



Biology of *Acerophagus texanus* (Howard, 1898) (Hymenoptera: Encyrtidae), a parasitoid of *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae)

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Abstract

Acerophagus texanus (Howard) (Hymenoptera: Encyrtidae) is a primary endoparasitoid of *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae). The parasitoid was described in 1898. It has been little studied and much of its biology remained unexplored. In the present investigation, several biological aspects of this species, such as the life cycle, fecundity, and longevity were studied under laboratory conditions. The parasitoids were collected from rambutan trees infested with *F. virgata* in Chiapas, Mexico, and transferred to the laboratory to establish a colony. Insects used in the experiments were obtained from a mass rearing of this colony. The results indicated that *A. texanus* is a koinobiont, gregarious endoparasitoid, with a pre-ovipositional period of 3 days. The life cycle from egg to adult, was 15 ± 0.4 days at 26.5 °C. The mean longevity of females fed on bee honey was 9.5 ± 1.2 days, whereas unfed females survived for two days only. Hosts parasitized by *A. texanus* died four days after parasitization, just when entering the 2nd larval instar of the parasitoid. The average progeny produced by mated females was 12.6 individuals per host, and the sex-ratio was biased to females in a proportion of 5:1. Unmated females can reproduce parthenogenetically, producing only males as progeny.

Keywords Development · Fertility · Longevity · Chalcidoidea · Mealybug · Biological control

Introduction

Rambutan (*Nephelium lappaceum* L.) is one of the most important cash crops in the South of Chiapas, Mexico (Castillo-Vera et al. 2017). A wide array of insect pests threatens rambutan production. Mealybugs (Hemiptera: Pseudococcidae) are among the most serious pests, as they feed on the sap and may transmit viruses, as the pineapple wilt-associated virus transmitted by two species of mealybugs (Sether et al. 1998). The invasive species, *Ferrisia virgata* Cockerell is widely distributed and can be found in high densities in rambutan orchards (Villatoro-Moreno et al. 2016). Mealybugs are difficult to control with conventional insecticides,

since they have cryptic habits and are protected by waxy hydrophobic hairs covering their bodies (McCorquodale and Hodges 2017). Use of other control methods is needed, such as biological control, which represents an important alternative against mealybugs in rambutan orchards. In this sense, encyrtids are the most efficient parasitoids for mealybug management in the Neotropics (Colmenarez et al. 2018). The parasitic wasp *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae), for example, has been successfully used against the pink hibiscus mealybug, *Maconellicoccus hirsutus* Green (Kairo et al. 2000). Similarly, the papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink has been efficiently controlled by the hymenopteran parasitoid *Acerophagus papayae* Noyes & Schauff as biological control agent (Amarasekare et al. 2009).

Many parasitoids are recorded to parasitize *F. virgata* (Watson 2016). From them, *Acerophagus texanus* (Howard) (Hymenoptera: Encyrtidae) is the most promising natural enemy because it is easy to rear in the laboratory, reproduces quickly, and has presented the highest levels of parasitism in the field (Noyes 2010). In Mexico, relatively few studies focussed on mealybugs natural enemies, particularly on *A.*

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texanus parasitizing *F. virgata* (Debach and Warner 1969). Recently, this parasitoid was reported parasitizing *F. virgata* in Chiapas, Mexico in rambutan orchards as a member of a complex of several parasitoids (Castillo-Vera et al. 2021). The objective of the present study was to investigate the biological aspects, longevity, and fertility of *A. texanus* parasitizing *F. virgata* under laboratory conditions.

Material and methods

Biological material

Mealybug mummie's were collected from a rambutan orchard in Frontera Hidalgo, Chiapas (14°45'7.47" N, 92°11'23.53" W; 52 m asl). Specimens were collected during several days and transferred to the laboratory to establish a laboratory colony. In the laboratory, pupae were placed into plastic containers (2 L) for adults' emergence. Newly emerged parasitoids were fed with bee honey and subsequently transferred to rearing cages containing *F. virgata*. Nymph and adult individuals of this mealybug were obtained from a colony established on courgette (*Cucurbita pepo* L.). The taxonomic keys described by Rosen (1969) were used to identify *A. texanus* and voucher specimens were deposited in the Natural History Museum, London (UK). Both rearing and bioassays were performed at 26.5 ± 2 °C, $70 \pm 10\%$ relative humidity, and a 10 h light/14 h dark photoperiod.

