Title

Impact of the Temperature on the Phenology of Diaphorina citri (Hemiptera: Liviidae) and on the Establishment of Tamarixia radiata (Hymenoptera: Eulophidae) in Urban Areas in the Lower Colorado Desert in Arizona

Permalink

https://escholarship.org/uc/item/5k46g2fk

Journal

Environmental Entomology, 48(3)

ISSN

0046-225X 1938-2936

Authors

Gomez-Marco, Francesc
Gebiola, Marco
Baker, Bobby G
et al.

Publication Date

2019-06-01

DOI

10.1093/ee/nvz048

Peer reviewed

Impact of the temperature on the phenology of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae) and on the establishment of *Tamarixia radiata* (Hymenoptera: Eulophidae) in urban areas in the lower Colorado desert in Arizona

Francesc Gomez-Marco[1], Marco Gebiola[1], Bobby G. Baker[2], Richard Stouthamer[1], Gregory S. Simmons[3]

[1]Department of Entomology, University of California, Riverside, CA 92521, USA.

[2]United States Department of Agriculture, Animal and Plant Health Inspection Service, Yuma, Arizona 85364, USA

[3]United States Department of Agriculture, Animal and Plant Health Inspection Service, Salinas, California, USA.

Running title: Phenology of *D. citri* in Arizona

Abstract

The invasive pest *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) was first detected in Arizona in 2009. Since late 2013, the parasitoid *Tamarixia radiata* (Hymenoptera: Eulophidae), the main biocontrol agent of *D. citri*, has been released as part of a biological control program on citrus grown in urban areas of two western Arizona counties in the lower Colorado desert environment. Here we report a three-year survey aimed at assessing the impact of the release of *T. radiata* on the phenology of *D. citri* under these climate conditions. We also monitored the phenology of *D. citri* as part of this assessment on different citrus host species. We show that the high summer temperatures in the Arizona desert halt the development of *D. citri* for about three months every year which appears to have limited the establishment and impact of *T. radiata*. At survey sites distant from release areas the parasitism rates over the season ranged from 0% to 75% and on average peaked around 50% in 2016 but it was low or absent in 2015 and 2017 respectively. We discuss the consequences of this phenology of *D. citri* in the desert areas for the prospects of long-term establishment of *T. radiata* and the management of this key citrus pest.

Keywords: *ACP, biological control, population dynamics, degree-day, citrus*

Introduction

Diaphorina citri Kuwayama (Hemiptera: Liviidae), commonly known as Asian citrus psyllid (ACP), is a worldwide citrus pest that is able to vector the phloem-dwelling bacterium, *Candidatus* Liberibacter asiaticus, which causes a lethal and incurable citrus disease known as citrus greening or Huanglongbing (HLB) (Bové 2006, Grafton-Cardwell et al. 2013). In the United States (USA), *D. citri* has established in almost all the southern states (Alabama, Arizona, California, Florida, Georgia, Louisiana, Mississippi, South Carolina and Texas) and Hawaii (Grafton-Cardwell et al. 2013, Mead and Fasulo 2013). HLB has been detected in all these states except for Arizona, where the vector *D. citri,* was first detected in San Luis in October 2009 without signs of HLB infections (Mead and Fasulo 2013). Following this discovery, a part of Yuma County and an area of 32 Km around Lake Havasu City in Mohave County were immediately placed under Federal quarantine for *D. citri* by the United States Department of Agriculture. While the entire state of Arizona is currently a quarantine area for *D. citri.* the pest is only found in the citrus producing areas in Western Arizona and in some residential areas with backyard citrus (ADA, 2018, USDA 2018).

D. citri populations in commercial citrus are controlled using insecticides. However, the use of insecticides has proven not feasible to control *D. citri* populations in the residential environment because of resistance to ongoing treatments by residents, high costs of ongoing treatments and difficulties of operating long-term pesticide treatments in residential areas (Grafton-Cardwell et al. 2011, Hoddle and Pandey 2014). Consequently, biological control is currently the only viable management option. In an effort to reduce populations of *D. citri* and the possible establishment of HLB in urban areas from impacting nearby commercial citrus, a classical biological control program was started in late 2013. *Tamarixia radiata* (Waterson) (Hymenoptera: Eulophidae), a host specific parasitoid of *D. citri,* was started with releases on residential citrus in two areas of Arizona where the psyllid was originally found, Yuma County and Mohave County (Lake Havasu City). This parasitoid is able to parasitize *D. citri* nymphs from the 3rd to the 5th instar, preferring the last two instars (Chen and Stansly 2014). *Tamarixia radiata* had a significant effect on reducing *D. citri* densities in urban citrus in Southern California (Kistner et al. 2016a, Kistner et al. 2016b), although its impact varied across locations and time.

Abundance and migration data analyses of insect pest populations are key to achieve successful pest management programs (Pedigo and Rice 2014). As temperature drives insect development and behavior, the phenology of its life stages can be predicted by degree-days (DDs) accumulation (Cammell and Knight 1992, Nietschke et al. 2007, Gómez-Marco et al. 2016, Milosavljević et al. 2018). These predictions may be used to control pest populations by targeting specific life stages, thus informing management decisions directed, for example, at enhancing the efficacy of the control agents (Broatch et al. 2006, Logan et al. 2006). Additionally, predicting the pest phenology with DD models integrated on larger databases could improve pest management programs across different geographic regions (Jones et al. 2010), and biocontrol programs in particular, as they may be more affected by climate patterns and how pest population dynamics evolve over time/temperature (Milosavljević et al. 2018).

To evaluate the long-term establishment of *T. radiata* and its impacts on *D. citri* populations in this biological control program there is a need to understand the fluctuations in population dynamics of *D. citri*. Modeling the different *D. citri* life stages most vulnerable to parasitism or predation will result in a more precise targeting of the pest in different locations (Milosavljević et al. 2018). By providing a tool to assist with the development of sustainable management practices, these models based on temperature (DD accumulations) may then help halting the spread and establishment of *D. citri* in Arizona.

In this study we present the results of a three-year evaluation of our work to release and establish *T. radiata* on citrus trees in two urban areas of western Arizona. We document the lack of establishment, parasitism levels and the phenology of *D. citri* populations in these areas on different citrus hosts. Additionally, using DD accumulations, we estimate phenology models for each *D. citri* stage (eggs, nymphs and adults). We then discuss the relevance of our findings and models to the management of *D. citri* in Arizona and how these may limit the success of *T. radiata* as an agent for a classical biological control program in the warmest citrus areas of Arizona.

Material and Methods

Study sites

D. citri populations were monitored on citrus trees in 32 urban sites located in two Counties (Yuma County and Mohave County, Arizona) every year from 1 January 2015 to 31 December 2017 (the two first years of the program, 2013 and 2014, were excluded from the analyses because low number of sites and the surveys started on different dates). The sites from Yuma County were located in three different zones (Yuma Valley [North-West], San Luis [South] and Yuma Gila [North-East]), whereas all the sites from Mohave County were located in Lake Havasu City. Each site had one or two of the following citrus species: grapefruit (*Citrus x paradise* Macfad.), lemon (*Citrus limon* [L.] Osbeck), lime (*Citrus aurantifolia* [Christm.] Swingle), orange (*Citrus sinensis* [L.] Osbeck) and tangelo (*Citrus x tangelo* J.W. Ingram and H.E. Moore)) for a total of 187 study trees (Table 1). All trees were more than five years old and were not sprayed with insecticides throughout the study.

Meteorological data

Hourly air temperatures were obtained from the nearest meteorological station for each site using the data archive of the Arizona Meteorological Network (AZMET, available at: https://cals.arizona.edu/azmet/az-data.htm). The following meteorological stations were used: Yuma Gila, Yuma Valley, Yuma South and Mohave-2. Temperature data were used to calculate the cumulative DD for each *D. citri* stage (see *Data analyses* section).

