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Authors: Logarzo, Guillermo A., Virla, Eduardo G., Triapitsyn, Serguei V., and Jones, Walker A.

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BIOLOGY OF *ZAGELLA DELICATA* (HYMENOPTERA: TRICHOGRAMMATIDAE), AN EGG PARASITOID OF THE SHARPSHOOTER *TAPAJOSA RUBROMARGINATA* (HEMIPTERA: CLYPEORRHYNCHA: CICADELLIDAE) IN ARGENTINA

GUILLERMO A. LOGARZO[1], EDUARDO G. VIRLA[2], SERGUEI V. TRIAPITSYN[3] AND WALKER A. JONES[4]
[1]USDA-ARS South American Biological Control Laboratory
USDA-ARS SABCL 3130 Buenos Aires Place, Washington D.C. 20521-3130.

[2]CONICET-PROIMI, Avenida Belgrano y Pasaje Caseros, T4001MVB
San Miguel de Tucumán, Tucumán, Argentina

[3]Department of Entomology, University of California, Riverside, CA 92521, USA

[4]Beneficial Insects Research Unit, USDA-ARS, Kika de la Garza Subtropical Agricultural Research Center
2413 E. Highway 83, Weslaco, TX 78596, USA

ABSTRACT

Research on biological control of glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* (Say) started in the 1990s. This sharpshooter, vector of Pierce's disease bacteria *Xylella fastidiosa* Wells, expanded its geographical distribution and it has become a very serious pest problem in several crops, especially grapes, in southern California. In 2000, a survey of sharpshooter egg parasitoids was initiated in Argentina. Fourteen species of egg-parasitoids were collected. We report here on laboratory studies of adult longevity, oviposition preference, sex ratio, and development time of *Zagella delicata* De Santis. Field result of the incidence on its hosts and seasonal occurrence also are provided. *Zagella delicata* produced one adult per host egg. The overall results indicated that 72.5% of the sharpshooter eggs exposed to *Z. delicata* were parasitized. Wasps emergence was 43.8%. In host plant searching preference tests, *Z. delicata* females parasitized 66.7% of host eggs on sugar cane, 57.0% of eggs on corn and 4.5% on citrus leaves. The development time (from oviposition to adult emergence) averaged 23.5 ± 1.2 days. The average adult longevity was 10.3 ± 5.8 days. Females lived longer than males (females: 12.2 ± 5.6 days, males: 6.2 ± 3.7 days). The sex ratio in the laboratory was 1: 2.1 (males/females). In a hyperparasitism test, no adults of *Z. delicata* emerged from eggs previously exposed to *Gonatocerus tuberculifemur*. Seasonal sampling carried out in San Miguel de Tucumán showed that *Z. delicata* occurred from spring to fall, with maximum abundance at the beginning of the spring, where 57.2% out of the 1568 sampled eggs were parasitized. Field and laboratory data suggest that *Z. delicata* could be a prospective biological control agent against other, exotic, proconiine sharpshooters including *H. coagulata*. However, the efficiency of *Z. delicata* is restricted to habitats dominated by grasses.

Key Words: glassy-winged sharpshooter, *Homalodisca coagulata*, Trichogrammatidae, egg parasitoid, biological control, *Zagella delicata*.

