EXPLORATION IN BELIZE FOR PARASITOIDS ATTACKING EGGS OF CITRUS WEEVILS, AND AN EVALUATION OF *PEDIOBIUS IRREGULARIS* AND *HORISMENUS BENNETTI* (HYMENOPTERA: EULOPHIDAE) AS POTENTIAL BIOLOGICAL CONTROL AGENTS OF *DIAPREPES ABBREVIATUS* AND *PACHNAEUS LITUS* (COLEOPTERA: CURCULIONIDAE)

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During July 16-23, 2001, Belize was explored for parasitoids attacking eggs of citrus weevils. The trip, organized by coauthors Peña and Hall and funded by a grant from the Florida Citrus Production Research Advisory Council (981-42E), was made in hopes of finding parasitoid species that could be introduced into Florida to boost biological control of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.), a major pest of citrus (*Citrus* spp.) and ornamental plants in Florida (Hall et al. 2001). No parasitoids are known to attack eggs of *D. abbreviatus* in Florida (Hall et al. 2001). Attempts in Florida to establish egg parasitoids that attack *D. abbreviatus* in Guadeloupe (*Ceratogramma etiennei* Delvare, Hymenoptera: Trichogrammatidae), Puerto Rico (*Quadrastichus haitiensis* Gahan) and the Dominican Republic (*Aprostocetus vaquitarum* (Wolcott)) (Hymenoptera: Eulophidae) have been made (e.g., see Hall et al. 2001, Peña et al. 2000).

Exploration in Belize, conducted by coauthors Hall and Eger, was confined to the Stan Creek District in the southern half of Belize where most of the country's citrus is grown. Five days were spent exploring five citrus groves near the town of Dangriga and one day exploring four groves near the city of Belmopan. The groves surveyed near Dangriga included Mullin's River grove (sweet oranges, *C. sinensis* (L.) Osbeck) about 5 km west of Dangriga; Buckshell's grove (grapefruit, *C. paradise* Macf.) 4 to 5 km west to southwest of Dangriga; two sweet orange groves 8 to 10 km southwest of Dangriga; and Bowman's grove (sweet oranges) to the south of Dangriga. The groves surveyed near Belmopan included the Werrie Head Resort Inn grove (sweet oranges) around 5 km to the west of the city.

Three weevil species were collected from citrus trees during the trip: *Exophthalmus vitticollis* Champion, *E. lunaris* Champion and *Tanymecus confusus* (Say) (identifications by coauthor O'Brien). The Diaprepes root weevil was not observed and was not known to occur in Belize. A total of 178 adult weevils thought to be *E. vitticollis* were collected during the trip; 138 of these were examined by O'Brien and confirmed as *E. vitticollis* (voucher specimens deposited with the Florida State Collection of Arthropods (FSCA) in Gainesville and with coauthor O'Brien's personal collection). Adult *E. vitticollis* were common at most collection sites and abundant at some. We encountered only two adult *E. lunaris* (Mullin's River grove) and six adult *T. confusus* (Buckshell's grove), and whether these species utilized citrus as a host was not known. *E. vitticollis* was previously known to be associated with citrus in Belize (Schauff 1987). The economic importance of citrus weevils in Belize was unclear, but it generally appeared that most growers did not consider weevils to be significant pests. Trees from which weevils were collected varied in apparent health and productivity, and it was possible that some trees that did not appear healthy were suffering from attack by weevil larvae.

The majority of time spent at each citrus location was devoted to searching citrus leaves for weevil egg masses. Specifically, we looked for weevil egg masses glued between two juxtaposing leaves (Hall et al. 2001). A total of 168 egg masses were found; these ranged from freshly deposited to possibly several weeks old. Leaves with eggs were carefully removed from trees and placed into plastic containers with ventilation screens. Each evening after collecting, leaves with eggs were removed from the containers and trimmed with scissors, leaving a minimum of leaf tissue around each egg mass. The masses were then placed into shell vials (1 dram) and plugged with a small wad

of tissue paper. Of the 168 egg masses collected, 121 were placed individually into shell vials while the remaining were placed 3 to 5 at a time into vials. The shell vials were then placed into a compartmentalized plastic box with snap lid. A moistened paper towel was laid on top of the plastic box, and the box and towel were slipped into a ziplock bag. Shell vials with egg masses were added to the plastic boxes each night. Observations after several days of collecting indicated that the humidity level inside the boxes was excessive (some leaf tissue showed beginning signs of mold); thereafter no moistened paper towels were placed inside the ziplock bags. Three such ziplock bags containing eggs in vials inside a plastic box were transported from Belize to Miami inside a small cooler with a blue ice pack. In Miami, the material was transferred to coauthor Peña and then into quarantine at the University of Florida Quarantine Center at Homestead. The importation into Florida was covered by USDA-APHIS PPQ Form 526 permit 37969.

