



Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) populations in Florida citrus

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ABSTRACT

Management of 'huanglongbing' (HLB) or the Asian form of citrus greening disease caused by the bacterium, *Candidatus Liberibacter asiaticus*, and spread by *Diaphorina citri* Kuwayama depends largely on the management of the vector insect. Seventeen experiments conducted during 2006–2007 included *D. citri* colonies initiated with eggs or first instar nymphs on young shoots protected with sleeve cages, sticky barriers or left unprotected through adult emergence to assess the relative impact of abiotic and biotic factors on psyllid populations. Temperatures dropped to 0 °C and killed most of the shoots and nymphs in February 2007. Spiders (Araneae) and insects in the families Coccinellidae, Blattellidae, Chrysopidae, Formicidae, Syrphidae, Anthocoridae and Miridae were observed on colonies or caught in sticky barriers. The ladybeetles *Olla v-nigrum*, *Curinus coeruleus*, *Harmonia axyridis*, and *Cycloneda sanguinea*, the cockroach *Blattella asahinai*, lacewings, *Ceraeochrysa* sp. and *Chrysoperla* sp., and spiders were most often encountered. Parasitism by *Tamarixia radiata* provided 1–3% nymphal mortality in relation to adult emergence although a maximum of about 25% was observed. Net reproductive rate (R_0), based on observed survivorship and temperature-dependent fecundity data was estimated to be 5- to 27-fold higher in the colonies protected with full cages compared to the unprotected colonies except during the freeze. At the extreme, R_0 was estimated in caged colonies at 125–285 in Jun 2006 whereas all nymphs disappeared in the unprotected colonies. Intermediate results were observed in colonies protected with sticky barriers or coarse mesh cages. These findings suggest that biotic factors play a vital role in regulating populations of *D. citri*. Their elimination through reckless pesticide use could increase pest pressure and enhance the spread of the disease. Therefore, integrated control programs based on conservation of natural enemies of *D. citri* through judicious use of insecticides and releases of new parasitoids are needed for sustainable management of pest and disease.

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1. Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is one of the most important insect pests of citrus in several tropical and subtropical regions of the world, and is also reported from related plant species in family Rutaceae (Halbert and Manjunath, 2004). Shoots with unfolded and tender leaves are needed for oviposition and nymphal development; whereas adults can survive, but not reproduce, on mature leaves (Shivankar et al., 2000; Michaud, 2004; Qureshi and Stansly, 2008). Nymphs and adults directly damage the plants by feeding on the young shoots and leaves, although the primary importance of *D. citri* is as a vector of the bacterium, *Candidatus Liberibacter asiaticus*, one of the causal organisms of Huanglongbing (HLB) or citrus greening disease (Garnier et al., 2000; Bové, 2006). Citrus greening is considered the most important disease of citrus, reducing fruit yield and quality and

severely debilitating citrus trees (Aubert et al., 1996; Roistacher, 1996; Bové, 2006). In the United States, *D. citri* was first discovered in Palm Beach County, Florida on hedges of orange jasmine, *Murraya paniculata* (L.) Jack. (Rutaceae) in 1998 (Halbert, 1998). It is now well established in citrus growing regions of Florida (Qureshi et al., 2009), Texas (French et al., 2001), and has most recently been detected in California (http://www.cdffa.ca.gov/egov/Press_Releases/Press_Release.asp?PRnum=08-057). Citrus greening disease was first identified from citrus groves in south Florida during 2005 (Halbert, 2005) and is spreading rapidly throughout the state (FDACS-DPI, 2008).

The development of *D. citri* is dependent upon temperature, with an optimum range of 25–28 °C (Liu and Tsai, 2000). Under controlled conditions, populations reared on *M. paniculata* at 10 °C and 33 °C failed to develop, whereas survival of the nymphal instars 3–5 was unchanged between 15 and 28 °C. Highest intrinsic rate of increase (0.1999) and net reproductive rates (292.2) were observed at 28 °C. Fecundity increased with increasing temperature, reaching a life time maximum of 748 eggs per female at

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28 °C. Mean developmental period from egg to adult varied from 49.3 days at 15 °C to 14.1 days at 28 °C (Liu and Tsai, 2000).

Biological and chemical methods of pest control and removal of disease-infected trees are tactics being employed to reduce the incidence of the pest and the disease in Florida (Michaud, 2004; Qureshi and Stansly, 2007; Rogers et al., 2008). Biological control has been an important component of citrus insect pest management in Florida (McCoy, 1985) and therefore a thorough understanding of its contribution to mortality of *D. citri* is essential (Stansly and Qureshi, 2008). Although natural enemies were not seen as key factors in regulating populations of *D. citri* in an early study conducted in southern Florida during 1998–1999 (Tsai et al., 2002), Michaud (2001) reported increased populations of a native predatory ladybeetle, *Olla v-nigrum* Mulsant, in response to the *D. citri* invasion. He found coccinellid beetles, *Harmonia axyridis* Pallas, *O. v-nigrum*, *Cycloneda sanguinea* L., and *Exochomus childreni* Mulsant as the most important predators of high-density nymphal populations of *D. citri*, followed by lacewings, *Ceraeochrysa* sp. and *Chrysoperla rufilabris* Burmeister, and the spider (Araneae), *Hibana velox* (Becker) during the course of three cohort studies conducted Sept–Oct 2001 and Jun–Jul 2002 and surveys of three groves in Lake Alfred, FL and one in Ft. Pierce, FL during Feb–Mar 2002 (Michaud, 2004). In a two year study in southwest Florida, *Curinus coeruleus* Mulsant, *H. axyridis*, *O. v-nigrum*, *C. sanguinea* L., lacewings and spiders were found in orange trees infested with *D. citri* (Qureshi and Stansly, 2008).

