Annals of the Entomological Society of America, 2016, 1–12
doi: 10.1093/aesa/saw053
Research article

Ecology and Population Biology

Abiotic and Biotic Mortality Factors Affecting Asian Citrus Psyllid (Hemiptera: Liviidae) Demographics in Southern California

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Received 25 March 2016; Accepted 27 June 2016

Abstract

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an invasive citrus pest in California that vectors a bacterium that causes the deadly citrus disease huanglongbing. From 2014–2015, 116 experimental *D. citri* cohorts were monitored to determine survivorship, life table parameters, and marginal rates of mortality of immature *D. citri* at three sites in Riverside County, CA. *D. citri* cohorts of ∼200 eggs were established on potted *Citrus volkameriana* (Rutaceae) plants. Field deployed cohorts were protected with fine or coarse mesh enclosures, sticky barriers, or left unprotected. Egg through adult emergence rates were used to assess the relative importance of biotic and abiotic factors on psyllid survivorship rates by life stage. Predation of immature *D. citri* by larvae of *Allograpta* sp. (Diptera: Syrphidae) and *Chrysoperla* sp. (Neuroptera: Chrysopidae) comprised 86% of all observed predation mortality. When protected from all other arthropods, parasitism by *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) comprised 21% of the total marginal rate of immature *D. citri* mortality from September to November in 2014 and 2015. Overall, *D. citri* net reproductive rates were reduced by 55–95% when exposed to natural enemies, indicating the importance of the classical biological control agent, *T. radiata*, and generalist predators in reducing *D. citri* densities in urban areas of southern California.

Key words: *Diaphorina citri*, biological control, generalist predator, life table, *Tamarixia radiata*

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was discovered in southern California in 2008 and is now widespread throughout the region (Civerolo 2015). *D. citri* is a notorious citrus pest because it vectors the phloem-dwelling bacterium, *Candidatus* Liberibacter asiaticus (CLas), which causes the deadly citrus disease huanglongbing (HLB), which can kill susceptible citrus varieties within 5–8 yr postinfection (Bové 2006, Halbert and Manjunath 2004, Hall et al. 2012). In the United States, *D. citri* has been detected in nine states and HLB has been detected in six of those states including California (Center for Environmental and Research Information Systems [CERIS] 2015). The *D. citri*–CLas complex has had profound impacts on the entire commercial US citrus industry (Hall et al. 2012), with *D. citri*-vectored CLas leading to > US$4 billion in economic losses in Florida alone (Farnsworth et al. 2014). Chemical and biological pest control as well as removal of CLas-infected trees are employed to control *D. citri* populations and reduce the rate of CLas spread in California (Grafton-Cardwell et al. 2015, Hornbaker and Kumagai 2016, Kistner et al. 2016a).

California's residential landscape provides an ideal habitat for *D. citri* to proliferate, as backyard citrus is largely devoid of insecticide treatments. Additionally, *D. citri* can feed and develop on many *Citrus* (Sapindales: Rutaceae) cultivars and the related curry leaf plant, *Murraya koenigii* (L.) Sprengel (Rutaceae), all of which are widely planted in the region (Kistner et al. 2016a). *D. citri* population growth is limited by the availability of new leaf (i.e. flush) growth on host trees (Hall et al. 2008). However, Kistner et al. (2016a) found that frequently flushing limes, lemons, and curry leaf plants can host urban *D. citri* populations year round in southern California.

Given that ∼36% of southern Californian residences have citrus (Hoddle and Pandey 2014) and CLas-infected trees have only been detected in urban areas (Kumagai et al. 2013, Hornbaker and Kumagai 2016), there is a serious threat of CLas transmission into commercial citrus production areas by *D. citri* migrating from unmanaged residential landscapes (Grafton-Cardwell et al. 2015). This movement increases the risk of CLas acquisition and spread which subsequently leads to HLB epidemics (Bassanezi et al. 2013). Therefore, vector management is a critical component of HLB prevention (Pelz-Stelinski et al. 2010, Bassanezi et al. 2013, Lewis-Rosenblum et al. 2015).

In an attempt to reduce *D. citri* densities in urban areas, classical biological control with host-specific parasitoids is being utilized (Hoddle and Pandey 2014, Bistline-East et al. 2015). In December 2011, releases of *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a host-specific parasitoid of *D. citri* sourced from Punjab Pakistan, began as part of a statewide classical biological control program with the intent of suppressing urban *D. citri* population growth and spread (Hoddle and Pandey 2014). As of December 2015, the California Department of Food and Agriculture had released ∼3,000,000 parasitoids at >4,000 sites across southern California (Rick A. Dunn, Citrus Research Board, unpublished data). *T. radiata* is an ectoparasitoid that can also kill *D. citri* nymphs through host feeding (Chien et al. 1995, Gómez-Torres et al. 2012). This widely used *D. citri* biological control agent has reportedly provided good levels of biological control in Réunion Island (Ètienne and Aubert 1980), Puerto Rico (Pluke et al. 2005a), India (Husain and Nath 1927), Taiwan (Chien and Chu 1996), and Guadeloupe (Ètienne et al. 2001). However, the impact of *T. radiata* on *D. citri* in Florida has not been sufficient to suppress *D. citri* populations to acceptable levels (Michaud 2004, Qureshi and Stansly 2009, Hall et al. 2012). In California, *T. radiata* parasitism rates are highly variable and the overall impact of this parasitoid on urban *D. citri* population growth and spread is uncertain at this early stage of the classical biological control program (Kistner et al. 2016a).

Naturally occurring enemies, especially generalist predators, provide varying degrees of *D. citri* control throughout the psyllid's native (Khan et al. 2014) and invaded ranges (Hall et al. 2012). There is general consensus that lady beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae), hover flies (Diptera: Syrphidae), and spiders (Araneae) are major predators of *D. citri* (Halbert and Manjunath 2004, Hall et al. 2012, Grafton-Cardwell et al. 2013). However, the magnitude of *D. citri* reduction provided by each group remains unclear. Lady beetles are the most abundant *D. citri* predators in Florida (Michaud 2004, Qureshi and Stansly 2009, Chong et al. 2010), Puerto Rico (Pluke et al. 2005b), Colombia (Kondo et al. 2015), and India (Batra et al. 1990). In California, predation by generalist predators has been shown to reduce immature *D. citri* densities, with green lacewing larvae consuming large numbers of eggs and nymphs (Goldmann and Stouthamer 2015). In contrast, there is little evidence that generalist predators limit *D. citri* populations in Iran (Rakhshani and Saeedifar 2013) or Pakistan (Vetter et al. 2013, Khan et al. 2014). In some situations, biological interactions, such as the development of mutualisms between *D. citri* and ants, may enhance *D. citri* survival in citrus ecosystems (Navarrete et al. 2013, Tena et al. 2013). Ants often protect honeydew-excreting hemipterans, like *D. citri*, from natural enemies in exchange for food, and these mutualistic interactions can have a negative impact on biological control (Powell and Silverman 2010). Navarrete et al. (2013) found that ants significantly reduced the efficacy of *T. radiata* in Florida citrus groves. In southern California, the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), inhabits >90% of urban properties where they readily tend urban *D. citri* colonies (Tena et al. 2013). Argentine ants are capable of reducing *T. radiata* parasitism rates by up to 80% (Tena et al. 2013) and may also prevent generalist predators from accessing *D. citri* colonies (Kistner and Hoddle 2015).

In addition to biotic factors, abiotic conditions also play a crucial role in *D. citri* population dynamics. *D. citri* development is intrinsically linked to temperature (Fung and Chen 2006), with an estimated optimal temperature range of 25–28 °C (Liu and Tsai 2000). Under laboratory conditions, *D. citri* reared at 28 °C exhibit the highest survivorship, shortest developmental times, and highest net reproductive rates (Liu and Tsai 2000). Field studies in Florida (Tsai et al. 2002, Hall et al. 2008) consistently found temperature to be an important abiotic factor driving *D. citri* densities. Similarly, Kistner et al. (2016a) found positive correlations between temperature and urban *D. citri* densities across Riverside and Los Angeles County, CA. Therefore, examining seasonal changes in *D. citri* survival and abundance under field conditions is important for developing effective psyllid management programs in California. To better understand the contribution of abiotic and biotic factors on *D. citri* population dynamics in southern California, we developed life tables for 116 experimental *D. citri* cohorts deployed over 15 mo across three sites. Varying degrees of natural enemy exclusion treatments were used to isolate different sources of mortality and *D. citri* natural enemy abundance and diversity associated with experimental cohorts were assessed.

