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ABSTRACT

Biological traits of a prospective candidate for biological control of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), in the United States are herein reported. The mymarid wasp, *Gonatocerus tuberculifemur* (Ogloblin), is an egg-parasitoid native to Argentina and its first known host is *Tapajosa rubromarginata* (Signoret), a species related to GWSS. Laboratory studies were made in Tucumán and Buenos Aires Provinces, Argentina. Seven generations were maintained in the laboratory, and only one adult emerged per host egg. The average parasitism rate was 71.6% of total eggs. Although eggs of all ages (4 to 190 h old) were parasitized, wasps did not emerge from eggs over 96 h old. The percentage of wasp emergence was 66.5% from eggs between 4 and 72 h old. Over the seven generations that *G. tuberculifemur* was reared, the parasitism rate ranged between 55-84%. This percentage of emergence increased as the parasitoid generations progressed. The duration of development from oviposition to adult emergence of *G. tuberculifemur* was 12.6 ± 1.8 days (range 11.4-13.0) at 22.5-27.5°C and 70-80% RH. The duration of development was significantly affected by sex and temperature. Males developed faster than females (12.2 vs. 12.8, respectively).The sex ratio was not significantly different from 1:1. Average adult longevity was 6.73 ± 3.93 days fed on honey. Male and female longevity was not significantly different. Oviposition and mating behavior are described.

Key Words: glassy-winged sharpshooter, *Homalodisca coagulata*, biological control, bionomics, *Gonatocerus*, Mymaridae, egg parasitoid

RESUMEN

Se informan los resultados del estudio de las características biológicas de la avispa Mymaridae *Gonatocerus tuberculifemur* (Ogloblin), un parasitoide de huevos, nativo de Argentina cuyo primer hospedador conocido es el sharpshooter *Tapajosa rubromarginata* (Signoret). Esta avispa es un candidato potencial para el control biológico de la chicharrita de alas cristalinas, *Homodalisca coagulata* (Say) (Hemiptera: Cicadellidae) en los Estados Unidos de Norteamérica. Esta especie fue estudiada bajo condiciones de laboratorio en las Provincias de Tucumán y Buenos Aires, Argentina. Siete generaciones fueron obtenidas, y solo un adulto emergió de cada huevo parasitado. El porcentaje promedio de huevos parasitados fue de 71.6%. A pesar que huevos de todas las edades testeadas (4-190 horas) fueron atacados por hembras de *G. tuberculifemur*, no emergieron avispas de huevos con mas de 96 horas de edad. El porcentaje de emergencia de avispas fue 64.1%. Durante las siete generaciones criadas el porcentaje de parasitsmo osciló entre 55 y 84% del total de huevos, este porcentaje se incrementó con el avance de las generaciones. El tiempo necesario para completar el desarrollo de *G. tuberculifemur* (desde huevo hasta adulto) fue de 12.6 ± 1.8 días (rango 11.4-13.0) a 22.5-27.5°C y 70-80% HR, y fue significativamente afectado por la temperatura y el sexo. Los machos necesitaron menos tiempo para desarrollar (12.2 días) que las hembras (12.8 días). La proporción de sexos no mostró diferencias significativas. La longevidad de los adultos alimentados con miel fue de 6.73 ± 3.93 días, y no mostró diferencias significativas entre los sexos. Se describe el comportamiento de oviposición y cópula.

Translation provided by the authors.

Most of the Auchenorrhyncha (Hemiptera) which are economically important to agriculture are vectors of plant diseases such as viruses and bacteria. The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* Say (Hemiptera: Cicadellidae: Proconiini), has recently become a major pest in California, primarily as a vector of *Xylella fastidiosa* Wells et al., which causes Pierce's disease in grape vines and also infects other crops. In California, wine and table grape producers are under threat due to the action of the GWSS in vectoring this pathogen. Biological control is an important component in the management of the GWSS (Morgan et al. 2000; Jones 2001). In general, Cicadellidae are not infected by pathogenic viruses, bacteria, and protozooa, however, they are infected by pathogenic fungi (Soper 1985). Waloff & Thompson (1980) and Denno & Roderick (1990) found that mortality produced by egg parasitoids was a "key factor" in Cicadellidae species. These parasitoids are one of the few taxa playing an important role in limiting leafhopper populations (Döbel & Denno 1993). Mymarid wasps are the best-known egg parasitoids of leafhoppers, and representatives of the family have been successfully utilized in several instances for the control of crop pests (Huber 1986; Meyerdirk & Moratorio 1987).

The egg parasitoid, *Gonatocerus tuberculifemur* (Ogloblin), was found in several surveys for egg parasitoids of Proconiini sharpshooters in Argentina during 2000-2004. Until 1986, 252 species of *Gonatocerus* were known, with 60 described from the Neotropics (Huber 1986). Of the 30 species described from Argentina, only three have known host records (De Santis 1957, 1979).