Life cycle

In order to study the life cycle of *A. texanus*, 98 three-day-old female parasitoids were individually placed into 7.5×1.5 cm vials with a 3rd instar nymph of *F. virgata* for 6–8 h. The parasitoid development was monitored for 15 consecutive days by dissecting 7 parasitized hosts, which were considered replicates, each 24 h after parasitism. Dissected specimens were examined on excavated slides using entomological pins to separate the parasitoid from the host's tissues. The preparation was stained with Giemsa (1%, one drop) to facilitate the observation of the parasitoid's immature stages. Larval development was recorded by capturing images with a digital camera (Canon EOS Rebel T6) mounted on a compound microscope (Olympus CX31). Larval growth was measured daily with a micrometre ruler. Images were processed with the ImageJ software program (version 1.52a) to determine the body size of the parasitoid (Rueden et al. 2017). Individuals were then treated with Karnovsky's fixative (3% glutaraldehyde, 2% formaldehyde in 0.1 M phosphate buffer, pH 7.4) and labelled for analysis by scanning electron microscopy (SEM).

Parasitoid specimens for SEM examination were prepared according to the following protocol: samples were washed by

distilled water in an ultrasonic cleaning bath (5 times, 5 min. per wash). Next, the samples were dehydrated in increasing series of ethanol concentrations (30, 50, 70, 90, and 100%; for 30 min. at a time). The final concentration (100%) was repeated once. After passing the specimens for the critical point drying (Tousimis, Samdri-795), they were secured on aluminium mounting stubs with a double sided carbon tape. Next, the samples were coated with gold–palladium in a sputter coater (Denton Vacuum Desk II) and immediately examined under SEM (JEOL, NeoScope JCM-7000).

Adult survival

The survival of *A. texanus* adult females was studied under three different feeding regimes: (i) deprived of food, (ii) fed with ten μ l of water, and (iii) fed with ten μ l of bee honey, offered as drops inside the vial. Each treatment included 50 individuals, which were considered replicates. Each treatment was set up with newly emerged females that were individually placed into 7.5×1.5 cm vials. The number of living and dead individuals was recorded daily for each treatment and the experiment was completed until the death of the last individual.

Fecundity

The reproduction of *A. texanus* was studied in mated and virgin females. In the first case, females were kept with males for 72 h to favour mating, and in the second, females were kept alone, without any contact with males. Females of both groups were 3 days old and were fed with bee honey. *F. virgata* hosts were individually offered to each parasitoid female for six hours for oviposition, which was used once. Subsequently, the hosts were isolated for daily observation until the emergence of the parasitoid's offspring. The clutch size, sex, and development time from parasitization to adult emergence were recorded.

