



## Digital video surveillance of natural enemy activity on *Diaphorina citri* (Hemiptera: Liviidae) colonies infesting citrus in the southern California urban landscape



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### ABSTRACT

The Asian citrus psyllid, *Diaphorina citri*, is a significant economic pest of citrus because it vectors a bacterium that causes the lethal citrus disease huanglongbing. From 2015 to 2016, digital video recorders were used to identify natural enemy species attacking sentinel immature *D. citri* colonies deployed monthly at three urban sites in southern California. Natural enemy activity during both daylight and nighttime hours was recorded. To examine the impact of ant-hemipteran mutualisms on biological control efficacy, we compared natural enemy interactions with *D. citri* colonies that were either fully exposed or protected from walking arthropods with a sticky barrier. Spiders (Araneae), syrphid larvae (Syrphidae), predatory mites (Phytoseiidae), green lacewing larvae (Chrysopidae), thrips (Thripidae), and the introduced parasitoid, *Tamarixia radiata*, were the dominant natural enemy groups visiting immature *D. citri* colonies over the course of the study. A significant proportion of syrphid and green lacewing larvae, and *T. radiata* visits to the sentinel colonies resulted in *D. citri* attacks. Spiders rarely attacked immature *D. citri*. Argentine ants, *Linepithema humile*, were the most common arthropod visiting *D. citri* colonies across all study sites and ants were active day and night. When ant access to *D. citri* colonies was restricted, natural enemy abundance and attack rates increased, particularly those by syrphids and *T. radiata*. This study highlights the importance of natural enemies and ant-psyllid interactions in the biological control of *D. citri* in urban citrus ecosystems. Therefore, conservation of natural enemies and ant control should be considered for the sustainable management of this pest-disease complex.

### 1. Introduction

Naturally-occurring predatory and parasitic arthropods can reduce pest populations and subsequent loss in crop yields. This can result in a concomitant reduction in insecticide applications in both agricultural and urban settings (Van Driesche et al., 2008). However, the efficacy of beneficial arthropods in limiting pest populations varies both spatially and temporally, and mortality from natural enemies, especially from predation, is often difficult to quantify due to the limitations of commonly employed monitoring methods. For example, beat sampling and pitfall traps are time-specific, cost-effective methods of indirectly assessing predatory taxa associated with pest populations. However, predatory taxa caught in traps may not necessarily attack pests of

interest (Lang, 2000; Grieshop et al., 2012). Molecular techniques can detect the presence of pest antibodies and DNA in predator digestive tracts, but cannot quantify predation frequency, predator behavior, or periodicity of attack (Sheppard and Harwood, 2005). Molecular analyses of gut contents is a type of “lottery” that necessitates the collection and testing of lots of material to get “hits” which identify the few individuals that fed on the target pest. Detection rates may be further reduced by quick digestion times and low retention rates of molecular signatures used to identify consumed pest species (King et al., 2008). Direct human observations can provide important information on the identity and impact of natural enemies. This type of data collecting is time-limited and humans cannot continuously document natural enemy activities over a 24 h period for several consecutive days (Rosenheim

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et al., 1999; Costamagna and Landis, 2007).

Continuing advances in digital video recording technology are providing opportunities to better estimate natural enemy attacks on pest populations through the use of small, weatherproof, digital video recorders (“DVRs”) that are affordable and easy to use (Grieshop et al., 2012). Digital videography is a useful tool in biological control research as it allows the unambiguous identification of key taxa directly attacking pests and assists with quantification of mortality rates from contemporaneous factors (Meyhöfer, 2001; Merfield et al., 2004; Frank et al., 2007; Grieshop et al., 2012). Furthermore, videography can be used to assess periodicity of activity and the number of prey items attacked by natural enemies (Varley et al., 1994; Schenk and Bacher, 2002), and it can identify natural enemy species that were previously unknown to be important biological control agents (Zou et al., 2017).

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an important pest of citrus because it vectors the bacterium, *Candidatus Liberibacter asiaticus* (CLas), a causal agent of the deadly citrus disease huanglongbing (HLB). Huanglongbing is the most destructive disease of citrus worldwide, reducing fruit yield and quality of many susceptible *Citrus* (Sapindales: Rutaceae) cultivars (Halbert and Manjunath, 2004; Grafton-Cardwell et al., 2013). Throughout the 1990s, *D. citri* invaded citrus growing countries in Central and North America with subsequent CLas detections occurring in Mexico, Belize, Cuba, and the USA (Hall et al., 2013). In Florida alone, the *D. citri*–CLas pathosystem has led to over US\$4 billion in estimated net losses since their initial detections in 1998 and 2005, respectively (Farnsworth et al., 2014).

In California, *D. citri* and CLas were first detected in 2008 and 2012, respectively. *D. citri* has subsequently become widespread across the residential landscape of southern California (Kistner et al., 2016a). In contrast, CLas distribution is significantly more limited but confirmed infections in urban citrus are increasing (Kumagai et al., 2013; Hornbaker and Kumagai, 2016). *D. citri*–CLas presents a serious threat to the major commercial citrus production area of the San Joaquin Valley where approximately 75% of California citrus is grown (Bassanezi et al., 2013; Grafton-Cardwell et al., 2015). This risk exists because CLas transmission into commercial citrus production areas can occur by *D. citri* migrating from infected urban citrus (Kumagai et al., 2013; Hornbaker and Kumagai, 2016). Because of high costs and public resistance to pesticide applications in urban areas, biological control with a focus on the classical biological control agent, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), is the dominant *D. citri* management tactic for the vast majority of residential citrus trees in California (Hoddle and Pandey, 2014). *Tamarixia radiata*, a host-specific ectoparasitoid of *D. citri* native to Asia (Gómez-Torres et al., 2012), has been introduced into several countries invaded by *D. citri* including Taiwan (Chien and Chu 1996), Réunion Island (Étienne and Aubert, 1980), and the USA (Halbert and Manjunath, 2004). While *T. radiata* alone has provided insufficient biological control of *D. citri* populations in Florida (Michaud, 2002; Qureshi and Stansly, 2009), there is evidence that *T. radiata* may be contributing to reductions in urban *D. citri* densities in southern California (Kistner and Hoddle, 2015; Kistner et al., 2016a).

Exclusion studies indicate that generalist predators provide substantial biological control of *D. citri* populations even though direct evidence such as identification of the remains of prey bearing unique attack signatures from a particular predator species are hard to discern (Michaud, 2004; Qureshi and Stansly, 2009; Kistner et al., 2016b). In Florida, coccinellids are considered important predators of immature *D. citri* (Michaud, 2004; Chong et al., 2010) and Kistner et al. (2016b) observed significant psyllid reductions from syrphid fly and lacewing larvae predation in California. However, research on natural enemies and biological control of *D. citri* have been dominated by diurnal observations where mortality from predation, for example, is inferred based on potential predator presence rather than direct observation of a successful predation event (Grafton-Cardwell et al., 2013; Hall et al.,

2013). Therefore, the extent to which each observed taxonomic group that comprise the potential predator guild reduces *D. citri* densities remains unclear. For instance, the impact of some predator guilds such as spiders and lacewing larvae may be underestimated given these arthropods often forage at night (Meyhöfer, 2001; Merfield et al. 2004; Van Driesche et al., 2008), a time when human observers are typically not active. Other biological interactions, like intraguild predation and ant-hemipteran mutualisms, may also affect *D. citri* population dynamics. In Florida, intraguild predation by coccinellids reduces mortality rates that would be attributable to *T. radiata*, but the net effect of these interactions on biocontrol efficacy and resulting *D. citri* densities is uncertain (Michaud, 2004).

