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Status of biological control by egg parasitoids of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus in Florida and Puerto Rico

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Abstract. Eggs of *Diaprepes abbreviatus* (L.) were routinely monitored in citrus groves at ten locations in Florida during 1997 and 1998 to study egg parasitism. One citrus location was studied in Puerto Rico. No native parasitoids were recovered from 1,337 *D. abbreviatus* egg masses studied in Florida citrus. In contrast, an average of 35.5% (range 12.5 to 68.8%) parasitism of egg masses was reported in Puerto Rico. The parasitoids *Aprostocetus gala*, *Horismenus* spp, and *Quadrastichus haitiensis* were recovered from the eggs of *D. abbreviatus* studied in Puerto Rico. The *Horismenus* parasitoids were suspected hyperparasitoids. Releases of the parasitoid *Ceratogramma etiennei* from Guadeloupe were initiated during 1998 at each of the Florida research sites. By the end of 1998, *C. etiennei* had been recovered from *D. abbreviatus* eggs at two of nine locations in Florida citrus. The parasitoid was recovered from 1 of 34 egg masses at one of these locations during the month of September and from 3 of 34 egg masses at the other location during the month of November. Whether or not *C. etiennei* establishes itself at one or more locations in Florida remains to be seen.

Key words: citrus, classical biological control, egg parasitoids, root weevils, *Ceratogramma etiennei*, *Diaprepes abbreviatus*

Introduction

The root weevil *Diaprepes abbreviatus* (L.) is an important citrus pest in Florida (Hall, 1995; McCoy et al., 1995). Larvae of the weevil can cause substantial damage to roots resulting in decreased production and sometimes

plant death (Woodruff, 1968). Larvae and adults are highly polyphagous (Simpson et al., 1996). Adult female *D. abbreviatus* place their eggs with an adhesive in a single layer in a niche fabricated by juxtaposing leaves (Howden, 1995) or other available surfaces (Wolcott, 1933). Neonates fall to the ground and enter the soil where they feed on progressively larger roots as they grow (Browning et al., 1995). The larvae can infest and destroy the root systems of both seedlings and mature citrus trees (Beavers et al., 1979). Damage to roots is often in the form of girdling, which can cause the death of small citrus trees (Woodruff, 1968) and probably larger trees. In addition to direct damage, larval feeding provides infection sites for plant pathogens, notably *Phytophthora* spp., that can contribute to tree mortality and reductions in productivity (Rogers et al., 1996).

Management strategies currently advocated for an infestation of *Diaprepes abbreviatus* in Florida citrus include chemical sprays for adult and egg control, entomopathogenic nematodes for control of larvae, tactics to control *Phytophthora* if this fungal pathogen is present in an infested grove, and carefully managed tree fertilization and irrigation practices to prevent tree stress (Bullock et al., 1999). A management program employing all of these control tactics is expensive, and general observations indicate that the program may reduce but not prevent economic losses due to the weevil. Additional and alternative management tactics are therefore being researched.

Biological control by egg parasitoids may hold some potential as an additional component of a management program for the weevil in Florida citrus. Although no indigenous egg parasitoids have been reported to attack *Diaprepes abbreviatus* in Florida, reports and museum specimens from Puerto Rico, Dominican Republic, Dominica and other Caribbean and West Indian countries indicate that five parasitoid species may attack eggs of *D. abbreviatus: Ceratogramma etiennei* Delvare (Hymenoptera: Trichogrammatidae) in Guadeloupe; *Aprostocetus gala* (Walker) (= *Aprostocetus vaquitarum* (Wolcott)) (Hymenoptera: Eulophidae) in Puerto Rico, Guadeloupe, and Dominica; *Quadrastichus* (previously placed in *Tetrastichus*) *haitiensis* Gahan (Hymenoptera: Eulophidae) in Guadeloupe, Puerto Rico, and Dominica; *Q. fennahi* Schauff (Hymenoptera: Eulophidae) in St Lucia and Barbados; and *Brachyufens osborni* (Dozier) (Hymenoptera: Trichogrammatidae) in Puerto Rico, Dominican Republic and Andros (Etienne and Delvare, 1991; Schauff, 1987; Pinto and Viggiani, 1991) [questions have been raised regarding whether some of these parasitoid species actually parasitize *D. abbreviatus*]. The parasitoid *Horismenus bennetti* Schauff (Hymenoptera: Eulophidae) has been recovered from eggs of *D. abbreviatus* in the Caribbean and West Indies, but it is thought to be a hyperparasite of *Quadrastichus* spp. (Schauff, 1987). The economic benefits of biological control by parasitoids

of *D. abbreviatus* eggs are not known, however, *D. abbreviatus* is generally considered an important pest in the Caribbean and West Indies in spite of the presence of egg parasitoids.