D. citri population dynamics and phenology model data collection

Trees were monitored monthly at each site throughout this three-year study period. Each tree was divided into four cardinal quadrants (North, East, West and South) that were examined for *D. citri* life stages on each sampling date. At each quadrant, three flushes were randomly selected and the number of *D. citri* eggs (counted and estimated), 1st to 3rd instar nymphs and 4th to 5th instar nymphs, adults (the count of this *D. citri* adults are used only for the calculation of the total population in the Figure S2, *see below*) and the number of nymphs parasitized by *T. radiata* were recorded. Eggs and nymphs counts were used to assess the cumulative *D. citri* egg and instar load for each surveyed tree over time (Ruppel 1983) (see *Data analyses* section). In order to evaluate the *D. citri* adult populations, 64 yellow sticky traps (Scentry Biologicals Inc. [23 x 28 cm], Billings, Montana, USA) were placed on citrus trees (Monzo et al. 2015) at sites about 1.6 Km apart from the survey sites (48 in Yuma County and 16 on Lake Havasu City) and the number of *D. citri* adults were counted and traps replaced every two weeks in Yuma County and every month in Lake Havasu City.

To evaluate the infestation levels of *D. citri* on each individual tree over time, the average monthly numbers of *D. citri* eggs, 1st to 3rd and 4th to 5th instar nymphs, and adult counts (from the yellow sticky traps), were converted to corresponding insect-days (Ruppel 1983, Milosavljević et al. 2018). These data were used to calculate the cumulative population load of each *D. citri* life stage over time for each tree (Milosavljević et al. 2018).

Host preference

To analyze whether *D. citri* populations were affected by host plant species (grapefruit, lemon, lime, orange and tangelo), the maximum *D. citri*-days recorded at each surveyed tree was analyzed by Kruskal Wallis test.

Phenology model

The percentage of total cumulative *D. citri* egg, 1st to 3rd and 4th to 5th instar nymphs and adult days were calculated over each of the three sampling years (2015 to 2017) and then regressed against accumulated daily temperatures to model the population dynamics of each development stage in relation to degree days. Degree days (DDs) accumulations, based upon air temperature at each study location, were calculated for each survey year from a biofix date of 1

January using the direct calculation method (Milosavljević et al. 2018). The lower development threshold values used for *D. citri* eggs, 1st to 3rd nymphs, 4th to 5th nymphs and adults were 8.96, 10.83, 10.7 and 11 ˚C respectively (Liu and Tsai 2000). The upper development threshold values used for *D. citri* eggs, nymphs, and adults were 33, 33, and 41°C, respectively (Liu and Tsai 2000, Hall et al. 2011). Cumulative *D. citri* load based on accumulated DDs was modeled using a sigmoid distribution (Logan 1988):

$$y = N.max * \left(\frac{e^{(b*(x-a))}}{1 + e^{(b*(x-a))}} \right)$$

Where *y* is the percent cumulative *D. citri*-days, *x* is the observed cumulative DDs, *a* is the pivot point for the symmetric sigmoid function, *b* is the slope of the straight line delimiting the exponential period and *N.max* is the theoretical maximum cumulative *D. citri*-days percentage (Table 2). Separate, sigmoid distribution models were obtained for each *D. citri* life stage and year using R (R core development team 2018). Models were fit across all surveyed trees with presence of *D. citri* stages each year as the development of *D. citri* life stages did not differ significantly between citrus species (see results).

Detection time of D. citri populations

The DDs corresponding with the first *D. citri* adult detections on the yellow sticky traps where used to obtain the detection time and quantity of the *D. citri* in the studied areas. Data from 2015 were excluded from this analysis because traps were gradually put in place throughout the year. This data was plotted on maps using the R packages “ggplot2” and “ggmap”.

Mass rearing and releases of T. radiata

Tamarixia radiata wasps were reared at UCR as described by Soper (2014). Briefly, *D. citri* were reared on curry leaf plants, *Murraya koenigii* (L.) Sprengel (Sapindales: Rutaceae).

These wasps were collected from different regions of Pakistan (Hoddle 2012) and the rearing is based on several isofemale colonies to maintain genetic variability (Stouthamer 2015). Beginning in late December 2013, about 1,600-2,000 wasps were shipped from UCR to USDA-APHIS in Yuma every week by overnight mail service for a total release of 366,983 wasps by the end of 2017. *T. radiata* were shipped at a temperature between 15- 18˚C in plastic tubes containing 200 wasps each provided with 70% honey on paper toweling strips. Upon arrival. wasp, releases were made as early in the day as possible. Each vial was then opened inside the foliage of a release tree and wasps were gently tapped out (one release/month/zone). Release sites were located at least ~1.6 Km from the survey sites and yellow sticky traps sites. Each release site had at least two healthy and regularly watered citrus trees per site, had current or recent *D. citri* populations and were not treated with systemic pesticides. If the site was treated with foliar spray, the release was skipped until the following month. After release, the number of wasps dead in the vials was recorded.

Parasitism by T. radiata

Percentage parasitism (total number of parasitized 4th to 5th instar nymphs / [(total number of non-parasitized 4th to 5th instar nymphs + total number of parasitized 4th to 5th instar nymphs) x 100]) was calculated for each study site and date. Sample periods with no *D. citri* 4th and 5th instars nymphs present were excluded from percentage parasitism analyses because *T. radiata* parasitizes almost exclusively these nymphal stages.

Results

Population dynamics of D. citri in Arizona

For each of the surveyed years all *D. citri* stages (eggs, nymphs and adults) were present at two different time periods, from early spring until early summer and in smaller numbers in fall (Fig. 1A, B, C and D). In the first period (spring) the earliest date when the *D. citri* eggs were detected was 48 Julian Days (JD) (February 17) in 2016 and the latest was at 185 JD (July 4) in 2017. *D. citri* nymphs had the same pattern for the same period in spring. Young (1st to 3rd instar) and old (4th and 5th instar) *D. citri* nymphs appeared the earliest at 62 JD (March 2) in 2016. The latest day that young nymphs were present was at 200 JD (July 19) in 2017 and at 172 JD (June 21) for old nymphs (Fig. 1B and C). The abundance of *D. citri* adults differed among years, with 2016 being the year with the highest abundance (Fig. 1D). Adults of *D. citri* were first detected in traps in winter (19 JD; January 19) for all years, but adult populations did not increase until around 60 JD (end of February). The presence of *D. citri* (all the stages) on the second peak (fall) was only noticeable in 2015 (Fig. 1A, B, C, D and Fig. S2). Populations of *D. citri* were extremely low near the limit of detection, during the three months of summer every year (Fig. S2).

Effects of citrus species on the D. citri populations

Although *D. citri* populations (eggs, 1st to 3rd and 4th to 5th instar nymphs) varied across study years (Fig. 1), no significant differences were found within the same life stage among the five host citrus species studied (grapefruit, lemon, lime, orange and tangelo) in terms of maximum cumulative *D. citri*-days from 2015 to 2017 (Fig. 2) (Maximum cumulative eggs-day; $\chi^2 = 3.128$, $P = 0.537$, Maximum cumulative 1st to 3rd instar by variety; $\chi^2 = 3.678$, $P = 0.451$ and Maximum cumulative 4th to 5th instar $\chi^2 = 6.993$, $P = 0.136$).

Detection time of D. citri populations

The distribution of first detection dates (measured in DD) of *D. citri* adults did not show a clear pattern in 2016 and 2017. The earliest *D. citri* adults were detected on four trees, all located in North-West of Yuma area, at 10 cumulative DDs (6 January) in 2016, whereas on one tree, in the same area, at 12 cumulative DDs (5 January) in 2017 (one *D. citri* adult per trap both years). Overall, *D. citri* adults arrived earlier in 2016, at 229.708 ± 37.483 SE cumulative DDs (around 24 February) and 172.25 ± 36.23 SE cumulative DDs (around 17 February) in Yuma County and Lake Havasu City, respectively. In 2017, *D. citri* adults were detected on the yellow sticky traps on average at 826.857 ± 110.203 SE (around 29 April) and 495.125 ± 123.027 SE (around 1 April) in Yuma County and Lake Havasu City, respectively.

