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# Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida

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## Abstract

The survival rate of nymphs of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, was estimated in central Florida citrus groves by reiterative, non-destructive sampling of newly expanding terminal shoots on grapefruit trees. Feeding by *D. citri* nymphs often resulted in the abscission of leaves and even whole shoots. Shoot elongation was abated when death of the apical meristem occurred. Key predators of nymphal psyllids were the coccinellid beetles *Harmonia axyridis* Pallas, *Olla v-nigrum* Mulsant, *Cycloneda sanguinea* L., and *Exochomus childreni* Mulsant. Two lacewings, *Ceraeochrysa* sp. and *Chrysoperla rufilabris* Burmeister, a spider, *Hibana velox* (Becker), and the introduced parasitoid *Tamarixia radiata* (Waterston) contributed additional mortality. Exclusion of primary predators from infested terminals with field cages doubled the duration of psyllid infestation, improved parasitoid survival by 40-fold, and improved psyllid maturation success (first instar to adult) by 120-fold. The results indicate that coccinellid beetles are the most important biological control agents of high-density *D. citri* populations in central Florida and that intraguild predation causes >95% mortality of immature stages of *T. radiata*.
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*Keywords:* Ceraeochrysa sp.; *Chrysoperla rufilabris*; *Cycloneda sanguinea*; *Diaphorina citri*; *Exochomus childreni*; *Harmonia axyridis*; *Hibana velox*; *Olla v-nigrum*; *Tamarixia radiata*

## 1. Introduction

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the primary vector of citrus greening disease caused by the bacteria *Liberobacter asiaticum*. Consequentially, it is an economically important pest of citrus wherever greening disease occurs. Nymphal stages of *D. citri* develop exclusively on newly expanding shoots of citrus and related species of Rutaceae (Shivankar et al., 2000), although adults can survive for extended periods by feeding on mature leaves. In contrast to nymphs of the South African citrus psyllid, *Trioza erytreae* (Del Guercio), that develop in leaf pits, *D. citri* nymphs inject a feeding toxin that causes considerable leaf and shoot distortion on citrus, often leading to the abatement of terminal elongation, and the abscission of leaves and

whole shoots (Mead, 1976). A single *D. citri* nymph feeding for <24 h on a citrus leaf induces a distinctive and permanent deformation that becomes increasingly evident as the leaf expands (Michaud, unpublished). This characteristic damage is a useful tool for estimating psyllid activity because it remains evident long after nymphs have been eliminated by predation or other sources of mortality.

*Diaphorina citri* has been present in Brazil since the 1940s (Costa Lima, 1942) and in the Caribbean and Central American regions since the 1990s (Shivankar et al., 2000). The psyllid was first discovered in Stuart, Florida, on hedges of Jasmine Orange, *Murraya paniculata* (L.) Jack., in 1998 (Halbert et al., 1998). More than three years were required for *D. citri* to spread throughout the citrus-growing regions of Florida, although many large infestations were observed during this period (Michaud, 2002). The economic impact of *D. citri* on citrus in Florida has been mitigated by the absence of greening disease. However, heavy psyllid infestations in newly-planted groves and nurseries have

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required pesticide applications to avert direct damage to young trees (Timmer, 2002).

Initial field observations revealed a numerical response by a native ladybeetle, *Olla v-nigrum* Mulsant, to infestations of *D. citri* in the early stages of its invasion (Michaud, 2001). Other preliminary work in Florida reported a wide range of predatory insects that fed on *D. citri* nymphs and developed successfully, including predators as diverse as lacewings (Neuroptera: Chrysopidae), spiders (Aranae), and hoverflies (Diptera: Syrphidae) (Michaud, 2002). Subsequently, Michaud and Olsen (2003) examined the suitability of *D. citri* nymphs as prey for six coccinellid species: *Curinus coeruleus* Mulsant, *Exochomus childreni* Mulsant, *Harmonia axyridis* Pallas, *O. v-nigrum* Mulsant, *Cycloneda sanguinea* L., and *Coelophora inaequalis* (F.). A diet of *D. citri* nymphs supported successful larval development in the first five species, and the first four species reproduced well feeding on *D. citri* as adults. However, both *C. coeruleus* and *E. childreni* are Chilocorine species with significantly lower reproductive rates and longer generation times than the Coccinelline species *H. axyridis* and *O. v-nigrum*, factors that may limit their ability to respond numerically to developing psyllid infestations (Michaud and Olsen, 2003).

Two exotic parasitoids of Asian origin were introduced as part of a classical biological control program against the ACP in Florida in 2000, *Diaphorencyrtis aligarhensis* (Shafee, Alam and Agaral) (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (Hoy and Nguyen, 2001). Although *D. aligarhensis* has not established, *T. radiata* is now widely distributed in the Florida citrus ecosystem (Michaud, 2002). The distribution of *T. radiata* throughout Florida appears to have been facilitated by the inadvertent transport of the parasitoid on *D. citri*-infested *M. paniculata* by the discount nursery trade (S. Halbert, personal communication). This inference is supported by the recent recovery of *T. radiata* from *D. citri*-infested citrus in the lower Rio Grande valley of Texas where it had never been intentionally released (Michaud, unpublished). The psyllid was discovered in a Texas nursery in September 2001 on potted *M. paniculata* originating in Florida (French et al., 2001), material that apparently served to effectively deliver both pest and parasitoid. There have been no published reports to date of any native parasitoids attacking *D. citri* nor of any native hyperparasitoids attacking *T. radiata*.

This study was undertaken with two primary objectives: (1) to estimate the survival rate of immature *D. citri* under central Florida field conditions, and (2) to estimate the relative abundance of the various generalist predators feeding on *D. citri* nymphs. A secondary objective was to assess the relative contribution of the introduced parasitoid *T. radiata* to mortality of *D. citri* nymphs.