Few studies have been published on the bionomics of *Gonatocerus* (Miura 1979, 1990; Sahad 1982). There is no information on the bionomics of *G. tuberculifemur*. We studied aspects of its biology (egg-laying behavior, egg viability, duration of developmental stages, sex ratio, and longevity) reared under laboratory conditions on its natural host, *Tapajosa rubromarginata* (Signoret).

Materials and Methods

The studies were carried out in Planta Piloto de Procesos Industriales Microbiológicos, CONICET, San Miguel de Tucumán, Tucumán Province, and at the USDA-ARS, South American Biological Control Laboratory, Hurlingham, Buenos Aires Province. Laboratory studies were conducted to asses the effects of temperature on development time, progeny sex ratio, adult longevity, oviposition, and mating behavior.

Parasitoid Culture

A culture of *G. tuberculifemur* was initiated in the laboratory by collecting 20 egg masses of *T. rubromarginata* on Johnson grass, *Sorghum halepense* (L.), in an open field in San Miguel de Tucumán, Tucumán Province, in September 2002. Additional collections were made when necessary. The female *Tapajosa rubromarginata* lays her eggs in parallel rows in groups of 3 to 32 eggs per mass (n = 68, mean = 15.0; SD = 6.0) just under the epidermis layer (E. G. V. unpublished data).

In the laboratory, field-collected (by sweeping) females of *T. rubromarginata* placed in polyethylen-terephtalathe (PET) cylindrical cages (35 cm high × 18 cm diam.) on maize leaves were used to obtain host eggs. Potted maize plants (pot of 6.3 dm^3) in the vegetative stage (four to eight leaves) were checked daily for eggs. When egg masses were detected, the sharpshooters and the PET cages were removed, and the corn leaf was introduced into a 20-cm high × 2-cm diam glass tube with 1-3 mated *G. tuberculifemur* females (24-48 h old) for 24 h. Each glass tube top was fitted with a cotton plug, which was moistened with water and honey as needed. After five days, the parasitized egg masses were removed from the leaf and transferred to a Petri dish with wet tissue paper and covered with clear plastic food wrap to prevent desiccation, and to keep wasps from escaping. Parasitized egg masses were checked daily to ensure leaf quality until the emergence of the adult wasps.

Not all exposed eggs were parasitized, so to estimate percent parasitism, host eggs that changed to brownish or reddish after five to seven days were considered "parasitized", while those developing eyespots were considered "unparasitized". The number of leafhopper nymphs and wasps that emerged from the exposed eggs were counted daily. Host age acceptability was studied at six different ages: 4, 24, 48, 72, 96, and 190 h.

The total time required for the development from egg to adult emergence, and progeny sex ratios were measured at 4 temperatures: 22.5°C ± 1.3, 24.5°C ± 1.3, 26.0°C ± 1.3, and 27.5°C ± 1.3 in 875 individuals (457 males, 504 females) from 102 attacked egg masses. Differences in the duration of the development between male and females were analyzed by a two-factor ANOVA.

Adult longevity was estimated based on the observation of 114 individuals (56 females and 58 males). Adults were kept individually in vials (7 cm length × 1 cm diameter) without host material, but with honey for food. The experiment was carried out under room temperature (22.9°C ± 8°C) at 70-80% RH with ambient natural light providing, a summer photoperiod of approximately 15 L: 9 D. Differences between male and females longevity were analyzed by a t test. The effect of temperature on sex ratio was analyzed by one-way ANOVA.

Voucher specimens were deposited in the entomological collections of M. Lillo Institute (Tucumán, Argentina) and the University of California at Riverside (Riverside, California, USA).

RESULTS

Gonatocerus tuberculifemur is solitary, producing only one adult per host egg. During the study, 102 out of 142 egg masses exposed were attacked, producing 961 wasps from 1500 parasitized eggs out of 2095 exposed.

The average parasitism rate for all eggs was 71.6%; however, it differed according to egg age (Table 1). Although eggs of all ages were parasitized, wasps did not emerge from eggs aged 96-190 h old. The percentage of wasp emergence was 64.1% (from eggs between 4 and 72 h old). The maximum percentage of wasp emergence was obtained from 48-h-old eggs (71.5%). The average of host nymph emergence for all egg ages was 12.2%; increasing to 65.7% in eggs aged 190 h. No noticeable effect of host egg age was observed on the sex ratio, longevity, or on development time. Most of the *G. tuberculifemur* adults emerged between 10:00 and 15:00 h. A similar observation was reported by Sahad (1982) studying *Gonatocerus* sp. attacking *Nephotettix cincticeps* Uhler in Japan.