Phenology models

The percentage of the cumulative *D. citri* eggs, nymphs and adult days were paired with accumulated DDs for all sampled sites (trees) for the three years of study (Table 2, Fig. 4). The seasonal dynamics of each life stage was dependent of the temperature and time. Logistic models described over 70% of the variation of the cumulative *D. citri* different life stages, except for adults (43%) and 1st to 3rd instars (61%), both in 2015 (Table 2). Fifty percent (the half way point of the exponential increase) of *D. citri* eggs were predicted to occur, from the 1 January biofix point, at 1258 (May 16), 922 (April 22, Fig. 3) and 1291 (May 20) DDs on 2015, 2016 and 2017, respectively (Table 2). The appearance of 50% of 1st to 3rd instars nymphs was predicted to occur at 854 (April 29), 1017 (May 16, Fig. 3) and 1061 (May 20) DDs on 2015, 2016 and 2017, respectively. Similarly, the increase to 50% of the 4th and 5th instar nymphs populations was predicted to occur at 987 (May 10), 985 (May 12, Fig. 3) and 1259 (May 31) DDs on 2015, 2016 and 2017, respectively. For the adults, the appearance of half of the total population was predicted to occur at 752 (April 21), 692 (April 18, Fig. 3) and 890 (May 5) on 2015, 2016 and

2017, respectively (Table 2). The accumulation of 50% of the eggs was predicted earlier in 2016, consistently with the earlier detection of the adults in the same year.

Parasitism by Tamarixia radiata

A total of 82,071, 98,474 and 86,872 *T. radiata* parasitoids were released in 2015, 2016 and 2017 respectively. The shipping mortality of *T. radiata* was always below 7%. Parasitism rates by *T. radiata* on *D. citri* populations were different among years, peaking on average at around 50% from April (90 JD) to June (180 JD) of 2016. The average parasitism in 2016 was 12.52 ± 3.79% (SE) when the suitable hosts (4th and 5th instar nymphs) were present, from beginning of March (62 JD) to end of June (175 JD) (Fig. 5). However, parasitism rates were low in 2015 (~1.4% in fall) (Fig. 5) and no parasitism was recorded in 2017.

Discussion

In this study we examine the population dynamics of *D. citri* in citrus trees from urban areas of the Arizonan deserts, notably showing that *D. citri* populations were at undetectable levels in summer for about three months every year. This disappearance may be due to the usual high summer temperatures in Yuma County and Mohave County (Lake Havasu City) (Fig. S1). Interestingly, Sales et al. (2018) found that experimental heat waves compromise sperm functionality in a model insect *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Consequently, the heatwaves that occur in desert areas, as the ones from our study, may also affect the reproductive success of the *D. citri* adults in summer (Fig. S1).

We also confirmed that the cumulative temporal populations of *D. citri* life stages infesting citrus can be predicted using deterministic DD models (adjusted $R^2 > 0.7$; except for

2015 since surveys and trapping started in spring), confirming the usefulness of this approach. The models developed here suggest a time when each psyllid life stage should likely be abundant. The predictions obtained in our models were similar to those obtained by Milosavljević et al. (2018) for urban citrus trees in Southern California. The main difference is that the exponential increase phase of *D. citri* populations occurred earlier in Arizona (April-May) than in Southern California (May-July) (Milosavljević et al. 2018). As a result, the maximum populations were reached earlier in Arizona than in Southern California. However, as commented above, *D. citri* populations do not increase during summer in the Arizonan desert, as happens in Southern California (Milosavljević et al. 2018). In this three-year survey we found that urban citrus grown in Arizona support lower year-round *D. citri* densities compared to urban citrus grown in Southern California (e.g., Fig. S2). Two factors may explain this: i) *D. citri* populations are extremely reduced in the heat of Western Arizona summers because thermal maximum temperature for development are exceeded; ii) Citrus have reduced flushing periods under these summer weather conditions. These factors make the risk of *D. citri* and the possible harboring of HLB in urban citrus in Yuma County a much less of a threat for the commercial citrus compared to the situation in Southern California.

The phenology of *D. citri* described here and the lack of availability of summer hosts appears to have affected the success of the efforts to establish *T. radiata* in this classical biological control program. In 2016, when *D. citri* was more abundant, parasitism of 4th and 5th nymphs was much higher than in 2015, whereas no parasitism was detected in 2017. These observations are consistent with previous studies that show *T. radiata* parasitism rates vary significantly depending on geographic area, season, and availability of appropriate life stages for parasitism (Tsai et al. 2002, Michaud 2004, Qureshi and Stansly 2009, Kistner et al. 2016a, Milosavljević et al. 2018). The parasitoid was recovered at least once at 23 of 32 survey sites, and in one instance at a location ~24 Km away from the closest release site. Sequencing of a fragment of the mitochondrial gene COI confirmed that *T. radiata* recovered at this non-release and non-survey site were indeed from UCR (data not shown). As the survey sites were distant from the release sites, the 2016 recoveries suggest that *T. radiata* established for part of the year or one winter but it is unclear if they are capable of permanent establishment as shown by the absence of parasitism throughout 2017. This would be consistent with the fact that *D. citri* may not be able to reproduce under the summer desert temperatures, thus depriving the specific parasitoid *T. radiata* of hosts for a prolonged period. Continued surveys in subsequent years of these areas after releases stop may confirm lack of permanent establishment or if *T. radiata* is present in numbers so low as to often be undetectable.

Because of these conditions of few hosts available during the summer months, continuing with release efforts to establish this parasitoid in the hottest Western parts of Arizona is not likely to increase the odds of permanent establishment. After five years of releasing *T. radiata* in this area for a total of more than 460,000 have been released. This is a large number relative to other classical biological control programs and should have been sufficient for establishment. Given that long-term establishment at sufficient parasitoid densities does not appear to be a realistic goal in Western Arizona, other measures for possible control or management for control of *D. citri* in these urban areas could be considered. First, it is not clear that these populations, if unmanaged, represent a significant risk to commercial citrus production. Our data show for two of three survey years *D. citri* numbers were low during the spring months and adult and nymphal populations were at near undetectable levels for all of the three survey years during the summer, a pattern which continued into the fall and early winter of each year. Whether or not these urban populations represent a risk to commercial citrus will depend on how close specific *D. citri* infested urban citrus trees are to production areas, their quality as hosts, and if a given year's environmental conditions favor high *D. citri* population growth. Decisions to manage *D. citri* on urban citrus trees will depend on these factors and the availability of control tools. It is likely that only urban trees nearest to commercial citrus production areas would need management as *D. citri* dispersal distance is limited within a year. In the California program for example, residential buffer treatments on urban citrus trees and ornamental citrus relatives are only applied to urban areas within 400-800 m buffer zone around commercial citrus (CDFA 2018). If HLB were to be detected in urban citrus in these areas that would change this risk appraisal.

The effect of augmentative or inoculative releases of *T. radiata* have not been studied. However, if augmentative or inoculative releases of *T. radiata* were deemed to be an effective management strategy releases should be made, based on the models, when suitable nymph instars are present. DDs models for each *D. citri* life stage can inform the timing of releases of natural enemies or of pesticides treatments (Milosavljević et al. 2018). These pest management choices will target specific *D. citri* life stages to decrease the maximum pest populations. For example, our models suggest that inoculative releases of *T. radiata* in urban citrus desert areas in Arizona should be concentrated before ~984 DDs (i.e., mid-May), when *D. citri* nymphs susceptible for parasitism were already in the exponential population increase phase, and again in the fall ~4113 DDs (i.e., end of October). In addition, using the framework developed by Milosavljević et al. (2018) for California, and applied in this work for Arizona, DDs models could be extended to areas situated in hot desert regions similar to the one studied here. As this approach is applicable to a totally different bioclimatic area, it would be possible to develop a web-based decision support system for *D. citri* management by citrus growers in other similar regions in Arizona, California and Mexico.