## 2. Materials and methods

### 2.1. Damage assessment surveys

Oviposition by female psyllids on spring flush in 2002 was diffuse and asynchronous, precluding any opportunity to monitor a cohort of developing psyllids. Instead, three groves in Lake Alfred, FL, and one grove in Ft. Pierce, FL, were surveyed in late February and early March 2002 for psyllid damage, live psyllids, and foraging natural enemies. A series of newly expanding terminal shoots were examined on each of a series of 10–20 flushing trees in each grove. The following data were recorded: the proportion of expanding terminals on each tree with psyllid damage, but no live nymphs (=% terminals damaged and uninfested), the proportion of terminals with live psyllid nymphs and/or adults (=% terminals infested), the number of psyllid adults and nymphs per infested terminal, and the number of foraging natural enemies. Data on psyllids and damage were compared among groves by one-way ANOVA followed by LSD with $\alpha = 0.05$. The relative abundance of the primary coccinellid species was compared within and among groves by means of a $(2 \times r)$ contingency table analysis and the $\chi^2$ test ($\alpha = 0.05$).

### 2.2. Cohort studies

Two synchronous cohorts of *D. citri* were observed simultaneously in two different grapefruit groves approximately two miles apart in Lake Alfred, FL from September 19 to October 3, 2001. A third cohort was observed in one of these same groves in late summer, 2002. A series of newly expanding terminal shoots infested with psyllid eggs were selected, tagged with flagging tape, and numbered in each of the two groves, respectively (99 terminals on 20 trees in Block 1 and 75 terminals on 15 trees in Block 2). The third cohort was followed from June 26 to July 19 in Block 2 and consisted of 100 infested terminals on 20 trees (5 per tree). These separate studies are referred to as Cohorts 1, 2, and 3, respectively. In Cohort 3, an additional 20 terminals (one on each of the 20 trees) were enclosed in field cages. Each cage was made out of a 2 L clear plastic soda bottle with the bottom and top cut off to yield a flexible, transparent cylinder. Nylon muslin sleeves were glued on to each end of the cylinder with clear, weatherproof silicon. The apertures of the muslin were ca. 2 mm square and readily permitted the entry of foraging *T. radiata* females while effectively excluding the larger predators. A double length of 15-G wire was secured to the inside of the cage with packing tape so it could be wrapped around a branch to support the cage once in place over a shoot. The sleeves were then gathered and tied, top and bottom. Shoots were able to grow and expand within the cages without restriction and the

upper sleeve could be opened to permit examination of the shoot and removal of maturing psyllids.

The number of flushed terminals and the proportion infested was tallied for each sampled tree on the first day of each cohort study. Summing the number of infested terminals over all sampled trees and dividing it by the sum of all available expanding terminals (infested + uninfested) provided an estimate of the percentage of terminals infested at the beginning of each study. Stages of terminal growth were recorded daily in five arbitrary categories as defined in Michaud and Browning (1999) (feather, feather/expanding, expanding/hardening, hardening, and hard) and the length of each terminal was measured. Daily temperature data were recorded both inside the tree canopy and on an exposed terminal branch using HOBO data loggers (Onset Computer, P.O. Box 3450, Pocasset, MA 02559-3450). In the third cohort, an additional data logger was placed inside an exclusion cage on an exposed branch.

Psyllid eggs could not be counted in a non-destructive manner because they were typically embedded within the folds of young, fragile leaves. Therefore, egg predation was not quantified, although such predation was observed and noted. Counts of first instar nymphs were made during the days following egg hatch. All terminals were examined on a daily basis and all insect life stages were counted on each terminal.

The number of psyllid adults maturing on each terminal was determined by removing all teneral adults and fifth instar exuviae from each terminal on a daily basis. When the number of exuvia was greater than the number of adults present, it was assumed that additional adults had molted and been dislodged from the terminal within the previous 24 h. Previous field observations (Michaud, unpublished data) indicated that teneral adults tend to feed in place on their nascent terminal for 24–48 h before taking flight. Unlike preceding molts, the final molt from fifth instar nymph to adult psyllid results in an exuvium that adheres to the leaf. Furthermore, grapefruit terminals are in advanced stages of development by the time psyllids are maturing and are no longer attractive to reproductive adults that might otherwise confound counts of newly molted adults. In addition, gravid females are easily distinguished by their swollen, orange abdomens. Survival (first instar to adult) was estimated for each cohort by dividing the total number of adults maturing by the total number of first instar nymphs.

To improve estimates of relative abundance, counts of predators observed on branches adjacent to sampled terminals were recorded along with those present on tagged terminals. Sources of mortality for psyllid nymphs (terminal abscission, predation, and parasitism) were inferred whenever possible. When a whole terminal abscissed, abscission was attributed as the source of mortality for all nymphs present on the previous sampling date. Any decline in numbers of psyllid nymphs

from one day to the next was attributed to removal by predators. Adult *T. radiata* females were tallied when they were directly observed foraging on terminals and psyllid mortality due to parasitism was estimated by the number of mummies formed. It should be noted that female *T. radiata* also kill some psyllid nymphs by host-feeding prior to oviposition (Chien et al., 1995), and this mortality was indistinguishable from losses attributed to other forms of predation. The relative abundance of the primary coccinellid species (adult-days and larva-days combined) was compared within and among cohorts by means of a $(2 \times r)$ contingency table analysis and the $\chi^2$ test ($\alpha = 0.05$).