Two parasitoid species in Florida citrus have been reported to attack eggs of the citrus root weevils *Pachnaeus litus* and *P. opalus*: *Brachyufens osborni* (Baranowski, 1960; Beavers et al., 1980) and *Trichogramma* sp. (Beavers et al., 1980). Neither of these parasitoids has ever been recovered in Florida from eggs of *Diaprepes abbreviatus*, which raises questions regarding reports that *B. osborni* parasitizes *D. abbreviatus* in Caribbean areas. The parasitoid *Quadrastichus haitiensis* is recognized as an important biological control agent of *D. abbreviatus* eggs in Puerto Rico (Wolcott, 1948; Woodruff, 1964). *Q. haitiensis* was imported from Puerto Rico and released in Florida citrus during 1969. Subsequently, the parasitoid was recovered from one *D. abbreviatus* egg mass from a release location near Apopka (Sutton et al., 1972) and from one *P. litus* egg mass from a release location near West Palm Beach (Beavers and Selhime, 1975). Further releases of the parasitoid were made during 1970 and 1971, but recovery efforts each year indicated the parasitoid did not establish (Beavers and Selhime, 1975). During 1978 in a citrus grove near Oakhill, Florida, in Volusia county, *Q. haitiensis* was recovered from egg masses of *P. opalus*, indicating the parasitoid was established in Florida (Beavers et al., 1980). However, weevil eggs screened in citrus during the 1980s and early 1990s produced no parasitoids (H. Browning, personal communication), suggesting that *D. abbreviatus* is currently not subjected to any biological control by egg parasitoids in Florida. In view of the potential for further releases of parasitoids from the Caribbean and West Indies, additional attempts at classical biological control of *D. abbreviatus* eggs in Florida citrus appear justified.

Reported here are the results of a survey conducted to determine whether eggs of *Diaprepes abbreviatus* in Florida citrus are attacked by indigenous parasitoids and, if so, to what extent. Similar research was conducted in Puerto Rico for comparison. Releases of the exotic parasitoid *Ceratogramma etiennei* from Guadeloupe were subsequently initiated in Florida citrus. *C. etiennei* was chosen for releases because co-author Peña had established a colony and had received clearance to release the parasitoid. The outcome of early recovery attempts for this parasitoid is discussed.

Materials and methods

Eggs of *Diaprepes abbreviatus* were monitored for parasitoids during 1997–1998 in Florida citrus at ten locations (eight counties): Moore Haven (Glades Co.); Homestead (Dade Co.); Denaud (Hendry Co.); Vero Beach site #1

Table 1. Citrus sites sampled during the study

Location	Scion	Rootstock	Soil type	Tree age	Grove size
Alturas, FL	Hamlin	Swingle	Astatula fine sand	9–10 yrs	4 ha
Apopka, FL	Hamlin	Carrizo	Sandy loam	3 yrs	1 ha
Denaud, FL	Mandarins, sweet oranges	Mixed	Pinada Fine Sand	Mixed	25 ha
Ft Pierce, FL #1	Red grapefruit	Cleopatra	Winder loamy sand	11 yrs	32 ha
Ft Pierce, FL #2	Hamlin	Carrizo	Immokalee sand	6 yrs	12 ha
Homestead, FL	Lime	Wide collection	Rockdale	1–25 yrs	1 ha
Plant City, FL	Hamlin	Cleopatra Swingle	Orlando fine	5–12 yrs	5 ha
Moore Haven, FL	Fallglo, Valencia	Swingle	Immokolee sand	6–7 yrs	1 ha
Vero Beach, FL #1	White, red grapefruit	Cleopatra Swingle	Winder fine sand	11–29 yrs	3 ha
Vero Beach, FL #2	Navels, red grapefruit	Sour orange	Winder fine sand	8–25 yrs	4 ha
Isabela, PR	Valencia	Cleopatra	Cotto	3–4 yrs	2 ha

(Indian River Co.); Vero Beach site #2 (Indian River Co.); Ft Pierce site #1 (St Lucie Co.); Ft Pierce site #2 (St Lucie Co.); Apopka (Orange Co.); Alturas (Polk Co.); and Plant City (Hillsborough Co.). One location was studied near Isabela in Puerto Rico during 1998–1999. Table 1 presents basic information on the study sites. No insecticides were applied during the study period at any of the locations researched.

