BIOLOGICAL CONTROL

Methodology of Mass Multiplication of *Telenomus podisi* Ash. and *Trissolcus basalis* (Woll.) (Hymenoptera: Scelionidae) on Eggs of *Euschistus heros* (Fab.) (Hemiptera: Pentatomidae)

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Metodologia de Multiplicação Massal de *Telenomus podisi* Ash. e *Trissolcus basalis* (Woll.) (Hymenoptera: Scelionidae) em Ovos de *Euschistus heros* (Fab.) (Hemiptera: Pentatomidae)

RESUMO - Estudos de laboratório foram conduzidos com o objetivo de desenvolver uma metodologia de multiplicação massal de *Telenomus podisi* Ash. e *Trissolcus basalis* (Woll.) em ovos de *Euschistus heros* (Fab.) e compará-la à metodologia utilizada no programa de controle biológico dos percevejos-pragas da soja no Brasil [*T. basalis* criado em ovos de *Nezara viridula* (L.)]. Em um primeiro experimento, foi determinado que massas de ovos aleatoriamente distribuídas foi o método mais adequado de exposição dos ovos de *E. heros* ao parasitismo. Em um segundo experimento, massas de ovos de *E. heros* foram aleatoriamente coladas em cartelas de papelão e expostas a *T. podisi* e *T. basalis*. Os parasitóides multiplicados nos ovos de *E. heros* apresentaram índices de parasitismo (cerca de 99%) similares ao observado para *T. basalis* multiplicado em ovos de *N. viridula* (99,7%). As taxas de emergência foram superiores a 80% para ambos parasitóides em ovos de *E. heros* e superior a 95% para *T. basalis* em ovos de *N. viridula*. Posteriormente, índices de emergência similares (> 78%) foram observados nas cartelas de papelão amarradas nas plantas de soja. O comportamento de cópula dos parasitóides não variou. A razão sexual da geração subsequente de *T. podisi* e *T. basalis* gerados em ovos de *E. heros* foi igual à de *T. basalis* criado em ovos de *N. viridula*. Os resultados deste estudo indicam que a metodologia desenvolvida é simples, eficiente e pode tornar-se importante ferramenta no programa de controle biológico dos percevejos da soja.

PALAVRAS-CHAVE: Insecta, *Glycine max*, parasitóide de ovos, criação massal, percevejo marrom

ABSTRACT - Laboratory studies were carried out to develop a methodology for the mass production of *Telenomus podisi* Ash. and *Trissolcus basalis* (Woll.) on eggs of *Euschistus heros* (Fabr.) and to compare it with the methodology presently used in the biological control program of soybean stink bugs in Brazil [*T. basalis* reared on *Nezara viridula* (L.) eggs]. In a first experiment, egg masses randomly distributed were shown the most adequate method of exposure of *E. heros* to parasitism. In a second experiment, egg masses of *E. heros* were randomly glued on cardboard cards and exposed to parasitism by *T. podisi* and *T. basalis*. The parasitoids multiplied on *E. heros* eggs showed parasitism (ca. 99%) similar to that observed with *T. basalis* reared on *N. viridula* eggs (99.7%). Emergence rates were higher than 80% for *T. podisi* and *T. basalis* multiplied on *E. heros* eggs and 95% for *T. basalis* reared on *N. viridula* eggs. Later, similar emergence rates (> 78%) were observed on cardboard cards tied on soybean plants. No differences were detected between courtship behavior of *T. podisi* and *T. basalis* emerged from *E. heros* eggs compared to *T. basalis* emerged from *N. viridula* eggs. The sex ratios in the offspring of *T. podisi* and *T. basalis* emerged from *E. heros* eggs were similar to that exhibited by the offspring of *T. basalis* emerged from *N. viridula* eggs. The results suggest that the developed methodology is simple, efficient and can be an important tool in biological control program of soybean stink bugs.

KEY WORDS: Insecta, *Glycine max*, egg parasitoid, mass rearing, brown stink bug

The seed suckers (Hemiptera: Pentatomidae) are the most important group of insect pests of soybean in Brazil. *Nezara viridula* (L.) (southern green stink bug), *Euschistus heros* (Fab.) (neotropical brown stink bug) and *Piezodorus guildinii* (West.) (small stink bug) are the more frequent species which constitute the stink bug complex. These insects cause substantial reduction in soybean yields and seed quality (Panizzi & Slansky 1985).

Chemical insecticides are the most usual control method, but its massive use increases production costs and usually results in reduction of beneficial insects, pests resurgence and leads to other environmental problems (Panizzi *et al.* 1977, Shepard *et al*. 1977). Among alternative control methods, biological control agents are potentially very useful in soybean integrated pest management programs (Orr 1988).

In Brazil, *Trissolcus basalis* (Woll.) was found parasitizing stink bug eggs for the first time in 1979 (Corrêa-Ferreira 1980, 1986). Nowadays, *T. basalis* is the most important agent used in biological control programs for soybean stink bugs (Corrêa-Ferreira 1993). The mass release of *T. basalis* to control the sink bug complex has been developed in large and continuos river basin areas. In Paraná State, a mean of 300,000 *T. basalis* adults are multiplied and released in approximately 18,020 hectares of soybean per year (Corrêa-Ferreira *et al*. 2000).