Ants protect *D. citri* nymphs in exchange for secreted honeydew and these mutualistic interactions reduce parasitism rates by *T. radiata* in both Florida (Navarrete et al., 2013) and California (Tena et al., 2013; Schall and Hoddle, 2017). In addition, this mutualistic relationship may prevent access to *D. citri* colonies by generalist predators. Continuous video observation of these biological interactions in the field can help determine both the frequency and overall impact of these types of interactions on *D. citri* survival rates (Meyhöfer, 2001).

To develop a more comprehensive understanding of which arthropods are associated with *D. citri*, we employed digital video recorders equipped with infrared LEDs to continuously document diurnal and nocturnal insect activity associated with patches of immature *D. citri* infesting potted citrus plants that were deployed in urban citrus ecosystems. The aims of this study were to: 1) determine the identity, activity periodicity, diversity and abundance of predators and parasitoids attacking immature *D. citri* colonies, and 2) quantify the effect of the ant-hemipteran mutualism on natural enemy abundance and attack rates on immature *D. citri*.

## 2. Materials and methods

Two residential study sites, Lochmoor (33°95' N; 117°31' W; 420 m) and Jurupa (33°99' N; 117°50' W; 214 m), and an experimental citrus orchard, the Biocontrol Grove (33°58' N; 117°19' W; 359 m) at the University of California Riverside (UCR), all located in Riverside, County California USA, were used for digital videography studies. Both *D. citri* and *T. radiata* were well established at these sites when this study commenced. The number of mature *Citrus* (Rutaceae) trees varied across sites with 10 trees at Jurupa, 40 trees at Lochmoor, and 550 trees at the Biocontrol Grove. No foliar or systemic insecticide applications were applied to sites throughout the duration of the study.

### 2.1. Digital video system design

The digital videography set up employed for these studies used a Raspberry Pi Model B minicomputer with 512MB of RAM. DVR units consisted of six major components: 1) Raspberry Pi NoIR camera, 2) infrared LED bulbs, 3) SD card, 4) real time clock, 5) Raspberry Pi Model B, and 6) a USB flash drive (Fig. 1a). The Raspbian operating system installed on an 8 GB SD card was used to run the cameras. The motherboards, SD cards, and cameras were manufactured by Raspberry Pi (Raspberry Pi Fdn, Caldecote, UK). The cameras were 5MP infrared cameras illuminated by 2 LED infrared bulbs for night time recordings. The camera's focal length was modified to record in macro which kept arthropods visiting *D. citri* patches in focus. A real time clock, powered by a CR2032 battery, was installed in the motherboard, enabling date and time stamps for each recording (AB Electronics UK LTD, Swanage, UK). All recordings were saved onto a 128 GB USB flash drive (Corsair Components Inc., Fremont, CA, USA) which could store approximately 130 h of recordings.

The digital recording system was powered with 12 V, 50 Ah rechargeable SLA AGM batteries (Universal Power Group Inc., Coppel, TX, USA) housed in waterproof plastic bins (46L × 31.1H × 29.2W cm) (Fig. 1b). The 12 V power from the battery

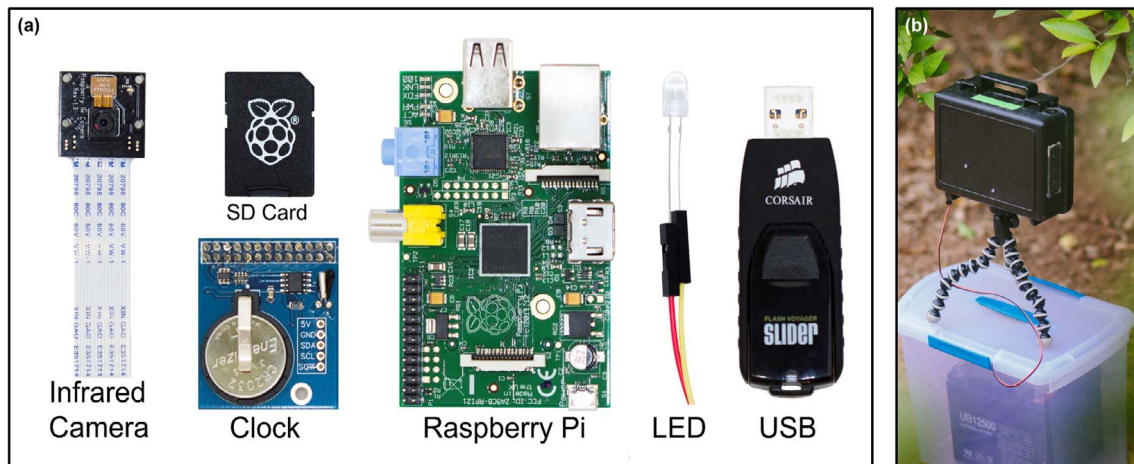


Fig. 1. Photos of a) Digital video recorder (DVR) components and b) DVR unit in a weather proof case powered by 12 V battery.

was transferred through positive and negative alligator clips to two shield communication wires linked to a 12 DC power adaptor connected to a USB car charger adaptor (Zeimax LLC, Hackensack, NJ, USA) which directly powered the DVR motherboard via a USB connection. DVR units were housed in a weather proof polypropylene case (21.6L × 14H × 6.4W cm) (Pelican Products Inc., Torrance, CA, USA). Units were mounted with adjustable camera tripods (Joby, Petaluma, CA, USA), allowing them to be easily positioned around experimental citrus plants infested with *D. citri* cohorts. Camera units and batteries were attached to concrete blocks with security cables to deter opportunistic theft (Kistner et al., 2016c).

## 2.2. Deployment of *D. citri* cohorts

Cohorts of *D. citri* eggs oviposited onto flush growth of *Citrus volkameriana* were monitored with digital video recorders to document arthropod interactions with immature stages of *D. citri*. At the start of each experiment, two *C. volkameriana* plants containing ~200 *D. citri* eggs were established in the laboratory (see Kistner et al., 2016b for setup procedures) and placed on stands (i.e., plastic buckets: 33 (diameter) × 37 (height) cm) at each study site. One potted plant was left completely exposed to allow free access to *D. citri* colonies by all natural enemies and ant mutualists. The second plant had a sticky barrier (Tangle foot® insect barrier, Contech Enterprises Inc., Victoria, Canada) applied across the entire circumference of the base of the pot. The purpose of the sticky barrier was to prevent access to *D. citri* colonies by walking arthropods thereby enabling assessment of ant interference with natural enemy activity. For consistency of terminology, we will now refer to the former treatment as no treatment and the latter as the sticky barrier treatment. To capture seasonal variation in arthropod abundance and activity level, these exposure experiments were repeated monthly beginning in January 2015 and ending in March 2016 (Table 1).

