. citri* densities ($P > 0.05$).

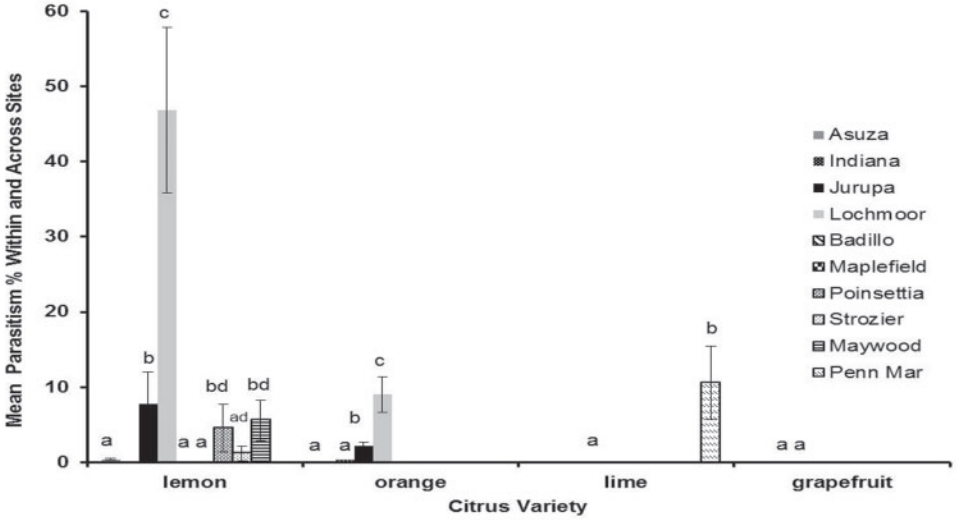

Fig. 4. Mean (± SE; untransformed) percentage parasitism for *T. radiata* attacking *D. citri* nymphs from 2013–2015 across eight sites with lemons, four sites with oranges, two sites with limes, and two sites with grapefruits. Different letters within a citrus variety indicate significant differences between varieties (Tukey HSD test, $P > 0.05$). Percentage parasitism for each citrus variety was averaged for sites with multiple trees.

Discussion

This work is the first major study to document *D. citri* phenology, *T. radiata* activity, and flush phenology on five different types of host plants in southern California. Although we did find significant declines in *D. citri* populations across the majority of our survey sites since the introduction of *T. radiata*, solid evidence for *T. radiata*-driven suppression of *D. citri* populations was not detected using the survey methods employed. Urban *D. citri* densities varied greatly across and within sites, host plants, and years. *D. citri* populations consistently peaked in the fall and spring, but the magnitude of these seasonal fluctuations varied spatially and temporarily. Our results indicated that lime consistently supported higher immature *D. citri* densities while curry leaf plants supported the largest numbers of adults. We suspect that greater frequency and intensity of flush growth allowed limes to support higher densities of *D. citri* eggs and nymphs while a combination of flush availability and olfactory cues attracted more *D. citri* adults to curry leaf plants. Previous studies indicate that the volatile chemical composition of curry leaf plants (Robbins et al. 2012) and closely related orange jasmine plants, *Murraya paniculata* (L.) Jack, act as strong *D. citri* attractants (Patt and Setamou 2010). All *D. citri* life stage densities were positively correlated with temperature, suggesting that citrus growing in warmer inland sites may consistently host higher *D. citri* densities. While parasitism rates during the course of the study were low, seven out of the eleven study sites exhibited significant decreases in adult *D. citri* numbers since the biological control program with *T. radiata* began in California. The reasons for this decline are not well understood and further studies (e.g., life table analyses) that quantify the effects of biotic and abiotic factors affecting urban *D. citri* densities and phenology are warranted.

The seasonal availability of flush growth greatly influences the phenology of *D. citri*, as this pest oviposits and small nymphs develop almost exclusively on young flush (Hall et al. 2008). In our study, increases in *D. citri* egg, first–third instar, and adult densities consistently coincided with flush abundance which in turn was limited by temperature. Generally, flush growth decreased with increasing average temperature and all four citrus varieties exhibited major flushing events from January to March in 2012, 2013, and 2014. However, our surveys indicated that flush growth was present at study sites at some level year round, and given the wide variety of citrus plants (and curry leaf plants), varying climatic conditions (e.g., temperature), and differing levels of maintenance (e.g., irrigation, pruning, and fertilization) in California's urban gardens, it is likely that available flush is highly heterogeneous throughout the year and across different areas. This heterogeneity may be important for sustaining *D. citri* and *T. radiata* populations in southern California. For instance, limes, curry leaf plants, and lemons produced flush at regular intervals, thereby enabling urban *D. citri* populations to reproduce almost year round at our study sites. Additionally, variation within and among *D. citri* life stage densities on different host plants were likely due in part to host plant quality and the influence of temperature on developmental rates (Liu and Tsai 2000, Fung and Chen 2006).