The parasite recovery methodology was to, at least once a month, cage ovipositing female weevils onto bouquets of citrus leaves for 24–48 h. The caged bouquets of leaves usually consisted of both mature and expanded flush leaves. One bouquet of leaves was caged per tree within the basal third of the canopy, with up to five trees receiving a cage across an area of 50 to 100 trees (about 0.25 ha). One to five wild adult weevils of each sex were released into each cage. After the 24–48 h period, cages and adults were removed and

leaves with eggs were tagged. Four days later, these leaves with eggs were excised and taken to a laboratory. Individual egg masses (still intact on leaf tissue) were placed into a 30 ml glass vial with a screw cap, and then the vials were placed into a humidified plastic bag on a lab bench under ambient conditions (among the laboratories holding eggs, mean 24.4 °C, SEM 0.57, maximum 26 °C and minimum 22 °C). One week later the caps were removed from the vials, the opening of each vial was plugged with cotton wrapped in a piece of tissue paper, and then the vials were placed back onto a bench under ambient conditions. The vials were examined for neonate larvae and emerged parasites every 4 to 7 days over a 28-day period, during which time the cotton plugs were kept moist. Number of eggs per mass, percent egg hatch, and percent eggs parasitized were determined. For reporting purposes, data from 2 sites sampled near Vero Beach were combined and data from 2 sites near Ft Pierce were combined.

Releases of *Ceratogramma etiennei* were initiated in Florida during May 1998. The parasitoid was imported from Guadeloupe by co-author J. Peña and reared at Homestead (Peña et al., 1998) and Gainesville. Peña planned a separate publication to review specific rearing procedures for *C. etiennei*. Briefly, the general methods employed by Beavers and Selhime (1975) to propagate *Quadrastichus haitiensis* were used to propagate *C. etiennei*. Adult *Diaprepes abbreviatus*, field-collected from limes and silver buttonwood (*Conocarpus erectus* L. variety *sericeus* Fors. ex DC (Combretaceae)), were held in a laboratory for oviposition on wax paper. A number of strips of wax paper with fresh eggs were then exposed to adults of *C. etiennei* (generally at a ratio of 2 to 8 masses per female parasitoid) in a glove box. All rearing was conducted at 24–26 °C, 12:12 L:D photoperiod, 75–81% RH. Progeny were field-released either by hanging wax paper strips with nearly-mature parasitoid pupae in trees, by placing wax paper strips with nearly-mature parasitoid pupae in paper emergence bags hung in citrus trees, or by releasing newly-emerged adults into trees. In cases where adults were released, they were aspirated and released (no honey or other adult food was supplied to the newly-emerged adults). Releases of *C. etiennei* were made within the same 50- to 100-tree areas in which we surveyed for parasitoids of *D. abbreviatus* eggs, and the releases sometimes coincided with periods of time during which we were sampling for egg parasitoids (i.e., weevil eggs on tagged leaves were available to parasitoids).

Parasitoid specimens observed during the study were identified by Dr John Pinto (Department of Entomology, University of Riverside, Riverside, CA) and Dr Michael Schauff (USDA-ARS, c/o National Museum Natural History, Washington, DC).