## 2.3. Videography

Potted plants assigned to their respective treatments were placed on the same stationary stand at each site over the duration of the study to avoid potential confounding effects from filming location. Cameras were positioned 12.7 cm away from the focal *D. citri* colonies situated on infested citrus flush. One camera was assigned per potted plant. To ensure consistent recording over time, we employed a portable HDMI monitor (Adafruit, New York City, NY, USA) which allowed for on-site visual displays of focal *D. citri* colonies. Horizontal camera distance from focal *D. citri* colony was measured using a ruler. Cameras filmed *D. citri* colonies continuously at 960H (i.e., 960 × 480 pixels resolution).

Camera units were checked every 3–4 days, at which point batteries and USB flash drives were changed. Experimental plants were thoroughly watered at this time. Video from the USB flash drive was copied onto an external 4 TB hard drive (Western Digital Technologies Inc., Irvine, CA, USA) and archived by date, site, and treatment. All videos were backed up on a second external hard drive using the same method as described above. Since natural enemies often attack different insect life stages, filming of an experimental cohort ended when all ~200 focal *D. citri* had either been killed, disappeared, or reached adulthood. Termination decisions were made via human examination of experimental cohorts on potted plants at field sites. Since *D. citri* adults are highly mobile, we did not attempt to record arthropod interactions with adult psyllids. A total of 60 experimental cohorts were filmed (n = 20 per site and n = 10 per treatment) over the course of this study for a total of 19,200 h of digital data.

## 2.4. Video analysis

Digital video files were watched using a VLC Media Player (version 2.2.2., Video LAN Org., Paris, France) which permitted viewing at four times normal speed and frame by frame analysis. From the recordings, each time an arthropod visited focal *D. citri* colonies, the species, number of visits, and the time and date of each visit was recorded. Visiting arthropods were categorized into families for insects and orders for non-insect invertebrates. When possible, visiting arthropods were classified at the genus and species level. A visit was defined as an event in which a potential natural enemy (i.e., predator or parasitoid) came within one centimeter of an immature *D. citri* (Grieshop et al., 2012). We also used camera data to quantify the frequency of interactions and to distinguish between arthropods that acted as predators or parasitoids, from those that did not interact with immature *D. citri*. An attack from a visiting arthropod that resulted in the death of an immature *D. citri* by consumption was classified as a predation event. Likewise, an attack by *T. radiata* was classified as a female parasitoid standing on top a *D. citri* nymph for at least 3 min (Chen and Stansly, 2014). It was not possible to consistently categorize the nature of ant interactions with *D. citri* nymphs (i.e. tending, guarding, harvesting honey dew) due to large numbers of ants visiting *D. citri* cohorts.

## 2.5. Statistical analysis

For all statistical analyses, only complete 24 h data recordings were assessed as there were some cases of camera failure over the course of the study due to a combination of extreme weather events, technological malfunctions, and human interference. Rainy days were also excluded as arthropods were not active during these periods. We

**Table 1**  
 Experimental *D. citri* cohort deployment start and end dates, total morphotaxa visits per cohort, and total hours filmed per cohort across all sites and treatments over the course of the study. Bold values are summed across the entire duration of the study (2015–2016).

Site	No Treatment										Sticky Barrier																				
	D. citri cohort start and end dates					# of visits per morphotaxa					Hours filmed					D. citri cohort start and end dates					# of visitation per morphotaxa					Hours filmed					
	F	A	S	Tr	P	Th	C	O	F	A	S	Tr	P	Th	C	O	F	A	S	Tr	P	Th	C	O	F	A	S	Tr	P	Th	C
Biocontrol Grove	31 Mar to 23 Apr 2015	107	1	3	2	12	29	0	0	144	31 Mar to 19 Apr 2015	0	0	10	3	8	11	0	0	240											
	21 May to 31 May 2015	104	10	1	0	10	13	14	3	240	21 May to 18 Jun 2015	3	2	20	1	5	1	3	0	384											
	18 Jul to 8 Aug 2015	221	6	1	1	1	8	0	78	384	18 Jul to 30 Jul 2015	237	1	22	6	0	9	0	3	240											
	29 Aug to 19 Sep 2015	155	17	1	1	2	1	1	37	456	29 Aug to 19 Sep 2015	5	4	0	5	5	0	1	2	408											
	3 Oct to 14 Oct 2015	120	9	1	0	5	0	0	0	216	3 Oct to 29 Oct 2015	3	14	2	34	1	0	0	5	600											
	14 Nov to 24 Nov 2015	15	11	0	1	20	0	0	0	240	14 Nov to 24 Nov 2015	10	5	0	0	6	1	0	7	288											
	5 Dec to 23 Dec 2015	17	13	0	0	33	8	0	5	360	5 Dec to 23 Dec 2015	0	3	0	0	2	2	2	0	504											
	2 Jan to 10 Jan 2016	4	3	0	0	12	5	0	0	144	2 Jan to 28 Jan 2016	5	6	0	0	0	0	0	5	240											
	6 Feb to 4 Mar 2016	75	5	31	0	0	0	0	2	480	6 Feb to 4 Mar 2016	0	0	16	0	3	0	0	15	528											
	19 Mar to 29 Mar 2016	38	3	1	0	1	0	0	1	216	19 Mar to 31 Mar 2016	0	7	0	0	0	0	0	4	120											
	<b>31 Mar 2015 to 29 Mar 2016</b>	<b>856</b>	<b>78</b>	<b>39</b>	<b>5</b>	<b>96</b>	<b>64</b>	<b>15</b>	<b>126</b>	<b>2880</b>	<b>31 Mar 2015 to Mar 31 2016</b>	<b>263</b>	<b>43</b>	<b>70</b>	<b>49</b>	<b>30</b>	<b>24</b>	<b>6</b>	<b>41</b>	<b>3552</b>											
	Lochmoor	2 Feb to 15 Feb 2015	115	35	4	2	19	8	9	0	168	2 Feb to 20 Feb 2015	1	4	0	0	0	0	1	0	120										
		19 Mar to 7 Apr 2015	143	9	4	3	17	11	1	0	120	19 Mar to 27 Mar 2015	10	5	14	12	4	14	7	2	216										
		2 May to 28 May 2015	170	12	7	5	3	18	10	5	504	2 May to 6 June 2015	7	3	10	17	1	10	2	2	408										
		22 Jun to 9 Jul 2015	208	32	0	0	0	0	0	1	456	22 Jun to 16 Jul 2015	10	2	0	3	11	0	0	0	480										
		31 Jul to 25 Aug 2015	473	17	1	3	0	0	0	1	552	31 Jul to 11 Aug 2015	8	1	2	2	7	0	0	0	264										
5 Sep to 11 Sep 2015		71	0	0	2	2	0	0	0	120	5 Sep to 24 Sept 2015	0	0	0	0	2	0	0	0	360											
10 Oct to 2 Nov 2015		114	18	0	3	0	0	0	0	408	10 Oct to 5 Nov 2015	3	0	0	20	0	0	6	4	528											
14 Nov to 28 Nov 2015		17	7	0	38	0	0	0	0	168	14 Nov to 28 Dec 015	1	0	0	23	1	0	0	1	696											
2 Jan to 19 Jan 2016		2	1	0	0	1	0	0	0	192	2 Jan to 28 Jan 2016	0	0	0	0	0	0	0	0	312											
4 Feb to 20 Feb 2016		0	1	0	0	0	0	5	1	96	4 Feb to 10 Mar 2016	0	0	0	0	0	0	0	0	480											
<b>2 Feb 2015 to 29 Mar 2016</b>		<b>1313</b>	<b>132</b>	<b>16</b>	<b>56</b>	<b>42</b>	<b>37</b>	<b>25</b>	<b>8</b>	<b>2784</b>	<b>2 Feb 2015 to 10 Mar 2016</b>	<b>40</b>	<b>15</b>	<b>26</b>	<b>77</b>	<b>26</b>	<b>24</b>	<b>16</b>	<b>9</b>	<b>3864</b>											
Jurupa		15 Jan to 1 Feb 2015	13	0	0	0	0	0	3	0	120	15 Jan to 26 Feb 2015	16	1	0	0	4	0	0	0	192										
		10 Mar to 24 Mar 2015	148	18	6	0	41	7	9	12	216	10 Mar to 25 Mar 2015	19	9	65	1	3	8	4	0	216										
		22 Apr to 28 Apr 2015	93	3	8	0	7	0	0	0	120	22 Apr to 30 May 2015	7	4	4	0	0	0	0	0	336										
		12 Jun to 19 Jun 2015	218	33	3	0	19	0	0	3	192	12 Jun to 2 Jul 2015	265	24	82	0	1	0	0	2	480										
		25 Jul to 1 Aug 2015	344	12	1	0	0	0	0	0	168	25 Jul to 6 Aug 2015	224	12	55	0	0	0	0	1	288										
	22 Aug to 10 Sep 2015	319	27	0	0	0	0	0	0	312	22 Aug to 8 Sep 2015	282	33	0	0	0	0	0	0	336											
	26 Sept to 10 Oct 2015	255	13	0	0	0	0	0	4	288	26 Sept to 20 Oct 2015	148	13	3	0	2	0	0	0	552											
	31 Oct to 17 Nov 2015	223	9	0	0	0	0	0	0	264	31 Oct to 28 Dec 2015	271	7	1	0	0	0	0	0	624											
	9 Jan to 2 Feb 2016	73	3	0	0	2	1	0	0	336	9 Jan to 2 Feb 2016	16	1	0	1	0	0	0	0	504											
	20 Feb to 29 Feb 2016	213	16	0	0	0	1	0	0	480	20 Feb to 1 Mar 2016	1	0	0	1	0	0	0	0	96											
	<b>15 Jan 2015 to 29 Feb 2016</b>	<b>1899</b>	<b>134</b>	<b>18</b>	<b>0</b>	<b>69</b>	<b>9</b>	<b>12</b>	<b>19</b>	<b>2496</b>	<b>15 Jan 2015 to 1 Mar 2016</b>	<b>1249</b>	<b>104</b>	<b>210</b>	<b>3</b>	<b>10</b>	<b>8</b>	<b>4</b>	<b>3</b>	<b>3624</b>											