D. citri population dynamics in urban areas were driven by host plant growth and climatic conditions. Increases in *D. citri* egg, first–third instar, and adult densities coincided with flush, and favorable environmental conditions, especially temperature, which was consistently correlated with *D. citri*-days across all life stages. This combination of pronounced periods of flush growth interspersed with lesser flush periods and temperatures able to support *D. citri* growth resulted in psyllid populations being present but patchily distributed temporally and spatially across sites year round. Consequently, *D. citri* densities peaked in April–May and July–October when average daily temperatures consistently fell within *D. citri*'s optimal temperature range of 25–28°C (Liu and Tsai 2000). Rainfall was low throughout the duration of the study and was not correlated with percentage flush growth (California was experiencing a significant drought over the course of these studies and all experimental trees were irrigated by home owners). However, rainfall was negatively correlated with *D. citri* fourth–fifth instar-days, suggesting that precipitation events may adversely affect larger nymphs, possibly by dislodging them from host trees. It is plausible that observed differences among sites in *D. citri* densities are driven, in part, by environmental conditions. For example, overall *D. citri* densities were consistently higher at Lochmoor and Jurupa, two Riverside County sites, whose average maximum temperatures were ~2.5°C higher than Los Angeles County sites. Laboratory studies indicate that *D.*

citri reared at warm optimal temperatures (i.e., 25–28°C) exhibit enhanced survival, reduced developmental times, and increased fecundity (Fung and Chen 2006, Liu and Tsai 2000). Further, field studies in Florida found positive correlations between temperature and *D. citri* densities (Tsai et al. 2002, Hall et al. 2008). However, since 8 of our 11 study sites were located Los Angeles County, the relationship between study site, temperature, and *D. citri* densities may be influenced by an over representation of sites from this area. To better address the role of climatic factors and *D. citri* population densities through time, future studies should examine urban *D. citri* phenology across a wider geographic gradient using an east (hot and arid) to west (cool and humid) transect with a balanced distribution of sites and citrus varieties to assess the effects of California's varied climate on pest population growth.

Differences in urban garden care may also contribute to observed differences in *D. citri* densities across residential sites, as host plant quality varied greatly among different sites (Kistner, personal observation). Homeowners that regularly prune trees, which promotes flush growth, may inadvertently increase psyllid densities due to increased flush availability (Kistner and Hoddle 2015a). Similarly, regularly trimmed and well managed ornamental orange jasmine hedges in Florida produce the highest *D. citri* densities compared to citrus because regular pruning promotes flush growth (Tsai et al. 2002, Chong et al. 2010, Hall and Rohrig 2015). In addition to pruning, watering and fertilization may enhance host plant quality, and when coupled with mixtures of different citrus varieties with varying flush patterns could result in higher urban *D. citri* densities than would be observed in commercial citrus groves which are uniformly planted and managed. If this is the case and heterogeneous urban environments do support greater year round *D. citri* populations, this may be beneficial for the persistence of biological control agents like *T. radiata*, but it could also increase the risk of *C*Las acquisition and transmission by psyllids.

The low average year-round parasitism rates by *T. radiata* observed in this study were consistent with similar findings from Florida (Tsai et al. 2002, Michaud 2004, Qureshi and Stansly 2009). Three different hypotheses may explain low observed parasitoid activity. First, over half the sites monitored for the duration of the study were never purposely inoculated with *T. radiata* and this parasitoid migrated to these nonrelease sites, possibly from nearby designated release sites. At these nonrelease sites, our results suggest that *T. radiata* failed to provide significant *D. citri* control. A notable exception was Penn Mar, a site in Los Angeles County, where parasitoid activity averaged 11% and was consistent over all three years of the study. Likewise, Lochmoor, a site in Riverside County, was the only designated release site where *T. radiata* flourished with percentage parasitism averaging 18.6% from 2013 to 2015. Interestingly, average overall parasitism rates were 35% higher at sites where *T. radiata* was deliberately released compared to nonrelease sites that the parasitoid invaded. Second, viable urban *T. radiata* populations may not have established in some release areas due to low numbers of individuals released (e.g. propagule size; Beirne 1975) in conjunction with too few discrete release events (e.g. propagule number; Beirne 1975) that may not have coincided with *D. citri* life stages susceptible for host feeding and parasitism. Due to the limited availability of parasitoids during the early stages of this classical biological control program, propagule size and release frequency over the course of this study were low, averaging eight releases of ∼159 individuals per designated release site. In addition, propagule size and number of releases were not consistent across release sites with some sites receiving more parasitoids (i.e., Lochmoor) or more frequent releases (i.e., Asuza) than others (see Table 1 for release summaries). Poor timing of releases may have hindered *T. radiata* establishment at some sites. For example, multiple December and January releases were conducted at Azusa, Rodeway Inn, and Jurupa. At this time densities of *D. citri* fourth–fifth instars, preferred stages for parasitism, were low. Finally, another invasive urban pest, the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), may be interfering with *T. radiata* suppression of *D. citri* (Kistner and Hoddle 2015b, Tena et al. 2013). Argentine ants infest > 90% of all southern California properties where they actively tend and protect up to 55% of *D. citri* colonies. This mutualistic relationship can reduce *T. radiata* parasitism rates by as much as 80% (Tena et al. 2013). In the context of this study, Argentine ants may have contributed to the lack of observable parasitoid activity at Maplefield and Azusa, two designated release sites in Los Angeles County, as they had persistent year-round ant activity. Argentine ant exclusion experiments in California are needed to assess the importance of ant-*D. citri* mutualisms on *T. radiata*'s efficacy as a biological control agent.