Tsai et al. (2002) and Michaud (2004) reported only 1–2% parasitism by *Tamarixia radiata* (Waterston), an ectoparasitoid of *D. citri* nymphs imported from Taiwan and south Vietnam, and released in Florida to improve the biological control of *D. citri* (Hoy et al., 1999). Parasitism rates of *T. radiata* calculated based on adult emergence of pest and parasitoid were found to be variable in a study conducted in 28 citrus groves across Florida during 2006–2007, averaging less than 20% during spring and summer but increasing during fall to 39% in Sept. and 56% in Nov in central and southwest Florida, respectively (Qureshi et al., 2009). The entomopathogenic fungus, *Hirsutella* near *citriiformis* Speare, is also known to attack *D. citri* in Florida; however, its contribution is not well documented (Meyer et al., 2007).

Observations of natural enemy impact and the need to develop sustainable pest and disease management programs warranted more detailed investigations to estimate the contribution of natural mortality factors to population growth of *D. citri* in the field. Evaluations through direct observation of insect colonies established under different conditions have proven to be useful in similar investigations (Southwood, 1978; Elkington et al., 1992; Van Driessche et al., 1994; Naranjo and Ellsworth, 2005; Xiao et al., 2007). Net reproductive rate (R_0) or the lifetime reproductive output of a single female is a population parameter often used to analyze the impact of mortality in cohorts (Birch, 1948; Bellows and Van Driessche, 1999; Carey, 2001). The objectives of our studies were to estimate the overall contribution of abiotic and biotic factors to natural mortality of *D. citri* cohorts or generations at different times and simultaneously identify the sources related to such mortality.

2. Materials and methods

2.1. Location and experimental conditions

All experiments were conducted in a 28 ha experimental citrus orchard at the Southwest Florida Research and Education Center, University of Florida – IFAS, Immokalee, FL (26°28'N, –81°26'W) during 2006–2007. The experimental trees were five to ten year old sweet orange *Citrus sinensis* (L.) Osbeck 'Valencia' and were not treated with insecticides before or during the course of the

study. Blocks containing experimental trees were planted at the rate of 323 trees ha⁻¹ on double-row raised beds, irrigated by micro-sprinklers and otherwise subjected to conventional cultural practices (Jackson, 1999). Climatic data were obtained from a weather station located within 500 m of the study blocks (www.fawn.ifas.ufl.edu). Minimum, maximum, and mean daily temperature averaged 9.5–22.7 °C, 23.7–33.6 °C, and 16.5–26.8 °C, respectively, and relative humidity averaged 66–84% during the study period (Fig. 1).

2.2. Cohort establishment and experimental procedures

Experiments were initiated on 23 Jan, 13 Mar, 24 Apr, 8 May, 16 Jun, 3 and 31 Jul, 21 Aug, 18 Sept, 16 Oct, 13 Nov, and 4 Dec in 2006, and 5 Jan, 13 Feb, 13 Mar, 16 Apr, and 11 May in 2007. The first three experiments in 2006 were conducted on 10 year old trees and the remainder on 5–6 year old trees. We use the term 'cohort' for the synchronous age-class of insects in the grove and 'colony' for nymphs of uniform age cohabiting a single shoot. Each experiment began with 20–40 young shoots infested with a colony of *D. citri* eggs or neonates that were examined at least three times a week until no nymphs were left. Shoots were infested with feral populations except when insufficient infested shoots were available for experiments initiated on 13 Nov and 4 Dec 2006 and 5 Jan 2007. At those times, gravid females from greenhouse colonies were caged on the shoots for 24–48 hr to obtain eggs. Infested shoots were selected on multiple trees and covered individually with 15.24 × 30.48 cm (6 × 12 in.) sleeve cages made from fine mesh organdy for 24–48 h to allow for sufficient expansion of tightly appressed leaves in the bud in order to observe all eggs and nymphs. Cages were removed and all eggs and neonate nymphs were counted on each shoot. Older instars were either removed or shoots containing them were not selected. Selected shoots were tagged with flagging tape, numbered, and randomly distributed across one to three exclusion treatments and an unprotected control. Ten infested shoots were allocated to each treatment.

Three exclusion techniques or treatments were used to evaluate the contribution of natural factors, particularly predators and parasitoids to the mortality of *D. citri* populations. Sleeve cages as described above were used to completely protect against predators and parasitoids. The same size cage made with coarse (1 mm²) mesh organdy was employed on 10 colonies in experiments initiated 8 May, 16 Jun, 3 Jul, 31 Jul, and 6 Dec in 2006 and all experiments in 2007 to allow *T. radiata* individuals, but not larger insects to enter the cage. A third exclusion treatment, included from 13 Mar 2006 onward, consisted of a sticky barrier (Tangle foot[®] insect trap coating, The Tanglefoot Company, Grand Rapids, Michigan 49504) applied to a five centimeter portion of the branch to stop crawling insects from reaching the psyllid colonies. The colonies used as control were without any exclusion barrier of any kind (Smith and DeBach, 1942).