RESUMEN

Las investigaciones sobre el control biológico de *Homalodisca coagulata* (Cicadellidae: Proconiini) fueron iniciadas en la década del 90. Esta chicharrita, vector de la bacteria *Xylella fastidiosa*, expandió su área de distribución geográfica y aumentó su abundancia transformándose en una seria plaga de varios cultivos, especialmente en la vid, en el sudeste de California. En el año 2000 fue iniciada una exploración de parasitoides de huevos de chicharritas proconiinas en Argentina, donde fueron colectadas 14 especies de parasitoides. Aquí se reportan los resultados de campo y laboratorio de uno de estos parasitoides, *Zagella delicata* De Santis, aportando información sobre su bionomía (longevidad de adultos, preferencia de oviposición, proporción de sexos y duración del desarrollo), incidencia sobre su hospedador en el campo, y ocurrencia estacional. *Zagella delicata* produjo un solo adulto por huevo. En general, parasitó el 72.5% de los huevos y emergiendo avispas del 43.8% de los huevos parasitados. En las pruebas de preferencia de oviposición de planta hospedadora, las hembras de *Z. delicata* parasitaron 66.7% de los huevos en caña de azúcar, 57.0% de los huevos en maíz y fueron atacados 4.5% de los huevos depositados en *Citrus*. La duración del tiempo de desarrollo (desde huevo a adulto) fue de 23 ± 1.2 días. La longevidad promedio de los adultos fue de 10.3 ± 5.8 días. Las hembras vivieron más que los machos (hembras: 12.2 ± 5.6; machos: 6.2 ± 3.7 días). La proporción de sexos en el laboratorio fue de 1: 2.1 (machos/hembras). En los estudios de hyperparasitismo, ningún adulto de *Z. delicata* emergió de huevos

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previamente expuestos al mymárido *Gonatocerus tuberculifemur*. El muestreo estacional realizado en San Miguel de Tucumán mostró que *Z. delicata* aparece desde la primavera hasta el otoño, con máxima abundancia a principios de la primavera, donde el 57.2% de los 1568 huevos muestreados estaban parasitados. Los estudios de campo y laboratorio sugieren que *Z. delicata* tiene potencialidades como agente de control biológico de otras chicharritas proconiinas exóticas incluyendo a *H. coagulata*. Sin embargo, *Z. delicata* está circunscripta a hábitats dominados por gramíneas.

Translation provided by the authors.

In the early 1990s, the glassy-winged sharpshooter *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) established in California, USA. It has become a very serious problem as an efficient vector of Pierce's disease (*Xylella fastidiosa* Wells) (Blua et al. 1999). The insect is native to the southeastern USA and northeastern Mexico. Although parasitization of *H. coagulata* can reach 100% in California, it seems that natural enemies cannot control the pest (Triapitsyn et al. 1998; Triapitsyn & Phillips 2000). Although the glassy-winged sharpshooter appears to be adapting to California environment, it is not certain that native parasitoids will be as effective there as they are in their co-evolved native range. Thus, egg parasitoids of closely related hosts were sought from areas in South America where subclimate types and habitats were similar to that in California.

In order to identify candidates for a neo-classical biological control program against this pest, a survey of proconiine sharpshooter egg parasitoids was initiated in South America in 2000 (Jones 2001). Most of the collection efforts were carried out in northwestern Argentina with sentinel egg masses of *Tapajosa rubromarginata* (Signoret), a native South American proconiine sharpshooter. *Tapajosa* is close related to *Homalodisca*; both genera have the posterior meron exposed and are included in the same group due to their phylogenetic proximity (Young 1968). This survey resulted in collections of nine different species of the genus *Gonatocerus* Nees (Hymenoptera: Mymaridae), at least one species of *Paracentrobia* Howard, two species of *Oligosita* Walker, and two species of *Zagella* Girault (Hymenoptera: Trichogrammatidae).

To date, there are nine described species of *Zagella* in the New World; three are from the Nearctic region (Triapitsyn 2003), and six from the Neotropical region (De Santis 1957, 1970, 1997). The only known hosts of a *Zagella* species are *H. coagulata* and *Oncometopia orbona* (Fabricius) (Cicadellidae: Proconiini) for *Z. spirita* (Girault) in the USA (Triapitsyn & Phillips 2000; Triapitsyn 2003). Of the two *Zagella* species collected from *T. rubromarginata* eggs in Argentina, *Z. delicata* was the most abundant in terms of frequency of occurrence and population density. The other, tentatively identified as *Z. platensis* (De Santis) by Triapitsyn, was much less abundant.

Zagella delicata De Santis was described from a single female without information on its host association(s) (De Santis 1970). Detailed taxonomic and biological studies are indispensable for a biological control program. Essential biological information about a *Zagella* species has not been available before this study on the biology, geographic distribution, and hosts. We report the results of laboratory and field studies on *Z. delicata*, providing information on its bionomics (i.e., adult longevity, oviposition preference, sex ratio, development time), the incidence of its host in the field, and seasonal occurrence of the parasitoid. The possibilities of using this egg parasitoid as a potential agent for biological control of *H. coagulata* in the USA are discussed.