F, Formicidae; A, Araneae; S, Syrphidae; Tr, *T. radiator*; P, Phytoseiidae; Th, Thripidae; C, Chrysopidae; O, Other.

employed non-parametric Kruskal-Wallis tests, followed by a Mann-Whitney U pairwise comparisons, to determine differences in the number of visits between morphotaxa groups at each site. To distinguish between predatory taxa that frequently consume immature *D. citri* and those that only occasionally consume *D. citri*, we conducted Fisher's exact tests. This test compared the total number of visits and total number of attacks by each major predator taxon in a 2 (visits and attacks) by 5 (five predatory taxa) contingency table. The total number of observed visits and attacks for each predatory taxa were summed over the entire duration of the study. Given the uneven timing of behavioral observations across sites and treatments (Table 1), a total of six Fisher's exact tests were conducted for each site and treatment combination. If the contingency table was significant ( $P < 0.05$ ) indicating a significant difference in the frequency of visits relative to attacks by the five predatory taxa overall, pairwise comparisons were made between taxa using  $2 \times 2$  contingency tables to determine the most recurrent taxa attacking immature *D. citri*. To determine patterns in diel activity, morphotaxa visitation data collected hourly over a 24 h period by filming the same experimental cohort were assessed using Repeated Measures ANOVA. To avoid temporal pseudo replication, number of hourly morphotaxa visits was set as the response variable and hour was set as a fixed predictor variable with repeated measures (i.e. sample day) taken on cohorts as a random variable. For the morphotaxa syrphid, only visits from larvae, which are predatory, were included in the repeated measures analysis. The number of morphotaxa visits to focal *D. citri* colony per hour were log transformed prior to analysis (Zar, 1999). All statistical analyses were performed using the statistics program, R.3.2.5 (R Development Core and Team 2016).

### 3. Results

#### 3.1. Arthropod community

A total of 8 major morphotaxa groups interacting with *D. citri* were identified through video monitoring of *D. citri* colonies (Fig. 2). The frequency of visits varied significantly across morphotaxa at the Biocontrol Grove (Fig. 2a;  $H = 36.83$ ,  $df = 7$ ,  $P < 0.001$ ), Lochmoor (Fig. 2b;  $H = 29.62$ ,  $df = 7$ ,  $P < 0.001$ ), and Jurupa (Fig. 2c;  $H = 87.31$ ,  $df = 7$ ,  $P < 0.001$ ). Across all sites, Argentine ants, *Linepithema humile* (Mayr), were the dominant visitors to no treatment *D. citri* cohorts (Fig. 2) and comprised > 99% of all ants observed visiting *D. citri* cohorts over the course of this study. Dark rover ants, *Brachymyrmex patagonicus* (Mayr), were the only other ant species filmed interacting with *D. citri* and were only observed at the Biocontrol Grove in October 2015. At Jurupa, ant visits to immature *D. citri* were significantly higher when compared to visits made by all other morphotaxa groups (Fig. 2c;  $P < 0.001$ ). Consequently, the sticky barrier treatment resulted in an increase in the average proportion of visiting predatory and parasitic morphotaxa and a decrease in ant abundance (Fig. 2). Nevertheless, this exclusion treatment was less effective at Jurupa compared to the other two sites where ants comprised over a half of the total visits compared to the other morphotaxa groups (Fig. 2c). Although taxa abundance varied across sites and treatments, the most commonly recorded natural enemy taxa groups were Araneae, *T. radiata*, Syrphidae, Phytoseiidae, Thripidae, and Chrysopidae (Fig. 2). Ghost spiders (Anyphaenidae), jumping spiders (Salticidae), and yellow sac spiders (Eutichuridae) were abundant at all three study sites. Adult hover flies (Syrphidae) in the genus, *Allograpta*, were commonly filmed laying eggs near focal *D. citri* colonies at all three sites. Likewise, adult green lacewings (Chrysopidae) in the genus, *Chrysoperla*, were also observed visiting *D. citri* cohorts at all three sites. All filmed occurrences of hover fly and green lacewing larvae could not be identified beyond the level of family.

Uncommon predatory insect taxa included lady beetles (Coccinellidae), minute pirate bugs (Anthocoridae), and earwigs (Forficulidae). Adult lady beetles composed of the species *Harmonia*

*axyridis* (Pallas) and *Cycloneda* spp. were filmed consuming immature *D. citri* at Lochmoor. The brown garden snail, *Cornu aspersum* (Müller), was commonly observed at the Biocontrol Grove. These snails were filmed consuming the flush of sentinel citrus plants infested with immature *D. citri* on both treatment types since their mucus secretions enabled them to cross the sticky barriers. Snail consumption of *D. citri* cohorts was rarely recorded at the other two urban sites. At the Biocontrol Grove, *C. aspersum* was commonly recorded after rainfall events and comprised 6% and 15.5% of the average proportion of taxa observed visiting sentinel *D. citri* colonies receiving either no treatment or a sticky barrier, respectively (Fig. 2a).