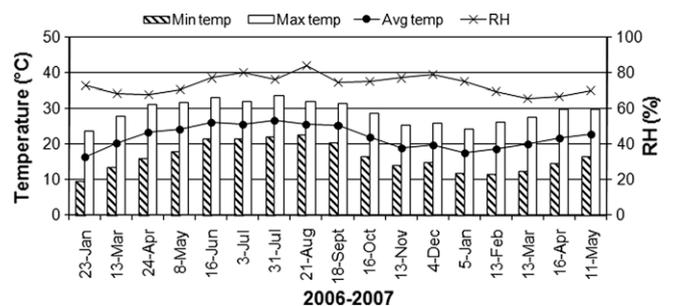


Fig. 1. Minimum, maximum and average temperature (°C) and relative humidity (%) during experiments initiated on different dates, 2006–2007.

Shoots were examined with a 10× hand lens Mondays, Wednesdays and Fridays except on holidays. All eggs, nymphs, and any emerging adults or their exuviae were counted on each observation. Adults, exuviae, and any additional eggs were removed following each observation. Mortality was estimated directly by the disappearance of nymphs or indirectly by their emergence as adults, and considered as generational mortality (Naranjo and Ellsworth, 2005). Any predators or parasitoids observed on the shoots or sticky barrier were counted and removed from the latter. Additionally predator populations were assessed beginning 8 May 2006 on each experimental tree by searching the foliage for 1 min at approachable heights around the canopy.

Overall rates of parasitism by *T. radiata* within study groves were assessed by collecting 4th and 5th instar *D. citri* nymphs that were reared through to emergence of adult psyllids or parasitoids in the laboratory. Shoots containing the nymphs were held in glass cylinders with the stems in water and covered with ventilated containers held at ambient temperature in the laboratory for at least 2 weeks to allow adults of *D. citri* and *T. radiata* to emerge.

2.3. Statistical analysis

Survival for each colony was calculated as: (no. adults emerged/max. no. immatures observed) × 100. The net reproductive rate (R_0) was calculated for each colony as the product of nymphal survival to adulthood and fecundity (Bellows and Van Driesche, 1999; Xiao et al., 2007) assuming a sex ratio of 1:1 using fecundity at the appropriate temperature range as reported by Liu and Tsai (2000). Mean generation time (T) was estimated as the sum of egg incubation time (Liu and Tsai, 2000) and nymphal developmental time through 50% adult emergence as observed in each colony, plus the pre-reproductive period as reported by Wenninger and Hall (2007). The rate of nymphal development to adulthood for colonies that developed in full cages and were therefore not influenced by predators or parasitoids was calculated as the reciprocal of days to 50% adult emergence, and analyzed for relationship with temperature.

Numbers of immature and adult predators were combined to compare relative abundance of different groups (ladybeetles, lacewings, and spiders) and ladybeetles species (*C. coeruleus*, *H. axyridis*, *O. v-nigrum*, and *C. sanguinea*). Data were transformed by $\log(x + 1)$ for analysis. Parasitism rates (%) from field collected laboratory reared nymphs were calculated as: (no. adults emerged *T. radiata*/total no. adults emerged *D. citri* + *T. radiata*) × 100 (Stansly et al., 1997; Qureshi et al., 2009). A comparable index was calculated from the number of mummies and adult psyllids developing in colonies in the field.

Data were analyzed for normality using the Shapiro-Wilk W test and normality plots using the univariate procedure (Shapiro and Wilk, 1965; Shapiro et al., 1968; SAS Institute, 2004). Data with normal distribution were analyzed using analysis of variance (ANOVA) and GLM procedure (SAS Institute, 2004). Predator data collected over time on the same trees during a cohort were analyzed using repeated measures ANOVA and PROC MIXED procedure (SAS Institute, 2004). Treatment means were compared using the least significant difference (LSD) at a significance level of 0.05 (Littell et al., 1996). Non-normal data were analyzed by using the non-parametric Kruskal–Wallis test and pairwise treatment comparisons made with the Mann–Whitney U -test (Hollander and Wolfe, 1973; SPSS, 2004). Actual means ± SEM are presented for all data.

3. Results

3.1. Cohort survival

Young nymphs generally disappeared rapidly from unprotected colonies causing rapid divergence from the situation in small mesh

cages, most notably in 2006 (Fig. 2). Differences during 2007 were less pronounced, and non-existent during the February cohort when most flushes and nymphs were killed by a freeze during which temperatures dipped to 0 °C for two successive nights during the experiment. Most loss in unprotected colonies resulted from the disappearance of instars 1–3 which took about 12 days to develop (Fig. 2). Disappearance of these nymphs by day 12 was more marked (76–100%) between Jan and Oct 2006 than Nov–Dec (29–31%). More nymphs were lost by day 12 during 2006 (76–92% Jan–May) than the same period in 2007 (36–76%) when insecticide use on citrus had accelerated in the region. Few nymphs were found trapped in the sticky barrier indicating that losses were not due to dispersal (data not presented).

