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# EFFECTS OF HOST AGE, FEMALE PARASITOID AGE, AND HOST PLANT ON PARASITISM OF *CERATOGRAMMA ETIENNEI* (HYMENOPTERA: TRICHOGRAMMATIDAE)

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ABSTRACT

Parasitism of *Diaprepes abbreviatus* (L.) eggs by *Ceratogramma etiennei* Delvare as influenced by host age, age of the female parasitoid, and host plant preference was evaluated under laboratory or greenhouse tests. Percent parasitism of *D. abbreviatus* eggs by *C. etiennei* decreased as eggs matured. The optimal age of *C. etiennei* for successful parasitism ranged from 1-2-d old. Host plant leaf thickness, leaf pubescence, and plant strata probably played a role on the parasitism by *C. etiennei*. This parasitoid is diurnal and spent approximately 5 min searching for eggs laid in cryptic locations, 46 min parasitizing an egg mass and 24 min resting. This biological information is relevant in evaluating the potential of *C. etiennei* in the classical biological control of *D. abbreviatus*.

Key Words: parasitism, *Ceratogramma etiennei*, *Diaprepes abbreviatus*, host age, parasitoid age, host plants, host preference, host-finding behavior

RESUMEN

Se estudió el efecto de edad del hospedero, edad del parasitoide, y preferencia del parasitoide a la planta huésped en el parasitismo exitoso de *Ceratogramma etiennei* Delvare sobre huevos del picudo *Diaprepes abbreviatus* (L.). El porcentaje de parasitismo disminuyó al incrementar la edad de la postura. La edad óptima de *C. etiennei* para realizar un parasitismo exitoso es 1-2 d de edad. *Ceratogramma etiennei* demostró cierta preferencia a la planta huesped en terminos de grosor de la lámina foliar, pubescencia de hojas y estrato de la planta donde se encuentren las posturas de *D. abbreviatus*. *Ceratogramma etiennei* es diurna y gasta aproximadamante 5 minutos buscando las posturas, 46 min parasitizando y 26 min descansando. La información generada durante este estudio puede ayudar a evaluar el potencial de *C. etiennei* como agente de control biológico de *D. abbreviatus*.

Translation provided by the authors.

The Diaprepes root weevil, *Diaprepes abbreviatus* (L.), is one of the major pests of citrus, vegetables, and ornamentals in Florida, Puerto Rico, and the West Indies (Beavers et al. 1983; Figueroa & Roman 1990; Sirjusingh et al. 1992). *Diaprepes abbreviatus* adults feed on the foliage of many plant species belonging to at least 30 families (Simpson et al. 1996; Adair et al. 1998). After mating, the females deposit their eggs between host plant leaves glued together with an adhesive produced by the female (Richman et al. 1983). The eggs hatch in 7-10 d and the larvae drop to the surface of the ground and feed on the roots of most host plants (Woodruff 1964; Whitcomb et al. 1982). A lack of native parasitoids attacking this weevil in Florida (Hall et al. 2001) and past failures to establish exotic parasitoids against this weevil (Beavers et al. 1980), justify further efforts to introduce, release, and evaluate candidate parasitoids for Florida (Peña et al. 1998; Peña & Amalin 2000). One of these candidates is *Ceratogramma etiennei* Delvare (Hymenoptera: Trichogrammatidae), a highly specific parasitoid to Diaprepes in Guadeloupe (Etienne et al. 1990). It was introduced into Florida from Guadeloupe in 1997 (Peña et al. 1998) and released from 1998-2000 in citrus, ornamental fields, and natural habitats infested with *D. abbreviatus*. Subsequent to release, *C. etiennei* was recovered from lime, *Citrus aurantifolia* (Christman) Swingle, and pigmy palms, *Phoenix roebelenii* O'Brien in south Florida (Table 1). However, none were recovered from 2001-2002 in the same locations. The reason for its disappearance is unknown.

This paper provides some of the information that may be involved in the host selection process by *C. etiennei*, such as host age, age of the parasitoids, and host plant preference.