Materials and Methods

Study Sites

Experimental *D. citri* cohorts were deployed at two residential sites, Lochmoor (33° 95′ N, 117° 31′ W, 420 m) and Jurupa (33° 99′ N, 117° 50′ W, 214 m), as well as an experimental citrus grove, the University of California—Riverside (UCR) Biocontrol grove (33° 58′ N, 117° 19′ W, 359 m) located in Riverside, County, CA. Both *D. citri* and *T. radiata* were established at these sites at the start of study. Mature *Citrus sinenis* (L.) Osbeck 'Valencia' trees were present at each site. No foliar or systemic insecticide applications were applied to sites throughout the duration of the study. Temperature and relative humidity data were recorded hourly at each site with HOBO Pro v2 data loggers placed in trees within 50 cm from experimental cohorts (1 logger per site: U23-001, Onset Computer Corporation, Bourne, MA). Precipitation data were obtained from weather stations within 2–6 km of each study site (The Weather Underground 2015). Climate data were used to prepare average summaries for temperature, humidity, and rainfall for the durations of all *D. citri* cohort experiments conducted across the three sites (Fig. 1).

Maintenance of *D. citri* Cohorts

D. citri used for the life table experiments were sourced from CLas-free colonies that were initiated from southern California-collected material and maintained in the Insectary and Quarantine Facility (IQF) at UCR in compliance with CDFA permit number 2870. *D. citri* were reared on potted *Citrus volkameriana* V. Ten. & Pasq plants (25 cm tall) which were obtained as rooted seedlings < 2 yr of age (Willits and Newcomb Inc. Arvin, CA). Seedlings were reared in greenhouses at UCR Agricultural Operations (see Bistline-East et al. 2015 for management practices). To initiate an experimental cohort, ∼15–20 colony-sourced *D. citri* adults were added to eight plants that were pruned to produce heavy flush growth and allowed to oviposit for 3–4 d. All ovipositing adults in IQF rearing rooms were maintained under constant conditions at 29°C, 40% RH, and a photoperiod of 14:10 (L:D) h. At the end of this period, adults were removed, and eggs were counted. To ensure accurate counting individual flush shoots were numbered and eggs per shoot were counted using an LED 10x hand lens (MG7810, ASC Scientific, Carlsbad, CA). Eggs per shoot were summed across all shoots to

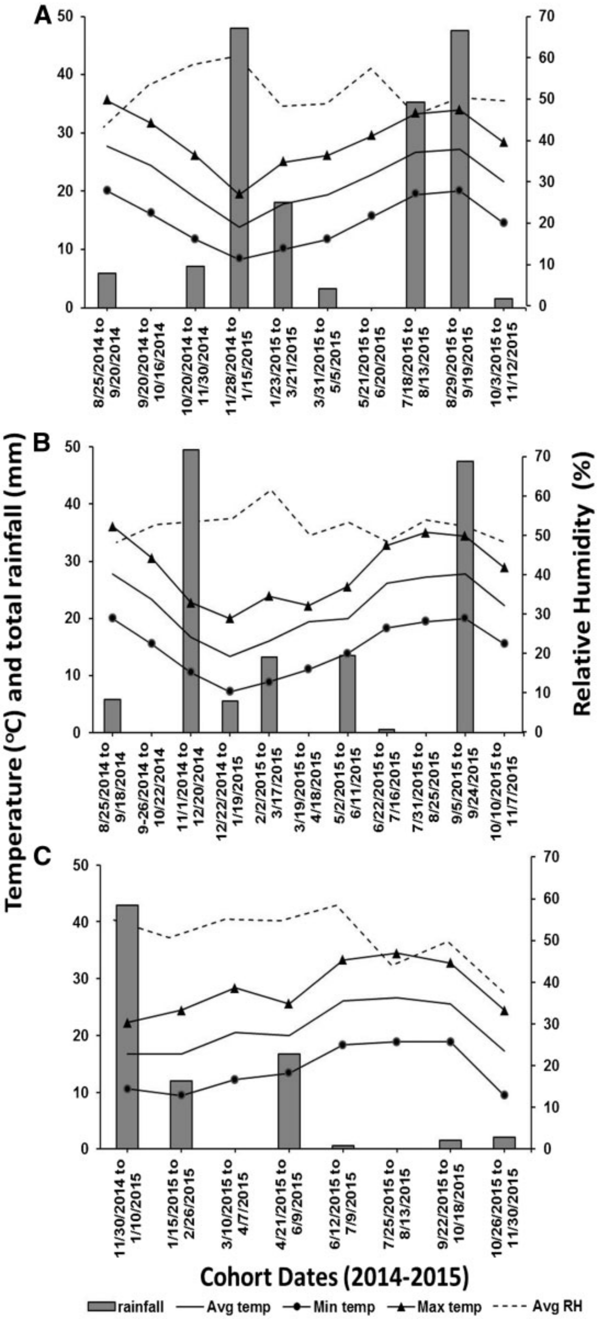

Fig. 1. Mean minimum, maximum, average temperatures, relative humidity (%), and total rainfall (mm) during cohort experiments initiated on different dates at (**A**) the UCR Biocontrol grove, (**B**) Lochmoor, and (**C**) Jurupa in Riverside County, CA.

determine the total egg load per plant. Since egg load per plant varied, excess eggs were removed either by using a fine-hair paintbrush or by removing egg infested flush growth until plants hosted ~200 (± 10%) eggs. Plants infested with newly hatched nymphs were excluded from the study. Four *C. volkameriana* plants containing ~200 eggs were then placed in four plastic cages and immediately moved from IQF to their respective experimental field site.

Cohort Establishment and Population Monitoring

Experimental cohorts were initiated monthly at all three sites from August 2014 to November 2015. *C. volkameriana* potted plants infested with experimental *D. citri* eggs were placed on four stands (i.e., plastic buckets: 33 (diameter) by 37 (height) cm) situated beneath the lower canopy of two Valencia orange trees at each site. Nearby tree foliage was pruned monthly to ensure that no citrus foliage touched the potted plants or experimental enclosures. Buckets were secured to the ground with steel tent pegs and plants were secured to the bucket using 25 cm long bungie cords. Four treatments were employed to assess natural enemy impact, especially generalist predators and parasitoids, on *D. citri* survival. We employed similar exclusion treatments utilized by Qureshi and Stansly (2009) in Florida. First, an enclosure (15 by 30 cm) made of fine mesh (95 μm^2 holes) organdy was placed over potted plants, which was supported using two 28 cm long sticks inserted into potting soil. This control treatment excluded all natural enemies thereby enabling us to determine *D. citri* survivorship rates in the absence of natural enemies (i.e., determine baseline mortality rates from natural causes and abiotic factors). Second, enclosures (15 by 30 cm) made of course mesh organdy (3 mm^2 holes) were applied to potted plants using the same procedure described above with the intent of excluding large predators while still allowing entry of small natural enemies like *T. radiata*. A third exclusion treatment consisted of a sticky barrier (Tangle foot insect barrier, Contech Enterprises Inc., Victoria, Canada) applied liberally around the entire circumference of plant pots to prevent access to *D. citri* cohorts by walking arthropods, including Argentine ants. A sticky barrier was also applied to pots with plants enclosed by course mesh. Fourth, potted plants with no mesh or sticky barriers were fully exposed to allow free access to *D. citri* life stages by all natural enemies and ant mutualists. This uncaged treatment enabled us to determine total biotic mortality from both *T. radiata* parasitism and predation from generalist predators. Plants were examined every other day using a 10x hand lens and numbers of *D. citri* by life stage (eggs, first-third instars, fourth-fifth instars, and adults) were recorded per treatment. Any additional *D. citri* eggs were removed following each observation and potted plants were thoroughly watered as needed. Counts continued until all immature *D. citri* in experimental cohorts had died from unknown causes, disappeared, been parasitized or consumed, or emerged as adults. Adult *D. citri* emerging into enclosures as well as fifth-instar nymphal exuviae left on twigs/leaves were counted and removed at each inspection. Fifth-instar nymphal exuviae were used to estimate adult survival in uncaged treatments. Comparisons between mean adult and fifth-instar nymphal exuviae counts in fully enclosed treatments found a 3% difference in estimated adult numbers, indicating a 97% nymphal exuviae recovery rate. Nymphal exuviae counts were an effective method for estimating adult emergence in uncaged treatments. Additionally, arthropods associating with immature *D. citri* cohorts were assessed every other day by searching each experimental potted plant's foliage for one minute. Arthropod surveys on experimental plants were conducted between 0800 and 1300. Predators, parasitoids, and ants observed on *D. citri* colonies during one min surveys or trapped in sticky barriers were counted and removed from the latter. Any unknown arthropods or those trapped in the sticky barrier were preserved in 95% ethanol in labeled vials and identified. Parasitism by *T. radiata* was assessed by counting *D. citri* mummies. Mummies are easily identified by their dark brown coloration, silken threads securing mummy to plant surface, and an exit hole in the thorax region created by emerged adult *T. radiata* (Chen and Stansly 2014). Predation of nymphs by hover fly and green lacewing larvae was often directly observed and could be assessed by counting partially consumed cadavers (E.J.K., personal observation). A total of 116 experimental cohorts ($n = 29$ per treatment) were monitored over the course of the study and assigned to different seasons based on their start and end states (Suppl. Table 1 [online only]). Seasonal experimental cohorts were defined as follows: 1) Winter cohorts were deployed and monitored between 21 December 2014 to 18 March 2015, 2) Spring cohorts were deployed and monitored between 19 March 2015 to 20 June 2015, 3) Summer cohorts were deployed and monitored between 25 August to 20 September 2014 and 21 June 2015 to 22 September 2015, 4) Fall cohorts were deployed and monitored between 23 September to November 30 2014 and 23 September to 21 December 2015. Eight cohorts at Jurupa initiated in September and October 2014 and four spring cohorts at the UCR Biocontrol grove initiated in April 2015 were excluded from analyses due to the failure of exclusion treatments to keep natural enemies out.

