POTENTIAL IMPACT OF LADY BEETLES ON *DIAPHORINA CITRI* (HOMOPTERA: PSYLLIDAE) IN PUERTO RICO

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Abstract

The first detections of Asian citrus psyllid, *Diaphorina citri* Kuwayama, in North America occurred almost simultaneously in Florida and in the Caribbean (Guadeloupe). Damaging populations on citrus have been reported in Florida but not in Puerto Rico where the psyllid was first detected in 2001, having probably arrived with its parasitoid, *Tamarixia radiata* Waterston. In an effort to identify additional sources of mortality, the relative abundance of coccinellid species was estimated on 180 citrus trees from early April to early July 2003. The most abundant species were *Coelophora inaequalis* F. (38.8%) and *Cycloneda sanguinea limbifer* L. (31.3%), and the least abundant were *Cladis nitidula* F. (5.9%), *Coleomegilla innonata* Mulsant (4.8%), *Chilocorus cacti* L. (2.1%), *Scymnus* sp. (5.9%), *Hippodamia convergens* Guerin (2.4%), and *Cryptolaemus montrouzieri* Mulsant (8.8%). These eight species were collected from citrus production areas in Puerto Rico for a laboratory study of feeding behavior. All eight consumed *D. citri* nymphs, with *C. innonata* consuming psyllids at a greater rate than *C. cacti* or *Scymnus* sp. Choice tests showed that *C. inaequalis* and *C. s. limbifer* preferred brown citrus aphid (BCA) *Toxoptera citricida* to *D. citri* as prey, whereas *C. nitidula* and *C. cacti* (both Chilochorini) preferred *D. citri*. Our results suggest that coccinellid species could play an important role as predators of the psyllid in Puerto Rico and contribute to its natural control.

Key Words: Asian citrus psyllid, Coccinellidae, predator, relative abundance, feeding behavior, natural control

Resumen

La primera identificación en Norte América del psílido asiático del cítrico, *Diaphorina citri* Kuwayama, se produjo casi simultáneamente en Florida y Guadeolupe. Se habían detectado daños en cítrico en Florida pero no en Puerto Rico, donde el psílido fue descubierto por primera vez en el 2001, llegando posiblemente junto con su parasitoide *Tamarixia radiata* Waterston. En un intento por encontrar posibles fuentes adicionales de mortalidad para este insecto plaga, se estimó la abundancia relativa de especies de cocinélidos en 180 árboles de cítrico desde principios de Abril hasta principios de Julio del 2003. Las especies más abundantes fueron *Coelophora inaequalis* F. (38.8%) y *Cycloneda sanguinea limbifer* L. (31.3%), y las menos abundantes fueron *Cladis nitidula* F. (5.9%), *Coleomegilla innonata* Mulsant (4.8%), *Chiloccorus cacti* L. (2.1%), *Scymnus* sp. (5.9%), *Hippodamia convergens* Guerin (2.4%) y *Cryptolaemus montrouzieri* Mulsant (8.8%). Estas ocho especies fueron capturadas de áreas de producción citrícola de Puerto Rico para el estudio en laboratorio de su comportamiento alimenticio. Las ocho especies consumieron ninfas *D. citri*, pero *C. innonata* consumieron un mayor número de ninfas por hora comparado a *C. cacti* o *Scymnus* sp. Los ensayos de elección de presa mostraron que *C. inaequalis* y *C. s. limbifer* prefirieron como presa al áfido negro del cítrico (BCA) *Toxoptera citricida* en lugar de *D. citri*, mientras que *C. coerulus* y *C. cacti* (ambos Chilocorini) prefirieron como presa a *D. citri*. Nuestros resultados sugieren que estas especies de cocinélidos podrían jugar un papel importante como depredadores del psílido en Puerto Rico y contribuir a su control natural.

Translation provided by the authors.

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, was first described from Taiwan in 1907. It has been present in Brazil since the 1940s (Lima 1942) but was not detected in Florida until 1998 (Halbert et al. 1998) and in Texas until September 2001 (French et al. 2001). In the Caribbean, the psyllid was first reported from Guadeloupe (Etienne et al. 1998) and subsequently detected in Puerto Rico in May, 2001 (Halbert & Nuñez 2004; P. Stansly, unpublished data). *Diaphorina citri* spread rapidly throughout commercial citrus areas in Florida and heavy populations were observed, especially in the south. In contrast, infestations in Puerto Rico and Texas are typically light (unpublished data and V. French, pers. comm.).