Seasonal differences in *D. citri* cohort start and end dates, total hours filmed, and total number of morphotaxa visitation events across sites and treatments are summarized in Table 1. Overall, visitation events occurred less frequently in the winter months (Dec-Feb) compared to other seasons across all sites and treatments (Table 1). Although Argentine ants visited *D. citri* cohorts year round, visitation frequency was highest from Mar-Oct with peak periods of visitation varying across sites (Table 1). Spiders (Araneae) were observed regularly visiting *D. citri* cohorts over this 12 month period (Table 1). Jan was the only time in which *T. radiata* was absent from all three sites (Table 1). At Lochmoor and the Biocontrol Grove, the number *T. radiata* visits from Oct–Nov 2015 accounted for more than 50% of all *T. radiata* visits over the course of the study (Table 1). Syrphid larvae were mostly observed from Feb–Aug with peak visitation frequency varying across sites and treatments (Table 1). Green lacewing (Chrysopidae) larvae activity levels peaked in spring (Mar–May), and were observed less frequently in summer (June–Aug), fall (Sept–Nov), and winter (Dec–Feb) months.

#### 3.2. Predator and parasitoid activity

There were significant differences in the frequency of visits in which an attack was observed by five natural enemy taxa and between individual pairs of taxa with the exception of the sticky barrier treatment at Lochmoor (Table 2). Although thrips (Thripidae) and predatory mites (Phytoseiidae) were frequent visitors to *D. citri* cohorts, they were too small for predatory activity, if it occurred, to be recorded. Overall, spiders (Araneae) were the most common predatory visitors at *D. citri* cohorts, but were rarely observed attacking (i.e. consuming) immature *D. citri*. In contrast, syrphid larvae, *T. radiata*, and green lacewing larvae were the most commonly observed morphotaxa attacking immature *D. citri* with their visitation frequency and attack rates (i.e. percentage of visits in which an attack was observed) varying greatly between treatments and across sites over time. At the Biocontrol Grove, *T. radiata*'s attack rate increased from 0% in no treatment *D. citri* cohorts to 30.6% when the sticky barrier treatment excluded ants (Table 2). In contrast, *T. radiata* attack rates were similar between treatments at Lochmoor, but overall attack rates were high compared to syrphids at this site (Table 2). At Jurupa, green lacewing larvae were the major predatory morphotaxa observed attacking no treatment *D. citri* cohorts. In contrast, syrphids were the only morphotaxa observed attacking *D. citri* protected from walking arthropods with the sticky barrier treatment at the Jurupa site (Table 2).

#### 3.3. Diel rhythm

Formicidae, Syrphidae, *T. radiata*, and Araneae were the only morphotaxa visiting *D. citri* with sufficient frequency to statistically analyze patterns in diel activity. For graphical presentation of visits, interaction hours were summed across all video clips and averaged across all individual cohorts filmed at their respective sites and assigned treatments (Fig. 3). Overall, the four morphotaxa groups showed clear patterns of diel activity that were consistent across all three study sites. Argentine ants (Formicidae) visited *D. citri* during both the photophase (6:00–20:59 h) and scotophase (21:00–5:59 h), but the effect of hour was significant at the Biocontrol Grove ( $F_{23,2199} = 2.15$ ,  $P = 0.001$ ),

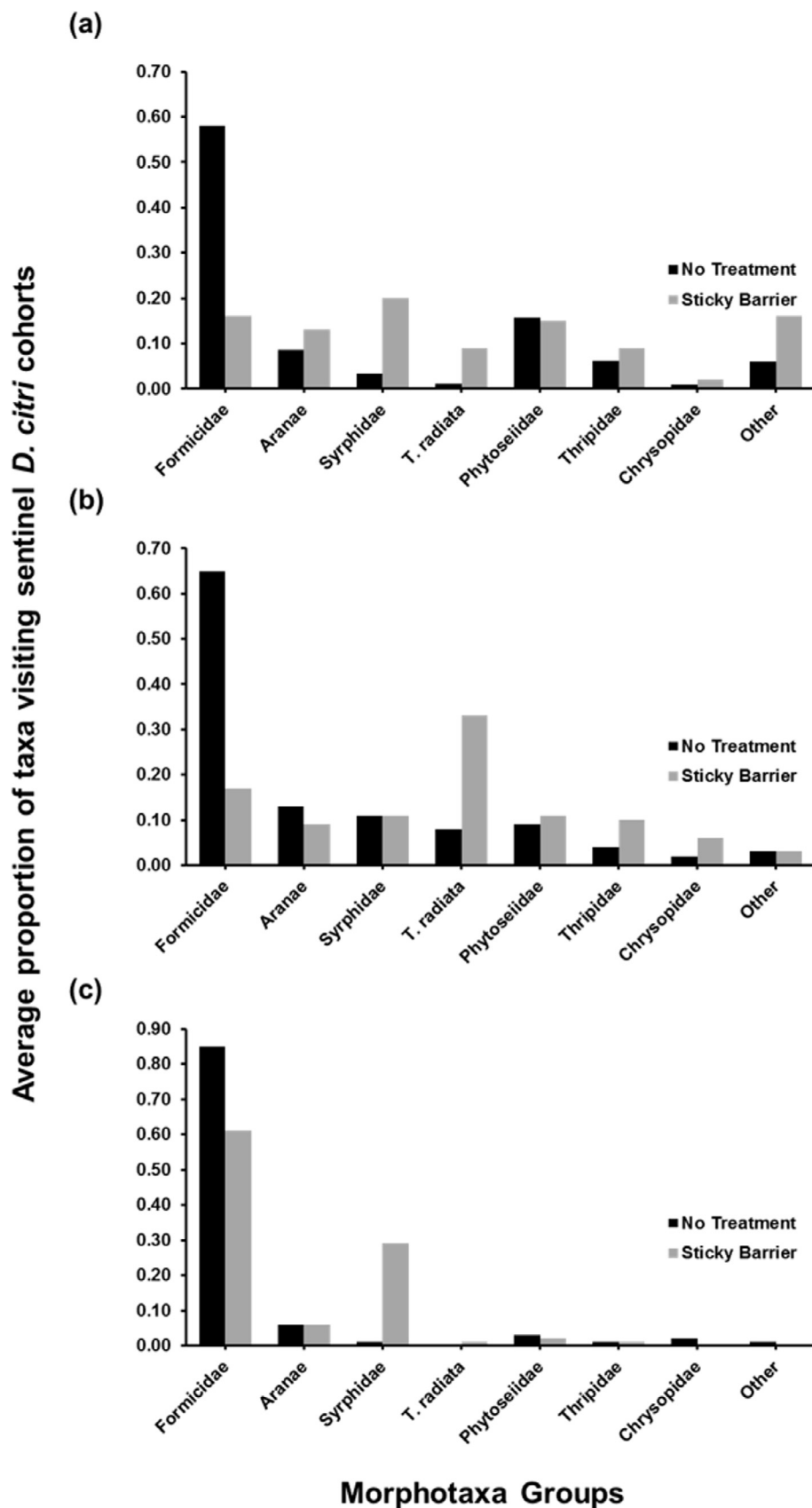


Fig. 2. The average proportion of taxa observed visiting sentinel *D. citri* cohorts under no and sticky barrier treatments at the (a) Biocontrol Grove, (b) Lochmoor, and (c) Jurupa sites in Riverside County, California. The “Other” category is comprised of uncommon predatory taxa including Coccinellidae, Anthocoridae, Mantidae, Forficulidae, and the brown garden snail, *Cornu aspersum*.