Data Analysis

Demographic Growth Parameters

Egg to adult survivorship rates were calculated for each cohort (i.e., number of emerged adults/initial number of eggs used to generate that cohort) and averaged across seasons from 2014–2015. Life tables were constructed for each individual *D. citri* cohort per treatment. Development and survivorship data were used to determine the l_x (number of original cohort entering each life stage) and d_x (number of original cohort dying at each life stage), which were summed across all experimental cohorts within a season at each site. These summed parameters were then used to calculate q_x (mortality rate of each life stage) and mean d (duration of each life stage in days) per season at each of the three study sites (Suppl. Tables 2–4 [online only]). Assuming a constant 1:1 sex ratio, the net reproductive rate (R_o) was calculated as a product of the fraction of females surviving to adulthood (Carey 1993) and fecundity (Bellows and Driesche 1999) at the appropriate temperature range as reported by Liu and Tsai (2000). Mean generation time (T_c) was estimated as the sum of egg incubation time (Liu and Tsai 2000), nymphal developmental time through 50% adult emergence as observed in each cohort (Qureshi and Stansly 2009), and the prereproductive period (Wenninger and Hall 2007). The intrinsic rate of natural increase (r_m) and population doubling time (T_d) were also calculated for each cohort (Carey 1993, Bellows and Driesche 1999).

Marginal Probabilities of Mortality

To isolate the magnitude of different contemporaneous mortality sources (i.e., unknown mortality, predation, parasitism, and disappearance), marginal rates of mortality were calculated. The marginal rate of mortality is the number of individuals that would die from a factor in the absence of all other contemporaneous mortality agents (Elkinton et al. 1992), which provides an estimate of mortality rates from competing mortality factors. For instance, intraguild predation of parasitized *D. citri* nymphs by generalist predators may obscure contemporaneous mortality from parasitism (Michaud 2004). The marginal probability of mortality from Elkinton et al. (1992) was calculated as:

$$m_i = 1 - (1 - d)^{d_i/d}$$

where m_i is the marginal probability of mortality from the *i*th cause, d_i is the death rate from the *i*th cause, and d is the death rate from all causes combined. The marginal probabilities for each mortality factor by life stage for each experimental cohort treatment were calculated.

Statistical Analysis

The three sites were treated as replicates given that no significant between site differences were detected when comparing mean annual R_o (H = 2.89; df = 2; $P = 0.24$), T_c (H = 6.25; df = 2; $P = 0.10$), r_m (H = 1.74; df = 2; $P = 0.42$), and T_d (H = 0.81; df = 2; $P = 0.67$) life table parameters generated from experimental cohorts. Prior to statistical analyses, all life table parameters and marginal attack rates were subjected to the jackknife procedure using the bootstrap package in R 3.2.0 (Efron and Tibshirani 1993); a commonly employed method in population growth statistics to test the precision of a parameter of interest. The jackknife method omits one observation at a time from the original data set and recalculates the statistic of interest (Meyer et al. 1986). All life table parameters were analyzed using the nonparametric Kruskal–Wallis test followed by the Mann–Witney U-test to determine pairwise treatment and seasonal comparisons. The Kruskal–Wallis test was also employed to determine differences in marginal attacks rates for each mortality factor per life stage within each season. All Kruskal–Wallis and Mann–Witney U-tests were performed using the statistics package in R.3.2.0 (R Development Core Team 2015).

Results

Experimental Cohort Survival

Regardless of season, *D. citri* eggs in "no exclusion" cohorts ($n = 29$) consistently exhibited low adult recruitment, averaging a 2.6% survival rate to adulthood (range: 0 to 23.7%) over the course of this study (Fig. 2). Year round egg survival to first–third instars was greatly enhanced by exclusion treatments with an average annual reduction of only 25.8% compared to the 63.8% reduction in egg survival observed in the "no exclusion" treatment. First–third instars exhibited lower survival rates in natural enemy accessible "walking arthropods excluded" and "no exclusion" treatments (Fig. 2). "Full exclusion" treatments greatly enhanced immature *D. citri* survival with adult recruitment averaging 57.8% (range: 2.8 to 89.8%) for these completely protected cohorts (Fig. 2). However, this natural enemy protection was far less beneficial for *D. citri* in winter (Fig. 3A). In winter, inclement weather resulted in only 20% of "full exclusion" *D. citri* eggs reaching adulthood (Fig. 3A) while 63% of "full exclusion" *D. citri* eggs set up in spring months (Fig. 3B) survived to adulthood. Overall, immature *D. citri* survival to adulthood peaked in the summer across all three exclusion treatments (Fig. 3C). As summer transitioned into fall, survival of "full exclusion" *D. citri* cohorts decreased by 12.8% (Fig 3C-D) as average daily temperatures began to decline (Fig. 1). *T. radiata* decreased survival of immature *D. citri* in "*T. radiata* access" cohorts by an additional 47.9% when compared to "full exclusion" cohorts (Fig. 2). Increased *D. citri* mortality by *T. radiata* was due to a combination of both parasitism and host feeding. Survival of "*T. radiata* access" cohorts from egg to adult averaged 30.2% (range: 0 to 79.1%), with *T. radiata* induced mortality peaking in spring (Fig. 3B) and fall (Fig. 3D). Survival of "walking arthropods excluded" immature *D. citri* was low, averaging 16.5% (range: 0 to 77.1%) over the course of the study (Fig. 2).