Our data also show that *D. citri* do not have a preferred citrus host species, as the phenology and density of *D. citri* eggs and nymphs being observed across sites and years was similar on the five citrus species studied (grapefruit, lemon, lime, orange and tangelo). This confirms previous findings (Tsai et al. 2002, Nava et al. 2007, Kistner et al. 2016a), except for lime (*Citrus* × *aurantiifolia* Swingle), which supported higher *D. citri* densities in a previous survey (Kistner et al. 2016a). The lack of variation in host plant suitability for oviposition and nymph development and attractiveness to adult *D. citri* suggest that DDs models for *D. citri* populations may be developed independently of the citrus species infested by the pest in Arizona. However, cumulative temporal *D. citri* populations consistently coincided with leaf flushing patterns over time in other areas (Hall et al. 2008, Kistner et al. 2016a, Milosavljević et al. 2018) and the availability of young plant tissue on citrus host trees should be taken into account to evaluate *D. citri* population growth in Arizona.

First detection of *D. citri* adults, in 2016 and 2017 (Fig. 3), does not clarify if every year new adults invade the desert areas in Arizona from surrounding areas, or if a few *D. citri* life stages survive the summer temperatures. The rate of increase of *D. citri* population growth may be dependent on the number of adults that overwinter in these areas to start the next generation and these in turn are dependent on how many *D. citri* adults survive the previous summer. We have shown that summer temperatures in this area cause a severe population bottleneck for *D. citri* populations persisting into the fall. Understanding how and where *D. citri* survive during the summer months may be important for managing the risk of *D. citri* near commercial citrus. Trap and visual inspection counts of *D. citri* adults show that while the numbers are extremely low, some adults can be found during the summer months. How they survive is uncertain, though it is known in the native range of *D. citri* in Pakistan that adults manage to survive on citrus where summer temperature can peak at 45˚C (Khan et al. 2014). There is evidence that there may be a period of heat acclimation which allows adult *D. citri* to better adapt for high summer heat survival (Hall and Hentz 2014). This may be occurring in the dry hot desert citrus production areas of Arizona, California and Mexico along with *D. citri* adults finding cooler places with suitable micro-climatic niches within these areas where there may be higher survival. Future surveys will be conducted in such areas to possibly identify niches where *D. citri* may survive.

Acknowledgements

We want to thank all the technical assistance in the field provided by the USDA-APHIS team located in Yuma. We also thank Luis Cayuela for teaching FG how to obtain the models used in this manuscript. This work was supported by grants from the USDA-MAC program and USDA-NIFA Hatch funds to RS

References Cited

[ADA] Arizona Department of Agriculture. 2018. https://agriculture.az.gov/pests-pest-control/agriculture-pests/citrus-pests/asian-citrus-psyllid. Retrieved on December 12, 2018.

Bayles, B. R., S. M. Thomas, G. S. Simmons, E. E. Grafton-Cardwell, and M. P. Daugherty. 2017. Spatiotemporal dynamics of the Southern California Asian citrus psyllid (Diaphorina citri) invasion. PloS ONE. 12: e0173226.

Bové, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

Broatch, J. S., L. M. Dosdall, G. W. Clayton, K. N. Harker, and R.-C. Yang. 2006. Using degree-day and logistic models to predict emergence patterns and seasonal flights of the cabbage maggot and seed corn maggot (Diptera: Anthomyiidae) in Canola. Environ. Entomol. 35: 1166-1177.

Cammell, M. E., and J. D. Knight. 1992. Effects of climatic change on the population dynamics of crop pests. Adv. Ecol. Res. 22: 117-162.

California Department of Food and Agriculture (CDFA). 2018. Action plan for Asian Citrus Psyllid and Huanglongbing (citrus greening) in California. http://www.cdfa.ca.gov/citruscommittee/docs/ActionPlan.pdf. Retrieved on December 27, 2018.

Chen, X., and P. A. Stansly. 2014. Biology of *Tamarixia radiata* (Hymenoptera: Eulophidae), parasitoid of the Citrus greening disease vector *Diaphorina citri* (Hemiptera: Psylloidea): a mini review. Fla. Entomol. 97: 1404-1413.

Gómez-Marco, F., A. Tena, J. A. Jaques, and A. U. García. 2016. Early arrival of predators controls *Aphis spiraecola* colonies in citrus clementines. J. Pest. Sci. 89: 69-79.

Grafton-Cardwell, E., J. Morse, and B. Taylor. 2011. Asian citrus psyllid treatment strategies for California-Arizona. Citrograph. 5: 5-10.

Grafton-Cardwell, E. E., L. L. Stelinski, and P. A. Stansly. 2013. Biology and management of Asian citrus psyllid, vector of the Huanglongbing pathogens. Ann. Rev. Entomol. 58: 413-432.

Hall, D. G., M. G. Hentz, and R. C. Adair. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. Environ. Entomol. 37: 914-924

Hall, D. G., E. J. Wenninger, and M. G. Hentz. 2011. Temperature studies with the Asian citrus psyllid, *Diaphorina citri* Kuwayama: cold hardiness and temperature thresholds for oviposition. J. Insect Sci. 11: 1-15.

Hall, D. G. and M. G. Hentz. 2014. Asian citrus psyllid (Hemiptera: Liviidae) tolerance to Heat. Ann. Entomol. Soc. Am. 107: 641-649.

Hoddle, M. S. 2012. Foreign exploration for natural enemies of Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae), in the Punjab of Pakistan for use in a classical biological program in California USA. Pak. Entomol. 34: 1-5.

Hoddle, M. S., and R. Pandey. 2014. Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina citri* (Hemiptera: Liviidae: Euphyllurinae: Diaphorinini) in California. J. Econ. Entomol. 107: 125-136.

Jones, V. P., J. F. Brunner, G. G. Grove, B. Petit, G. V. Tangren, and W. E. Jones. 2010. A web-based decision support system to enhance IPM programs in Washington tree fruit. Pest. Manag. Sci. 66: 587-595.

Khan, S. Z., M. J. Arif, C. D. Hoddle, and M. S. Hoddle. 2014. Phenology of Asian citrus psyllid (Hemiptera: Liviidae) and associated parasitoids on two species of citrus, kinnow mandarin and sweet orange, in Punjab Pakistan. Environ. Entomol. 43: 1145-1156.

Kistner, E. J., R. Amrich, M. Castillo, V. Strode, and M. S. Hoddle. 2016a. Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of Southern California. J. Econ. Entomol. 109: 1047-1057.

Kistner, E. J., N. Melhem, E. Carpenter, M. Castillo, and M. S. Hoddle. 2016b. Abiotic and biotic mortality factors affecting Asian citrus psyllid (Hemiptera: Liviidae) demographics in Southern California. Ann. Entomol. Soc. Am. 109: 860-871.

Lactin D L, Holliday N J, Johnson D L, Craigen R. 1995. Improved rate of model of temperature-dependent development by arthropods. Environ. Entomol. 24: 68-75.

Liu, Y. H., and J. H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Ann. Appl. Biol. 137: 201-206.

Logan, J. A. 1988. Toward an expert system for development of pest simulation models. Environ. Entomol. 17: 359-376.

Logan, J. D., W. Wolesensky, and A. Joern. 2006. Temperature-dependent phenology and predation in arthropod systems. Ecol. Model 196: 471-482.