**Table 2**

The number of visits and total attacks by common natural enemies on *D. citri* colonies that were fully exposed (No Treatment) or protected from walking arthropods (Sticky Barrier) across three sites in Riverside County, California (2015–2016).

Site	Predator Taxa	No Treatment			Sticky Barrier		
		# Visits	# Attacks	% <sup>a</sup>	# Visits	# Attacks	% <sup>a</sup>
Biocontrol Grove	Aranae	78	7	8.97ab	43	1	2.33a
	Syrphidae	39	7	17.95ac	70	22	31.43b
	<i>T. radiata</i>	5	0	0.0ab	49	15	30.61b
	Phytoseiidae	96	–	– <sup>b</sup>	30	–	– <sup>b</sup>
	Thripidae	64	–	– <sup>c</sup>	24	–	– <sup>c</sup>
	Chrysopidae	15	6	40.0c	6	1	16.67ab
	Other	163	27	16.56ac	41	8	19.51b
	OR <sup>d</sup> , P		0.23, < 0.01		13.35, < 0.01		
Lochmoor	Aranae	132	2	1.52a	15	0	0.0a
	Syrphidae	16	1	6.25ab	21	1	4.67a
	<i>T. radiata</i>	56	7	12.5b	77	8	10.39a
	Phytoseiidae	42	–	– <sup>b</sup>	26	–	– <sup>b</sup>
	Thripidae	37	–	– <sup>c</sup>	24	–	– <sup>c</sup>
	Chrysopidae	25	8	32.0b	16	0	0.0a
	Other	8	3	37.5b	9	0	0.0a
	OR <sup>d</sup> , P		20.52, < 0.001		0.37, 0.69		
Jurupa	Aranae	134	0	0.0a	104	0	0.0a
	Syrphidae	18	0	0.0ab	210	60	28.57b
	<i>T. radiata</i>	–	–	–	3	0	0.0ab
	Phytoseiidae	69	–	– <sup>b</sup>	10	–	– <sup>b</sup>
	Thripidae	9	–	– <sup>c</sup>	8	–	– <sup>c</sup>
	Chrysopidae	12	2	16.67b	4	0	0.0ab
	Other	19	2	10.53b	3	0	0.0ab
	OR <sup>d</sup> , P		0.0, < 0.01		0.0, < 0.001		

Taxa with the same letters in the % column were not significantly different in pairwise comparisons.

<sup>a</sup> Percentage of visits in which an attack was observed (# attacks/ # visits × 100%).

<sup>b</sup> Phytoseiidae were too small for their activity to be recorded accurately from the video image.

<sup>c</sup> Thripidae were too small for their activity to be recorded accurately from the video image.

<sup>d</sup> Fischer's exact test odds ratio indicating a significant difference in the frequency of visits relative to attacks by the five predatory taxa overall.

Lochmoor ( $F_{23,1984} = 3.10$ ,  $P < 0.001$ ), and Jurupa ( $F_{23,1746} = 3.31$ ,  $P < 0.001$ ), respectively. The number of ant visits peaked during the photophase and ant visitation was more frequent at Jurupa compared to the two other study sites (Fig. 3a). Spider (Aranae) visitation rates also varied significantly by hour at all three sites (Biocontrol Grove:  $F_{23,1047} = 2.32$ ,  $P < 0.001$ ; Lochmoor:  $F_{23,1191} = 1.97$ ,  $P = 0.004$ ; Jurupa:  $F_{23,1312} = 3.43$ ,  $P < 0.001$ ; respectively). While spiders (Aranae) visited *D. citri* more frequently during the scotophase, they were also observed during the photophase (Fig. 3b). Syrphid larvae activity peaked in the scotophase, but larvae were also seen consuming *D. citri* during the photophase (Fig. 3c). Adult syrphids were only recorded on *D. citri* patches during the photophase when they were ovipositing. Overall, 65.5% to 74.1% of all syrphid larvae visits occurred during the scotophase, and this periodicity of hourly visitation was significant at the Biocontrol Grove ( $F_{23,421} = 1.95$ ,  $P = 0.005$ ), Lochmoor ( $F_{23,262} = 1.79$ ,  $P = 0.02$ ), and Jurupa ( $F_{23,715} = 2.33$ ,  $P < 0.001$ ). *T. radiata* visited *D. citri* colonies exclusively during the photophase with peak activity occurring at 13:00 h at both the Biocontrol Grove ( $F_{23,500} = 4.59$ ,  $P < 0.001$ ) and Lochmoor ( $F_{23,763} = 6.37$ ,  $P < 0.001$ ). Likewise, *T. radiata* visits at Jurupa were only observed during the photophase, but visits were too infrequent to statistically analyze patterns in diel activity (Fig. 3d).

#### 3.4. Additional observations

Although both syrphid and lacewing larvae were occasionally filmed consuming immature *D. citri*, green lacewing larvae tended to consume more *D. citri* individuals per visit compared to syrphid larvae. Green lacewing larvae typically consumed 50–78% of visible *D. citri* nymphs within a 1–3 h period of digital recording. In contrast, syrphids would consume an average 16–35% of visible *D. citri* nymphs within a 1–3 h recording period. Ants were observed tending *D. citri* colonies and

protecting them from potential predators. When allowed unrestricted access to *D. citri* cohorts (i.e. no treatment), ant attendance of *D. citri* nymphs peaked in Jul with the percentage of ant visits resulting in *D. citri* attendance ranging from 14.28% to 18.89%. Ants were also filmed removing syrphid larvae and adult coccinellids from *D. citri* colonies. Over the entire course of study, instances of intraguild predation were documented on just three separate occasions. At Lochmoor in Jul 2015, an adult coccinellid was recorded consuming two syrphid larvae. In May 2015, a minute pirate bug (Anthocoridae) was filmed consuming a green lacewing larva at Jurupa. At the Biocontrol Grove in Oct 2015, a brown garden snail consumed two parasitized *D. citri* nymphs that had undergone color change upon mummification.

#### 4. Discussion

This study found that urban citrus has an active and diverse natural enemy community that helps regulate populations of *D. citri* in southern California. Argentine ants were the most abundant morphotaxa visiting immature *D. citri* cohorts and were frequently observed tending *D. citri* nymphs. Consequently, the number of predatory and parasitoid morphotaxa visits to *D. citri* cohorts increased when ant access was restricted with a sticky barrier compared to unprotected cohorts receiving no treatment. The three most abundant natural enemies attacking *D. citri* were predatory syrphid and green lacewing larvae and the minute parasitoid, *T. radiata*. Spiders, although frequent visitors to *D. citri* patches, were not significant *D. citri* predators. Taken together, our results suggests that the conservation of natural enemies of *D. citri* should be incorporated into an ongoing integrated management strategy for this pest and CLAs in California.