Despite low average year-round parasitism rates, *T. radiata* may be contributing to *D. citri* population declines at some California residencies (Kistner and Hoddle 2015a). Parasitism rates of fourth–fifth instars often exceeded 50% during periods of peak parasitoid activity. Consequently, mean cumulative *D. citri* fourth–fifth instar-days, *T. radatia*'s preferred life stage for oviposition (Gomez-Torres et al. 2012), were significantly lower in 2014 compared to 2013 across all 11 sites. Furthermore, adult psyllid densities at five Los Angeles County sites declined by 68% over a 3-yr period since the introduction of *T. radiata* into this area. Similarly, psyllid densities at two Riverside County sites declined by ∼50% from 2013 to 2015 following *T. radiata* releases. An additional mortality factor that *T. radiata* is imposing on *D. citri* populations is host feeding by female parasitoids. Laboratory studies indicate that this can be very high with an estimated 500 nymphs over a single female's lifetime (Chien et al. 1995). In addition, Skelley and Hoy (2004) found that 57% of *D. citri* nymphs were killed by *T. radiata* host feeding compared to the 36% parasitized under laboratory conditions. The combined impact of host feeding and parasitism equated to a 93% total reduction in *D. citri* numbers. Unfortunately, due to difficulty in detecting and accurately identifying from field collected samples, psyllid mortality from host feeding by *T. radiata* females could not be quantified in these studies. Regardless, our results provide no definitive evidence that *T. radiata* is currently limiting urban *D. citri* populations across southern California and additional phenology surveys at later stages of this ongoing classical biological program will be warranted. Other biotic factors, such as generalist predators, may also influence *D. citri* densities at urban sites.

D. citri is subjected to varying degrees of control by natural enemies throughout its native (Khan et al. 2014) and invaded ranges (Hall et al. 2012). In Florida, ladybeetles (Coleoptera: Coccinellidae) may be the most important natural enemy group attacking immature *D. citri* (Michaud 2004, Chong et al. 2010, Qureshi and Stansly 2009). Likewise, naturally occurring predators may significantly reduce *D. citri* densities in California (Goldmann et al. 2014). For example, Kistner and Hoddle (2015b) found green lacewing larvae (Neuroptera: Chrysopidae) and hover fly larvae (Diptera: Syrphidae) can reduce immature *D. citri* by as much as 93% in some instances. Further studies (e.g., life table analyses or videography of *D. citri* cohorts) examining natural enemy impact on urban *D. citri* population suppression are needed. Reduced vector densities, especially of mobile *D. citri* adults, is a crucial component of HLB management since bacterium transmission rates decrease as a function of decreasing *C*Las-infected *D. citri* feeding on susceptible host plants (Pelz-Stelinski et al. 2010). Reducing psyllid densities in urban areas, where HLB is currently present and spreading, may decrease the rate at which HLB appears in California's commercial orchards because fewer *D. citri* from potentially infected urban sources carrying CLas will be available to migrate into uninfected commercial citrus groves.

In conclusion, this study provides a crucial snapshot of both urban *D. citri* population dynamics and efficacy of *T. radiata* at the early stages of an ongoing *D. citri* management program in urban areas in southern California that is relying heavily on classical biological control. Our results indicate that *D. citri* densities are strongly linked to seasonal changes in flush growth in conjunction with temperature and that *D. citri* populations have declined at multiple California residences since the introduction of *T. radiata*. Given that *T. radiata* propagule pressure was low over the course of the study, it is likely that *T. radiata* has not yet reached its full potential as a classical biological control agent targeting *D. citri* in urban areas. Mass production and release of *T. radiata* in California has increased substantially since its initial introduction in Dec. 2011. For instance, <5,000 parasitoids were released across Los Angeles County in 2012 when this project began, while >200,000 parasitoids were released in 2014, the final year of these surveys (Richard A. Dunn, Citrus Research Board, unpublished data). Furthermore, there are often significant time lags between the release, establishment, and subsequent top down control of the target pest by a biological control agent (Van Driesche et al. 2008). We recommend repeating these monitoring surveys in 5–8 yr, thereby providing a comparison of *T. radiata* efficacy during the early and later stages of this classical biological control program in urban areas in southern California.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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