Over seven generations (142 masses, 2095 eggs exposed), the parasitism rate ranged between 55-84%, and the emergence of wasps from 45 to 87% (Table 2). The emergence rate increased over generations, from 58.5% during the first generation, to 86.3% for the seventh generation.

The immature stages of the parasitoid produced changes in the host egg. In the first three days it was not possible to identify the parasitized eggs through changes in coloration, except for tiny dark oviposition marks in the leaf cuticle and/or chorion. Host eggs containing parasitoid larvae (2-5-days-old) had distinctive light brown coloration. After 4-7 days, the whole egg became orange or red. When the parasitoids reached the pupal stage (6-12 days), it turned to dark brown or black.

The duration of development from oviposition to adult emergence of *G. tuberculifemur* was 12.6 ± 1.8 days (range 11.4-13.0). The duration of development of *G. tuberculifemur* was influenced by temperature (F = 32.130; df = 3, 861; P = 1.09E-19), by sex (F = 21.082; df = 1, 861; P = 5.05E-06), and by the interaction between temperature and sex (F = 2,888; df = 3, 861; P = 0.03). Overall, males developed faster than females (Table 3). At 22.5 and 24.5°C, males did not show differences in duration of development (Table 3). On the other hand, duration of development of females was not different at 26 and 27.5°C. The sex ratio was slightly female biased at 1.1: 1 (n = 504 females, 457 males), without being different (F = 2.308; df = 3, 98; P = 0.0812).

Adult longevity was 6.73 ± 3.93 days, showing high variability. Few individuals were able to survive more than 15 days. There was no difference in longevity by sex (Fig. 1) (t = -0.464; df = 1; P = 0.32). About 50% of the adults died on the 7th day.

Parasitoid mating behavior was observed as follows. Immediately upon emergence, males rushed for the females and mating occurred as soon as they managed to reach the females and position themselves appropriately. It was common to observe as many as 2-4 males around one female. Each mating lasted 4-10 seconds. One male was observed trying to mate with a female while she was emerging.

No premating or preovipositional period was observed; the oviposition of *G. tuberculifemur* began immediately after adult emergence. They searched rapidly over the leaves, tapping the surface constantly with the tips of the antennae on a host egg mass. It was not determined whether host location was by random search or by directional cues. However, females found the eggs very quickly. Any time that a female was caged with a plant containing an egg mass, the female located the egg mass and began oviposition within 30 seconds; this behavior was observed at least 30 times when females were caged. The process of oviposition was initiated after the host was examined; the female positioned the tip of the abdomen on the host egg, the ovipositor was then extruded and inserted through the leaf cuticle. As a general behavior, once oviposition began, a female continued laying eggs on the remaining eggs in the mass. The main cause of oviposition interference was the arrival of another female at the egg mass; as a consequence, one of them abandoned the egg

TABLE 1. EFFECT OF *TAPAJOSA RUBROMARGINATA* EGG AGE ON PERCENT PARASITISM BY *GONATOCERUS TUBERCULIFEMUR*.

Egg age (hours)	No eggs exposed (no. masses)	No. parasitized eggs (%)	No. emerged wasps (%)	No. emerged nymphs (%)
4	232 (19)	121 (52.7)	76 (62.8)	51 (22.0)
24	1222 (74)	863 (70.6)	578 (67.0)	180 (14.7)
48	394 (29)	340 (86.3)	243 (71.5)	2 (0.5)
72	158 (15)	122 (77.2)	64 (52.5)	0 (0.0)
96	54 (3)	51 (94.4)	0 (0.0)	0 (0.0)
190	35 (2)	3 (8.6)	0 (0.0)	23 (65.7)
Total	2095 (142)	1500 (71.6)	961 (64.1)	256 (12.2)

TABLE 2. NUMBER OF PARASITIZED EGGS AND WASP EMERGENCE OF *GONATOCERUS TUBERCULIFEMUR* OVER SEVEN GENERATIONS REARED ON *TAPAJOSA RUBROMARGINATA* UNDER LABORATORY CONDITIONS.

Generation	No. egg masses	No. eggs exposed	No. eggs parasitized (%)	No. wasps emerged (%)	No. nymphs emerged(%)
I	30	447	359 (80.3)	210 (58.5)	36 (8.1)
II	28	331	279 (84.3)	137 (49.1)	15 (4.5)
III	19	338	186 (55.0)	84 (45.2)	42 (12.4)
IV	19	241	183 (75.9)	126 (68.8)	4 (1.7)
V	10	151	104 (68.9)	90 (86.5)	27 (17.9)
VI	21	238	178 (74.8)	132 (74.2)	40 (16.8)
VII	15	349	211 (60.5)	182 (86.3)	92 (26.4)

mass to continue looking for other egg masses in the container. Superparasitism was observed in the laboratory when 1 or 2 females were placed in a container. In both cases, only one wasp emerged from each egg.