Statistical analysis

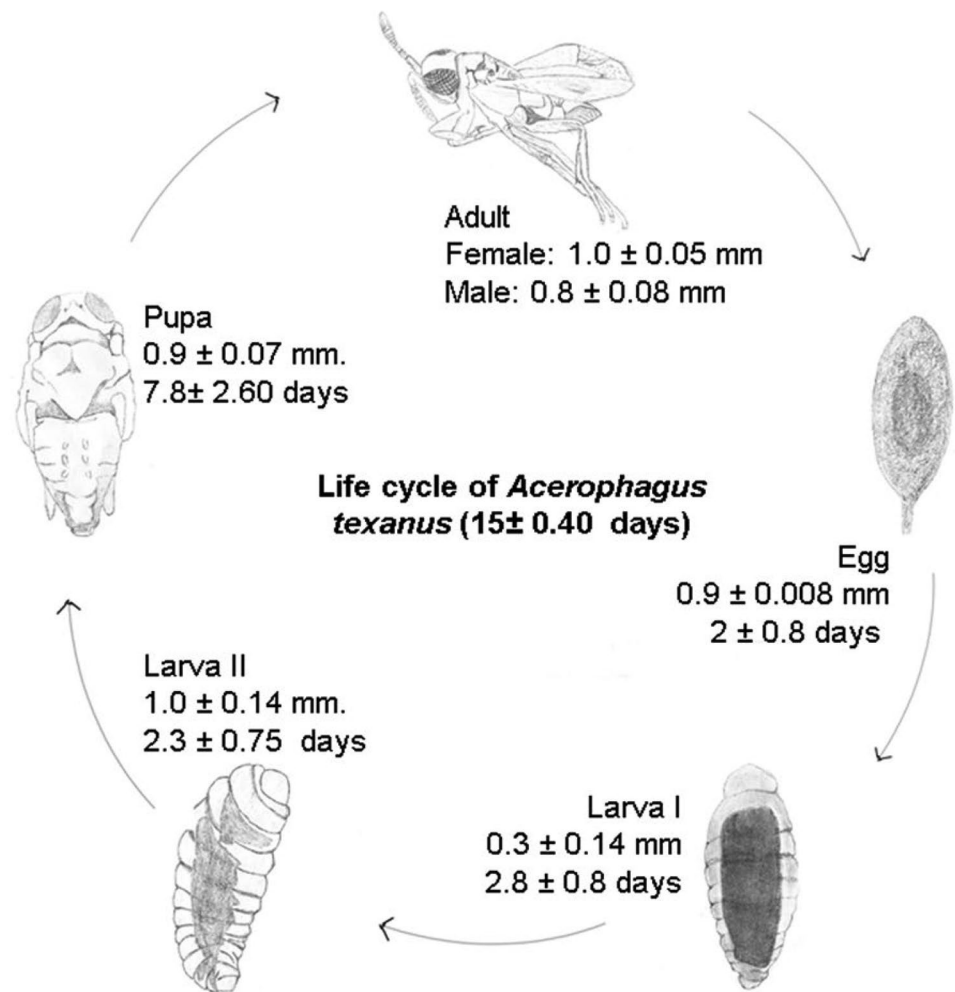
Descriptive statistics were used to analyse the parasitoid size and developmental time. Principal component analysis was applied to determine the instars of the parasitoid. Kaplan–Meier survival analysis was performed for female parasitoids longevity. All analyses were conducted using the R project software package (version 3.6.2; R Core Team 2019).

Results

Life cycle

The life cycle from egg to adult of *A. texanus* averaged 15 (± 0.40) days (Fig. 1). The parasitoid's external morphology

Fig. 1 Life cycle of the parasitoid *Acerophagus texanus* (26.5 ± 2 °C and $70 \pm 10\%$ relative humidity)



is presented in Fig. 2. Different developmental stages of the parasitoid are shown in Fig. 3 and described below.

Acerophagus texanus eggs are 1 mm (± 0.1) long, slightly oval, translucent, with a short pedicel. Because of the eggs' small size and translucent shell, observations were complicated. The mean incubation time was two days ($n=235$). Egg colour changed to light orange 2 days after oviposition as approached the eclosion. The mature egg remained encyrtiform and exhibited a rounded pedicel with an intense orange colour (Fig. 3b). Various changes in the parasitized hosts were also observed during this 2-day period. On the second day after parasitization, a marked decrease in the host mobility was noted compared to the first day.