## 3. Results

### 3.1. Damage assessment surveys

A total of 70 trees was examined in four groves to characterize feeding damage to expanding terminals and assess residual psyllid populations in spring 2002. The data for mean numbers of adults and nymphs per tree and mean percentage of terminals damaged are depicted in Fig. 1. The proportion of uninfested terminals with
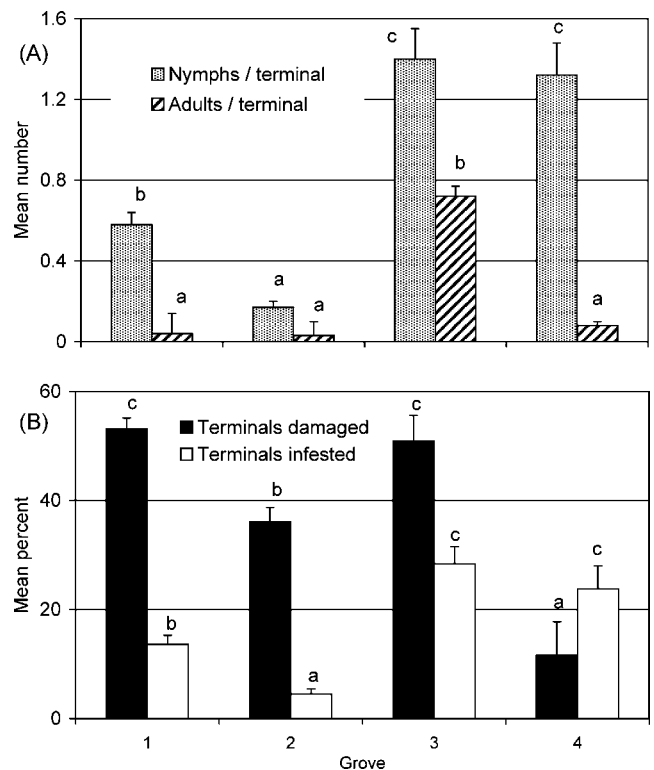


Fig. 1. Mean numbers (±SEM) of psyllid nymphs and adults per expanding citrus terminal (A) and mean percentages of terminals (±SEM) damaged and infested (B) from four citrus groves in Florida during spring, 2002. 1: Lake Alfred, grove 1, 2/25/02; 2: Lake Alfred, grove 2, 2/25/02; 3: Ft. Pierce, 3/7/02; 4: Lake Alfred, grove 3, 3/9/02. Columns bearing the same letter were not significantly different within groups ($\chi^2$ test, $P > 0.05$).

Table 1
Frequency of natural enemies observed in four Florida citrus groves surveyed for *D. citri* in spring, 2001

| Parameter/species | Stage sampled | Grove 1 Lake Alfred 2/25/02 | Grove 2 Lake Alfred 2/25/02 | Grove 3 Ft. Pierce 3/07/02 | Grove 4 Lake Alfred 3/09/02 |
|---|---|---|---|---|---|
| No. trees sampled | | 20 | 20 | 20 | 10 |
| % Terminals infested with *D. citri* ($\pm$ SEM) | | $13.6 \pm 1.67$b | $4.5 \pm 0.94$a | $28.4 \pm 3.11$c | $23.8 \pm 4.21$c |
| Aranae | | | | | |
| *Hibana velox* | Nymphs | 0 | 0 | 4 | 0 |
| Coleoptera: Coccinellidae | | | | | |
| *Brachiacantha dentipes* | Adults | 0 | 0 | 1 | 0 |
| *Coelophora inaequalis* | Adults | 0 | 0 | 2 | 0 |
| *Curinus coeruleus* | Adults | 0 | 0 | 2 | 0 |
| *Cycloneda sanguinea* | Adults | 22 | 19 | 11 | 10 |
| | Larvae | 0 | 0 | 16 | 1 |
| *Exochomus childreni* | Adults | 4 | 0 | 0 | 0 |
| *Harmonia axyridis* | Adults | 50 | 4 | 8 | 1 |
| | Larvae | 0 | 15 | 2 | 0 |
| *Olla v-nigrum* | Adults | 8 | 3 | 2 | 0 |
| | Larvae | 0 | 0 | 24 | 0 |
| Neuroptera: Chrysopidae | | | | | |
| *Chrysoperla* spp. | Larvae | 0 | 0 | 0 | 1 |
| *Ceraeochrysa* spp. | Larvae | 1 | 1 | 0 | 4 |
| Diptera: Syrphidae | | | | | |
| *Allograpta obliqua* | Adults | 1 | 0 | 4 | 2 |
| Hymenoptera: Eulophidae | | | | | |
| *Tamarixia radiata* | Mummies | 0 | 0 | 0 | 1 |

some psyllid feeding damage approached or exceeded 50% in three of the four groves surveyed, but live psyllids were present on less than 30% of terminals in the same groves. There were significant differences among groves with respect to the mean number of adults per terminal ($F = 24.912$; 3, 66 df; $P < 0.001$), the mean number of nymphs per terminal ($F = 8.092$; 3, 66 df; $P < 0.001$), the percentage of terminals damaged ($F = 19.109$; 3, 66 df; $P < 0.001$), and the percentage of terminals infested ($F = 21.186$; 3, 66 df; $P < 0.001$). The frequencies of natural enemies observed on the sampled trees are presented in Table 1. Proportional abundance of the four primary coccinellid species (adults and larvae combined) were significantly different in contingency table analyses ($\chi^2$ test, $P < 0.05$): Grove 1: *H. axyridis* > *C. sanguinea* > *O. v-nigrum* = *E. childreni*; Grove 2: *H. axyridis* = *C. sanguinea* > *O. v-nigrum* = *E. childreni*; Grove 3: *C. sanguinea* = *O. v-nigrum* > *H. axyridis* = *E. childreni*; Grove 4: *C. sanguinea* > *H. axyridis* = *O. v-nigrum* = *E. childreni*. There was no significant difference in abundance of *E. childreni* across the four groves sampled. Differences in relative abundance of the other three species among groves were as follows: *C. sanguinea*: Grove 3 = Grove 2 > Grove 1 > Grove 4; *H. axyridis*: Grove 1 = Grove 2 > Grove 3 = Grove 4; *O. v-nigrum*: Grove 3 > Grove 1 = Grove 2 = Grove 4.