Field Demography

Exclusion treatments protecting immature *D. citri* cohorts resulted in increased net reproductive rates (R_o) and intrinsic rates of natural increase (r_m), and reduced population doubling times (T_d) (Table 1). The annual mean net reproductive rate, Ro, in "full exclusion" *D. citri* cohorts was 20-fold higher than unprotected "no exclusion" *D. citri* cohorts. (H = 38.26; df = 3; $P < 0.001$; Table 1). Likewise, the intrinsic rate of natural increase, r_m, was significantly lower, being reduced by 66.7 to 81.3% as *D. citri* cohorts were increasingly exposed to natural enemies (H = 39.14; df = 3; $P < 0.001$; Table 1). Population doubling time, Td, was significantly longer for "no

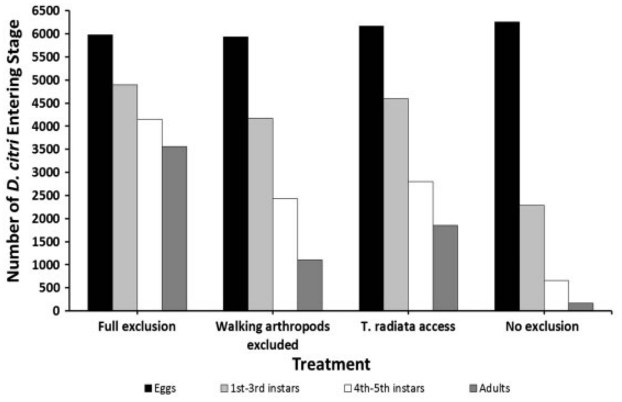

Fig. 2. Total numbers of experimental *D. citri* entering each life stage summed across: (A): Full exclusion, (B) *T. radiata* access, (C) Walking arthropods excluded, and (D) No exclusion treatments ($n = 29$ per treatment). *D. citri* cohort numbers counts were summed across the three sites in Riverside County, CA, beginning in August 2014 and ending in November 2015.

exclusion" *D. citri* cohorts (H = 9.74; df = 3; $P = 0.02$) when compared to cohorts benefiting from some level of exclusion (Table 1). *D. citri* cohorts assigned partial exclusion treatments, "*T. radiata* access" and "walking arthropods excluded," generally exhibited survival rates and demographic values that were intermediate to full and no exclusion cohorts (Table 1; Fig. 2). The magnitude of effects between exclusion treatments varied across seasons (Table 2). "No exclusion" *D. citri* cohorts had significantly lower net reproductive rates compared to the two partial exclusion treatments in spring, summer, and fall (Table 2). In winter, the partial and no exclusion treatments exhibited no difference in their net reproductive rates or intrinsic rate of increase (Table 2). Net reproductive rates (R_o) of "full exclusion" cohorts averaged 171.40 and ranged from a low of 1.4 in January 2015 to a high of 301.9 in July 2014. Generation time (T_c) ranged from a low of 22.1 days in September 2015 to a high of 52.9 days in January 2015 and did not vary across treatments (H = 2.30; df = 3; $P = 0.316$; Table 1) indicating that higher summer temperatures in the "full exclusion" treatment had minor effects on *D. citri* developmental time.

Marginal Probabilities of Mortality

Treatment effects on marginal rates of mortality varied across *D. citri* life stages and seasons (Table 3). In "no exclusion" cohorts, the marginal rate of mortality for disappearance in eggs consistently comprised the greatest proportion of overall mortality across all life stages and seasons.

Winter

Over winter, *D. citri* egg and first–third instar mortality was high across all treatments because of prolonged exposure to cold temperatures and occasional rainfall events (Fig. 1, Table 3). The marginal probability of mortality for predation was significantly higher for first–third instars in the "walking arthropods excluded" treatments (H = 9.81; df = 3; $P = 0.02$) and fourth–fifth instars in both the "walking arthropods excluded" and "no exclusion" treatments (H = 13.95; df = 3; $P < 0.01$; Table 3). No mortality from parasitism was detected during the winter months (Table 3).

Spring

Marginal probability of mortality for egg disappearance was significantly higher in the "no exclusion" cohorts compared to cohorts receiving some form of arthropod exclusion treatment (H = 13.15;

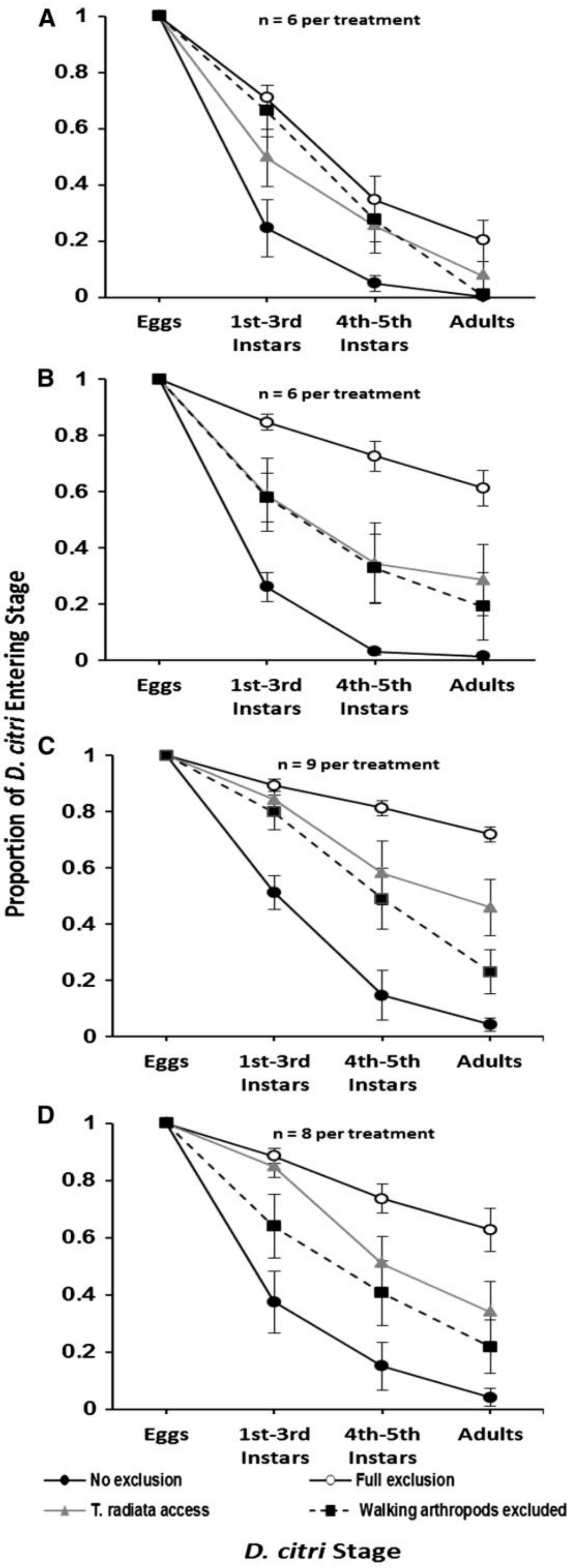

Fig. 3. Mean survivorship curves (± SE) of *D. citri* life stages in experimental cohorts that isolated different mortality factors across: (**A**) Winter, (**B**) Spring, (**C**) Summer, and (**D**) Fall. Experimental cohort population counts from three sites in Riverside County, CA, were averaged across the four seasons beginning in August 2014 and ending in November 2015.

Table 1. Mean ± SE jackknifed life table parameters of experimental *D. citri* cohorts that were fully exposed (No exclusion), or protected with full cages (Full exclusion), partial cages (*T. radiata* access), and sticky barriers (Walking arthropods excluded) over 2014–2015

Treatment	Net reproduction rate (R_o)	Generation time (T_c)	Intrinsic rate of increase (r_m)	Doubling time (T_d)
No exclusion	8.20 ± 2.66a	27.14 ± 1.57a	0.03 ± 0.01a	17.12 ± 6.51a
Full exclusion	171.30 ± 20.04b	33.88 ± 1.75a	0.16 ± 0.01b	5.63 ± 0.49b
T. radiata access	76.64 ± 17.59c	32.81 ± 2.1a	0.11 ± 0.02bc	9.14 ± 3.00b
Walking arthropods excluded	45.35 ± 12.23c	27.87 ± 1.60a	0.09 ± 0.01c	12.49 ± 4.67ab

Data were pooled across three experimental sites ($n = 29$ for each treatment) in Riverside California. Life table parameters differences between treatments were analyzed using Kruskal–Wallis test followed by a Mann–Whitney U test. Means within a column followed by a different letter are significantly different ($P \leq 0.05$; Bonferroni Correction).