Mead, F., and T. Fasulo. 2013. Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Insecta: Hemiptera: Psyllidae). University of Florida, Gainesville.

Michaud, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29: 260-269.

Milosavljević, I., R. Amrich, V. Strode, and M. S. Hoddle. 2018. Modeling the phenology of Asian Citrus Psyllid (Hemiptera: Liviidae) in Urban Southern California: effects of environment, habitat, and natural enemies. Environ. Entomol. 47: 233-243.

Monzo, C., H. A. Arevalo, M. M. Jones, P. Vanaclocha, S. D. Croxton, J. A. Qureshi, and P. A. Stansly. 2015. Sampling methods for detection and monitoring of the Asian citrus psyllid (Hemiptera: Psyllidae). Environ. Entomol. 44: 780-788.

Nava, D. E., M. L. G. Torres, M. D. L. Rodrigues, J. M. S. Bento, and J. R. P. Parra. 2007. Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures, pp. 709-715, J. Appl. Entomol. 131: 709-715.

Nietschke, B. S., R. D. Magarey, D. M. Borchert, D. D. Calvin, and E. Jones. 2007. A developmental database to support insect phenology models. Crop. Prot. 26: 1444-1448.

Pedigo, L. P., and M. E. Rice. 2014. Entomology and pest management. Waveland Press.

Qureshi, J. A., and P. A. Stansly. 2009. Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus. Biol. Control 50: 129-136.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.r-project.org/

Ruppel, R. F. 1983. Cumulative insect-days as an index of crop protection. J. Econ. Entomol. 76: 375-377.

Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, Ł., Hebberecht, L., Thomas, P., Franco, A. and Gage, M.J. 2018. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. Nature communications. 91; 4771.

Soper, A. 2014. Preserving genetic variability in mass-rearing *Tamarixia radiata* to control ACP. Citrograph: 1-6.

Stouthamer, R. 2015. Breeding high quality parasitoids for ACP control. Citrograph: 62-65.

Tsai, J. H., J. J. Wang, and Y. H. Liu. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Fla. Entomol. 85: 446-451.

[USDA, APHIS] United States Department of Agriculture. Animal and Plant Health Inspection Service. 2018. https://www.aphis.usda.gov/aphis/ourfocus/planthealth/plant-pest-and-disease-programs/pests-and-diseases/citrus-health-response-program/ct_citrus_greening

Figure legends

Fig. 1. Population dynamics of means of cumulative insect-days of *D. citri* (A) eggs, (B) 1st to 3rd instar nymphs, (C) 4th and 5th instar nymphs pooled across trees with *D. citri* presence of any stages on each year per site (trees with no presence of *D. citri* throughout each year were not used for this calculation, Table 1) and (D) adults. Immature *D. citri* stages data were obtained

from visual surveys from 2015 throughout 2017, whereas adult data were obtained from yellow sticky traps placed on citrus trees throughout the same time period (2015, n= 24; 2016, n=30; 2017, n=25).

Fig. 2. Mean (±SE) maximum cumulative *D. citri* egg, 1st to 3rd instar, 4th and 5th instar nymphs and adult-days from 2015 to 2017 on five citrus species (grapefruit, lemon, lime, orange and tangelo). No significant differences were found between hosts plants within the same life stage based on the Kruskal Wallis test.

Fig. 3. Detection time (measured in cumulative DDs) of *D. citri* adults to Yuma County and Lake Havasu City (Mohave County), Arizona in 2016 and 2017. The cumulative DDs were calculated with the *D. citri*-adults development thresholds (Liu and Tsai 2000). The DDs continuum was break for each month (2016; January = 70 DDs, February = 265 DDs, March to December > 507 DDs, for 2017; January = 70 DDs, March = 487 DDs, April = 820 DDs and May to December > 1220 DDs). The size of the bubble indicates the number of *D. citri* adults counted at that time.

Fig. 4. Cumulative insect-days for each *D. citri* development stage in relation to cumulative degree-days for 2016. Shown are cumulative insect-days of *D. citri* (A) eggs (B) 1st to 3rd instar nymphs (young nymphs) (C) 4th and 5th instar nymphs (old nymphs) and (D) adults. The points represent the raw data and the curves show the best-fit logistic model for each *D. citri* life stage.

Fig. 5. Percentage parasitism trends for *T. radiata* attacking *D. citri* nymphs on citrus trees from Arizona (Yuma County and Mohave County) in 2015 and 2016. No parasitism was found in 2017. Only trees with suitable *D. citri* nymphal stages are shown in the figure.

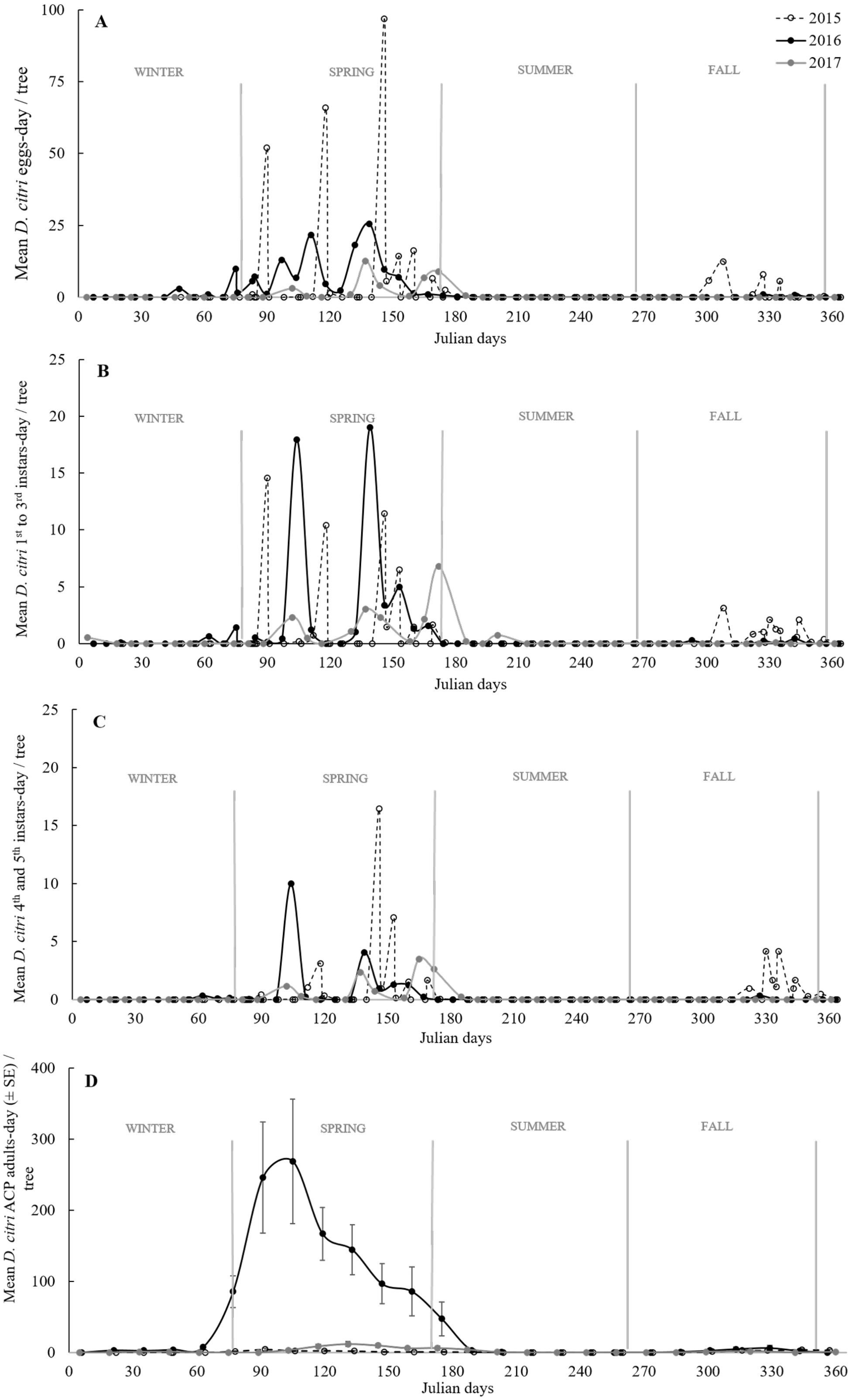
A
Mean D. citri eggs-day / tree
2015
2016
2017
WINTER
SPRING
SUMMER
FALL
Julian days
B
Mean D. citri 1st to 3rd instars-day / tree
C
Mean D. citri 4th and 5th instars-day / tree
D
Mean D. citri ACP adults-day (± SE) / tree
0
30
60
90
120
150
180
210
240
270
300
330
360

Fig. 1.