*Allograpta obliqua* Say is a syrphid species previously observed attacking *D. citri* in the region and capable of completing development on a diet of *D. citri* nymphs (Michaud, 2002). Adults of this species were observed inspecting citrus terminals in three of these groves, but no syrphid eggs were observed on infested terminals. However, no terminals were observed with more than 5 psyllid nymphs, probably too few to elicit syrphid oviposition or permit maturation of a syrphid larva.

*3.2. Cohort studies*

All three cohorts were initiated when a high proportion of expanding terminals in the grove were infested: 83.7, 92.9, and 87.4% for Cohorts 1, 2, and 3, respectively. Within-canopy ambient temperatures for the duration of both study periods are reported in Fig. 2. The mean temperature ($\pm$SEM) was $24.7 \pm 0.2\,°C$ during the first study period and $25.8 \pm 0.1\,°C$ during the second study period. There were significant differences among temperatures recorded within the canopy, on exposed terminals and within exclusion cages during the second study period ($F = 10.9$; 2, 1044 df; $P < 0.001$). Temperatures averaged $1.5\,°C$ higher in exclusion cages than within the canopy, and $1.3\,°C$ higher than on exposed terminals (LSD, $P < 0.05$). Total rainfall was 1.68 cm during the first study and 6.03 cm during the second study.

Data on initial numbers of first instar nymphs and final numbers of adults maturing per terminal are
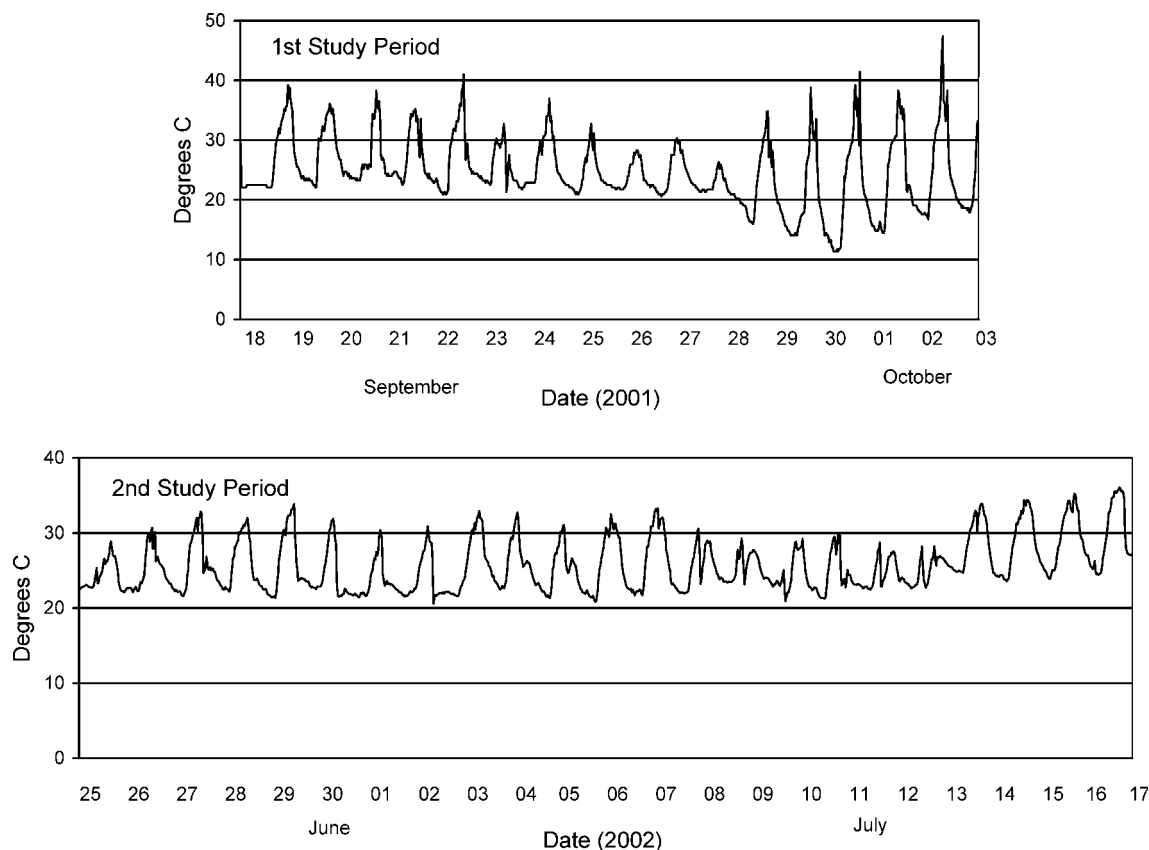
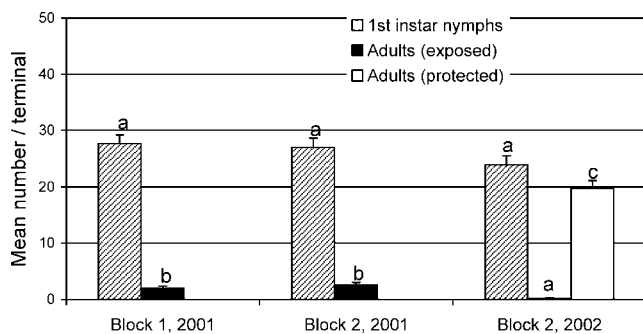Fig. 2. Ambient temperature data within the canopy for the two cohort study periods in Lake Alfred, Florida.