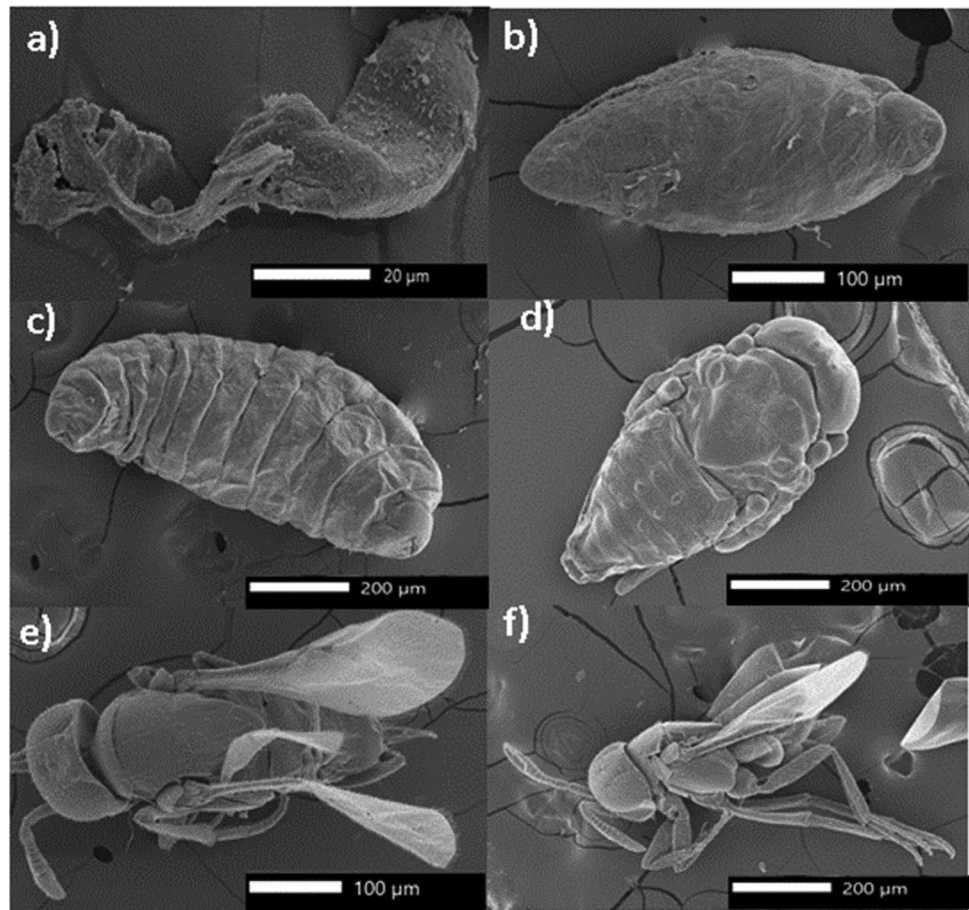
Total larval stage lasted 5 days on average. Principal component analysis indicated that larval size could be grouped in 2 instars. The first and second instars were 0.34 mm (± 0.14) and 1 mm (± 0.1) in length, respectively (Fig. 2). The first instar larva has a translucent, slightly elongated, and rounded body (different from the typical egg shape) with a yellow-orange interior. The second instar larva had

well-differentiated larval segments (Fig. 2c) with yellow external borders that allowed to observe its brown-coloured interior. The first instar lasted 2 days, during which time, the larva remained attached to the filamentous tissue of its host. On the third day after parasitization, the host stopped moving, and on the fourth day -which coincided with the beginning of the second larval instar- the host died.

The pupa is white but changes to yellow as it develops. The eyes also displayed colour changes during pupal development: they were initially white, but subsequently became yellow-orange, and finally turned brown. By that time, the pupa's appendices were clearly defined. The size of the larva was slightly reduced (10%) when transformed to pupa. The pupal stage lasted about 7 days.

Emergence of adult parasitoids occurred at 13–17 days post-oviposition. Adults of *A. texanus* exhibited an obvious sexual dimorphism. Females were larger than males: females measured 1.09 mm (± 0.1 , $n=10$), while males were 0.8 (± 0.1 , $n=8$). Moreover, the female body had a dark yellow appearance (Fig. 3g), whereas for the males were

Fig. 2 Development stages of the parasitoid *Acerophagus texanus*: **a)** egg with pedicel and host remains; **b)** newly emerged larva; **c)** larva II; **d)** pupa; **e)** adult dorsal view (female); **f)** adult lateral view (male)



light-yellow (Fig. 3h). Females had a small typical encyrtid ovipositor that is retracted into the abdomen.

Adult survival

Type of food had a significant effect on the survival of *A. texanus*. Females fed on bee honey had a significantly longer survival than the other treatments ($\chi^2 = 124$; $df = 2$, $p < 0.0001$). The mean survival for bee honey-fed females was 9.5 ± 1.2 days, whereas individuals deprived of food and fed with only water, lived 2.0 ± 0.2 and 2.5 ± 0.2 days, respectively. The longest survival (18 days) was registered for females fed with bee honey.