Diaphorina citri feeds and reproduces on citrus and additional hosts belonging to the subfamily Aurantioidea of the family Rutacea including jasmine orange, *Murraya paniculata* L., which is widely grown in Florida, Puerto Rico, and elsewhere as an ornamental plant. Feeding damage on citrus by large psyllid populations causes shoot distortion and abscission of the growing terminals. The psyllid also can vector the bacterium *Candidatus* Liberibacter spp., causal agent of citrus greening or 'huanglongbing' (Xu et al. 1988a). Citrus greening is considered to be the most serious disease of citrus in Asia; it has recently been reported from Brazil (Coletta-Filho et al. 2004), and poses a serious threat to citrus in Florida and Puerto Rico.

Two parasitoids have been imported and released in North America for control of *D. citri*, the endoparasitic encyrtid *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) and the ectoparasitic eulophid *Tamarixia radiata* (Waterson). *Tamarixia radiata* established itself in Florida and apparently arrived spontaneously in Puerto Rico (unpublished data) and Texas (French et al. 2001).

Predators in the family Coccinellidae (Coleoptera) have been shown to be responsible for a considerable degree of natural control in Florida (Michaud 2004). Michaud (2001) also presented field data indicating that *Olla v-nigrum* Mulsant and *Harmonia axyridis* (Pallas), coccinellid species capable of feeding and reproducing on *D. citri*, increased in relative abundance in psyllid-infested citrus groves. The following study was undertaken to provide baseline data on relative abundance of coccinellid species in Puerto Rican citrus groves and to evaluate the ability of the most abundant species to feed on *D. citri*.

Materials and Methods

Relative Abundance of Coccinellids on Citrus

Observations were carried out from early April to early July 2003, in citrus groves at the Agricultural Experimental Station in Adjuntas (18'10"N, 66'29"W. 457 m). The citrus groves of Adjuntas are well established and cover a significant proportion of both sides of the valley in which the experimental station is located. Because of the altitude and cooler weather, new growth in citrus is largely restricted to the main flush in the first half of the year. This is the time that population outbreaks of herbivorous insects such as aphids occur, with the subsequent increase of lady beetle numbers. The sampling period was designed to encompass this period of greater lady beetle activity. Thirty citrus trees were randomly selected and inspected between 8.00 a.m. and 12.00 a.m. every other week for a total of 180 trees, based on direct counts of all coccinellid adults, larvae, or pupae encountered. The relative abundance of each species was calculated as a proportion of the total coccinellids counted.

Prey Acceptability and Prey Preference

Psyllid nymphs used in the experiments were collected from a greenhouse colony established from field-collected individuals and reared on *Murraya panniculata* at the Río Piedras Agricultural Experiment Station. Nymphs of brown citrus aphid (BCA) *Toxoptera citricida* (Kirkaldy) were collected from infested citrus flushes at the Adjuntas Agricultural Experimental Station and used directly in the experiments. Coccinellid adults of the various species were collected from citrus groves at the Adjuntas Agricultural Experimental Station in Puerto Rico during the period from April to June, 2003 and maintained on *Ephestia kuehniella* Keller (Lepidoptera: Pyralidae) eggs at Adjuntas until needed. *Chilocorus cacti*, *Cladis nitidula*, *Cryptolaemus montrouzieri*, and *Scymnus* sp. could not be reared on *E. kuehniella* eggs, and so were collected directly from the field when needed for experiments.

The following eight species of cocinellids were tested for prey acceptance and prey preference in choice and no-choice tests: *Cycloneda s. limbifer* L., *Coelophora inaequalis* F., *Cladis nitidula* F., *Chilocorus cacti* L., *Coleomegilla innonata* Mulsant, *Cryptolaemus montrouzieri* Mulsant, *Hippodamia convergens* Guerin, and *Scymnus* sp. Individual adult coccinellids were starved for 24 h and then confined in Petri dishes (5 cm diam.) with one of the following prey configurations:10 psyllid nymphs only, 10 aphid nymphs only, or a combination of 5 psyllid nymphs and 5 aphid nymphs. The life stages of the two prey species were chosen to be of similar size (usually third-instars). Petri dishes were lined with white paper to assist with the visual assessment of feeding events. An experiment was judged to have been completed in the choice tests once the adult coccinellid had consumed all of one or the other prey species in the Petri dish. In the no-choice tests, the experiments were terminated after 7 h. Ten replicates of the tests were carried out and each adult coccinellid was tested only once. All experiments were carried out under controlled conditions at 25 ± 1°C and 75 ± 10% RH.