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TABLE 1. RECOVERY OF *CERATOGRAMMA ETIENNEI* IN FLORIDA, 1998-2000.

| County | Year | Commodity | Mean % parasitism |
|---|---|---|---|
| Miami-Dade | 1998 | Citrus, Guava, Ornamentals | 0.0 |
| | 1999 | Citrus | 20.2 |
| | | Guava | 0.0 |
| | | Ornamental | 75.7 |
| | 2000 | Citrus | 35.3 |
| | | Ornamental | 27.6 |
| Broward | 1998 | Citrus, Ornamental | 0.0 |
| | 1999 | Citrus, Ornamental | 0.0 |
| St. Lucie | 1999 | Citrus | 0.0 |
| Hendry | 1999 | Citrus | 0.0 |
| | 2000 | Citrus | 0.0 |

## MATERIALS AND METHODS

*Ceratogramma etiennei* used in this study was a Guadeloupe strain collected from *D. abbreviatus* eggs by J. Etienne. The colony was reared in a laboratory at 26.5 ± 1°C, 12:12 L:D, and approx 78% RH, on eggs of *D. abbreviatus* laid on strips of wax paper (Etienne et al. 1990). Adult *D. abbreviatus* used as a source of eggs were obtained from ornamentals in Homestead, Florida. Weevils were placed in Plexiglas® cages containing water, foliage of *Conocarpus erectus* L., and strips of wax paper.

### Host Age Preference

Laboratory test. One to 5-d-old *D. abbreviatus* egg masses on strips of wax paper were placed randomly in an experimental arena made of translucent plastic containers (70 mm high × 70 mm long × 20 mm wide). A mated 1-d-old female *C. etiennei* was introduced into each container, provided with honey and water and host eggs of various ages. The strips with egg masses were removed after 24 h and transferred to test tubes (12 × 75 mm) separately. Tubes were plugged with Kimwipes® tissue and held 10 days for parasitoid emergence. The number of eggs per mass used in this experiment ranged from 50-100 and was replicated 20 times. Parasitized eggs were counted and percent parasitism was computed by dividing the number of parasitized eggs by the total number of eggs on each wax paper strip. Weevil eggs parasitized by *C. etiennei* have a golden chorion which is characteristic of successful parasitism (Amalin, pers. observations).

### Greenhouse Test

Strips of wax paper containing 1- to 5-d-old *D. abbreviatus* eggs were randomly stapled to the upper side of leaf on 6-mo old potted *C. erectus* (height ranging from 50-65 cm) placed into a nylon mesh screen (91 cm wide × 91 cm long × 122 cm high) cage supported on a PVC frame. Ten 1-d-old mated female *C. etiennei* were released into the cage. The egg masses were collected after 24 h and processed in the same way described in the laboratory test. The test was replicated five times. Parasitized eggs from each egg mass were counted and the percent parasitism computed.

### Effect of Female Parasitoid Age on Parasitism

One-day-old mated female parasitoids were provided a 3-d-old weevil egg mass on wax paper strips for 24 h in test tubes (12 × 75 mm). Egg masses were replaced daily for 9 days (i.e., until females were 10 d old). This experiment was replicated 20 times. Exposed egg masses were collected and processed in the previously described manner. Parasitized eggs from each egg mass were counted and the percent parasitism computed.

### Choice Test on Selected Host Plants

Four 1-yr-old host plants, namely, lime (*Citrus aurantifolia*), green buttonwood (*Conocarpus erectus*), silver buttonwood (*Conocarpus erectus* variety *sericeus* Fors. ex. DC), and pigmy palm (*Phoenix roebelenii*) were tested to determine their effects on parasitism by *C. etiennei*. Four plants, one per species were placed into screen cages (3 m × 2 m × 1 m) along with 100 adult weevils (50:50 ♂:♀). The plants were removed after 3 d, leaving twenty egg masses per host plant. All plants were then placed in another screen cage (1 m × 1 m × 1.22 m) and arranged in a 1 m × 1 m square. About 1000 1-d-old *C. etiennei* adults (1:0.60 ♀:♂ ratio) were released within the cage in the middle of the square. Egg masses found on each host plant were collected three days later and processed as above. The experiment was replicated three times. Percent parasitism was computed. Leaf thickness and pubescence were also compared between the test host plants.