Fig. 3. Mean numbers (±SEM) of first instar *D. citri* nymphs (hatched bars), maturing adults (solid bars), per exposed grapefruit terminal in three cohorts. Observations were made daily from 19 September to 3 October (Blocks 1 and 2, 2001) and 26 June to 19 July (Block 2, 2002). The open bar shows percentage adults maturing per terminal (±SEM) for terminals protected by exclusion cages in the third cohort. Bars bearing the same letter were not significantly different among cohorts for nymphs and adults, respectively ($\chi^2$ test, $P > 0.05$). The number of adults maturing on protected terminals in Cohort 3 was compared only to the number of adults maturing on exposed terminals in the same cohort.

compared among the three cohorts in Fig. 3. There was no significant difference among cohorts in the number of first instar nymphs per terminal ($F = 1.739$; $2, 272$ df; $P = 0.178$). However, there were significantly fewer adults maturing per exposed terminal in Cohort 3 in summer 2002, than in either of the cohorts followed in fall, 2001 ($F = 8.571$; $2, 272$ df; $P < 0.001$; LSD, $\alpha = 0.05$). A total of 190 of 2745 first instar nymphs matured to adulthood in Cohort 1 (survival $= 6.9\%$ or 1.91 adults/terminal) compared to 189 of 2046 in Cohort 2 (survival $= 7.8\%$ or 2.52 adults/terminal) and 21 of 2420 in Cohort 3 (survival $= 0.9\%$ or 0.21 adults/terminal). In comparison, a mean of 19.8 adults matured per caged terminal in Cohort 3, significantly more than on exposed terminals in the same cohort. Terminals in Cohort 2 remained infested with psyllids for significantly longer than did terminals in Cohort 1 in fall 2001 ($7.9 \pm 0.35$ days vs. $6.0 \pm 0.34$ days; $F = 13.841$; $1, 172$ df; $P < 0.001$). The mean duration of terminal infestation in Cohort 3 was $9.2 \pm 0.42$ days, significantly longer than Cohort 2 ($F = 5.776$; $1, 174$ df; $P = 0.017$). The mean duration of infestation was $18.5 \pm 0.73$ days for protected terminals in Cohort 3, significantly longer than for exposed terminals ($F = 83.308$; $1, 118$ df; $P < 0.001$). One field cage was penetrated by a single larva of *E. childreni* that consumed a large proportion of psyllid nymphs before detection; this replicate was excluded from analysis.

Infestation by *D. citri* nymphs appeared to have a negative impact on the growth of grapefruit terminals.

Female *D. citri* puncture leaf tissues to embed the egg pedicels during oviposition. This injury sometimes resulted in the blackening and death of damaged tissues, the abscission of apical leaves prior to expansion, and substantial egg mortality that could not be quantified. Plant responses to nymphal feeding ranged from abscission of individual leaves, to death of the apical meristem, and even complete abscission of the terminal. Estimates of nymphal mortality resulting from the abscission of whole terminals were 6.4, 12.8, and 2.1% in the 3 cohorts, respectively. In Cohort 3, the final length of hardened terminals with meristems killed by nymphal feeding was $13.0 \pm 2.0$ cm (mean $\pm$ SEM) compared to $24.3 \pm 1.0$ cm for infested terminals with intact meristems ($F = 21.557$; 1, 78 df; $P < 0.001$). Data on the frequencies of meristem death, leaf abscission, and terminal abscission are given in Table 2. There was no significant difference between exposed and caged terminals in the frequency of meristem death in Cohort 3 ($F = 1.802$; 1, 117 df; $P = 0.182$), but significantly more caged terminals suffered leaf abscission ($F = 6.097$; 1, 117 df; $P = 0.015$).

Data for the relative abundance of natural enemies observed in the three cohorts are given in Table 3. Contingency table analysis revealed that *H. axyridis* was the most abundant predator in Cohort 2, was tied for most abundant with *O. v-nigrum* in Cohort 1, and was tied with both *O. v-nigrum* and *E. childreni* for most abundant in Cohort 3. *C. sanguinea* was second in abundance in Cohort 1 and Cohort 3 and was tied for second with *O. v-nigrum* in Cohort 2. *E. childreni* was the least abundant in Cohort 1 and was not detected in Cohort 2. The anyphaenid spider *Hibana velox* Becker was a notable presence with 55 arthropod-days; all other predators occurred in relatively low abundance. Comparisons of relative abundance of coccinellid species across cohorts revealed the following differences: *C. sanguinea*: Cohort 1 = Cohort 2 > Cohort 3, *E. childreni*: Cohort 3 = Cohort 1 > Cohort 2, *H. axyridis*: Cohort 1 > Cohort 2 > Cohort 3, *O. v-nigrum*: Cohort 1 = Cohort 2 = Cohort 3.

Considerable egg predation was observed in all three cohorts, but could not be quantified due to the difficulty of counting egg masses non-destructively. For example, four terminals with egg masses tagged in Cohort 3 on day one had to be replaced on day two because predators had consumed them completely. The majority of psyllid nymphs simply disappeared during periods between daily counts. To the extent that such disappearance might be attributed to removal of nymphs by predators, predation would account for 92.3% mortality in Cohort 1 (2534 of 2745), 87.0% mortality in Cohort 2 (1780 of 2046), and 96.9% mortality in Cohort 3 (2344 of 2420). When data were subjected to a bivariate correlational analysis of 'number of psyllid adults maturing per terminal' versus 'total predator-days' Pearson Correlations (two-tailed) of 0.850, 0.052, and −0.61 were obtained for the three cohorts, respectively, all non-significant ($P > 0.05$). However, in 48 of 273 exposed terminals in the three cohorts, one or more coccinellid larvae and/or adults were directly observed consuming the last psyllid nymph(s) on a terminal on its final day of infestation (Cohort 1: 22; Cohort 2: 14; Cohort 3: 13). In an additional 39 cases, a coccinellid larva was the last remaining insect on a terminal that had had live psyllid nymphs present the previous day (Cohort 1: 20; Cohort 2: 11; Cohort 3: 8). By comparison, in only one case was a *Chrysoperla* larva observed feeding on the last remaining psyllid nymphs (in Cohort 1) and in 2 cases, a *Ceraeochrysa* larva (both in Cohort 2). In 11 cases, the last nymphs remaining on a terminal formed mummies (Block 1, 2001: 3; Block 2, 2002: 8). In one case, *H. velox* was observed consuming the last nymphs remaining on a terminal (Cohort 2) and in 3 cases (also Cohort 2) this species was the last remaining predator on a terminal that had live psyllid nymphs the previous day. In four cases where *H. velox* was the sole predator observed directly feeding on nymphs on a terminal for two successive days, its mean consumption (estimated by the number of nymphs missing) was only 2.5 over the 24 h period.