Analysis

For determining the differences in coccinellid abundance, the square root transformation was used and then Tukey's multiple comparison test applied in Proc GLM in SAS (1999). For the feeding data comparisons in the combined host experiments, normality was examined by the Shapiro-Wilk test and plot functions of Proc Capability (SAS 1999). The paired analysis was conducted based on the signed rank test in the same SAS procedure. For the consumption rate comparisons in the single host experiments, normality was determined by Proc Capability. Tukey's multiple comparison test in Proc GLM was then used to compare coccinellid consumption.

RESULTS

Relative Abundance of Coccinellids

A total of eight species of coccinellids were found in citrus groves of the Adjuntas experimental research station during the course of the survey; *C. inaequalis*, *C. s. limbifer*, *C. innonata*, *C. cacti*, *C. nitidula*, *Scymnus* sp., *H. convergens*, and *C. montrouzieri*. The number of coccinellids observed was relatively constant over the 3-month study period (Fig. 1). *Coelophora inaequalis* and *Cycloneda s. limbifer* were the most common species found over the entire study period with the remaining species found on a regular basis, but in much lower numbers (Fig. 2).

Additionally, two species of Hymenoptera parasitized the coccinellids. *Homalotylus* sp. near *terminalis* Say (Hymenoptera: Encyrtidae) emerged from a *C. s. limbifer* pupa, while *Oomyzus* sp. near *scaposus* Thomson (Hymenoptera: Eulophidae) emerged from *C. montrouzieri* (larva) and *C. s. limbifer* (pupa) (determinations by M. W. Gates, pers. comm.).

Prey Acceptability

All eight coccinellid species in the no-choice tests fed on both host species with no rejection of any offered prey type, although variation in the quantity and rate of prey consumption was observed. During the allotted interval there were few differences in relative amounts of prey consumed in no-choice tests, with the possible exception of *C. nitidula*, which ate twice as many psyllids as aphids (Table 1). *Hippodamia convergens* demonstrated the highest rate of aphid consumption whereas *C. cacti*, *C. nitidula*, and *Scymnus* sp. showed the lowest rate (Table 2). The highest rate of psyllid consumption was observed with *C. innonata*, although not significantly different from the rates of all others except *C. cacti* and *Scymnus* sp. (Table 3).

Prey Preference

Two of the coccinellid species examined, *C. s. limbifer* and *C. inaequalis*, showed a strong preference for the brown citrus aphid, while *C. nitidula*, *C. cacti*, *C. innonata*, and *C. montrouzieri* showed significant preference for the Asian citrus psyllid (Table 4). The remaining two species, *H. convergens* and *Scymnus* sp. showed no preference for either prey species.

DISCUSSION

Of the seven species of Coccinellidae listed by Michaud (2004) as common in Florida citrus, only the exotic *Coelophora inaequalis* also was found

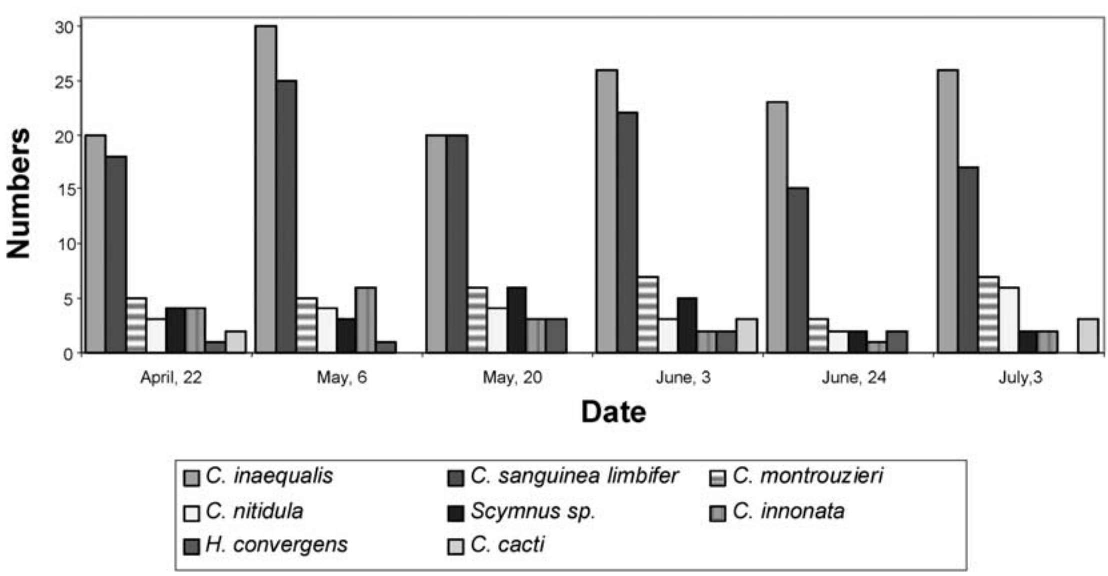

Fig. 1. Abundance of 8 coccinellid species during the 3-month sampling period in 2003 at the Adjuntas Agricultural Experimental Station in Puerto Rico.