### Effect of Plant Strata on Parasitism

Wax paper strips with two d-old weevils ($n$ = 5 per stratum) were stapled to the upper, middle

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and lower canopy of 6-mo-old potted *C. erectus* and introduced into a nylon mesh screen cage (91 cm wide × 91 cm long × 122 cm high) supported on PVC frames. Ten 1-d-old female and 5 male parasitoids were released inside the cage. Egg masses were collected after 24 h and placed in individual test tubes as described above. This test was replicated four times. The number of parasitized eggs was counted after 10 days.

### Host Finding Behavior Video

Host finding behavior of *C. etiennei* was observed in a Petri plate (60 × 15 mm) under a stereoscopic microscope with attached camera (Videoflex®) and connected to a television monitor (TV) (RCA®) and video recorder (RCA®). A 3-d-old *D. abbreviatus* egg mass and a 2-d-old mated *C. etiennei* female were placed in a plate for observation during daytime (1000 to 1400 h) and nighttime (1700 to 2100 h). The set-up was repeated five times with different individuals. The recorded data were collected, managed, and analyzed with the Observer Videopro 4.1 program (Noldus Information Technology®).

### Statistical Analysis

Data from selected experiments were analyzed for significant differences by the general linear model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Data transformations were performed on selected experiments. Means were compared by Duncan's multiple range test (DMRT).

## RESULTS

### Host Age Preference

Percent parasitism by female *C. etiennei* was significantly different among host age in both laboratory and greenhouse tests. Three-d-old weevil eggs were most acceptable to the parasitoids under laboratory and greenhouse conditions (Table 2).

### Effect of Female Parasitoid Age on Parasitism

Mean egg parasitism by female *C. etiennei* was affected by parasitoid age (Table 3). Percent parasitism significantly increased for 1- to 2-d-old females, but decreased thereafter. Parasitism reached a plateau when females were 3- and 4-d-old, and declined for 5- to 9-d-old females. By the 10th day, all parasitoids were dead.

### Choice of Host Plant

The highest percent parasitism of weevil eggs by *C. etiennei* was found on pigmy palm followed by green buttonwood and lime (Fig. 1). The lowest parasitism was recorded on silver buttonwood. Variation in parasitism for different host plants is probably influenced by the leaf thickness and pubescence. For instance, leaf thickness for silver buttonwood (0.48 ± 0.01 mm) and green buttonwood (0.45 ± 0.01 mm) is higher ($df$ = 3,76; $F$ = 81.44; $P$ = 0.001) than lime (0.23 ± 0.01 mm) and pigmy palm (0.23 ± 0.01 mm). At the same time, pubescence is higher for silver buttonwood (3186 ± 422 trichomes/mm$^2$) than green buttonwood (14.80 ± 1.58 trichomes/mm$^2$), lime, and pigmy palm (0.00 ± 0.00 trichomes/mm$^2$).

TABLE 2. EFFECT OF AGE OF *D. ABBREVIATUS* EGGS ON ACCEPTANCE BY *CERATOGRAMMA ETIENNEI* UNDER GREENHOUSE AND LABORATORY CONDITIONS.

| Egg age (days) | % Mean Parasitism ± S.E.* | |
|---|---|---|
| | Laboratory test | Greenhouse test |
| 1 | 0.53 ± 0.36 b | 1.87 ± 1.86 ab |
| 2 | 0.89 ± 0.59 ab | 3.46 ± 0.11 a |
| 3 | 2.15 ± 0.72 a | 3.38 ± 0.28 a |
| 4 | 1.76 ± 0.72 ab | 2.60 ± 1.30 ab |
| 5 | 0.40 ± 0.28 b | 0.00 ± 0.00 b |

*Means with the same letter are not different according to Duncan's multiple range test (DMRT) at $P \leq 0.05$. Data analysis was performed after log transformation.