Fecundity

Dissection of virgin females 3 days after the emergence as adults revealed the presence of a large number of eggs (about 40) in the ovaries. Mated females produced 12.6 ± 0.7 individuals per host (maximum recorded: 17 individuals per host). The progeny sex ratio was 5:1 (female: male). Unmated females produced only male progeny.

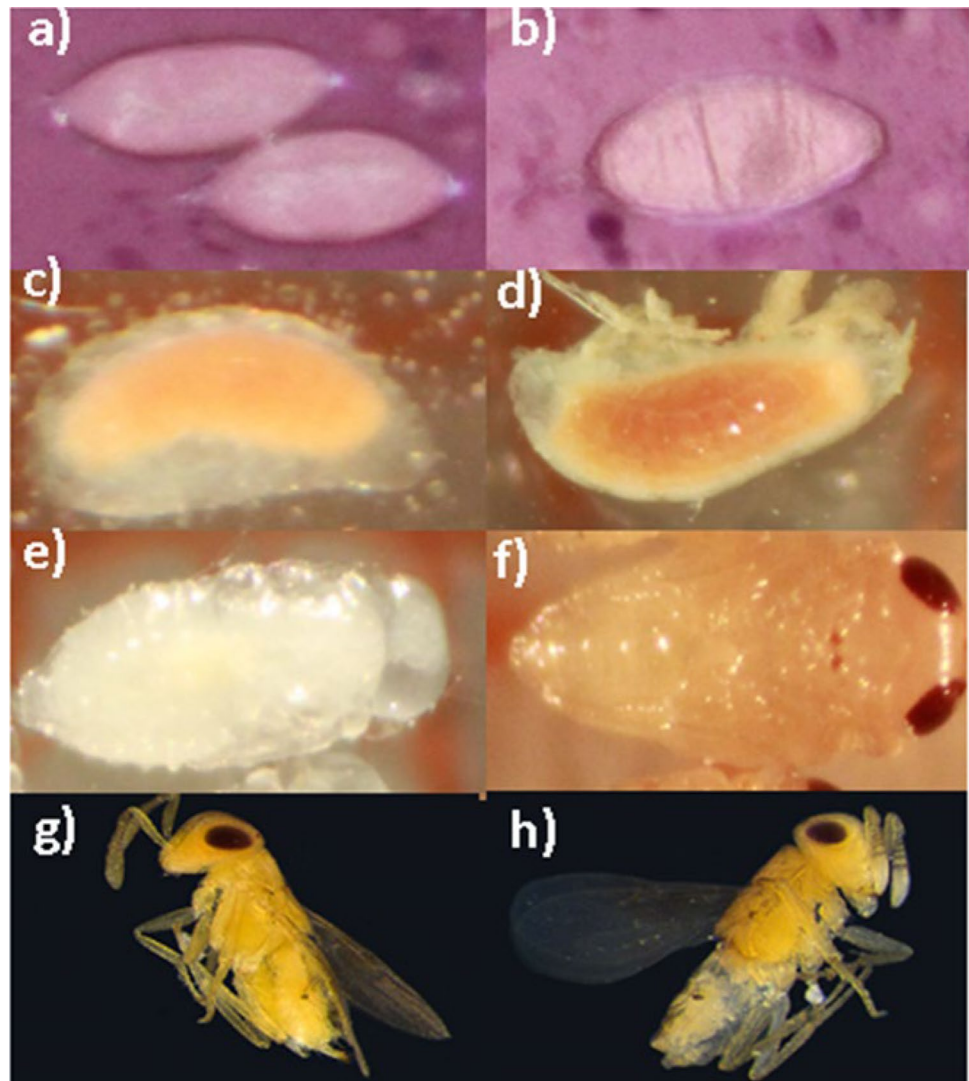
Males emerge from the puparium a few seconds earlier than females.