Materials and Methods

The studies on development time, sex ratio, adult longevity, and oviposition of *Z. delicata* on eggs of *T. rubromarginata* laid on different host plants were carried out at the USDA-ARS South American Biological Control Laboratory in Hurlingham, Buenos Aires Province, and at PROIMI, San Miguel de Tucumán, Tucumán Province. Field studies included (a) a seasonal sampling of egg masses in Tucumán Province for the entire 2002 growing season to estimate parasitoid incidence and occurrence, and (b) a survey with sentinel eggs in a wide geographical range between 22 and 42º LS in Argentina.

Laboratory Studies

The initial stock of *Z. delicata* was obtained by collecting 68 egg masses of *T. rubromarginata* on Johnson grass, *Sorghum halepense* (L.) Person, in an open field in Tafi Viejo, Tucumán Province, in January 2001. Additional collections of egg masses of *T. rubromarginata* on Johnson grass were made when necessary in a soccer field near PROIMI in San Miguel de Tucumán. The colony of *Z. delicata* was reared in the laboratory of PROIMI. About 5-7 wasps, both males and females, were placed in 20-cm high × 2-cm diameter glass tubes with 1-2 egg masses (6-15 eggs) of *T. rubromarginata* and left until the wasps died (approximately in 5-7 days. The eggs used in the experiment were 24-48 h old and were laid on the edge of the distal portions of corn leaves. For aer-

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ation, the glass tube top was either closed by a nylon mesh or the hole was fitted with cotton plugs that were moistened with water and honey as needed. After exposure, the egg masses were checked daily to ensure the freshness of the leaves until adult wasps emerged. Percentage of parasitism of the exposed eggs and percentage of wasps emerged were calculated on the two generations obtained in the laboratory. The effects of host plant species on the preference of *Z. delicata* to parasitize eggs of *T. rubromarginata* was tested on *Citrus* sp. (11 egg masses, 148 eggs), sugar cane (3 egg masses, 51 eggs) and corn (5 egg masses, 100 eggs). The experiments were conducted at room temperature (24.5 ± 6.2°C, 70-80% RH, photoperiod 14:10 h. L/D), and the colony was maintained under the same conditions.

Host eggs that changed to brownish or reddish after 5-7 d were considered "parasitized eggs", and those that developed eyespots of the host's nymphs were considered "unparasitized". The number of sharpshooter nymphs that hatched from the unparasitized eggs was counted daily. After 25 d, when parasitoid emergence was nearly complete, each leaf was dissected and the remaining host eggs were counted. By this time, most *Z. delicata* adults had either emerged or attained the pupal stage, so it was easy to distinguish parasitized eggs from unparasitized ones. The percentage of parasitism was calculated as follows:

% parasitism = (number of parasitized eggs/ number of host eggs offered) × 100.

Percentage of wasp emergence was calculated as follows:

% of wasp emergence = (number of wasps emerged/ number of offered eggs) × 100.

Test of Hyperparasitism

In field-collected egg masses of *T. rubromarginata*, it was common to observe the adults of *Z. delicata* and *Gonatocerus* spp. emerging from the same egg mass. *Zagella delicata* emerged about 10-15 days after *Gonatocerus*, suggesting it could be a hyperparasitoid of the latter, or that it is not an obligate primary parasitoid (Triapitsyn 2003). Considering that *Z. delicata* might be used as biocontrol agent, mixtures of primary parasitoids and hyperparasitoids often lead to erroneous host parasitoid records (Noyes 1994). We tested whether *Z. delicata* could develop in the eggs of *T. rubromarginata* parasitized by *Gonatocerus*. Seventy-two hours after an egg mass of this host laid in a sugar cane leaf, it was exposed to *Gonatocerus tuberculifemur* (Ogloblin). Then, the same egg mass was exposed to *Z. delicata* as explained above. Nine replications were carried out, with a total of 100 eggs exposed. Two egg masses (39 eggs) exposed to *Z. delicata* with no previous exposition to *G. tuberculifemur* served as control.