Psyllids mummified by *T. radiata* were rare in all cohorts. A total of 67 mummies formed on the 273 exposed terminals, contributing 1.3, 0.2, and 1.0% mortality of psyllid nymphs in the three cohorts, respectively. Mummies formed on only three of the 15 terminals where *T. radiata* females had been directly observed attacking *D. citri* nymphs in Cohort 3. Exclusion of the large predators in Cohort 3 increased the rate of mummy formation almost 20-fold (4.89 per terminal as opposed to 0.25 per exposed terminal) and increased mummy emergence 2-fold (72% as opposed to 36%).

Table 2
Observed responses of expanding grapefruit terminals to oviposition and nymphal feeding by *D. citri*

| Cohort, year | Death of meristem | Abscission of leaves | Abscission of whole terminal |
|---|---|---|---|
| 1, 2001 | 0 | 9/99 = 10.0% | 4/99 = 4.0% |
| 2, 2001 | 0 | 16/75 = 21.3% | 3/75 = 4.0% |
| 3, 2002 (exposed) | 14/100 = 14.0% | 5/100 = 5.0% | 1/100 = 1.0% |
| 3, 2002 (protected) | 5/19 = 26.3% | 4/19 = 21.1% | 0/20 |

Table 3
Natural enemies observed on, or adjacent to, three series of grapefruit terminals infested with *D. citri* in Lake Alfred, Florida expressed in "arthropod-days"

| Species/stage sampled | | Cohort 1, 2001 | Cohort 2, 2001 | Cohort 3, 2002 | Cohort 3, 2002 (Protected) |
|---|---|---|---|---|---|
| Aranae | | | | | |
| *Hibana velox* | Nymphs | 8 | 25 | 22 | 0 |
| Unidentified spiders[a] | | 6 | 15 | 6 | 0 |
| Coleoptera: Coccinellidae | | | | | |
| *Coleomegilla maculata* | Adults | 0 | 3 | 0 | 0 |
| *Cycloneda sanguinea* | Adults | 39 | 10 | 6 | 0 |
| | Larvae | 10 | 13 | 0 | 0 |
| *Exochomus childreni* | Adults | 14 | 0 | 18 | 0 |
| | Larvae | 3 | 0 | 9 | 0 |
| *Harmonia axyridis* | Adults | 30 | 50 | 16 | 0 |
| | Larvae | 41 | 15 | 9 | 0 |
| *Olla v-nigrum* | Adults | 19 | 11 | 21 | 0 |
| | Larvae | 61 | 20 | 12 | 0 |
| *Rodolia cardenalis* | Adults | 0 | 1 | 0 | 0 |
| Neuroptera: Chrysopidae | | | | | |
| *Chrysoperla* spp. | Larvae | 4 | 0 | 0 | 0 |
| *Ceraeochrysa* spp. | Larvae | 1 | 5 | 8 | 0 |
| Diptera: Syrphidae | | | | | |
| *Allograpta obliqua* | Larvae | 3 | 0 | 0 | 0 |
| *Ocyptamus fuscipennis* | Larvae | 1 | 0 | 0 | 0 |
| Hymenoptera: Eulophidae | | | | | |
| *Tamarixia radiata* | Adults | 2 | 1 | 17 | 5 |
| | Mummies | 37 | 5 | 25 | 93 |
| Hymenoptera: Formicidae[b] | | | | | |
| *Brachiamyrmex obscurion* | | 2 | 8 | 42 | 28 |
| *Camponotus floridanus* | | 9 | 12 | 4 | 2 |
| *Crematogaster ashmeadi* | | 0 | 0 | 1 | 5 |
| *Dorymyrmex bureni* | | 63 | 70 | 40 | 72 |
| *Monomorium floricola* | | 14 | 33 | 1 | 0 |
| *Paretrechina bourbonica* | | 8 | 5 | 0 | 0 |
| *Pseudomyrmex gracilis* | | 0 | 0 | 2 | 0 |
| *Solenopsis invicta* | | 2 | 1 | 8 | 8 |

Terminals were monitored daily from onset of oviposition to maturation of the last psyllid adult or, in the case of Cohort 3, until emergence of the last *T. radiata* mummy. Two series of terminals were followed in two separate blocks from September 20, to October 5, 2001 (*n* = 99 and 74, respectively). The third series was followed from June 26 to July 19, 2002 (*n* = 100) and included an additional set of terminals (*n* = 19) caged to exclude predators (= "Protected"). The mean number of days (±SEM) terminals remained infested with *D. citri* varied significantly among cohorts: Cohort 1 = 6.0 ± 0.34 < Cohort 2 = 7.9 ± 0.35 < Cohort 3 = 9.23 ± 0.42 < Cohort 3 (Protected) = 18.5 ± 0.73 (one-way ANOVA followed by LSD, α = 0.05).
[a] Unidentified spiders were only tallied when they were directly observed feeding on nymphs.
[b] Each observation of an ant species on a terminal was tallied as a single 'ant day'; ants were not counted.