In addition to egg masses of *E. vitticollis*, dead adult weevils infected by an entomopathogenic fungus were collected and returned to Florida. The diseased cadavers were collected on July 19 in an orange grove located about 10 km southwest of Dangriga. The dead weevils observed were widely distributed among larger numbers of live adults within the orange grove. All diseased weevils had died clutching a twig, a typical behavioral response of many insect hosts to invasion by a fungal pathogen. Diseased hosts had stalk-like structures protruding from the intersegmental areas of the body, typical of clavae formed by a *Cordyceps* (Samson et al. 1988). Each clava had a pinkish red head at the distal end. Until further mycological studies are completed at the ultrastructual level, the species will remain unknown. While in Belize, several diseased weevils were macerated in water and the mixture was sprayed onto new flushes of leaves, a common food source of adult citrus weevils. The treated leaves were then placed in a ventilated plastic box with about 20 adult weevils. After 3 days exposure under unknown ambient conditions, weevil behavior and mortality were assessed. None of the weevils exhibited abnormal behavior and none died. It was unknown, however, whether infective spores were in the original preparation or if environmental conditions were sufficient for infection to occur.

Two parasitoid species were recovered under quarantine from weevil eggs collected in Belize: *Pediobius irregularis* Kerrich and *Horismenus bennetti* Schauff (Hymenoptera: Eulophidae) (identifications by coauthor Evans) (voucher specimens placed with FSCA in Gainesville). *P. irregularis* had been identified previously as a parasitoid of *E. vitticolis* eggs in Belize (Schauff 1987), and *H. bennetti* had been identified previously as a suspect hyperparasitoid associated with egg parasitoids of citrus weevils in the Caribbean and West Indies (Schauff 1987) and known to occur in Belize (Etienne & Delvare 1991). A total of 280 individual adult parasitoids emerged from the material under quarantine; of 122 parasitoids formally inspected by coauthor Evans, 83 were *P. irregularis* and 39 were *H. bennetti*. Greater numbers of these parasitoids were obtained from material collected at Buckshell's grapefruit grove than from any other collection site. Few parasitoids were recovered from Bowman's grove, and none were recovered from material collected at the Weirrie Head Resort Inn grove. *P. irregularis* was the only parasitoid obtained from material collected at Mullin's River grove, although relatively few weevil eggs were collected from this site. Observations under quarantine indicated 87% of the weevil egg masses from Belize were parasitized. This percentage may not have been a good indicator of actual percent parasitism levels in the field, as it is possible that parasitized egg masses are easier to find than non-parasitized eggs (i.e., the adhesive associated with an egg mass between two juxtaposing leaves may be less apt to come apart if an egg mass is parasitized, increasing the chances of finding a parasitized mass). However, general observations indicated *E. viticollis* was subjected during July to at least moderate levels of parasitism in some locations such as Buckshell's grapefruit grove.

The potential of *P. irregularis* and *H. bennetti* as candidate parasitoids for *D. abbreviatus* and also for the citrus root weevil, *Pachnaeus litus* (Germ.) (another weevil pest of citrus in Florida) was evaluated under quarantine by coauthors Peña and Duncan. As adult parasitoids emerged from the Belize weevil eggs, some were placed into cages (clear plastic boxes, 0.03 m^3) along with weevil egg masses (one to three days old) which had been oviposited onto citrus leaves, leaves of green buttonwood (*Conocarpus erectus* L.), leaves of pigmy palm (*Phoenix roebelinii* O'Brien) or wax paper (some or all of these substrates as noted). Honey and water were provided in these cages as a food source for the adult parasitoids. With respect to the parent generation of *P. irregularis*, a ratio of 3 females to 1 male was observed. No parasitism of *D. abbreviatus* or *P. litus* eggs occurred by either endoparasitoid species during exposures made July 24-31 (Table 1). Low levels of parasitism by *P. irregularis* of both *D. abbreviatus* and *P. litus* eggs occurred during exposures made July 31-August 10, but the F_1 adult parasitoids recovered failed to parasitize the eggs of either weevil species (Table 2). The sex ratio of the F_1 parasitoids was not determined, but both sexes were produced from each weevil species. The F_1 parasitoids survived for a maximum of 17 days. No obvious predation by adult parasitoids on weevil eggs was observed, but this possibility was not investi-

TABLE 1 PARASITISM OUTCOMES IN CAGES CONTAINING EGG MASSES OF *DIAPREPES ABBREVIATUS* AND *PACHNAEUS LITUS* EXPOSED TO ADULT *HORISMENUS BENNETTI* AND/OR *PEDIOBIUS IRREGULARI*.