Results and discussion

Totals of 1,337 and 91 egg masses oviposited on citrus leaves by *Diaprepes abbreviatus* in Florida and Puerto Rico, respectively, were screened for parasitoids during the study (Table 2). A mean ± SEM of 53.8 ± 2.8 eggs per mass was observed in Florida and 49.8 ± 3.0 eggs per mass was observed in Puerto Rico (Table 2). Using our holding method in the lab, percentage egg eclosion per mass averaged 62.7 ± 3.7% among masses studied in Florida and 68.9 ± 7.9% among masses studied in Puerto Rico (Table 2). Percentages of eclosion per mass lower than 10% were occasionally observed. In cases of low percent hatch, the eggs usually looked normal at first, then turned milky white and later became brown and shriveled. In these cases it was possible that unfertile eggs had been laid, but this was not confirmed. No native parasitoids of *D. abbreviatus* eggs were recovered in Florida citrus; in contrast, a mean of 35.5 ± 11.9% parasitism of egg masses (range 12.5 to 68.8%) was reported in Puerto Rico (Table 2). With respect to parasitized masses observed in Puerto Rico, data were not collected on the percentage of parasitized eggs per mass. *Aprostocetus gala, Horismenus* spp., and *Quadrastichus haitiensis* were recovered from eggs of *D. abbreviatus* in Puerto Rico (identifications from co-author R. Franqui), but information on the relative abundance of these parasitoids was not recorded. However, samples of eggs thought to be *D. abbreviatus* from citrus (egg masses of this weevil morphologically resemble eggs of other citrus root weevil species) were obtained from Puerto Rico during the summer 1998, and parasitoid species recovered under quarantine in Gainesville, Florida, included predominantly *Q. haitiensis* but also *A. gala*, *Brachyufens osborni*, and *Horismenus bennetti*. As mentioned earlier, *H. bennetti* was a suspected hyperparasitoid. Because *B. osborni* in Florida citrus is known to attack *Pachneus* spp. but not *D. abbreviatus*, the presence of this parasitoid in the samples suggested either that Puerto Rican populations of *B. osborni* do parasitize *D. abbreviatus* in citrus or that some of the weevil egg masses studied were incorrectly identified as *D. abbreviatus* from citrus. Whether or not *B. osborni* parasitizes *D. abbreviatus* in citrus in the Caribbean area needs confirmation.

Over 62,000 adult *Ceratogramma etiennei* were released into Florida citrus during May–November 1998 (Table 3). The number of parasitoids released at a time varied and was dependent upon production. An average total of approximately 1,430 parasites (equivalent to about 5,720 parasites per ha) was released each month at each study location. Individual releases ranged from the equivalent of around 560 to around 1,200 parasites per ha. Usually 1 or 2 individual releases of the parasitoid were made monthly at each study site. A total of 1,181 *Diaprepes abbreviatus* egg masses were screened for parasitoids after releases were initiated, among which *C. etiennei*

Table 2. Results of the survey in citrus for parasitoids of *D. abbreviatus* eggs in Florida and Puerto Rico (a dash indicates no sampling was conducted)

Year	Loc[a]	Variable[b]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
1997	Ap	Egg masses	—	—	—	—	—	18	9	4	3	—	—
		Masses parasitized	—	—	—	—	—	0	0	0	0	—	—
	Dn	Egg masses	—	—	—	—	—	1	30	51	49	53	6
		Masses parasitized	—	—	—	—	—	0	0	0	0	0	0
	Hm	Egg masses	—	—	—	—	—	—	—	—	—	1	—
		Masses parasitized	—	—	—	—	—	—	—	—	—	0	—
	FP	Egg masses	—	—	—	—	—	—	—	20	27	19	—
		Masses parasitized	—	—	—	—	—	—	—	0	0	0	—
	MH	Egg masses	—	—	—	11	7	31	7	16	14	5	5
		Masses parasitized	—	—	—	0	0	0	0	0	0	0	0
1998	Al	Egg masses	—	—	1	5	5	—	18	1	52	—	—
		Masses parasitized	—	—	0	0	0	—	0	0	0	—	—
	Ap	Egg masses	—	—	—	21	17	16	8	9	14	3	—
		Masses parasitized	—	—	—	0	0	0	0	0	0	0	—
	Dn	Egg masses	—	—	—	11	13	—	—	—	—	—	34
		Masses parasitized	—	—	—	0	0	—	—	—	—	—	3[c]
	FP	Egg masses	—	—	—	—	53	66	51	28	48	30	7
		Masses parasitized	—	—	—	—	0	0	0	0	0	0	0
	Hm	Egg masses	—	—	2	2	16	5	—	6	—	8	—
		Masses parasitized	—	—	0	0	0	0	—	0	—	0	—
	MH	Egg masses	—	—	—	8	11	6	5	16	34	13	7
		Masses parasitized	—	—	—	0	0	0	0	0	1[c]	0	0
	PC	Egg masses	—	—	1	6	3	1	14	4	31	—	11
		Masses parasitized	—	—	0	0	0	0	0	0	0	—	0
	VB	Egg masses	—	—	—	29	15	23	31	47	35	21	28
		Masses parasitized	—	—	—	0	0	0	0	0	0	0	0
	PR	Egg masses	—	—	—	—	—	32	12	—	—	—	11
		Masses parasitized	—	—	—	—	—	22[d]	4[d]	—	—	—	3[d]
1999	PR	Egg masses	20	16	—	—	—	—	—	—	—	—	—
		Masses parasitized	5[d]	2[d]	—	—	—	—	—	—	—	—	—