Table 2. Mean ± SE jackknifed seasonal differences in life table parameters of experimental *D. citri* cohorts that were fully exposed (No exclusion), or protected with full cages (Full exclusion), partial cages (*T. radiata* access), and sticky barriers (Walking arthropods excluded) across three sites in Riverside County, CA (2014–2015)

Season	Treatment	Net reproduction Rate (R_o)	Generation time (T_c)	Intrinsic rate of increase (r_m)	Doubling time (T_d)
Winter[a]	Full exclusion	25.29 ± 4.92a	51.25 ± 2.42a	0.05 ± 0.01a	9.20 ± 0.95a
	No exclusion	1.09 ± 0.64b	51.86 ± 0.69a	0.007 ± 0.004b	29.80 ± 0.76b
	T. radiata access	1.63 ± 0.71b	50.04 ± 1.97a	0.02 ± 0.01b	30.13 ± 2.88b
	Walking arthropods excluded	1.49 ± 0.82b	49.63 ± 3.38a	0.01 ± 0.007b	15.37 ± 0.12c
Spring[b]	Full exclusion	194.01 ± 19.97a	37.35 ± 2.44a	0.13 ± 0.007a	4.71 ± 0.15a
	No exclusion	2.61 ± 1.71b	31.91 ± 3.21a	0.03 ± 0.01b	11.47 ± 2.33b
	T. radiata access	80.01 ± 33.06c	33.97 ± 0.37a	0.09 ± 0.03c	4.85 ± 0.34a
	Walking arthropods excluded	60.94 ± 36.30c	34.87 ± 1.03a	0.08 ± 0.002c	5.81 ± 0.72a
Summer[c]	Full exclusion	240.44 ± 17.33a	25.66 ± 0.51a	0.18 ± 0.02a	3.17 ± 0.11a
	No exclusion	13.13 ± 7.66b	23.81 ± 0.87a	0.09 ± 0.02b	5.26 ± 0.66b
	T. radiata access	141.87 ± 26.24c	24.14 ± 0.34a	0.17 ± 0.03ac	3.82 ± 0.53a
	Walking arthropods excluded	75.17 ± 29.75d	22.16 ± 1.15a	0.14 ± 0.02bc	4.32 ± 0.92b
Fall[d]	Full exclusion	166.74 ± 26.22a	30.11 ± 2.52a	0.21 ± 0.004a	4.22 ± 1.40a
	No exclusion	10.84 ± 7.11b	25.98 ± 1.34a	0.04 ± 0.01b	7.08 ± 1.34a
	T. radiata access	61.23 ± 8.89c	32.03 ± 2.48a	0.12 ± 0.02c	7.45 ± 1.72a
	Walking arthropods excluded	54.06 ± 17.86c	27.50 ± 1.20a	0.08 ± 0.03c	8.08 ± 2.47a

Within seasonal life table parameters differences between treatments analyzed using Kruskal–Wallis test followed by a Mann–Whitney U test. Means within a column followed by a different letter are significantly different ($P \leq 0.05$; Bonferroni Correction).

[a] Data were pooled across sites ($n = 6$ for each treatment).

[b] Data were pooled across sites ($n = 6$ for each treatment).

[c] Data were pooled across sites ($n = 9$ for each treatment).

[d] Data were pooled across sites ($n = 8$ for each treatment).

df = 3; $P < 0.01$; Table 3). Marginal rates of mortality from unknown causes were significantly higher for first–third instars in "*T. radiata* access" treatments compared to all other treatments (H = 8.34; df = 3; $P = 0.04$; Table 3). The marginal probability of mortality for predation was significantly higher for first–third instars in the "walking arthropods excluded" and "no exclusion" treatments (H = 17.26; df = 3; $P < 0.001$; Table 3). Marginal rates of mortality from parasitism were very low and no differences were observed between treatments (H = 3.1; df = 3; $P = 0.39$; Table 3).

Summer

Marginal rates of mortality from disappearance were significantly higher for eggs and first–third instars in the "walking arthropods excluded" and "no exclusion" treatments (H = 8.2; df = 3; $P = 0.04$; H = 9.14; df = 3; $P = 0.03$; respectively; Table 3). Marginal rates of mortality from unknown causes were 2- to 3-fold higher for first–third instars in the "*T. radiata* access" treatment compared to all other treatments (H = 15.82; df = 3; $P = 0.001$; Table 3). The marginal rate of mortality from predation was significantly higher for first–third instars in the "walking arthropods excluded" and "no exclusion" treatments (H = 13.72; df = 3; $P = 0.003$), with rates comparable to those in the spring cohorts (Table 3). The marginal rate of mortality from parasitism was significantly higher in the "walking arthropods excluded" treatment (H = 18.17; df = 3; $P < 0.001$), comprising ~16% of the total *D. citri* marginal rate of mortality for the "walking arthropods excluded" cohorts. Parasitism rates in the other three treatments were very low and no significant differences were observed between them (Table 3).

Fall

The marginal rate of mortality from egg disappearance was 4- to 7-fold higher in the "walking arthropods excluded" and "no exclusion" treatments (H = 9.62; df = 3; $P = 0.02$; Table 3). Like the previous spring and summer cohorts, the marginal rate of mortality from unknown causes was significantly higher for first–third instars in the "*T. radiata* access" treatment compared to all other treatments (H = 11.38; df = 3; $P = 0.01$; Table 3). Marginal rates of mortality from predation were significantly higher for first–third instars and fourth–fifth instars in the "no exclusion" treatment compared to all other treatments (H = 26.21; df = 3; $P < 0.001$; H = 17.40; df = 3; $P < 0.001$; respectively; Table 3). Marginal rates of mortality from parasitism were significantly higher for fourth–fifth instars in

Table 3. Number entering and dying by life stage, and mean ± SE jackknifed seasonal differences in marginal probability of mortality rates of experimental *D. citri* cohorts across three sites in Riverside County, CA (2014–2015)