3.2. Population parameters

Net reproductive rate (R_0), estimated for colonies protected with a full cage, averaged 191.1 ± 13.2 and ranged from a low of 73.7 in Jan 2006 to a high of 285.2 in Jun 2006, discounting the frozen cohort Feb 2007 (Table 1). In comparison, R_0 in unprotected colonies averaged 28.1 ± 5.2 , ranging from a low of zero in Jun 2006 to a high of 64.3 in Mar 2006 and Apr 2007. R_0 was significantly lower for all unprotected colonies compared to full cage colonies except Feb 2007 during the freeze (Table 1). Differences for the remaining colonies averaged 163 ± 15.3 with highest 285.2 in Jun 2006 when there was no survival in unprotected colonies and 161.3 in May 2007.

Net reproductive rate in colonies protected by sticky barriers and partial cages were generally intermediate between unprotected and full caged colonies (Table 1). Differences between sticky barrier and uncaged colonies were significant for 8 out of 16 experiments and between sticky barrier and full cage colonies for 13 out of 16 experiments. Similarly, 5 out of 10 and 6 out of 10 comparisons between partial and full cage colonies and between partial and no cage colonies, respectively, were significant. Four of 10 comparisons, all in 2006, between partial cage and sticky barrier exclusion were significant with higher R_0 in the partial cage, demonstrating more protection with this method.

The time required for 50% adult emergence varied with average temperature ($R^2 = 0.71$; $F = 35.85$; $df = 1, 16$; $P < 0.0001$), resulting in short generation times during warm seasons. Generation times were shortest (mean ± SEM = 18.8 ± 0.5 days) in Aug 2006 and longest (mean ± SEM = 39.03 ± 1.6 days) in Nov–Dec 2006 excluding the Feb 2007 cohort ($H = 121.57$; $df = 16$; $P < 0.0001$). Caged colonies consistently took more time to terminate than unprotected colonies (Fig. 2) despite expected higher temperature in the cages, probably because fewer individuals survived to emergence in unprotected cohorts.

3.3. Natural enemies

3.3.1. Predators

Spiders and insect predators in the families Blattellidae, Coccinellidae, Chrysopidae, Formicidae, Syrphidae, Anthocoridae and Miridae were observed on colonies or trapped in sticky barriers (Fig. 3). The Asian cockroach *Blattella asahinai* Mizukubo was the most often encountered species trapped in sticky barriers but never seen in colonies, presumably because of its nocturnal habits and residence in the litter during the day. Spiders were observed with about equal frequency trapped in sticky barriers or on colonies, whether uncaged or protected by barriers, which they presumably accessed by dropping from above or bridging. The lacewings *Ceraeochrysa* sp. and *Chrysoperla* sp. were most often seen as adults or larvae in control colonies or adults in sticky barriers, indicating that adults both walked and flew to psyllid-inhabited flush. Ants were only seen in unprotected colonies or trapped