	Exposed to both parasitoid species together 7/24 -7/31[a]		Exposed to adult *H. bennetti* 7/31-8/13[b]	
Weevil species and oviposition substrate	Total # of egg masses provided	Total # F_1 adult parasitoids produced	Total # of egg masses provided	Total # F_1 *H. bennetti* adults produced
D. abbreviatus eggs on citrus leaves	0	—	2	0
D. abbreviatus eggs on buttonwood leaves	196	0	184	0
D. abbreviatus eggs on pigmy palm leaves	60	0	84	0
D. abbreviatus eggs on wax paper	10	0	0	—
P. litus eggs on citrus leaves	2	0	4	0
P. litus eggs on buttonwood leaves	4	0	2	0
P. litus eggs on pigmy palm leaves	0	—	0	—
P. litus eggs on wax paper	11	0	0	—

[a]A mix of *H. bennetti* and *P. irregularis* adults (202 total) introduced during 7/24-7/31.
[b]70 *H. bennetti* adults introduced during 7/31-8/13.

TABLE 2. PARASITISM OUTCOMES IN CAGES CONTAINING EGG MASSES OF *DIAPREPES ABBREVIATUS* AND *PACHNAEUS LITUS* EXPOSED TO ADULT *PEDIOBIUS IRREGULARIS*, F_0 ADULTS FROM BELIZE AND F_1 ADULTS OBTAINED IN QUARANTINE.

	Exposed to adult *P. irregularis* 7/31-8/10[a]		Exposed to F_1 adult *P. irregularis* 8/24-8/31[b]	
Weevil species and oviposition substrate	Total # of egg masses provided	Total # F_1 adult parasitoids produced	Total # of egg masses provided	Total # F_2 *P. irregulars* adults produced
D. abbreviatus eggs on citrus leaves	9	8 (from 1 egg mass)	43	0
D. abbreviatus eggs on buttonwood leaves	259	5 (from 2 egg masses)	124	0
D. abbreviatus eggs on pigmy palm leaves	116	0	0	—
D. abbreviatus eggs on wax paper	0	—	0	—
P. litus eggs on citrus leaves	0	—	48	0
P. litus eggs on buttonwood leaves	10	0	0	—
P. litus eggs on pigmy palm leaves	8	16 (from 3 egg masses)	0	—
P. litus eggs on wax paper	0	—	0	—

[a]174 *P. irregularis* adults introduced during 7/31-8/10.
[b]31 *P. irregularis* adults introduced during 8/24-8/31.

gated because doing so would have jeopardized parasitoid recovery.

The research indicated *P. irregularis* held little promise as a candidate for a biological control program for either *D. abbreviatus* or *P. litus* and that *H. bennetti* held no promise at all. *H. bennetti* was not confirmed as a hyperparasitoid of *P. irregularis,* but this remained probable.

We thank the Belize Agricultural Health Authority (BAHA) for graciously hosting the trip, helping to obtain the required Belizean collection and exportation permits, for providing transportation and for collection assistance. Additional transportation and collecting assistance was kindly provided by the Citrus Research and Education Institute (CREI). We especially thank the following individuals for their support during this project: Orlando Sosa (BAHA), who served as our primary contact in Belize, and Francisco Gutierrez (CREI). We are grateful to Dr. Michael Tewes (BAHA) and Dr. Steven Williams (CREI) and their respective agencies for helping make this project possible. This research was partially supported by a grant from the Florida Citrus Producers Advisory Council (FCPRAC). Florida Agricultural Experiment Station Journal Series No. R-08996.

Summary

Two parasitoid species, *Pediobius irregularis* and *Horismenus bennetti*, were recovered from weevil eggs collected in citrus in Belize. *Exophthalmus vitticollis* was the principal weevil species observed in citrus during the trip. Under quarantine in Florida, *P. irregularis* parasitized eggs of the Florida citrus weevils *Diaprepes abbreviatus* and *Pachnaeus litus*, but few F_1 adults were recovered and no F_2 adults were produced. The research indicated *P. irregularis* held little promise as a candidate for a biological control program for either *D. abbreviatus* or *P. litus* and that *H. bennetti* held no promise at all.

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