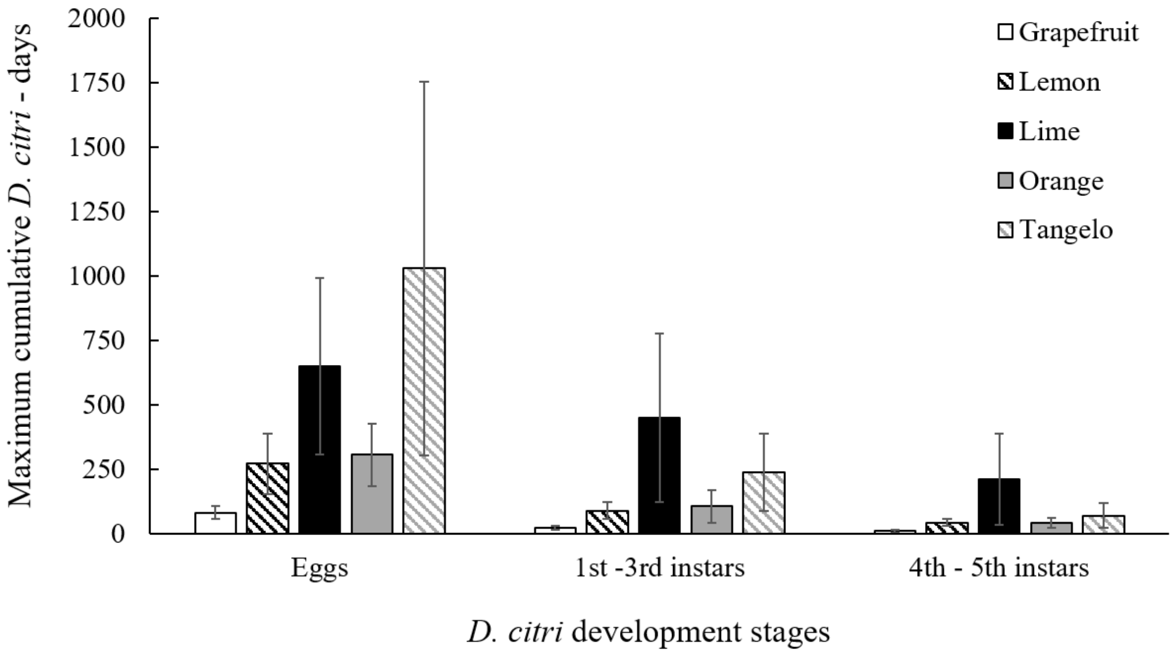
Maximum cumulative *D. citri* - days
2000
1750
1500
1250
1000
750
500
250
0
Grapefruit
Lemon
Lime
Orange
Tangelo
Eggs
1st -3rd instars
4th - 5th instars
D. citri development stages

Fig. 2.

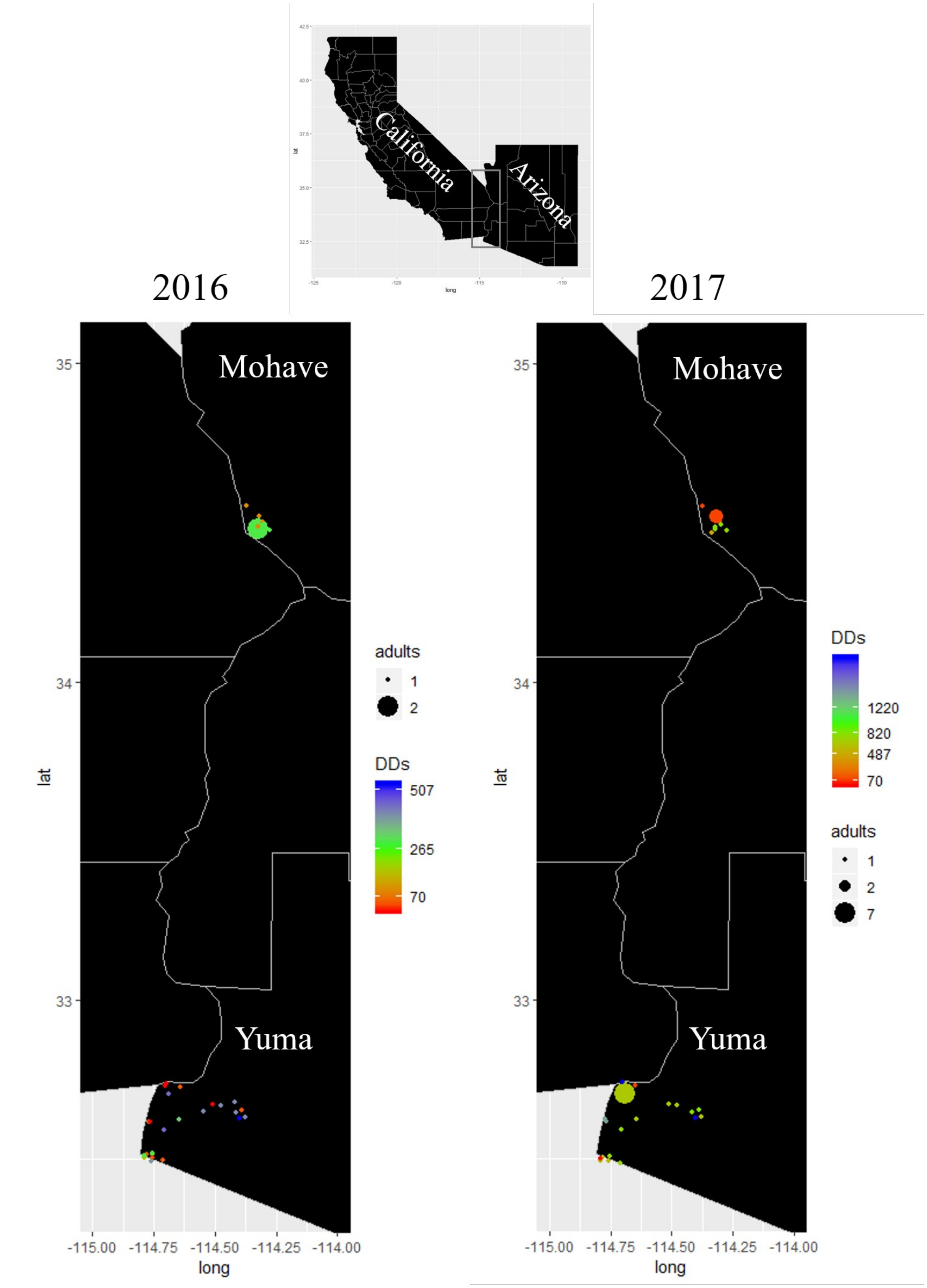
California
Arizona
lat
long
42.5
40.0
37.5
35.0
32.5
-125
-120
-115
-110
2016
2017
Mohave
Yuma
Mohave
Yuma
35
34
33
lat
long
-115.00
-114.75
-114.50
-114.25
-114.00
adults
1
2
DDs
507
265
70
DDs
1220
820
487
70
adults
1
2
7

Fig. 3.