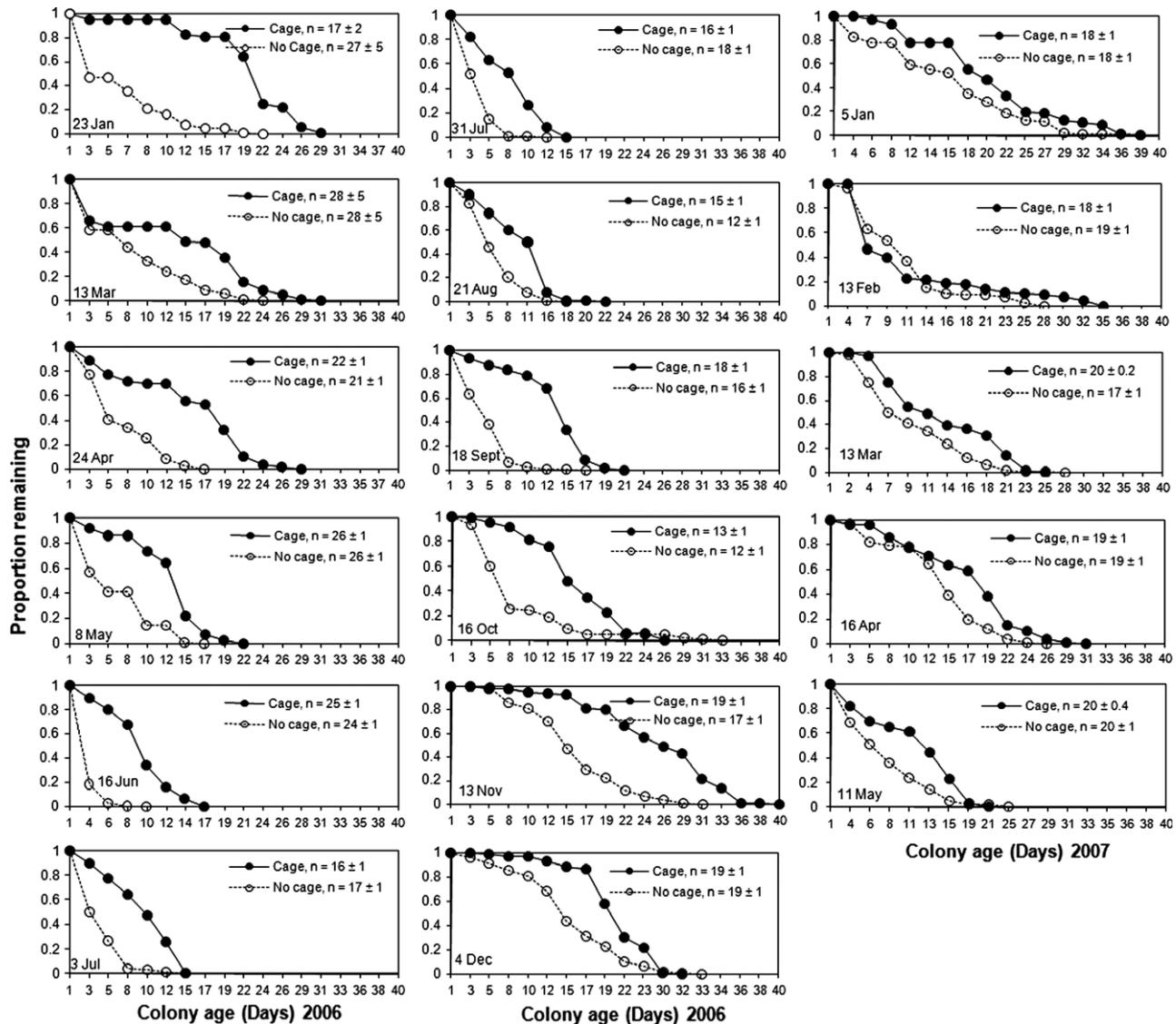


Fig. 2. Mean number of *Diaphorina citri* nymphs remaining through adult emergence in colonies established on shoots of *Citrus sinensis* that were fully caged or not caged (no exclusion barrier) to assess the impact of natural mortality factors on psyllid populations, 2006–2007. Dates in the figures indicate initiation of the experiment.

in sticky barriers which were effective in excluding them. Ladybeetles comprised of the species *O. v-nigrum*, *C. sanguinea*, *C. coeruleus*, and *H. axyridis* were the most frequently encountered group. Ladybeetle larvae were seen in control colonies and trapped in sticky barriers, but not in colonies protected by sticky barriers, indicating that they were unable to transverse this impediment.

The relative number of adults captured in sticky barriers was greatest for *C. sanguinea*, the species least well adapted to a psyllid diet (Michaud and Olsen, 2004). In contrast, *O. v-nigrum*, known as a psyllid specialist, was most predominantly observed as larvae in control colonies. More ladybeetle adults were trapped in sticky barriers than adults or larvae observed on colonies except *O. v-nigrum* for which more larvae were seen on the colonies (Fig. 3).

Ladybeetles were more abundant than lacewings and spiders among the three commonly observed groups during the 1 min visual search of tree canopy except in Aug and Dec 2006 when no differences were observed ($P = 0.05$, Fig. 4A). Numbers of ladybeetles declined from May through Aug and only a few were seen the rest of the year. In 2006, *O. v-nigrum* and *C. coeruleus* were the most commonly observed species followed by *C. sanguinea* and *H. axyri-*

dis (Fig. 4B). In spring 2007, *O. v-nigrum* was the only commonly observed species, with others present at low levels.

3.3.2. Parasitoids

Tamarixia radiata was the only parasitoid that emerged from *D. citri* nymphs. Parasitism in colonies was observed during Nov–Dec 2006 and Apr–May 2007, although a mere 1–3% of all nymphs were parasitized in any one experiment. This corresponded to 2–24% when considered relative to adult emergence. The greatest share of parasitism (%) occurred in colonies protected by sticky barriers, averaging 9.5 ± 9.5 and 4.3 ± 4.3 in Nov and Dec 2006 and 15.9 ± 8.2 and 24.4 ± 8.7 in Apr and May 2007. In contrast, parasitoids accounted for 2.5–6.3% emergence from unprotected colonies in Dec, Apr and May with none seen in Nov. Parasitized nymphs were observed in only one partial cage during Dec, accounting for $2.5 \pm 2.3\%$ of total emergence. Parasitism rates from field collected laboratory reared nymphs ranged between 0% and 14% (average = 4%) in spring and summer and 0–45% (average = 26%) in fall (Table 2). In 2006, parasitism declined from 14% in May to 2% in Jul and was not observed again until fall when

Table 1

Net reproductive rate (Mean ± SEM) of *Diaphorina citri* calculated based on fecundity (Liu and Tsai, 2000) and survival of nymphs in colonies that were unprotected (no exclusion barrier) or protected with full cages, partial cages and sticky barriers to assess the impact of natural mortality factors on psyllid populations, 2006–2007.