Adult longevity

Adult longetivy of *Z. delicata* was monitored twice a day using 84 individuals (57 females and 27 males) from a few h after emerging (within 12 h) until adult death. Observations were conducted on single wasps in individual vials without host material, but with honey as a food source. Differences between male and female longevity and duration of development were analyzed by a *t* test at the 0.05 level of significance (Statistica 5.0). The total time required for the development from egg to adult was based on 84 individuals.

Field Studies

To estimate parasitic activity a collection of egg masses was conducted in Tafi Viejo, Tucumán Province between 4 and 7 January 2001, when 68 egg masses (1018 eggs) were collected on Johnson grass. In addition, 12 samples were collected from September 2002 to April 2003 except in December (spring, summer and part of the fall) in San Miguel de Tucumán. We sampled a total of 6,253 eggs. For parasitoid emergence, each field-collected egg mass was placed in a Petri dish with the bottom filled with wet tissue paper. Each Petri dish was covered with food wrap to prevent eggs and leaves from dehydration and to keep the emerging wasps from escaping.

The survey with sentinel eggs was conducted from November 2000 to December 2002 in northwestern Argentina in areas that closely match the sub-climate types in the grape-growing regions of California. It also was conducted in the citrus-growing areas of eastern Argentina because it was suggested that the first generation of *H. coagulata* on citrus host plants is under poor natural control in California (Triapitsyn and Phillips 2000). Therefore sentinel egg masses in Argentina were produced on potted citrus plants. Field-collected females of *T. rubromarginata* were placed in sleeve cages on citrus plants. They were checked for presence of eggs daily, and when egg masses were detected, the sharpshooters were removed. Sleeves were kept on the plants to avoid uncontrolled parasitization from wild parasitoids. The sentinel eggs on the potted plants were placed in 41 selected sites in Tucumán, Jujuy, Misiones, Catamarca, La Rioja, Entre Ríos, Corrientes, San Juan, Salta, and Mendoza Provinces. Overall 8,000 eggs were exposed to parasitization.

RESULTS

Laboratory Studies

Zagella delicata is a solitary wasp parasitoid producing only one adult per host egg. The overall results showed that out of 353 eggs exposed to *Z. delicata* females, 72.5 ± 28.3% (Mean ± SD) were parasitized, and wasps emerged from 43.8 ±

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TABLE 1. PARASITIC ACTIVITY OF *ZAGELLA DELICATA* ON EGGS OF *TAPAJOSA RUBROMARGINATA* LAID ON THREE DIFFERENT PLANT SPECIES IN THE LABORATORY.

	Egg masses offered	Eggs offered	Egg masses attacked	*Z. delicata* emerged	Host nymphs emerged
Corn	5	10	5	57 (57.0 ± 14.5%)	29 (29.0 ± 17.9%)
Sugar cane	3	51	3	34 (66.7 ± 25.0%)	11 (21.6 ± 25.4%)
Citrus	12	148	1	7 (4.7 ± 2.0%)	69 (46.6 ± 4.7%)

41.4% of those. In the F1 progeny, 61.7 ± 40.3% of the exposed eggs were parasitized, and adult emergence was 26.9 ± 38.6%. In the F2 progeny, 94.5 ± 6.7% of the eggs were parasitized, and adult emergence was 85.0 ± 6.0%. Nymphs of *T. rubromarginata* emerged from 13.3 ± 28.7% of the exposed eggs.

In host plant searching preference test, *Z. delicata* females parasitized 34 host eggs (66.7 ± 25.0%) (3 egg masses) on sugar cane, 57 eggs (57.0 ± 14.5%) (5 egg masses) on corn and 7 (4.7 ± 2.2%) on citrus leaves (Table 1). In laboratory, *Z. delicata* females were able to find eggs of *T. rubromarginata* on citrus leaves. However, the wasps commonly failed to oviposit after several attempts. Citrus cuticle leaf is thicker than sugar cane and corn cuticle leaves, and probably cuticle thickness is a factor that interferes with oviposition of *Z. delicata*.