[a]Locations: Al=Alturas, Ap=Apopka, Dn=Denaud, FP=Ft Pierce, Hm=Homestead, MH=Moore Haven, PC=Plant City, VB=Vero Beach, PR=Puerto Rico.
[b]Variable: Egg masses is number of masses observed, Masses parasitized is number of masses parasitized.
[c]Parasitoids identified as *Ceratogramma etiennei*.
[d]Parasitoids identified as *Quadrastichus haitiensis, Aprostocetus gala* and *Horismenus* spp.

was recovered from one egg mass at Moore Haven during September and from three egg masses at Denaud during November (Table 2). With respect to the parasites recovered at Moore Haven, the egg mass parasitized had been present on a tree during 16–21 September and the closest *C. etiennei* release date was 26 August. Since adult *C. etiennei* live for 5 to 7 days with perhaps a maximum of 10 days (Amalin et al., unpublished), adult progeny from a previous release (perhaps the one on 26 August) probably parasitized this weevil egg mass at Moore Haven. This particular mass consisted of 42 eggs, all had turned brown a week after removing them from the tree, and 24

Table 3. Releases of *Ceratogramma etiennei*, approximate numbers of adults deployed (1998)[a]

Location	May	Jun	Jul	Aug	Sep	Oct	Nov
Alturas	1,000	500	1,000	500	1,000	500	1,000
Apopka	—	—	300	700	—	500	—
Denaud	400	—	2,000	1,800	7,450	2,100	—
Homestead	440	450	5,900	5,900	3,900	900	2,650
Ft Pierce	140	1,330	2,300	450	800	500	—
Moore Haven	—	130	540	2,200	—	4,800	1,000
Plant City	1,000	500	1,000	500	1,000	500	1,000
Vero Beach	—	250	—	260	1,070	725	—

[a]Mean of 73.3% (s.e. 1.75) of the adults were female based on 46 determinations during 1998.

produced parasites a week later (the remaining eggs shriveled). At Denaud, the parasitized egg masses had been present on trees on a *C. etiennei* release date (no data were collected from these masses with respect to numbers of eggs parasitized).

Based on the low incidence of *C. etiennei* recoveries during 1998, whether or not the parasitoid was establishing at any of the release sites in Florida could not be ascertained. Attempts to establish *C. etiennei* in the Dominican Republic failed (McCoy, unpublished information). Why *Quadrastichus haitiensis* and *C. etiennei* are well established in some Caribbean/West Indian areas and yet may fail to establish in other areas is not known. Beavers and Selhime (1975) speculated that differences in environmental conditions between Puerto Rico and Florida may have contributed to the failure of *Q. haitiensis* to establish on *D. abbreviatus* in Florida. Similarly, differences in climate may have contributed to the failure of *C. etiennei* from Guadeloupe to establish in the Dominican Republic and its possible failure in Florida. Other factors may influence the establishment of parasitoids for biological control of *D. abbreviatus* eggs in Florida including the availability of food sources (e.g., nectar or pollen) for adult parasites, the particular plant species on which weevils oviposit, and the seasonal availability of host eggs. In any case, *Q. haitiensis* from Puerto Rico and *C. etiennei* from Guadeloupe have now each been reared successfully on Florida populations of *D. abbreviatus* in the laboratory, indicating host compatibility.

Permanent establishment of *C. etiennei* or other egg parasitoids may be achievable in at least some areas in Florida, but whether or not egg parasitoids would reduce the economic importance of *D. abbreviatus* in citrus is not known. Unfortunately, information is lacking on the impact these parasitoids

may have against the economic importance of the weevil in the Caribbean and West Indies. The likelihood that egg parasitoids would be of major value in controlling *D. abbreviatus* in Florida is reduced given the fact that the weevil is considered to be an important pest in areas where egg parasitoids are already present (although it is possible in some of these areas that hyperparasitoids confound the benefits of the egg parasitoids to some extent).

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