Overall, the results from video surveillance of arthropods interacting with *D. citri* cohorts were consistent with findings from visual surveys that took place simultaneously at these study sites from

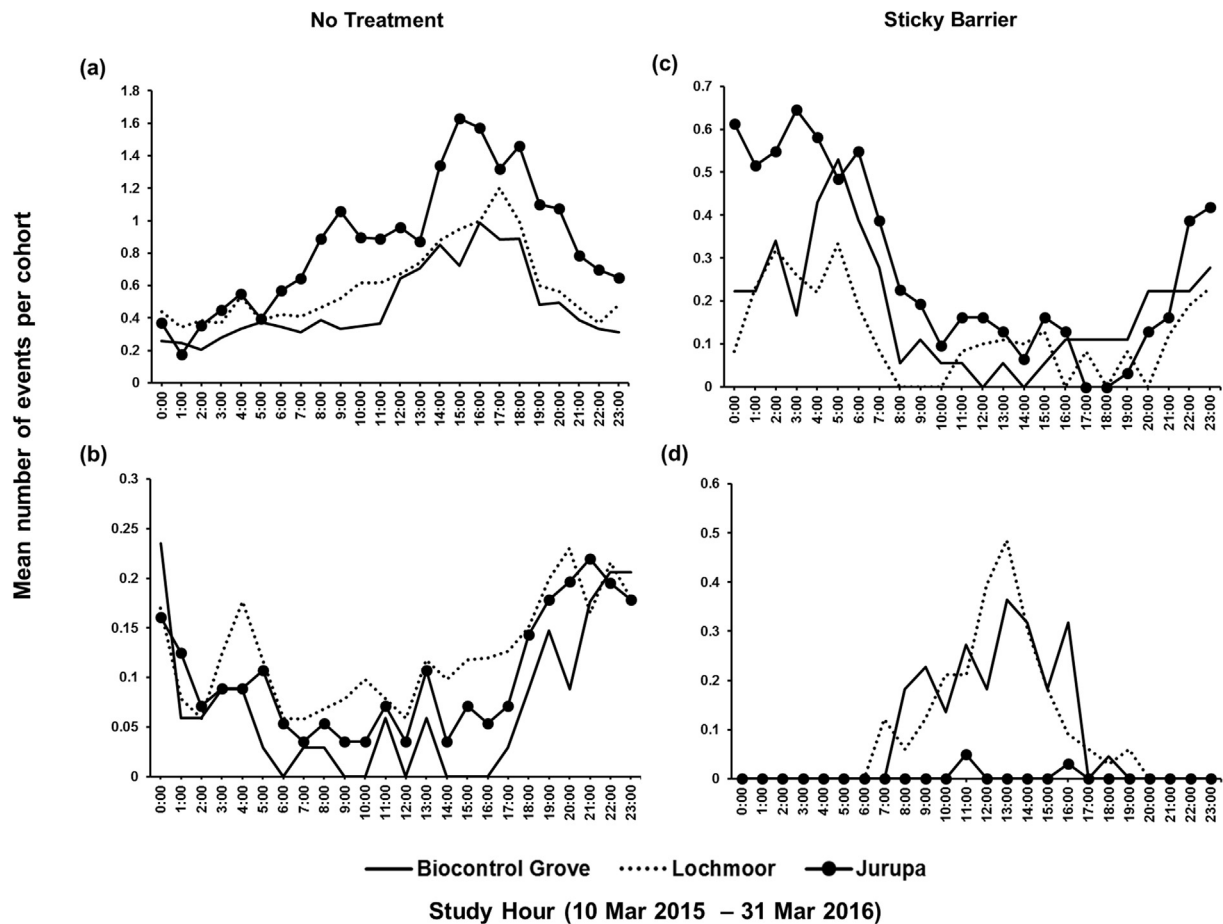


Fig. 3. The mean number of (a) Formicidae and (b) Araneae visiting *D. citri* cohorts fully exposed to walking arthropods (left column) and (c) Syrphidae and (d) *T. radiata* visiting *D. citri* cohorts protected from walking arthropods with a sticky barrier (right column) by study hour.

Jan–Nov 2015 (see Kistner et al., 2016b for procedure). This 24 h video surveillance study found syrphid larvae to be the most abundant natural enemy attacking immature *D. citri* and revealed that syrphid larvae were more active on *D. citri* patches at night. Vickerman and Sunderland (1975) also found that syrphid larvae abundance and predatory activity on wheat aphids peaked during the scotophase. We suspect that the observed nocturnal activity of syrphid larvae may explain why diurnal *D. citri* predator surveys do not report syrphids as an important *D. citri* predator (Michaud, 2004; Qureshi and Stansly, 2009; Chong et al., 2010; Khan et al., 2014). Our findings highlight the importance of predators with nocturnal foraging behavior as important natural enemies of *D. citri* as well as the utility of digital video surveillance in eliminating labor difficulties associated with night time sampling (Vickerman and Sunderland, 1975; Pfannenstiel and Yeagan, 2002).

Video surveillance revealed that peak *T. radiata* activity occurred at 13:00 h at the Biocontrol Grove and Lochmoor. In this study, digitally recorded *T. radiata* attacks were likely the result of either parasitism or host feeding (Tena et al., 2017). When host feeding, females use their ovipositor to puncture the nymphal cuticle and they then feed on the leaking hemolymph (Chen and Stansly, 2014). Laboratory studies suggest that host feeding comprises a larger portion of *T. radiata*'s *D. citri* killing capacity than parasitism (Chien et al., 1995; Skelley and Hoy, 2004). Camera magnification in this study was insufficient to distinguish between these two parasitoid behaviors. Interestingly, the high numbers of parasitoids filmed in the fall of 2015 was consistent with the large numbers of dead nymphs found among the *D. citri* cohorts filmed in this study (Kistner et al. 2016b). This finding suggests that host feeding by *T. radiata* in the field may be an important, but under

reported source of *D. citri* mortality. Future field video surveillance studies that employ a higher magnification camera could help elucidate under field conditions the *D. citri*-killing capacity of *T. radiata* in terms of both parasitism and host feeding.

In terms of the visitation frequency, spiders, syrphids, and *T. radiata*, were more common visitors to *D. citri* cohorts compared to green lacewing larvae with a ratio being 5:1; 4:1; and 2:1; respectively. While not observed as commonly on *D. citri* patches as syrphid larvae or *T. radiata*, green lacewing larvae consistently inflicted the highest mortality rates of all the natural enemy morphotaxa visiting the no treatment *D. citri* cohorts. Adult lacewings are nocturnal (Duelli, 2001) and were recorded visiting *D. citri* cohorts either at dusk or under complete darkness of the scotophase. However, lacewing larvae were observed attacking immature *D. citri* equally during the photo- and scotophase with peak activity occurring in the morning (3:00–9:00 h) and late afternoon-evening hours (15:00–21:00 h). These findings are consistent with a similar continuous video observation study of aphid predators by Meyhöfer (2001) and previous *D. citri* predator impact studies conducted in Riverside, County California (Goldmann and Stouthamer, 2014; 2015; Kistner et al., 2016b; Schall and Hoddle, 2017).