Developmental time (from oviposition to adult emergence) of *Z. delicata* averaged 23.5 ± 1.2 days (Fig. 1). Females and males did not show significant differences in developmental time (t = -1.1652, df = 1, $P > 0.05$) (females: 23.4 ± 1.2 days, males: 23.7 ± 1.1 days).

The average adult longevity was 10.3 ± 5.8 days. The analysis of adult longevity for females and males indicated significant differences, with females living longer than males ($t = 5.8717, P < 0.05$) (females: 12.2 ± 5.6 days, males: 6.2 ± 3.7 days).

The overall sex ratio of *Z. delicata* in the laboratory was 1: 2.1 (males/females). In F1 progenies it was 1: 3.2 and in F2 progenies 1: 1.9, respectively. In field-collected egg masses, the sex ratio was female biased, 1: 3.8 (27 males/103 females).

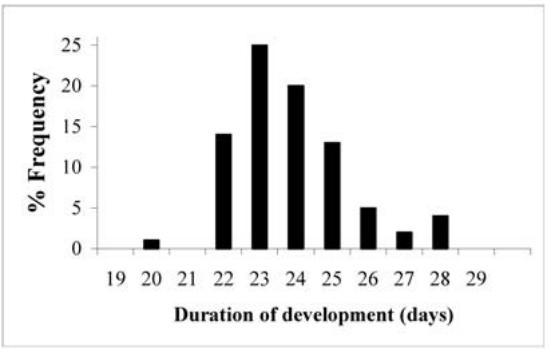


Fig. 1. Development time from egg to adult of *Zagella delicata* in the laboratory at room temperature.

Tests of Hyperparasitism

No adults of *Z. delicata* emerged from eggs previously exposed to *G. tuberculifemur*. Out of 100 exposed eggs, only 71 adults of *G. tuberculifemur*, and 2 nymphs of *T. rubromarginata* emerged. The remaining eggs yielded neither host nymphs nor parasitoids. Twenty seven adults of *Z. delicata* emerged from the 39 eggs used as control.

Field Studies

In the sample collected from Johnson grass in Tafi Viejo, 724 (71.1%) *T. rubromarginata* eggs were found to be attacked by a complex of egg parasitoids composed of several species of Trichogrammatidae and Mymaridae. *Zagella delicata* emerged from 626 eggs, or 86.5% of all parasitized eggs. Seasonal sampling carried out in San Miguel de Tucumán showed that *Z. delicata* occurred from September to March, with maximum abundance at the beginning of the season in October, where 57.2% out of the 1568 sampled eggs were parasitized (Table 2). From the 41 sites sampled in Argentina with sentinel eggs on citrus, *Z. delicata* adults were obtained in Santa Clara, Jujuy Province, and Tafi Viejo, Tucumán Province. In Santa Clara, 155 (61.7%) out of 251 exposed eggs were attacked by the parasitoid complex mentioned above. Sixty of these were parasitized by trichogrammatid species, including 38 (15.1%) by *Z. delicata*. In Tafí Viejo, 1,549 (47.0%) parasitoids emerged from 3,299 eggs exposed, but only 24 (1.5%) adults of *Z. delicata* emerged.

Distribution

In addition to Chacras de Coria in Mendoza Province (De Santis 1970), the locality where the type specimen was collected, *Z. delicata* also was collected in Tafi Viejo, Yerba Buena, and San Miguel de Tucumán in Tucumán Province as well as in Santa Clara, Jujuy Province, all in Argentina. Voucher specimens were deposited in Fundación e Instituto Miguel Lillo, San Miguel de Tucumán (IMLA), and Entomology Research Museum, University of California at Riverside (UCRC).

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TABLE 2. OCCURRENCE OF *ZAGELLA DELICATA,* OTHER TRICHOGRAMMATIDAE, AND *GONATOCERUS* SPP. (MYMARIDAE) IN EGGS OF *TAPAJOSA RUBROMARGINATA* ON JOHNSON GRASS AT SAN MIGUEL DE TUCUMÁN, ARGENTINA.