Season	Life Stage	Number entering stage l_x				Mortality factor	Number dying in stage d_x				Marginal probability of mortality ± SE			
		T_1	T_2	T_3	T_4		T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Winter[a]	Eggs	1,249	1,323	1,336	1,309	Unknown	211	87	198	121	0.25±0.06a	0.09±0.4b	0.17±0.04ab	0.11±0.03ab
						Disappear	316	234	431	876	0.31±0.09ab	0.18±0.04a	0.36±0.10ab	0.67±0.1b
	1st–3rd instars	722	1,002	707	312	Unknown	143	244	235	71	0.14±0.02a	0.18±0.06a	0.26±0.1a	0.06±0.03a
						Disappear	164	192	131	103	0.15±0.04a	0.15±0.04a	0.12±0.05a	0.09 ±0.03a
						Predation	0	201	0	56	0a	0.20±0.04b	0a	0.05±0.03a
	4th–5th instars	415	365	341	65	Unknown	87	88	161	12	0.11±0.03a	0.07±0.03a	0.21±0.08a	0.08±0.04a
						Disappear	92	137	85	21	0.13±0.06a	0.1±0.04a	0.08±0.02a	0.05±0.01a
						Predation	0	133	0	30	0a	0.17±0.08b	0.0a	0.1±0.05b
						Parasitism	0	0	0	0	0	0	0	0
	Adults	236	7	95	2									
Spring[b]	Eggs	1,300	1,298	1,321	1,378	Unknown	111	240	155	256	0.11±0.03a	0.23±0.05a	0.15±0.04a	0.22±0.04a
						Disappear	82	306	259	778	0.10±0.03a	0.26±0.07a	0.23±0.08a	0.64±0.06b
	1st–3rd instars	1,107	752	907	344	Unknown	112	93	246	50	0.11±0.03a	0.10±0.04a	0.22±0.04b	0.06±0.02a
						Disappear	35	49	109	118	0.04±0.01a	0.06±0.02a	0.12±0.06a	0.10±0.02a
						Predation	0	170	0	131	0a	0.19±0.07b	0a	0.15±0.04b
	4th–5th instars	960	440	552	45	Unknown	114	40	134	11	0.13±0.03a	0.04±0.01b	0.14±0.05a	0.04±0.01b
						Disappear	27	26	73	13	0.02±0.00a	0.06±0.02a	0.08±0.03a	0.03±0.01a
						Predation	0	88	0	9	0a	0.08±0.04a	0a	0.009±0.0a
						Parasitism	0	25	0	0	0a	0.03±0.01a	0a	0a
	Adults	819	261	345	12									
Summer[c]	Eggs	1,813	1,845	2,001	2,032	Unknown	47	56	107	119	0.03±0.01a	0.06±0.02a	0.11±0.05a	0.08±0.04a
						Disappear	130	322	188	853	0.08±0.02a	0.20±0.06b	0.14±0.06a	0.46±0.13b
	1st–3rd instars	1,636	1,467	1,706	1,060	Unknown	100	71	516	147	0.05±0.01a	0.08±0.02a	0.23±0.05b	0.12±0.04a
						Disappear	44	156	59	307	0.02±0.00a	0.12±0.03b	0.04±0.2a	0.20±0.04b
						Predation	0	232	0	292	0a	0.15±0.06b	0a	0.18±0.08b
	4th–5th instars	1,492	1,008	1,131	314	Unknown	68	104	105	107	0.04±0.01a	0.07±0.03a	0.06±0.02a	0.07±0.03a
						Disappear	8	54	20	22	0.004±0.0a	0.04±0.02a	0.02±0.0a	0.02±0.01a
						Predation	0	105	0	32	0a	0.08±0.04a	0a	0.03±0.01a
						Parasitism	0	248	109	61	0a	0.16±0.04b	0.06±0.03ab	0.05±0.02ab
	Adults	1,416	497	897	92									
Fall[d]	Eggs	1,613	1,471	1,506	1,537	Unknown	100	96	102	109	0.08±0.03a	0.08±0.04a	0.09±0.02a	0.08±0.02a
						Disappear	83	427	127	855	0.07±0.2a	0.38±0.12b	0.09 ±0.03a	0.60±0.13b
	1st–3rd instars	1,430	948	1,277	573	Unknown	122	120	420	136	0.1±0.04a	0.15±0.06ab	0.35±0.05c	0.13±0.04ab
						Disappear	20	99	85	155	0.02±0.01a	0.07±0.01b	0.08±0.02b	0.13±0.03b
						Predation	0	115	0	46	0a	0.08±0.03b	0a	0.03±0.01a
	4th–5th instars	1,288	614	772	236	Unknown	158	109	64	33	0.12±0.05a	0.12±0.05a	0.08±0.04a	0.05±0.02a
						Disappear	42	40	17	23	0.03±0.01a	0.05±0.02a	0.02±0.01a	0.06±0.03a
						Predation	0	0	0	114	0a	0a	0a	0.08±0.03b
						Parasitism	0	105	159	0	0a	0.13±06b	0.21±0.08b	0a
	Adults	1,088	340	518	66									

T1, full exclusion; T2, walking arthropods excluded; T3, *T. radiata* access; T4, no exclusion. Within-season mortality differences between treatments per life stage analyzed using Kruskal–Wallis test followed by a Mann–Whitney U test. Means within a row followed by a different letter are significantly different ($P \leq 0.05$; Bonferroni Correction). Disappear = unexplained disappearance. Unknown = undetermined source of mortality.

[a] Data were pooled across sites ($n = 6$ for each treatment).

[b] Data were pooled across sites ($n = 6$ for each treatment).

[c] Data were pooled across sites ($n = 9$ for each treatment).

[d] Data were pooled across sites ($n = 8$ for each treatment).

the "walking arthropods excluded" and "*T. radiata* access" treatments compared to the complete absence of *T. radiata* activity observed in other two treatments (H = 11.45; df = 3; *P* = 0 .001; Table 3).

Biological Interactions

Insect predators in the families, Anthocoridae, Chrysopidae, Coccinellidae, Formicidae, Forficulidae, Syrphidae, and Thripidae, and spiders and predatory mites were observed on *D. citri* patches or trapped in sticky barriers (Fig. 4). Arthropod abundance peaked in March through June 2015. The brown garden snail, *Cantareus aspersus* (Muller) was occasionally observed consuming flush infested with *D. citri* eggs and nymphs after rain fall events; these cohorts were excluded from analyses as snails traversed sticky barriers.

Ants (Formicidae) were consistently the most abundant arthropod group interacting with *D. citri* colonies and were found trapped in sticky barriers across all sites. Argentine ants comprised > 98% of all ant observations over the course of the study. Dark rover ants, *Brachymyrmex patagonicus* Mayr, were the only other ant species observed tending *D. citri* and were only found at the UCR Biocontrol grove in October 2015. While Argentine ants were observed year round, their abundance peaked from March to June 2015 at all three sites and declined from November 2014 to February 2015. Ant activity was also high from August to October 2014 and October to November 2015 at the UCR Biocontrol grove (Fig. 4A) and from August to October 2014 at Lochmoor (Fig 4B).

Two species of hover fly larvae (Syrphidae), *Allograpta obliqua* (Say) and *A. exotica* (Wiedemann), were the most abundant *D. citri* predators across all three sites. Hover fly larvae abundance peaked

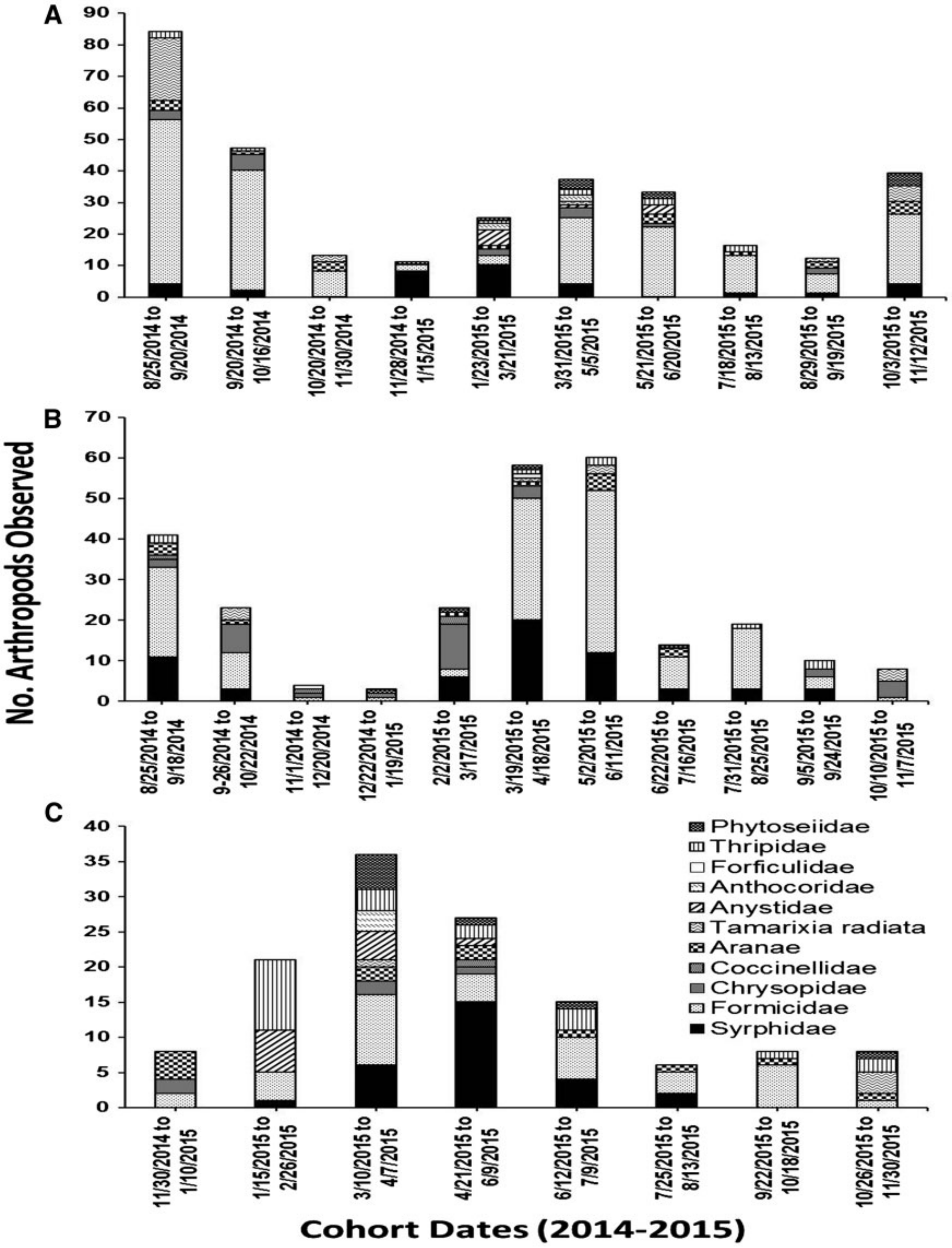

Fig. 4. Total number of arthropods observed on colonies of immature *D. citri* situated on potted *C. volkameriana* shoots or trapped in the sticky barriers used to exclude arthropods initiated on different dates at: (**A**) the UCR Biocontrol grove (*n* = 20), (**B**) Lochmoor (*n* = 22), and (**C**) Jurupa (*n* = 16) in Riverside County, CA.