Cohort initiation date ^a	No exclusion	Full cage exclusion	Partial cage exclusion	Sticky barrier exclusion	F or H	P
2006						
23-Jan	22.57 ± 7.53b	73.71 ± 19.20a	–	–	4.55	0.0329
13-Mar	64.27 ± 20.00b	169.84 ± 22.54a	–	125.83 ± 26.53a	7.12	0.0053
24-Apr	25.66 ± 12.39b	170.87 ± 38.43a	–	116.59 ± 23.80a	10.63	0.0009
8-May	52.66 ± 31.14c	239.23 ± 10.44a	188.16 ± 19.05b	62.78 ± 18.88c	22.9	<0.0001
16-Jun	0.00d	285.20 ± 13.84a	205.85 ± 32.59b	125.28 ± 31.83c	25.22	<0.0001
3-Jul	37.17 ± 13.87c	236.29 ± 10.34a	158.89 ± 26.04b	68.38 ± 16.43c	25.37	<0.0001
31-Jul	6.41 ± 4.35c	244.16 ± 26.57a	165.40 ± 34.78ab	135.01 ± 44.06b	10.27	0.0001
21-Aug	25.91 ± 11.49b	174.53 ± 27.90a	–	61.94 ± 24.16b	12.58	0.0004
18-Sep	4.47 ± 4.47c	261.75 ± 21.80a	–	105.60 ± 29.99b	30.87	<0.0001
16-Oct	14.00 ± 10.27c	170.46 ± 18.11a	–	96.99 ± 20.78b	25.32	<0.0001
13-Nov	13.24 ± 8.54c	194.07 ± 16.46a	–	84.01 ± 28.79b	22.58	<0.0001
4-Dec	33.05 ± 19.30b	176.53 ± 21.24a	139.14 ± 21.30a	37.87 ± 13.97b	16.88	<0.0001
2007						
5-Jan	50.74 ± 20.17b	190.15 ± 25.38a	105.16 ± 24.99b	45.43 ± 16.27b	9.3	0.0002
13-Feb	8.65 ± 8.65a	13.59 ± 9.47a	38.70 ± 20.32a	35.43 ± 13.19a	1.32	0.2868
13-Mar	7.89 ± 4.09c	131.46 ± 26.89a	65.06 ± 22.77bc	68.90 ± 23.89b	6.21	0.0024
16-Apr	64.34 ± 20.80b	150.83 ± 18.49a	99.90 ± 29.36ab	66.43 ± 18.41b	3.36	0.0334
11-May	27.64 ± 8.74b	188.94 ± 26.76a	51.13 ± 14.81b	45.30 ± 14.70b	15.35	0.0015

^a Data for colonies initiated on 8 and 11 May and 3 Jul analyzed using Kruskal–Wallis test followed by Mann–Whitney U test, all others using normal ANOVA followed by LSD test. Means within a row followed by a different letter are significantly different (P = 0.05). –, no treatment.

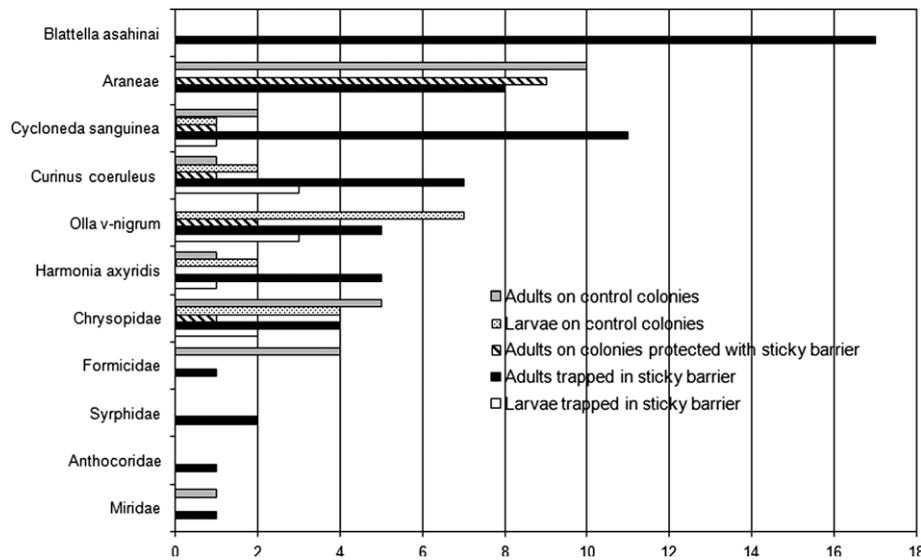


Fig. 3. Total number of predators observed on colonies of *Diaphorina citri* nymphs on *Citrus sinensis* shoots or trapped in the sticky barriers used to protect colonies initiated on different dates, 2006–2007. Note: *Blattella asahinai* and *Araneae* data presented as adults include some juveniles not recorded as separate age class.

26–45% was observed in Nov–Dec (Table 2). This was followed by a decrease to 4% in Jan 2007 and a slight increase to 9% in May.

4. Discussion

Impressive differences were observed in survival and consequently in estimated net reproductive rate between unprotected colonies compared to colonies protected with fine mesh cages. Sticky barriers and coarse mesh cages provided partial protection. These results suggested a leading role for predation among mortality factors impacting populations of *D. citri* (Table 1). Estimated R_0 for completely protected colonies compared to unprotected colonies was greater by an average 5-, 27-, 11-, and 6-fold during Jan–May, Jul–Sept, Oct–Dec in 2006 and Jan–May of 2007, respectively. Thus, *D. citri* showed great potential for population increase

in the absence of mortality factors impeded by the cage. Sticky barriers reduced R_0 an average 3-, 12- and 5-fold between Jan–May, Jul–Sept, and Oct–Dec of 2006 and 3-fold between Jan and May of 2007 compared to the unprotected colonies. This constituted an approximately 50% reduction compared to populations protected with complete cages and was attributable to predators that were observed on the colonies or caught in the sticky barrier (Fig. 3). The partial cage treatment was introduced to admit parasitoids but exclude predators. However, parasitism was negligible in control colonies and partial cages. Nevertheless, estimated R_0 was 4-fold greater in coarse mesh cages than unprotected colonies in May, Jul, and Dec 2006 and Jan–May 2007, and 26-fold greater in Aug 2006. The cause was mortality inflicted by small predators that penetrated the coarse mesh and were seen on the colonies such as early instar ladybeetle larvae and small spiders. Increased