DISCUSSION

Gonatocerus tuberculifemur can be considered as proovigenic, due to its ability to deposit eggs immediately after emergence (Flanders 1950). This characteristic is shared with *G. cincticipitis* Sahad (Miura 1990), and other members of the Mymaridae (Clausen 1940). *Tapajosa rubromarginata* is the first host recorded for this parasitoid.

The parasitism rate obtained in the laboratory (71.6%) was much higher than that obtained for *Gonatocerus* sp. (48.7%) parasitizing *N. cincticeps* (Miura 1979). The percentage of parasitized eggs that produced wasps (63.4%) was lower compared to that obtained by Virla (2001) on *Anagrus breviphragma* (80.5%), possibly due to differences in the rearing methods used. In the present study, egg masses were removed from the plant prior to parasitoid emergence, whereas in Virla's study, the host eggs were allowed to remain on the plant until wasp emergence. Rotting or desiccation of the eggs' host plant substrate leads to offspring death (Sahad 1984). Better humidity control may lead to higher wasp emergence rates.

Mymaridae show two behaviors regarding host suitability: true egg parasitoids that can attack and develop on newly laid eggs (before embryo development), and those that attack eggs in all their developmental stages. However, when eggs with advanced embryos are attacked, only some species are able to develop to the adult stage (Clausen 1940; Waloff 1979; Chantarasa-ard & Hirayima 1984). *Gonatocerus tuberculifemur* females were able to parasitize eggs of all development stages, but wasps did not emerge when eggs older than 96 h were attacked. Eggs older than 72 h were unsuitable for laboratory rearing and for field collection of parasitoids as "sentinel" eggs. Although parasitoid offspring cannot develop to the adult stage in eggs with well-developed embryos, the host egg is nevertheless killed. Further research is needed to establish whether *G. tuberculifemur* can attack eggs with well-developed embryos in the field.

The duration of development was significantly different between males and females. For *Gonatocerus* sp., Miura (1979) found no differences between males and females. Virla (2001) reported that sex affected development rate in *Anagrus* sp., but Meyerdirk & Moratorio 1987 found no differences in their studies with another *Anagrus* sp.

The observed ovipositional and mating behaviors, and the results for sex ratio and adult longevity obtained in this study, were similar to those

TABLE 3. EFFECT OF THE TEMPERATURE AND SEX ON DURATION OF DEVELOPMENT OF *GONATOCERUS TUBERCULIFEMUR* UNDER LABORATORY CONDITIONS.

	Males			Females		
Temperature (°C)[1]	*n*	Mean (days)	SD	*n*	Mean (days)	SD
22.5	130	12.7	2.1	105	13.7	0.8
24.5	194	12.7	1.7	212	13.3	1.6
26.0	76	12.0	1.6	118	12.0	1.9
27.5	57	11.5	1.3	69	12.0	2.0
Overall mean	457	12.2	1.8	504	12.8	1.8

[1]The duration of the development was influenced by temperature (F = 32.130; df = 3, 861; P = 1.09E-19), by sex (F = 21.082; df = 1, 861; P = 5.05E-06), and by the interaction between temperature and sex (F = 2,888; df = 3, 861; P = 0.03) (Two-way ANOVA).

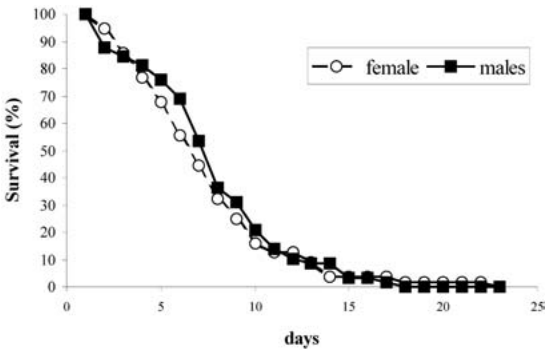

Fig. 1 Survival of males and females of *Gonatocerus tuberculifemur*.

reported for other *Gonatocerus* and *Anagrus* species (MacGill 1934; Clausen 1940; Miura 1979; Waloff 1979; Chantarasa-ard et al. 1984; Sahad 1982, 1984; Meyerdirk & Moratorio 1987).

A culture of *G. tuberculifemur* has been successfully maintained since March 2001 at the USDA-APHIS Mission Texas quarantine laboratory with eggs of a factitious host, *H. coagulata*, where the biology on this host egg is being studied. In the USDA-ARS SABCL and in the USDA-APHIS Mission laboratories the parasitoid host range is under study.

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