Interestingly, the adult stage of the most abundant predator taxon of *D. citri* at our study sites, syrphid flies, rely on floral resources (i.e. pollen and nectar) for nutrition. Therefore, the establishment of appropriate flowering plants that provide additional food and shelter to syrphids, and possibly *T. radiata*, may boost the fitness and in turn efficacy of these important *D. citri* natural enemies in citrus ecosystems (Landis et al., 2000; Wilkinson and Landis, 2005; Patt and Rohrig, 2017). For such a management tactic to be successful, flowering plants must be carefully chosen to provide additional resources for the target



natural enemy, and not for pests. Many hover flies and eulophids, like *T. radiata*, discriminate between plants based on floral structure, color, and types of sugars produced (Wilkinson and Landis, 2005; Patt and Rohrig, 2017). Buckwheat, *Fagopyrum esculentum* (Moench), may be a good candidate plant to boost populations of *D. citri*'s natural enemies. Several studies have found that planting insectary strips of buckwheat increase populations of hover flies and lacewings, which lead to further reductions of target aphid pests (van Rijn et al., 2013; Tschumi et al., 2016). In addition, preliminary laboratory studies have found that *T. radiata* that fed on buckwheat exhibited enhanced survival similar to individuals whose diet was supplemented with honey-water (Irvin and Hoddle, unpublished results).

Spiders were the most frequently filmed predatory taxa visiting unprotected *D. citri* cohorts, and the overwhelming majority of visits occurred at night. However, spiders were rarely observed consuming *D. citri* nymphs with only 1.52–8.97% of all visits resulting in a predation event. Video surveillance provides compelling evidence that spiders are not important predators of immature *D. citri* in California; a finding consistent with previous visual predator surveys in Florida (invaded range) (Qureshi and Stansly, 2009) and Pakistan (native range) (Vetter et al., 2013; Khan et al., 2014).

For honeydew producing hemipterans like *D. citri*, population dynamics can be complicated by ant-hemipteran mutualisms. Ant-hemipteran interactions alter the presence and efficacy of natural enemies associated with hemipterans (Hölldobler and Wilson, 1990; Styrsky and Eubanks, 2007). Argentine ants were the most frequently observed arthropods visiting immature *D. citri* cohorts and were often seen tending *D. citri* nymphs. These ants were also filmed forcibly removing natural enemies from *D. citri* colonies. Consequently, *D. citri* cohorts with restricted ant access (i.e. sticky barrier treatment), saw a 306% increase in syrphid larvae activity when assessed across all three study sites. Ants are known to reduce immature syrphid survival and oviposition by female syrphids (Amiri-Jami et al., 2017). Likewise, the number of *T. radiata* visits to *D. citri* cohorts more than doubled when ant access was restricted by sticky barriers. Studies have demonstrated that *T. radiata* parasitism rates decline in the presence of ant mutualists in Florida (Navarrete et al., 2013) and southern California (Tena et al., 2013, 2017; Schall and Hoddle, 2017).

Although our sticky barrier treatment reduced overall ant visitations to *D. citri* cohorts compared to those receiving no treatment, barrier efficacy varied across sites with only a 28.2% decrease in ant visits at Jurupa compared to the 72.4% and 73.8% decreases observed at the Biocontrol Grove and Lochmoor, respectively. Sticky barriers needed constant maintenance as trapped insect carcasses, leaf and twig fragments, and windblown dirt made bridges for ants to cross thereby enabling access to *D. citri* cohorts. Consequently, because of heavy ant activity and frequent bridging events, *T. radiata* was never observed attacking *D. citri* nymphs on sentinel plants deployed at Jurupa. In contrast, the observed 28.2% decrease in ant visits at Jurupa resulted in an 11-fold increase in syrphid activity on *D. citri* cohorts protected by a sticky barrier compared to no treatment cohorts. Furthermore, syrphid predation at Jurupa was only observed when *D. citri* cohorts were protected with a sticky barrier. Ant control using target specific liquid bait delivery systems should be considered an important component of *D. citri* management programs that have a high reliance on natural enemies for population suppression (Schall and Hoddle, 2017).

Intraguild predation can disrupt the efficacy of biological control programs targeting hemipteran pests (Hindayana et al., 2001; Meyhöfer, 2001). While rare, intraguild predation was observed at all three study sites over the course of these 12 month studies. On one occasion, parasitized *D. citri* nymphs, which contained developing *T. radiata*, were consumed by a brown garden snail. Unfortunately, the video surveillance system used in this study was unable to distinguish non-parasitized from parasitized *D. citri* nymphs that had not mummified and darkened. Given the abundance of generalist predators like syrphids and green lacewings on *D. citri* patches, intraguild predation of

developing *T. radiata* larvae and pupae within *D. citri* mummies may be common. For instance, *D. citri* life table analyses found that marginal rates of *T. radiata* parasitism were highest when all other arthropods were excluded (Kistner et al., 2016b). Predator exclusion studies by Michaud (2004) found that intraguild predation by coccinellids kills up to 95% of immature *T. radiata*. Additional manipulative field experiments examining intraguild predation within *D. citri* colonies are needed to clarify the significance of these complex natural enemy interactions.

While video surveillance can collect useful data that other survey techniques cannot, it has several disadvantages that should be noted. A major challenge to using digital video surveillance to monitor natural enemy activity against target pest groups is that processing recorded material requires a major time investment. This occurs because, in the absence of automated machine vision analyses, high levels of human input, such as the use of multiple technicians to review recordings and record data events of interest, are needed (Grieshop et al., 2012). Additional issues identified from our video analyses include camera resolution and magnification, human, weather, and lighting effects. Finally, technical malfunctions such as corrupt digital recorders, storage devices, and batteries that are unable to hold a charge between maintenance visits contribute to data loss.

In spite of these limitations, videography has great potential to allow highly detailed comparisons of natural enemy species composition and activity patterns across different habitats. With respect to the urban citrus ecosystem studied here, comparisons could be made between urban vs. commercial citrus habitats, and this could be refined further into different types of commercial citrus production such as organic vs. conventional, location, and citrus varieties grown. It is possible that natural enemy complexes associated with *D. citri* across these different habitats differ not only in species composition, but potentially in diel patterns of activity as well, which may be influenced by significant differences in temperature and humidity fluctuations over a 24 h period and citrus variety.

The results of this study emphasize the importance of natural enemies in reducing *D. citri* densities. By limiting urban *D. citri* population growth, natural enemies may help reduce the spread of CLAs and ultimately reduce economic losses from HLB in commercial orchards because of reduced vector densities. However, *D. citri* population reduction by natural enemies alone is unlikely to completely halt CLAs spread in California. Argentine ants were the dominant arthropod visiting sentinel immature *D. citri* at all three study sites. These hemipteran mutualists reduced the abundance of natural enemies and suppressed the efficacy of *T. radiata* and syrphids. To reduce disease risk from CLAs, integrated pest management programs should incorporate multiple complementary control tactics including insecticides, natural enemies, and ant suppression (Milosavljević et al., 2017). The use of selective insecticides coupled with judicious application timing and frequency can ensure that both chemical and biological control methods contribute to the suppression of *D. citri* densities. Employing a liquid baiting regime for Argentine ant management has proven to be a highly effective method for improving the biological control of *D. citri* and is recommended in heavily infested areas (Schall and Hoddle, 2017). Efficacy of natural enemies attacking *D. citri* may be further enhanced in urban, organic, and potentially conventional citrus, through the provisionment of floral resources on which predators and parasitoids can feed and shelter.

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