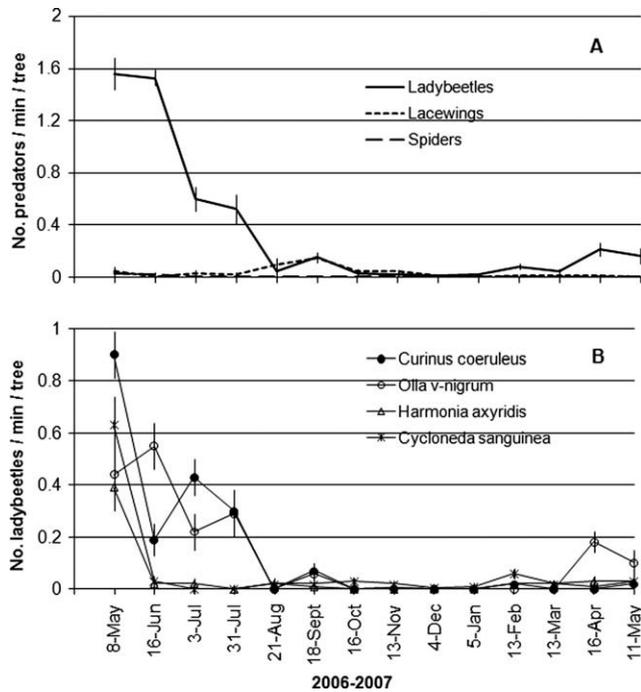


Fig. 4. Mean (\pm SEM) numbers of spiders, lacewings and ladybeetles per 1 min observation of foliage around *Citrus sinensis* trees that contained colonies of *Diaphorina citri* nymphs initiated on different dates, 2006–2007.

Table 2

Percentage parasitism by *Tamarixia radiata* from 4th and 5th instars nymphs of *Diaphorina citri* collected from *Citrus sinensis* trees in the experimental blocks and reared in the laboratory through adult emergence, 2006–2007.

Collection date	<i>D. citri</i> nymphs instars 4–5	Adults emerged			Parasitism ^a (%)
		<i>D. citri</i>	<i>T. radiata</i>	Total	
5/15/2006	238	31	5	36	13.89
5/19/2006	170	37	3	40	7.50
6/21/2006	120	47	2	49	4.08
7/10/2006	92	59	1	60	1.67
8/9/2006	120	27	0	27	0.00
8/23/2006	300	60	0	60	0.00
9/18/2006	41	16	0	16	0.00
11/14/2006	50	17	14	31	45.16
11/20/2006	68	17	6	23	26.09
12/1/2006	150	23	0	23	0.00
12/20/2006	482	88	44	132	33.33
1/10/2007	287	106	4	110	3.64
3/13/2007	192	42	0	42	0.00
4/16/2007	199	36	3	39	7.69
5/19/2007	215	31	3	34	8.82

^a Parasitism (%) calculated as the ratio of number of emerged adult *T. radiata* to the total combined number of adult *D. citri* and *T. radiata* that emerged from samples of 4th and 5th instars nymphs (Stansly et al., 1997; Qureshi et al., 2009).

R_0 seen in response to partial or complete exclusion reflected the action of some or all components of the predator complex on *D. citri* populations.

Temperature drove nymphal development rate but had little effect on mortality except for the freezing event in Feb 2007. Average temperatures during the experiments were within the range found suitable for survival and development of *D. citri* in the laboratory (Liu and Tsai, 2000). These authors reported a decline in the survivorship of young instars at temperatures above 28 °C. Very few dead nymphs were observed between Jun and Sept when maximum temperatures reached 32–33 °C, although some dead nymphs may have fallen off the shoots. Furthermore, we have ob-

served nymphs and adults of *D. citri* developing and surviving at temperatures between 35 and 40 °C in the greenhouse (J.A. Qureshi, personal observations). In contrast, different levels of protection resulted in great differences in survivorship, indicating that most mortality was biotic.

Ladybeetles were the most frequently observed visitors to colonies although spiders, cockroaches, lacewings, ants, syrphids, minute pirate bugs and mirids were also seen in the colonies or caught in sticky barriers. Ladybeetles were most common in the spring, declining during summer, and largely absent during winter (Fig. 4) in concert with flushing patterns and psyllid abundance (Qureshi et al., 2009). A reduction in ladybeetles and consequently psyllid mortality observed in 2007 compared to the previous year may have been due to the increased foliar applications of insecticides against *D. citri* that negatively impact beneficial insects (Rogers, 2008).