from March to June 2015, but were observed year round at the three study sites. Green lacewing larvae (Chrysopidae), comprised entirely of the species *Chrysoperla comanache* (Banks), were the second most abundant predator of *D. citri* nymphs. Hover fly and lacewing larvae were commonly seen consuming *D. citri* nymphs and comprised ~86% of all documented immature *D. citri* mortality from predation (Table 3).

Tamarixia radiata was the only parasitoid observed parasitizing *D. citri* nymphs over the course of the study and was the third most frequently observed natural enemy attacking *D. citri*. The parasitoid was completely absent from December 2014 to February 2015, but was commonly found on *D. citri* cohorts in March through May 2015 and August through November 2014 and 2015 (Fig 4). *T. radiata* was rarely seen at Jurupa (Fig. 4C). From July to August 2014 and June to August 2015, 16.5% of all surviving fourth–fifth instars assigned to either the "*T. radiata* access," "walking arthropods excluded," or "no exclusion" treatments were parasitized with percentage parasitism ranging from 0 to 83%. Parasitism in "no exclusion" cohorts peaked in summer with 19.1% of surviving fourth–fifth instars dying from parasitism (Table 3). Similarly, 16.3% of all surviving fourth–fifth instars in the partial and no exclusion fall cohorts were parasitized with percentage parasitism ranging from 0 to 78%. No parasitism was observed in the "no exclusion" cohorts from September to November in 2014 and 2015 (Table 3). Spiders, thrips (Thripidae), and predatory mites in families Aystidae and Phytoseiidae were observed less frequently than hover flies, green lacewings, and *T. radiata*. Ghost spiders (Anyphaenidae), jumping spiders (Salticidae), and yellow sac spiders (Eutichuridae) were common at the three study sites (Fig. 4). The large predatory mite, *Anysitis agilis* (Banks), was occasionally observed carrying away small *D. citri* nymphs from fully exposed cohorts set out at the UCR Biocontrol grove (Fig. 4A) and Jurupa (Fig. 4C). Adult and juvenile lady beetles composed of the species *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, and *Cycloneda* spp., were uncommon throughout the study, but were recovered from all three sites. Minute pirate bugs (Anthocoridae) were only found at the UCR Biocontrol grove (Fig. 4A) and Jurupa (Fig. 4C) while earwigs (Forficulidae) were only observed at Lochmoor (Fig. 4B).

Discussion

Our experimental results provide compelling evidence that natural enemies are inflicting impressive levels of mortality on immature stages of *D. citri* in southern California. "Full exclusion" treatments significantly enhanced *D. citri* survival, net reproductive rate (R_o), intrinsic rate of increase (r_m), and reduced population doubling time (T_d). Annual mean net reproductive rate was reduced by over 20-fold when immature *D. citri* were exposed to all natural enemies (i.e. no exclusion) compared to "full exclusion" cohorts that were fully caged and natural enemies were excluded access to eggs and nymphs. Low survival and reduced net reproductive rates in partial and no exclusion cohorts were due to a combination of natural enemy impacts, predation by generalist predators as well as parasitism and host feeding by *T. radiata*. Our results are consistent with the findings of similar *D. citri* predator exclusion studies conducted in Florida (Michaud 2004, Qureshi and Stansly 2009) and emphasize the importance of conserving natural enemies of *D. citri*.

The impact of seasonal variation on *D. citri* survival, developmental time, and net reproductive rates were most influential from December 2014 to February 2015 when the average minimum temperatures were below 10 °C across all three sites. Consequently, these prevailing cold temperatures and coinciding rainfall events reduced immature *D. citri* survival to adulthood in "full exclusion" cohorts by 80% and doubled the length of generation times when compared to summer cohorts. This outcome is unsurprising given that the minimum temperature development thresholds for *D. citri* were estimated at 10–13 °C (Liu and Tsai 2000, Fung and Chen 2006). In addition, average daily temperatures at this time ranged from 13.8 to 16.70 °C, well below *D. citri*'s estimated optimal developmental temperature range of 25–28 °C (Liu and Tsai 2000). Despite suboptimal temperatures for development, "full exclusion" winter *D. citri* cohorts were capable of minor population growth (i.e. net reproductive rates, $Ro, > 1$). However, since we deployed large numbers of *D. citri* eggs in the field during months when their numbers in southern California are naturally range from very low to completely absent in some regions (Kistner et al. 2016a), our *D. citri* demography data may be overestimating winter survival and reproductive rates. To determine whether naturally occurring *D. citri* populations can successful develop and reproduce year round at our study sites, monthly branch samples were taken from nearby (within 50 m) citrus trees at all three sites. *D. citri* eggs and nymphs were present on $< 10\%$ of the branches sampled while *D. citri* adults were present on 18% of all branches sampled from December 2015 to March 2016 (E.J.K., unpublished data). This finding suggests that *D. citri* can still reproduce and develop during southern California's winters at reduced capacities which supports previous phenology observations (Kistner et al. 2016a) and the results presented here.

From March through November, biotic factors strongly influenced *D. citri* population dynamics. Hover flies (*Allograpta* spp.) were consistently the most frequently observed *D. citri* predator on experimental cohorts. Over the course of these experiments, hover flies outnumbered the second most abundant predator, green lacewing larvae, by a ratio of 2:1. Hover fly larvae were frequently observed consuming *D. citri* nymphs especially in the spring when hover flies were most numerous. This observation was unexpected, as hover flies have not been previously reported as important *D. citri* predators (Michaud 2004, Qureshi and Stansly 2009, Chong et al. 2010, Khan et al. 2014). However, Catling (1970) noted that an *Allograpta* sp. was a major predator of the African citrus psyllid, *Trioza erytreae* (Del Guerico) and Michaud (2002) observed occasional attacks of *D. citri* nymphs in Florida by *A. oblique*, the most commonly observed *D. citri* predator at our field sites. Larvae of the green lacewing, *C. comanache*, were often seen consuming *D. citri* eggs and nymphs year round. These field observations support the results of *C. comanache* bioassays and gut content analyses targeting *D. citri* in organic citrus orchards in southern California (Goldmann and Stouthamer 2015). From March to June, hover fly and green lacewing larvae consumed up to 93% of *D. citri* nymphs in experimental cohorts to which these predators had access. *D. citri* nymphs hidden deep within flush growth or tucked in the fork between a branch and stem were typically the only individuals surviving to adulthood in uncaged, "no exclusion," cohorts.