### Effect of Plant Strata on Parasitism

Higher parasitism was detected in eggs found in the lower (85.15 ± 3.98) and middle strata (75.14 ± 3.44) rather than the upper portion (49.73 ± 4.51) of the plant ($df$ = 2,52; $F$ = 4.82; $P$ < 0.01) (Table 2). Peña et al. (unpubl.) found that *D. abbreviatus* deposits more eggs on the upper and middle plant canopy than on the lower canopy of silver buttonwood.

TABLE 3. PERCENT PARASITISM BY *CERATOGRAMMA ETIENNEI* AS INFLUENCED BY AGE OF THE PARASITOID.

| Age of female *C. etiennei* (days) | Mean % egg parasitism (± S.E.)* |
|---|---|
| 1 | 59.35 ± 3.71 b |
| 2 | 80.04 ± 2.90 a |
| 3 | 52.87 ± 2.79 b |
| 4 | 52.46 ± 2.50 b |
| 5 | 30.03 ± 3.58 c |
| 6 | 12.13 ± 2.50 d |
| 7 | 4.74 ± 1.63 de |
| 8 | 0.76 ± 1.10 e |
| 9 | 0.00 ± 0.00 e |

*Means with the same letter are not different according to Duncan's multiple range test (DMRT) at $P \leq 0.05$.

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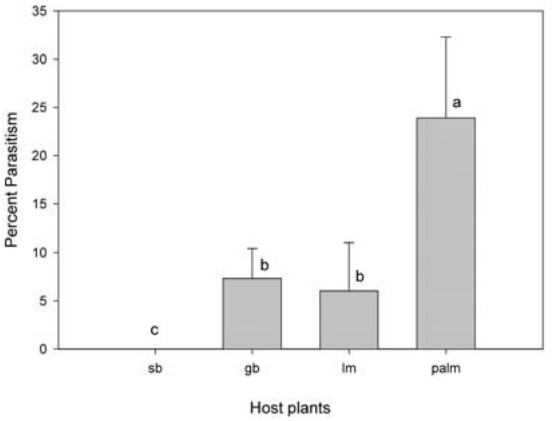



Fig. 1. Percent parasitism by *Ceratogramma etiennei* on various host plants. Notes: sb = silver buttonwood, gb = green buttonwood, lm = lime, and palm = pigmy palm. (Note: Bars with the same letter are not different according to Duncan's multiple range test (DMRT) at $P \leq 0.05$).

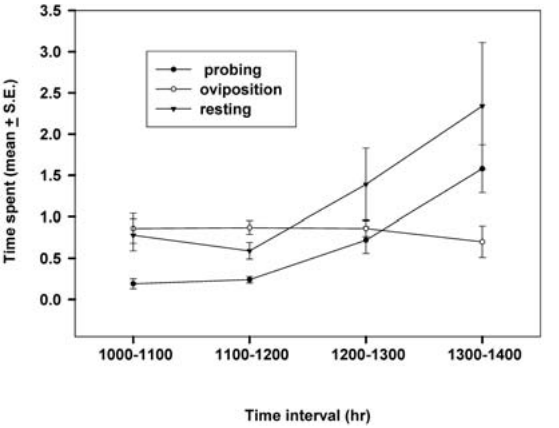



Fig. 2. Parasitism activities by *Ceratogramma etiennei* (a 4-h observation during daytime). The vertical scale showing time spent is in hours.