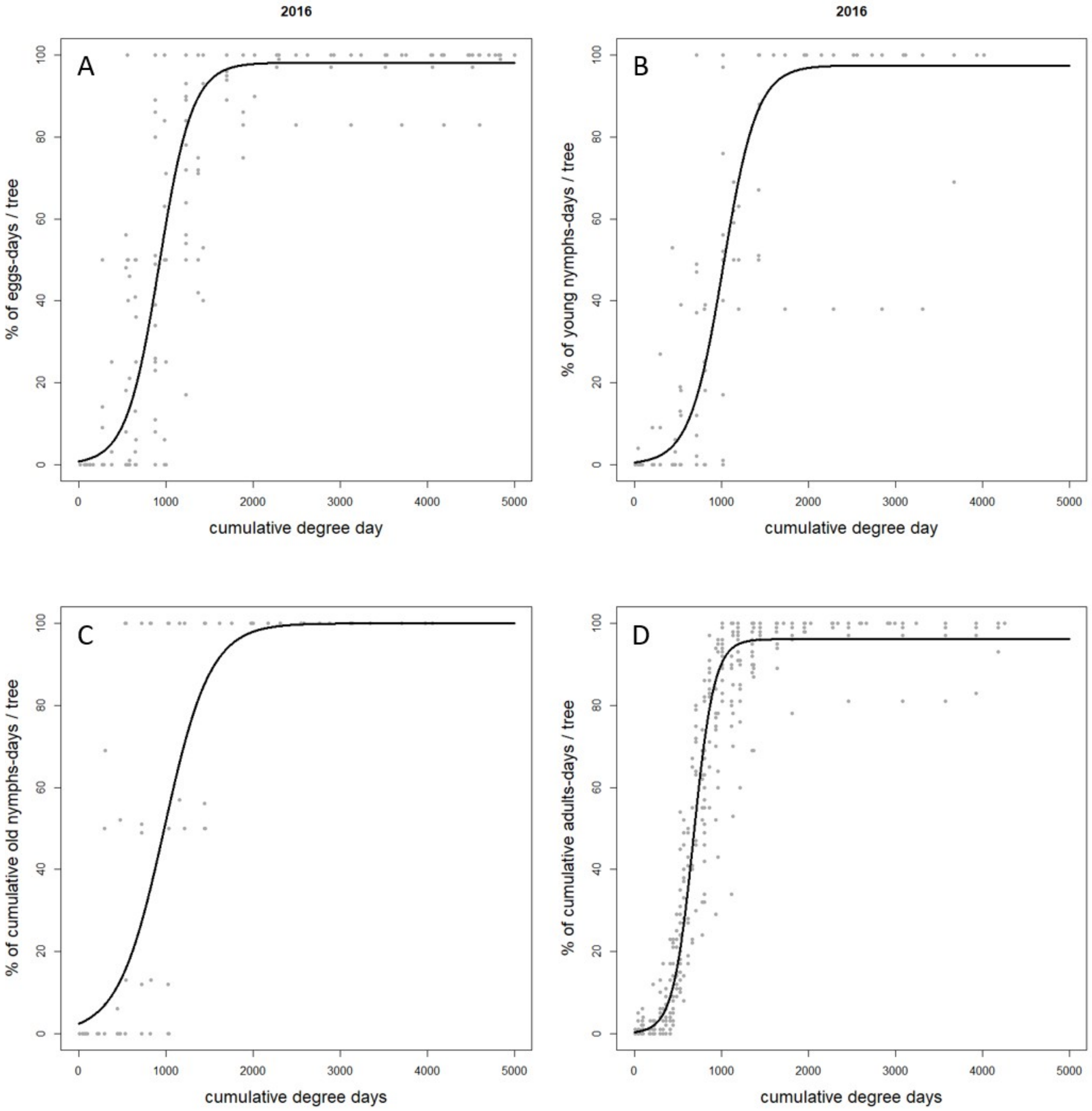
2016
A
% of eggs-days / tree
cumulative degree day
2016
B
% of young nymphs-days / tree
cumulative degree day
C
% of cumulative old nymphs-days / tree
cumulative degree days
D
% of cumulative adults-days / tree
cumulative degree days
0
20
40
60
80
100
0
1000
2000
3000
4000
5000

Fig. 4.

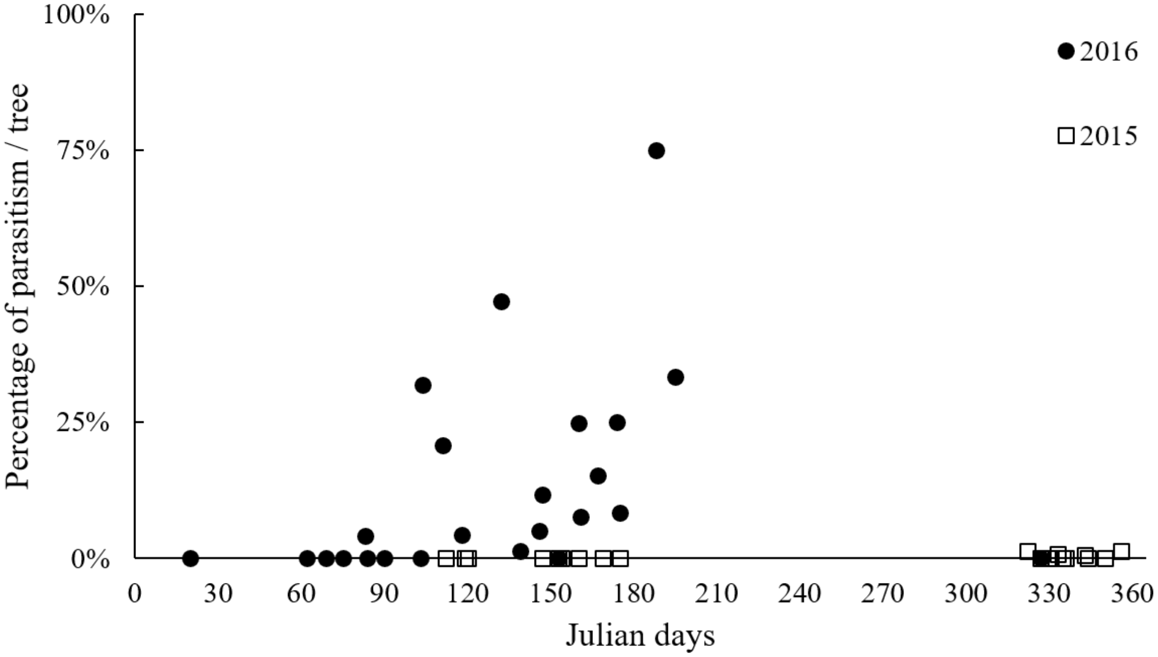
100%
75%
50%
25%
0%
Percentage of parasitism / tree
● 2016
□ 2015
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian days

Fig. 5.

Table 1. Number of trees with presence of *D. citri* stages (eggs, 1^{st} to 3^{rd} and 4^{th} to 5^{th} nymphs) for each citrus species surveyed from 2015 to 2017.