Discussion

In this study, aspects of the life cycle of *A. texanus* that hitherto have not been reported we described. Our findings indicate that *A. texanus* is a koinobiont and gregarious endoparasitoid with a life cycle of about 15 days. Females require a diet of bee honey to live for a maximum of 18 days. *A. texanus* had a preoviposition period of 3 days and can reproduce by arrhenotokous parthenogenesis. This wasp has 2 larval instars and exhibited a marked sexual dimorphism.

The life cycle of *A. texanus* was similar to other encyrtids of the same genus, such as *Acerophagus papayae*, which had the same number of instars as *A. texanus* (Nisha and Kennedy 2017). The number of instars in encyrtids varies from two to five, although 3 are more commonly reported (Pandey and Johnson 2006). The life cycle of *A. texanus* was shorter than that of its host *F. virgata*, whose duration can last up to 64 days (Lapis 1970).

Fig. 3 Immature stages of *Acerophagus texanus*: **a)** 1-day old egg; **b)** 2-day old egg; **c)** larva I; **d)** larva II; **e)** pupa on day 8; **f)** pupa on day 13; **g)** female; **h)** male



Longevity for adult females of *A. texanus* was similar to the reported value of 20 days for honey-fed *Acerophagus* nr. *coccois* (Beltrà et al. 2013). Female parasitoids, in general, require a sugar-rich diet to enhance their longevity (Villa et al. 2017). Another study indicated that females of *Anagyrus* nr. *sinope* attained the highest longevity when 50% of a bee honey solution was provided (Chong and Oetting 2006). In the case of *Anagyrus ananatis* Gahan, longevity increased up to 28 days when females were fed on a 50% bee honey solution (González-Hernández et al. 2005). In nature, it has been proved that parasitoids generally feed on honeydew, nectar, or sugar-rich substances (Wäckers et al. 2008). The increased longevity of *A. texanus* could therefore influence host-searching efficiency, which is an important attribute for natural enemies (Royer et al. 1999).

Newly emerged *A. texanus* females explored their host and even attempted to oviposit. These attempts, however, failed to produce progeny, since females emerged from the puparium without egg load. Our preliminary observations

indicate that females are synovigenic and have a preoviposition period of several days.

The most common mode of asexual reproduction in parasitoids is arrhenotokous parthenogenesis, in which biparental females and uniparental males are produced through haplodiploidy (Heimpel and De Boer 2008). Arrhenotoky offers an evolutionary advantage by producing male offspring at times when females are rare, and thus overcomes the need to spend time and energy in searching for a mate (Lanteri et al. 2010). Genetic variability is lower in parthenogenetically produced males because the offspring derived from the same individual, which could lead to a reduced adaptability to environmental factors (Heimpel and De Boer 2008).

Several encyrtid species prefer to parasitize completely developed nymphs rather than adults (Kapranas and Tena 2015) because the reproductive activity of the latter limits their suitability as hosts (Cadée and van Alphen 1997). Females of *A. texanus*, however, can parasitize both, nymphs and adults of *F. virgata*. Therefore, its potential as

a biological pest control agent is greater compared to parasitoids that restrict their host range to one stage only. Further research is necessary, however, to characterise and quantify the preference of *A. texanus* for each development stage of the host, especially considering that the larval development (greater longevity, reduced development time, smaller body) of several parasitoids is influenced by host stage (Badshah et al. 2016).

Effectiveness of a parasitoid as natural enemy derives from biological and ecological attributes such as host-searching efficiency, fertility, larval longevity, and sex ratio (Waage and Hassell 1982). The use of encyrtids as biological control agents of mealybugs has yielded some impressive results. For example, the control of cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) outbreaks with the parasitic wasp *Anagyrus lopezi* De Santis (Neuenschwander 2001). However, there is no information about the parasitism level of *A. texanus* to control mealybugs. For instance, the impact of releases of *A. texanus* to control *F. virgata* outbreaks in California was never evaluated (Bartlett 1978). In light of these results, *A. texanus* could be considered a promising candidate to control *F. virgata* in rambutan orchards of Chiapas, Mexico. Further field studies are still needed to confirm the efficacy of the parasitoid.

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Declarations

Competing interests The authors have declared that no competing interests exist.

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