Mortality from *T. radiata* parasitism was notable in the summer and fall, but negligible in spring. No evidence of *T. radiata* activity was detected from December 2014 to February 2015 when fourth–fifth instar *D. citri* nymphs, their preferred host life stage (Gómez-Torres et al. 2012), were almost completely absent at study sites. In experimental cohorts where natural enemies were either not excluded or partially excluded, only 2% of all surviving fourth–fifth instars were parasitized from March to May 2015. This low incidence of spring time parasitism may be due to reduced recruitment rates of fourth–fifth instars which coincided with increased abundance of generalist predators. Interference from Argentine ant may have also be reduced parasitism rates (Tena et al. 2013), as ants were actively observed tending experimental *D. citri* nymphs from March to November. From June to November (i.e. summer and fall seasons), only 16.4% of all fourth–fifth instars in the partial and no exclusion cohorts were parasitized, but total observed parasitism was as high as 24.6% in "walking arthropods excluded" cohorts deployed in the summer. The moderate rates of *T. radiata* parasitism found in our study are similar to those observed in Florida (Michaud 2004, Qureshi and Stansly 2009, Chong et al. 2010). Nevertheless, parasitism rates of fourth–fifth instars in individual cohorts often exceeded 50% from June to November with percentage parasitism reaching as high as 83% during this time. In the absence of other contemporaneous causes, parasitism comprised 21% of the total marginal rate of mortality in fall for "*T. radiata* access" cohorts, suggesting that *T. radiata* alone can reduce *D. citri* numbers in periods of peak parasitoid activity. The negative impact of *T. radiata* on immature *D. citri* survival may have been underestimated in this study given that the annual *D. citri* survival rate to adulthood in "*T. radiata* access" cohorts averaged only 30.1% (SE ± 0.05%). It is feasible that parasitoid host feeding (Chien et al. 1995) comprises a considerable proportion of the unexplained biotic mortality observed in our experimental *D. citri* cohorts. Laboratory studies suggest that *D. citri* mortality from host feeding by *T. radiata* exceeds that of parasitism by as much as 58% (Skelley and Hoy 2004). In the context of our manipulative field experiments, we suspect that a significant proportion of undetermined *D. citri* nymphal mortality may have been the result of host feeding by *T. radiata*. For example, the marginal rate of mortality from unknown causes of first–third instars was 2- to 3-fold higher in the "*T. radiata* access" treatments compared to the other treatments during the spring, summer, and fall seasons. This trend was most pronounced in the fall when mortality of first–third instars from unknown sources comprised 35% of the total marginal rate of mortality for all immature *D. citri* stages in the "*T. radiata* access" treatments. Given that visual *T. radiata* observations and parasitism rates peaked during this period, it is plausible that *T. radiata* were also killing *D. citri* nymphs via host feeding in fall. However, psyllid mortality from host feeding by *T. radiata* females was not confirmed with visual observations of *D. citri* cohorts in the field. Digital videography of immature *D. citri* colonies in field could help elucidate the importance of host feeding by female *T. radiata* in these urban environments (Kistner et al. 2016b).

The annual marginal rate of mortality for disappearance of *D. citri* eggs in the "no exclusion" treatment averaged 59% indicating the importance of egg predation. These findings are consistent with Michaud (2004) who also observed high levels of egg predation in central Florida. We suspect that predatory mites and thrips may be important egg predators in urban citrus in southern California. Predatory mites in the family Phytoseiidae were found on *D. citri* colonies at all three sites, but were never directly observed attacking *D. citri* eggs. The six spotted thrips, *Scolothrips sexmaculatus* (Pergande), a common predator of spider mites (Gilstrap and Oatman 1976), was found at the UCR Biocontrol grove and Jurupa where they may have opportunistically fed on *D. citri* eggs. Western flower thrips, *Frankliniella occidentalis* (Pergande), were observed at all three sites from March to April 2015. These omnivores will readily attack mite eggs and whitefly crawlers (van Maanen et al. 2012) so it is feasible they could consume *D. citri* eggs and small nymphs. Laboratory bioassays and manipulative field experiments are warranted to determine which of these small predators may be consuming *D. citri* eggs in southern California.

Spiders were the fourth most abundant natural enemy observed at our field sites where they were found in equal numbers on *D. citri* colonies and trapped in sticky barriers. Despite their abundance, we rarely observed spiders attacking *D. citri* nymphs. The ghost spider *Anyphaena pacifica* (Banks) was the only spider we observed actively consuming psyllid nymphs and a small number of nymphs were occasionally found trapped in webs. While several spider species have been reported to consume immature psyllids (Shivankar et al. 2000, Michaud 2002, 2004), there is little evidence that spiders are contributing significant levels of *D. citri* control in Florida (Qureshi and Stansly 2009). Generalist predators and *D. citri* phenology surveys in Pakistan revealed that peak spider abundance and vulnerable *D. citri* life stages did not coincide for long enough periods of time for effective biological control (Vetter et al. 2013, Khan et al. 2014). It is possible that these surveys are underestimating spider predation given the limitation of our diurnal visual observations. Nocturnal digital video recordings could help ascertain if spiders are important predators of immature *D. citri* in California (Kistner et al. 2016b).

Although the Argentine ant was the most abundant arthropod at our sites, annual survival and mean net reproductive rates of completely unprotected *D. citri* cohorts (i.e. no exclusion treatment) were 87% lower than cohorts assigned to treatments that excluded both ants and other walking arthropods. While Argentine ants were observed tending and guarding honey dew producing *D. citri* nymphs, eggs would not have benefitted from the presence of ants. Furthermore, major egg predators, like predatory mites, had full access to these unprotected *D. citri* cohorts which may explain why egg recruitment to first–third instars was 51% lower in "no exclusion" cohorts compared to those that receive some type of exclusion treatment. In addition, the negative impact of Argentine ant–*D. citri* mutualisms on biological control by naturally occurring enemies may have been reduced due to ongoing ant management at all three sites. Homeowners at Lochmoor and Jurupa periodically deployed commercially available liquid poison bait stations to suppress Argentine ant populations (E.J.K., personal observation). Argentine ant control experiments took place at the UCR Biocontrol grove from July through September 2014 and July through November 2015. Using a combination of thiamethoxam and the Argentine ant pheromone component (*Z*)-9-hexadecenal dissolved in a 25% sucrose solution, Argentine ant foraging activity was reduced by 40- to 60-fold as a result of these treatments (K. Schall, unpublished data). Similar target-specific bait delivery systems have proven to be effective in reducing Argentine ant populations in California's organic citrus groves (Greenberg et al. 2006). Manipulative field and laboratory studies are needed to examine the impact of Argentine ant on *D. citri* densities, parasitoid activity, and generalist predators.

This work is the first major study examining *D. citri* survival, demographic growth parameters, mortality factors, and associated generalist predators in southern California. Our estimated net reproductive rates and intrinsic rates of increase are likely overestimated given that they are based on uniform fecundity data from a laboratory study (Liu and Tsai 2000) and we assumed all individuals in our experimental cohorts that survived to adulthood successfully reproduced, which is unlikely. Individuals respond differently to environmental stressors and this variation in performance has been shown to impact future population growth (Ehrlén 2000). In addition, we did not take density-dependent effects into account when estimating *D. citri* demographic growth parameters. In natural insect populations, biotic mortality within a population can benefit surviving individuals by decreasing intraspecific competition (Oedekoven and Joern 2000). In this scenario, increased resource availability enhances the survival and fecundity rates of the remaining individuals which in turn could obviate negative effects of natural enemies on *D. citri* population growth. Therefore, *D. citri* demographic growth parameters as reported here should be viewed with some caution. Nevertheless, our study clearly demonstrates the importance of natural enemies in reducing *D. citri* populations in areas with no chemical control. Reducing *D. citri* densities and maintaining them at low densities in unmanaged urban areas, where *C*Las is present and spreading, is a crucial component of HLB management in California (Hornbaker and Kumagai 2016, Kistner et al. 2016a). Conserving and promoting robust natural enemy populations in urban citrus should help keep *D. citri* densities in check. At the same time, reducing Argentine ant densities in heavily infested areas is recommended, as it should enhance *D. citri* biological control by allowing natural enemies greater access to colonies of eggs and nymphs (Greenberg et al. 2006, Navarrete et al. 2013, Tena et al. 2013).

Acknowledgments

This research was supported, in part, by funds from the Citrus Research Board grant 5500-194, United States Department of Agriculture, Animal and Plant Health Inspection Service, Multi Agency Coordination grant 15-8130-0336-CA, and USDA-APHIS Citrus Health Response Program grant 11-0519-SF. We are grateful to Michael Pazanni at UC-Riverside Research and Economic Development for bridging funds. Douglas Yanega and Richard Vetter identified unknown arthropods and spiders, respectively. Kathryn Vega, Christina Hoddle, Victor Herrera, and Ruth Amrich provided field assistance. We thank Mamoudou Setamou and one anonymous reviewer for helpful feedback and comments. We are grateful to cooperating homeowners, and James Mickey, for generously allowing us to conduct these studies on their properties.

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