			Number of trees with *D. citri* presence for each stage		
				Nymphs	
Year	Citrus species	Total number of trees sampled / month	Eggs	1st to 3rd	4th and 5th
2015	Grapefruit	17	2	1	1
	Lemon	16	3	3	3
	Lime	6	-	-	-
	Orange	20	5	4	5
	Tangelo	6	3	2	2
	Total percentage (n=65)		20%	15%	17%
2016	Grapefruit	15	11	8	4
	Lemon	18	10	7	4
	Lime	5	4	4	4
	Orange	17	12	5	2
	Tangelo	7	4	4	3
	Total percentage (n=62)		66%	45%	27%
2017	Grapefruit	15	1	3	-
	Lemon	17	6	5	4
	Lime	5	1	1	1
	Orange	17	5	4	5
	Tangelo	6	2	2	2
	Total percentage (n=62)		25%	25%	20%

Table 2. The best-fit parameters of the logistic model for each *D. citri* developmental stage (eggs, 1st to 3rd instars, 4th and 5th instar nymphs and adults)

		Logistic parameters				
year	*D. citri* life stage	b ± SD	a ± SD	R^2	Degree days at N.max	Number of trees
2015	eggs	0.004 ± -	1257.600 ± -	0.79	2615	6
	1st - 3rd instars	0.006 ± 0.0003	853.526 ± 16.638	0.61	1785	5
	4th - 5th instars	0.009 ± 0.0007	987.081 ± 14.542	0.70	1536	6
	adults	0.005 ± 0.0002	751.929 ± 9.172	0.43	1938	20
2016	eggs	0.005 ± 0.0001	921.705 ± 8.831	0.88	1969	30
	1st - 3rd instars	0.005 ± 0.0001	1017.259 ± 9.905	0.90	1975	26
	4th - 5th instars	0.004 ± -	984.661 ± -	0.80	2391	15
	adults	0.000 ± 0.0002	692.105 ± 4.685	0.96	1365	35
2017	eggs	0.008 ± 0.0003	1290.546 ± 14.411	0.93	2500	15
	1st - 3rd instars	0.005 ± -	1061.205 ± -	0.91	2099	14
	4th - 5th instars	0.005 ± -	1259.301 ± -	0.96	2354	12
	adults	0.006 ± 0.0001	890.239 ± 6.791	0.86	2131	29

Supplementary Material

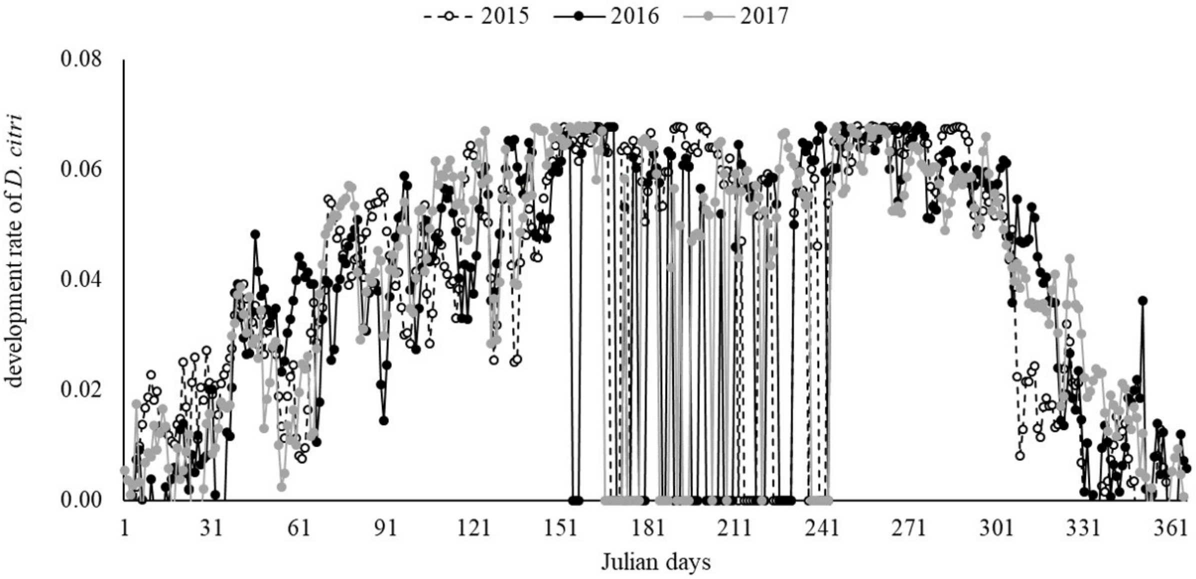

Figure S1. Developmental rate of *D. citri* calculated with the average temperature per day (average of the four zones of study; Yuma Valley, San Luis, Yuma Gila and Lake Havasu City) using the modified nonlinear model equation (Lactin 1995) calculated by Liu 2000 for the combined mature stages of *D. citri* ($p = 0.0777$, $T_{max} = 41.9700$, $\Delta = 12.6581$, $\lambda = -0.0914$, $R^2 = 0.94$). When the maximum temperature per day exceed the $T_{max} = 41.97$ ˚C the development rate was recalculated as 0.

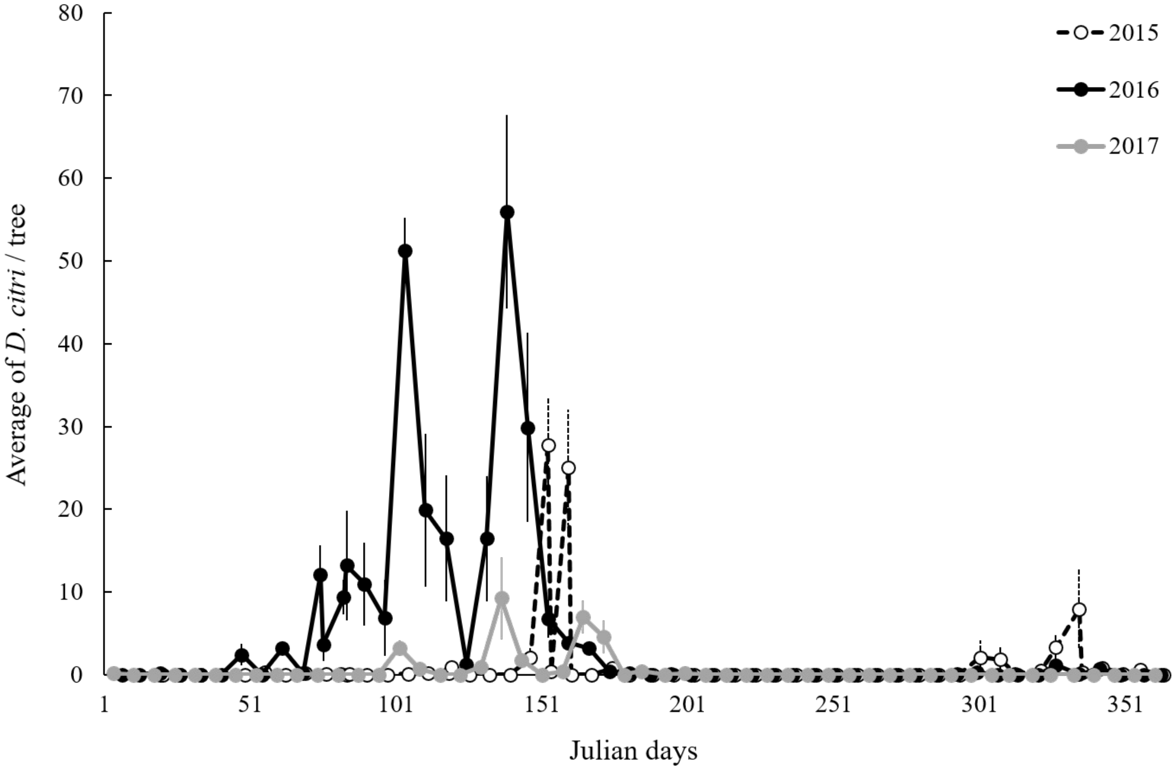

Figure S2. Average (±SE) of the total *D. citri* population (sum of all the stages) per citrus tree (n=~16) from urban areas in the four zones of study (Yuma Valley, San Luis, Yuma Gila and Lake Havasu City) in tree consecutive years (from 2015 to 2017).