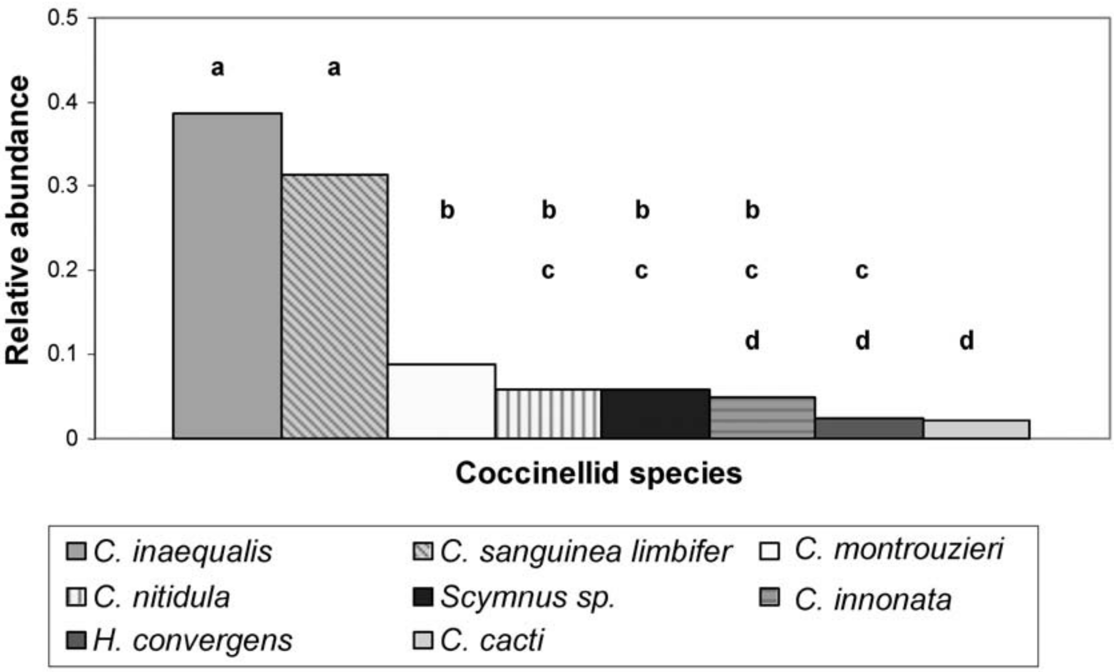

Fig. 2. Relative abundance of the coccinellid species caught at the Adjuntas Agricultural Experimental Station in Puerto Rico.

in this survey. However, *C. s. limbifer* was considered by Gordon (1985) as a subspecies of *C. sanguinea*, the most commonly encountered ladybeetle in Florida citrus groves prior to the invasion of *Harmonia axyridis* Pallas (Muma 1953; Michaud 2001; Michaud 2002). *Cycloneda limbifer* and *C. inaequalis*, both aphid-feeders, were the two most abundant species encountered in this study and also the most abundant reported from Puerto Rican citrus groves by Michaud and Browning (1999). The predominance of aphid feeders followed by scale feeders are characteristics shared by the coccinellid fauna in citrus groves of Florida and Puerto Rico.

TABLE 1. MEAN PERCENTAGE ± SD OF 10 PREY ITEMS CONSUMED IN NO CHOICE TESTS IN 7 H.

	Host species	
Species	BCA % eaten	ACP % eaten
H. convergens	94 ± 7%	80 ± 23%
C. innonata	84 ± 12%	83 ± 16%
C. s. limbifer	60 ± 32%	60 ± 13%
C. inaequalis	58 ± 26%	54 ± 21%
C. nitidula	30 ± 13%	65 ± 25%
C. montrouzieri	46 ± 21%	47 ± 21%
C. cacti	36 ± 9%	47 ± 8%
Scymnus sp.	16 ± 5%	16 ± 9%