Eight different ant species were observed on psyllid-infested terminals, usually harvesting honeydew (Table 3). All of these ants, even the large *C. floridanus,* were able to penetrate the field cages. Ants were frequently observed foraging side by side with adult and larval predators, but aggressive interactions between ants and predators were never observed. Workers of *Dorymyrmex bureni* (Trager) were observed carrying away *D. citri* nymphs on several occasions, as was a worker of *Pseudomyrmex gracilis* (F.) on one occasion. Teneral psyllid adults were attractive prey for workers of *D. bureni* that were observed carrying them around inside exclusion cages and attempting to pull them through the nylon sleeves on six separate occasions.

## 4. Discussion

### 4.1. Damage assessment surveys

All four groves sampled in spring 2002 had flushed terminals in similar stages of development, mostly in the newly expanding stages, but too mature to be suitable for psyllid oviposition. In three of the four groves, the mean number of uninfested terminals/tree with minor psyllid damage was greater than the mean number of infested terminals, suggesting that a great majority of juvenile psyllids suffered mortality in early stages of development. Coccinellids are typically most active in Florida citrus during the early spring months where they

feed on pollen and nectar in addition to homopteran pests (Michaud, 2000, 2001). The Lake Alfred grove sampled on 3/9/02 (Grove 4) was the only one where the mean number of terminals infested exceeded the mean number of terminals damaged but uninfested (Fig. 1). This same grove had the fewest natural enemies, lacking *H. axyridis* and *O. v-nigrum,* with only adult *C. sanguinea* present in any numbers (Table 1).

## 4.2. Cohort studies

### 4.2.1. Physical factors

The temperature averaged 1 °C higher during Cohort 3 than during the fall, 2001 cohorts, although daily thermal variation was greater in the fall due to cooler overnight temperatures. There was more than three times as much rainfall during the summer cohort as during the fall. Although it is impossible to assess what influence these factors had on rates of immature psyllid survival, observations made immediately before and after rain suggest that few nymphs were dislodged by rainfall events (J.P. Michaud, unpublished data), although this has been claimed as a source of mortality in previous work (Aubert, 1987). The higher summer temperatures no doubt accelerated the development of all insects, but temperature was not high enough to cause direct mortality to *D. citri* nymphs. Greenhouse colonies of *D. citri* can survive prolonged periods at temperatures exceeding 35 °C (J.P. Michaud, unpublished data). It seems unlikely that high temperatures were a limiting factor for the survival of either psyllid nymphs or their parasitoid in Cohort 3 because the survival of both was higher on caged terminals than on exposed terminals, even though the caged terminals experienced higher temperatures.

### 4.2.2. Plant responses

A blackening of the edges of grapefruit leaves early in the 'feather' stage of terminal expansion was noted only in Cohort 3 and appeared to result from heavy oviposition damage on terminals in early stages of development. The abscission of leaves and whole terminals in response to feeding by *D. citri* has been previously reported (Shivankar et al., 2000), although much of the Asian literature appears to confound symptoms of direct feeding damage with symptoms of greening disease. These observations indicate that direct feeding damage alone can result in the death of growing grapefruit meristems, leading to premature termination of shoot elongation. Abscission of leaves appeared to have little impact on psyllid nymphs that mostly feed along the stem, but abscission of whole terminals resulted in some mortality. The higher rate of leaf abscission on caged terminals relative to exposed terminals is likely a result of higher feeding pressure by psyllid nymphs on the protected shoots (Table 2). Furthermore, the death of growing meristems on some infested terminals, and the

reduced final length of these terminals compared to those with surviving meristems, suggests that feeding by psyllid nymphs can potentially have measurable impact on the growth of grapefruit trees.

### 4.2.3. Predation

The coccinellid beetles *H. axyridis* and *O. v-nigrum* were the most abundant predators in the cohort studies with the highest cumulative counts when larval-days and adult-days were combined for all three studies (152 and 144 arthropod-days, respectively). Both species are capable of utilizing psyllids for development and reproduction (Michaud and Olsen, 2003). *C. sanguinea* tallied only 78 larval- and adult-days combined in the cohort studies, but was either most abundant, or tied for most abundant, in three of the four groves surveyed in spring 2002. Because *C. sanguinea* does not diapause (Gordon, 1985) it may become active earlier in the season than species that diapause, although its inability to produce eggs while feeding exclusively on *D. citri* (Michaud and Olsen, 2003) probably limits its numerical response to psyllid populations.

The lack of a significant negative correlation between 'total predator-days' and 'no. psyllid adults maturing' on terminals in all three cohorts may not be meaningful. For example, if predators abandon terminals promptly once they have consumed all psyllid nymphs, they might have been observed less often on the terminals where they had the greatest proportional impact. Terminals with many nymphs might attract more predators than terminals with fewer nymphs, but still produce more psyllid adults if predator satiation were a factor. The numerous direct observations of predators consuming the last remaining nymphs on infested terminals, or remaining on 'clean' terminals that had been infested the day before, attest to their overall importance as sources of juvenile psyllid mortality. Furthermore, the predation of eggs would have contributed considerable additional mortality in a full life table analysis.

The fact that the survival rate of juvenile psyllids was increased by a factor of almost 120 when terminals were caged indicates the overwhelming importance of large predators as a key source of mortality for psyllid nymphs (Fig. 3). Inclusion of egg mortality would certainly yield even lower rates of psyllid survivorship in a full life table analysis. The mortality inflicted by ants is probably minor. Various ant species readily penetrated exclusion cages and foraged within them, but did not significantly depress juvenile psyllid survival relative to exposed terminals. Both *Cycloneda* and *Olla* spp. were referenced by Westigard et al. (1968) as important predators of the pear psylla, *Psylla pyricola* Foerster, in Oregon who "felt that they played a decisive part in control of the pear psylla in this particular orchard" in conjunction with other coccinellid species. In Cuba, Gonzalez et al. (2000) reported a similar guild of

predators as important in biological control of *D. citri*, including the coccinellids *C. sanguinea*, *Chilocorus cacti* (L.), and *E. cubensis* Dimn. and several lacewings and syrphids. Furthermore, Catling (1970) identified a series of predators attacking the African citrus psyllid, *T. eritreae* (Del Guercio), that are remarkably analogous to those attacking *D. citri* in Florida. These included a coccinellid, a series of trash-carrying lacewings, and a syrphid fly in the genus *Allograpta*. In the same paper, Catling (1970) described the results of a sleeve cage exclusion study in which the survival of developing *T. erytreae* was improved by a factor of two relative to terminals exposed to predation. Predator exclusion improved psyllid survival to a far greater degree in this study, suggesting that *D. citri* suffers considerably greater predation pressure in Florida than does *T. erytreae* in South Africa.