Host Finding Behavior Analysis

Video recording during the diurnal and nocturnal period showed that *C. etiennei* parasitizes its hosts only during daytime. Parasitism activities recorded showed three types of behavior from the time of host detection to departure from the host. These were probing (walking and antennation), oviposition (drilling and egg laying), and departure (resting). The average duration in hours of a single act of each behavior is as follows: walking (0.15 ± 0.10), antennation (0.08 ± 0.01), drilling (0.15 ± 0.01), egg laying (0.31 ± 0.02), and resting (0.24 ± 0.16). In probing, a female walked back and forth around the host, while drumming with its antennae. Once the egg mass was located, the female drilled a hole through the leaf and egg chorion to lay an egg within the weevil egg. Oviposition punctures were visible on parasitized eggs. The results of the 4 h observation period are shown in Fig. 2. Probing by the female was faster during the first 2 h of the act, but oviposition took longer initially. Resting time decreased in time. These observations suggest that host finding and acceptance (probing) was faster during first encounter with a fresh egg mass. Dissection of the parasitized eggs showed that more than one egg was deposited by *C. etiennei* periodically; however, only one parasitoid developed per host egg.

## Discussion

Results of our experiment on the host age preference showed that host age can affect choice of the parasitoids. *C. etiennei* appears to prefer younger eggs for parasitism. Similar result has been obtained on other trichogrammatids (Schmidt 1994). For instance, emergence rate of *Trichogramma chilonis* Ishii significantly decreased when eggs were older than 48 h at the time of encounter (Guang & Oloo 1990; Schmidt et al. 1999). The host age at the time of parasitism appears to have implications on fitness of progeny (Sequeira & Mackauer 1992, 1994) and parasitoids, which preferentially attack younger host stages (Hagvar & Hofsvary 1986; Sequeira & Mackauer 1988). The results obtained from the host age preference experiment provide relevant information regarding mass rearing of *C. etiennei.* To maximize *in vivo* parasitoid production, *C. etiennei* females should be provided with *D. abbreviatus* egg no older than 3 d.

The age of the parasitoid is also crucial on successful parasitism. A younger parasitoid is more fecund than the older ones. The effect of age of the parasitoid on their ability to parasitize their host has been documented on some parasitoids (Hentz 1998; Honda 1998). For instance, the optimum age for *Cotesia marginiventris* (Cresson), to successfully parasitize larvae of *Spodoptera frugiperda* (J.E. Smith) ranges from 48 to 96 h (Rajapakse 1992). *C. marginiventris* younger or older than the above age were not able to parasitize a host. Similar result has been shown in our experiment on the effect of female parasitoid age on parasitism, in which higher parasitism was exhibited by 1- to 2-d-old *C. etiennei*. Knowing the age of the parasitoids when they are most fecund is very important in deciding what age of the parasitoids to release in the field to obtain a meaningful level of parasitism.

Another factor that affects parasitism is the location of the host. Insect hosts in cryptic location provide a challenge for successful parasitism. For instance, *D. abbreviatus* eggs are deposited in between two leaves. The female parasitoid has to drill through the abaxial and adaxial surfaces of the leaf to reach *D. abbreviatus* eggs; therefore,

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successful oviposition may be facilitated when weevil eggs are deposited on leaves with thinner blades and non-pubescent foliage, such as pigmy palm. These leaf features might explain why *D. abbreviatus* eggs on silver buttonwood were the least parasitized among the four host plants. Variation in parasitism on various plants was also observed by Peter (1990). He found that maximum parasitism by *Apanteles taragamae* Viereck on *Diaphania indica* (Saunders) was recorded on two varieties of cucurbits with smoother leaves. Physiological factors (i.e., leaf odor) cannot be ruled out.

*Ceratogramma etiennei* search, find, and attack their host in cryptic locations, however, the cues they use to find *D. abbreviatus* eggs are still unclear. Short range and contact chemical cues associated with physical cues arising from the host plants or from the host are reported to affect host recognition and acceptance by some parasitoids. This might also be true for *C. etiennei*. Preliminary tests showed that chemical and physical cues play an important role in host recognition by *C. etiennei* (D. Amalin et al. unpublished data). The chemical cues can be found on scales from the weevil elytra, usually left behind near the egg mass by the female during oviposition, on adult feces, or on the substance produced by the female to cement the eggs between leaves. Further studies to identify the factors involving host recognition and host acceptance by *C. etiennei* are worth investigating.

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