The four ladybeetle species observed, *O. v-nigrum*, *C. sanguinea*, *C. coeruleus* and *H. axyridis*, are well documented predators of psyllid immatures. Michaud and Olsen (2004) showed that *O. v-nigrum*, *C. coeruleus* and *H. axyridis* developed and reproduced successfully on *D. citri* nymphs obtained from colonies on *M. paniculata*, whereas, *C. sanguinea* developed successfully but could not reproduce as adults. The greater abundance of *O. v-nigrum* compared to the other three species observed through presence of its larvae on the unprotected colonies and its populations in both years suggest dominance among Coccinellidae as predators of *D. citri* immatures. Survival of psyllid nymphs was reduced to zero in Jun 2006 when *O. v-nigrum* was most abundant (Fig. 4). Populations of this species were reported to increase in response *D. citri* invasion in Florida (Michaud, 2002). More *C. sanguinea* adults trapped in the sticky barriers than seen on the colonies indicated that they may walk away from infested shoots more often than other species. Large differences in R_0 between unprotected and protected colonies were observed in summer, and were probably due to combined efforts of ladybeetles with other predators which were common late in the season. Sticky barriers provided significant protection during this period, confirming that crawling predators were still active and likely responsible for the differences noted.

Spiders were another dominant group of predators observed in equal numbers on colonies and sticky barriers, although infrequently noted by visual observation of trees due to their cryptic habits. Spiders were seen in equal numbers on unprotected colonies and colonies protected with sticky barriers, indicating their arrival by dropping down, ballooning or bridging. Spiders were reported to feed on citrus psyllids by Shivankar et al. (2000) and Michaud (2002, 2004) signaled salticids and anyphaenids as possibly important predators of immature *D. citri*. Eighteen species of spiders, mostly from the family Salticidae, were identified as predators of *Trioxa erytrae* (DelGuercio) also known as African citrus psyllid (Hemiptera: Trioziidae) (Van den Berg et al., 1992). Reduced survival in the unprotected colonies at times when ladybeetles and lacewings were relatively scarce indicated that other predators such as spiders inflicted significant mortality.

The Asian cockroach, an introduced insect detected in Florida in 1986 and established in Georgia, Alabama, and Texas (Snoddy and Appel, 2008; Pfannenstiel et al., 2008) was the most frequently trapped insect in sticky barriers. The cockroaches live in the litter and move into the canopy at night where they may feed on young flush (Stansly et al., 1999). Asian cockroach has been reported to prey on brown citrus aphids in citrus (Persad and Hoy, 2004) and eggs of lepidopteran pests in cotton and soybean in Texas (Pfannenstiel et al., 2008). We observed very little impact on eggs and early instar nymphs of *D. citri* in cage studies (J.A. Qureshi, unpublished data) but further investigations are in progress. Other predators in the families Syrphidae, Anthocoridae and Miridae were seen in very small numbers on colonies or in sticky barriers.

The overall impact of parasitism by *T. radiata* was a mere 1–3% of the total mortality and therefore had a negligible effect on net reproductive rate. Parasitism was most frequently observed in colonies protected by sticky barriers, accounting for 24% of all adults emerged in May 2007. These results mirrored those of Michaud (2004) who observed less than 2% mortality from parasitism in unprotected colonies, but 20 times more in colonies protected from predators by open (2 mm) mesh cages, although overall mortality was less than in unprotected colonies. We used a smaller (1 mm) mesh that was penetrated only once by ovipositing *T. radiata*. However, the sticky barrier also appeared to provide partial protection from intraguild predation with a similar increase in parasitism but decrease in overall mortality. Nevertheless, parasitism rates seen from nymphs samples collected from the experimental blocks were generally higher than even colonies partially protected by sticky barriers, indicating that intraguild predation in general may be less prevalent than observed in the colonies. Parasitism rates from reared samples agreed with results from a state-wide study showing that parasitism rates were variable and generally low (<20%) during spring and summer but increased to 39–56% in fall (Qureshi et al., 2009). This finding warranted ongoing foreign exploration and possible augmentative releases of *D. citri* parasitoids.

Our results suggest that natural mortality factors, particularly predators and to lesser extent parasitoids, impose significant mortality on populations of *D. citri*. The estimated net reproductive rates based on fecundity data from a laboratory study (Liu and Tsai, 2000) are probably over-estimated given that adult survivorship and thus fecundity is likely to be lower in the field. In spite of biotic mortality, surviving populations have proved sufficient to cause economically significant disease transmission. On the other hand, elimination of biotic mortality by intensive use of insecticides could require an impractical level of efficiency against increased incidence of pest and disease that might not be cost effective and would likely be inductive of secondary pest outbreaks. A preferable alternative is conservation and enhancement of biotic mortality through the use of selective insecticides, application methods and timings, coupled with inoculative and/or augmentative release of natural enemies. Examples of selective insecticide use include (1) soil-applied systemic insecticides that avoid direct contact with natural enemies (Qureshi and Stansly, 2007, 2008), (2) dormant sprays directed at adults when trees are not flushing and predators largely absent (Stansly and Qureshi, 2008), and (3) selective insecticides such as horticultural mineral oils, lipid synthesis inhibitors and spinosyns (Stansly et al., 2002). At the same time, biological control might be enhanced by inoculative releases of new strains or species of parasitoids better suited to Florida conditions, augmentative release of *T. radiata* especially in spring, and possibly application of entomopathogens such as *Paecilomyces fumosus* and *Beauveria bassiana* (Hall, 2008; Qureshi et al., 2009). Sustainable management of psyllid and citrus greening will require an integrated strategy that includes biotic mortality components.

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