Our results showed that all ladybeetle species, with the exception of *C. nitidula*, consumed similar quantities of either host species presented separately. *Cladis nitidula* consumed more psyllids and also was the species showing greatest preference for *D. citri* when offered a choice between prey. Thus, choice and no-choice experiments were consistent in indicating *C. nitidula*'s preference for *D. citri* over *T. citricida*. A preference for *T. citricida* over *D. citri* was strongly expressed by *Cycloneda s. limbifer*, and to a lesser extent by *Coelophora inaequalis*, the two most abundant ladybeetles in this study, although both species consumed equal amounts of both prey when given no choice. Michaud & Olsen (2004) found that *Cycloneda sanguinea* also fed on *D. citri* as both larva and adult, but that larval development time was almost doubled compared to a diet of *E. kuehniella* and that female *C. sanguinea* stopped ovipositing following transferal to the *D. citri* diet. Further studies would be necessary see whether a *D. citri* diet negatively impacts the larval and reproductive performance of *Cycloneda s. limbifer* as it does *C. sanguinea*.

Prey suitability for Coccinellidae is a complex issue that goes beyond the scope of prey acceptance and prey preference studied here, and generalizations such as "aphid feeders" and scale feeders" are overly simplistic (Hodek 1996). Nevertheless, the species demonstrating the greatest degree of acceptance and preference for BCA,

TABLE 2. MEAN ± SD CONSUMPTION RATES OF COCCINELLIDS ON THE BROWN CITRUS APHID IN NO CHOICE TESTS.

Species	Mean # of BCA consumed/h	Standard deviation	Tukey's comparison			
H. convergens	1.54	0.64	a			
C. innonata	1.09	0.42	a	b		
C. s. limbifer	0.93	0.43		b	c	
C. inaequalis	0.78	0.40		b	c	d
C. montrouzieri	0.53	0.39		b	c	d
C. cacti	0.37	0.27			c	d
C. nitidula	0.30	0.26				d
Scymnus sp.	0.23	0.08				d

Note: different letters denote a significant difference at $P = 0.05$.

such as *C. s. limbifer*, *C. inaequalis*, and *H. convergens* were not surprisingly those considered to be aphid feeders. Those that preferred and/or most readily accepted ACP or showed no clear tendencies were either known to feed principally on other prey such as Diaspididae (*C. cacti*), and Pseudococcidae (*C. montrouzieri*), or had feeding habits that were largely undocumented (*C. nitidula*, *Scymnus* sp.).

Diaphorina citri was rarely encountered in Adjuntas during the course of this study, and ACP populations have remained generally low in Puerto Rico on both orange jasmine and citrus. Coccinellids typically respond to dense prey populations whereas parasitoids with narrow host ranges such as *T. radiata* are expected to track their host population at low densities. It is difficult to ascertain, in retrospect, why ACP never achieved high infestations in Puerto Rico as it has Florida; however, preliminary evidence suggests that *T. radiata* may be responsible for holding the psyllid in check in Puerto Rico (R. Pluke, unpublished data). In any case, the coccinellid guild present in Puerto Rican citrus, with its demonstrated ability to consume ACP and its similar mix of species in regard to feeding habits to the coccinellid guild in Florida, would likely respond positively to any future increase in psyllid numbers.

TABLE 3. MEAN ± SD CONSUMPTION RATES OF COCCINELLIDS ON THE ASIAN CITRUS PSYLLID IN NO CHOICE TESTS.

Species	Mean # of ACP consumed/h	Standard deviation	Tukey's comparison		
C. innonata	1.21	0.18	a		
H. convergens	1.14	0.33	a	b	
C. nitidula	1.14	0.74	a	b	
C. s. limbifer	0.88	0.55	a	b	
C. inaequalis	0.76	0.60	a	b	c
C. montrouzieri	0.67	0.30	a	b	c
C. cacti	0.50	0.32		b	c
Scymnus sp.	0.16	0.15			c

Note: different letters denote a significant difference at $P = 0.05$.

TABLE 4. MEAN PERCENTAGE (OF 5 HOST INDIVIDUALS) EATEN BY COCCINELLIDS IN CHOICE TESTS.

	Prey species			
Species	BCA % eaten	ACP % eaten	Preference	*P* value
C. s. limbifer	64	4	Aphid**	0.004
C. inaequalis	78	28	Aphid**	0.016
C. nitidula	8	76	Psyllid**	0.002
C. cacti	30	80	Psyllid**	0.031
C. innonata	46	72	Psyllid**	0.031
C. montrouzieri	32	68	Psyllid*	0.055
H. convergens	60	58	N/A	0.711
Scymnus sp.	31	31	N/A	1.000

Note: **significant at $P \leq 0.05$, *P significant at $P \leq 0.1$.

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