	Sampled eggs	*Z. delicata*	Other Trichogrammatidae[1]	*Gonatocerus* spp.
September	2722	0.8[2]	0.0	4.4
October	1568	57.2	1.5	0.4
November	248	16.5	4.8	2.4
January	370	19.2	27.6	4.3
February	559	21.1	17.7	7.2
March	611	9.3	17.8	21.9
April	175	0.0	3.4	19.4
May[3]	0	0.0	0.0	0.0

[1]Includes at least 3 different species in 3 genera: *Oligosita* (1), *Paracentrobia* (1 or 2), and *Zagella* (1).
[2]Percent of sampled eggs.
[3]No eggs were found in the field.

DISCUSSION

Biological characteristics of *Z. delicata*, a primary egg parasitoid of *T. rubromarginata*, have allowed us to estimate its overall potential as a biological control agent of *H. coagulata*. This is the first study that provides biological information on a species belonging to the genus *Zagella*. *Zagella delicata* was successfully reared under laboratory conditions for two generations before the colony was discontinued. The low percentage of emergence (35.4%) of *Z. delicata* adults in the laboratory was due to the damage (rotting or drying of the leaves and the host eggs) during the long preimaginal period of this parasitoid, thus complicating colony management. Most of the unemerged host eggs were parasitized, and nymphs of *T. rubromarginata* emerged from 13.3% of the exposed eggs. In F2 progenies, the percentage of adult emergence was higher than in F1 progenies because humidity control in the Petri dishes was improved. Females showed a strong preference for parasitizing host eggs on monocotyledon plants such as corn, Johnson grass, and sugar cane, whereas they hardly parasitized eggs laid on citrus. The low preference of *Z. delicate* to attack eggs laid on citrus plants is not a desirable characteristic for the use of this natural enemy in the control of glassy-winged sharpshooter. Regulation strategy for *H. coagulata* in California calls for its control in the citrus orchards. Also, the results of this survey with sentinel eggs of *T. rubromarginata* on citrus plants should be considered with caution because absence of *Z. delicata* in most of the samples does not necessarily mean that this species is absent in the sampled area. For example, in January 2001 in Tafí Viejo, 61.6% of *T. rubromarginata* eggs on Johnson grass in the field were parasitized, whereas at the same time and 1 km from that site, only 4% out of 99 sentinel eggs of the same host on citrus leaves were parasitized by *Z. delicata* (E. G. V. unpubl. data).

Sex ratios observed in the field and under laboratory conditions were similar, being female biased. The sex ratio in another trichrogrammatid species studied in Argentina, *Paracentrobia* sp. (misidentified as *P. subflava* (Girault) by L. De Santis), showed a predominance of females over males (Virla 1999).

Females of *Z. delicata* were able to parasitize more than 60% of the eggs in the laboratory, and about 61.6% in the field, suggesting great potential of this species as a biological control agent in some crops. The importance of synchronization of the occurrence (in time and space) of a parasitoid with its target host is often emphasized in biological control. *Zagella delicata* occurs in the field from September to April, during the same period as its host, *T. rubromarginata*, and has two generations for every generation of the host. Most importantly, it is the predominant natural enemy of *T. rubromarginata* early in the season (Fig. 2). Overall, field and laboratory data suggest that *Z. delicata* could be a prospective biological control agent against other, exotic,proconiine sharpshooters including *H. coagulata*. However, the efficiency of *Z. delicata* appears to be restricted to habitats dominated by grasses.

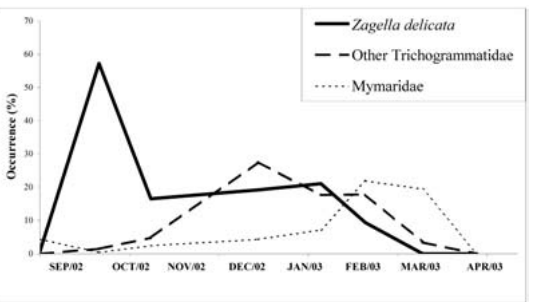


Fig. 2. Field occurrence of *Zagella delicata* and other egg parasitoids of *Tapajosa rubromarginata* on Johnson grass at San Miguel de Tucumán, Tucumán Province.

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