Spiders have often been reported as predators of citrus psyllids in the literature (Shivankar et al., 2000). In a comprehensive study of predators of *T. erytreae* in South Africa, Van den Berg et al. (1987) concluded that ''spiders appear to be the most important predators, followed by coccinellids.'' Van den Berg et al. (1992) reported eighteen species of spiders preying on *T. erytreae*, predominantly species in the family Salticidae. The unidentified spiders observed in these studies included a number of small Salticidae, but the most abundant spider was *H. velox* (Anyphaenidae), a species known to develop successfully on *D. citri* nymphs (Michaud, 2002). This study focused on sources of mortality to juvenile psyllids, but web-spinning species are of additional importance in trapping and killing adult psyllids.

### 4.2.4. Parasitism

In addition to parasitizing *D. citri* nymphs, female *T. radiata* kill other nymphs in the course of host-feeding (Chien et al., 1995), but even the combination of these two sources of mortality was insufficient for suppression of *D. citri* populations within the predator exclusion cages. Furthermore, *T. radiata* larvae and mummies suffered 95 and 64% mortality, respectively, due to intraguild predation on exposed terminals in Cohort 3. In some cases mummies disappeared completely; in other cases, remnants of dismembered mummies remained adhering to the terminal. Mortality of 28% of mummies within exclusion cages is most likely attributable to ant predation, because they were the only predators observed in the cages.

Although hyperparasitism could potentially limit the effectiveness of *T. radiata* in some regions, no hyperparasitoids of *T. radiata* have yet been detected in Florida (Michaud, 2002). Chien and Chu (1996) concluded that *T. radiata*'s efficacy against *D. citri* was unaffected by the seven hyperparasitoid species they found attacking it in Taiwan. Intraguild predation ap-

pears to be the primary source of mortality for immature *T. radiata* in central Florida, although the degree to which this will affect final levels of biological control is not yet clear. It is notable that reports of the effectiveness of *T. radiata* against *D. citri* come from small islands such as Reunion and Guadaloupe (Etienne et al., 2001), islands that probably lack the faunal diversity of larger land masses, including the wide range of generalist predators and large coccinellid species present in Florida citrus.

Chien et al. (1995) found that host utilization by *T. radiata* was inversely related to host density and that a density of between 2 and 9 fifth instar *D. citri* per female per day yielded a host utilization rate of 90–94% during the peak period of oviposition. *Tamarixia radiata* females are able to extend their lifespan by egg resorption, a trait that has been inferred as adaptive for synchronizing parasitoid reproduction with that of its host (Chien et al., 1994). However, the relatively small number of hosts killed per female per day by feeding and parasitism combined (Chien et al., 1995), and the long generation time of *T. radiata* relative to *D. citri,* are factors detracting from the parasitoid's impact on large populations of *D. citri*. Nevertheless, *T. radiata* is highly host-specific (Chien and Chu, 1996), and its proportional contribution to biological control might improve once psyllid populations have been reduced to lower densities by generalist predators.

### 4.2.5. Sources of error

Given the range of visible plant responses to psyllid oviposition and nymphal feeding, it is conceivable that other plant responses imperceptible to the observer were responsible for some reduction of psyllid nymphs that was attributed, by default, to predation. Nymphal mortality resulting from leaf abscission was difficult to assess and remains a factor of indeterminate significance in cohort studies. It is also possible that some psyllids matured on exposed terminals that escaped detection (for example if both a teneral adult and its exuvium were dislodged by wind action) and this might have lead to an underestimation of psyllid survival. However, most mortality was suffered by early instar nymphs; even if all disappearing fifth instar nymphs were assumed to have matured successfully, estimates of survival to adult would be improved only slightly. There is a further possibility that some disappearing nymphs emigrated to adjacent terminals. While psyllids were observed to change position on a terminal, nymphs were never observed traversing woody twigs between terminals. Furthermore, any significant movement of nymphs between terminals would have resulted in immigration as well as emigration, given the high proportion of terminals infested in these studies, but there were few examples of increases in numbers of nymphs on a terminal that could not be accounted for by hatching eggs.

It is also possible that cryptic or nocturnal predators contributed to predation. For example, chrysopid adults are known to be nocturnal and their larvae may share these habits to varying degrees (Szentkiralyi, 2001). Certainly, the naked larvae of *Chrysoperla* spp. are highly cryptic and tend to rest in secluded locations when they are not feeding, making them easily overlooked.

To conclude, *D. citri* nymphs are a generally acceptable and suitable prey for a wide range of generalist predators. Coccinellids were the most abundant predators of *D. citri* in both the spring surveys and in all three cohorts. Other predators such as spiders and lacewings contributed some mortality. The exclusion of these predators improved the survival of *D. citri* by a factor of 120, despite improving the survival of its primary parasitoid *T. radiata* by a factor of 40. Although *T. radiata* appeared to have little impact on psyllid survival and suffered heavy mortality from intraguild predation, it is possible that higher initial parasitoid populations would yield higher rates of parasitism, or that percentage parasitism might increase when psyllid populations are reduced to lower densities by generalist predators.

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