Eggs of *N. viridula* were used as hosts to multiplicate *T. basalis*. To obtain the host-eggs, adults of southern green stink bug are collected in the field, taken to the laboratory and maintained under controlled conditions (Corrêa-Ferreira 1985). However, changes in the dynamics of stink bug population and their parasitoids over the years (Panizzi & Corrêa-Ferreira 1997) have brought increasing difficulties in collecting *N. viridula* in the field, especially in low latitude regions (Cividanes & Parra 1994). As a consequence, the expansion of the biological control program for soybean stink bugs has been limited.

Presently, the brown stink bug *E. heros* is one of the most abundant stink bug in soybean fields in Brazil (Panizzi 1997, Costa *et al.* 1998). This specie can be easily reared under laboratory conditions and high rates of survivorship and fecundity can be obtained (Peres & Corrêa-Ferreira 2001). Additionally this specie is the preferential host of the important egg parasitoid *Telenomus podisi* Ash. (Corrêa-Ferreira 1986) and can be used as an alternative host for the multiplication of *T. basalis*. This study was conducted to develop a methodology for the mass multiplication of *T. podisi* and *T. basalis* on eggs of *E. heros*.

Material and Methods

The experiments were carried out under controlled environmental conditions (25 ± 2ºC, 65 ± 10% and 14 h L:10 h D). Two-day old *T. podisi* and *T. basalis* females previously copulated, fed on honey and without previous oviposition experience, were used in the experiments. Individualized colonies of *N. viridula* and *E. heros* were kept in a climatized room as a source of eggs, following the methodology described by Corrêa-Ferreira (1985). The eggs were collected daily and stored in the refrigerator under the temperature of 5ºC.

Methods of Exposure of *E. heros* Eggs to Parasitism. A preliminary test was conducted to determine the most appropriate method to expose *E. heros* eggs to parasitism. Approximately 40 eggs of *E. heros* and *N. viridula*, stored at 5ºC for up to 12 days were glued on a cardboard strip (3 x 1.5 cm). The cardboard strips were inserted in glass tubes (8 x 2.5 cm) containing four parasitoid females.

The stink bug eggs were exposed to parasitism in different ways: (1) *E. heros* egg masses with the operculum turned to the upside position (correct position); (2) *E. heros* egg masses with the operculum randomly turned upside, lateral side and upside down; (3) *E. heros* eggs separated and distributed randomly; and (4) *N. viridula* egg masses in correct position and only offered to *T. basalis* females (control).

After a parasitism period of 6h the cardboard strips were transferred to plastic petri dishes (9 x 1.5 cm) lined with moistened filter paper and kept under controlled conditions until adult emergence and parasitoid death. Those eggs that remained intact for several days after the emergence period were dissected to check their contents. Total parasitism was defined as the sum of eggs from which parasitoids emerged and parasitized eggs that did not result in parasitoid emergence.

The percentage of parasitism, adult emergence and sex ratio (number of females relatively to males and females produced) were assessed. A completely randomized design was used with four treatments, two insect species and 10 replicates.

***E. heros* Egg Masses Randomly Positioned on Cardboard Cards.** Based on the results of a preliminary test, stink bug egg masses stored at 5ºC for up to 20 days were glued in random position on a cardboard (8 x 4 cm), resulting in eggs distributed upside, lateral side and upside down positions. The cardboard, containing ca. 1200 *E. heros* or *N. viridula* eggs, was exposed to 40 parasitoid females for 12h in celluloid tubes (20 cm x 5 cm diameter). A completely randomized design was used with three treatments and five replicates: (1) *T. podisi* or (2) *T. basalis* parasitizing *E. heros* egg masses randomly located; (3) *T. basalis* parasitizing *N. viridula* egg masses with the operculum turned to the upside position (control).

After parasitism, the procedure was the same as described in the previous experiment. The percentage of parasitism, adult emergence and sex ratio (number of females relatively to males and females produced) were assessed.

Validation of Egg Parasitoids Multiplication Methodology on *E. heros* Egg Masses. Approximately 1200 eggs of *E. heros* and *N. viridula*, stored at 5ºC for up to 20 days (5ºC) were randomly glued on pieces of cardboard (8 x 4 cm) and offered to 40 parasitoid females for 24h. Three treatments were set: (1) *T. podisi* or (2) *T. basalis* parasitizing *E. heros* egg masses randomly positioned; (3) *T. basalis* parasitizing *N. viridula* egg masses with the operculum turned to the upside position (control). After parasitism, the procedure was the same as described in the previous experiment. A day before emergence of the adult parasitoids, the cardboards were wrapped in nylon netting and tied on the middle third of soybean plants

(with immature pods) cultivated in pots and kept inside the laboratory. When emergence appeared complete, the cardboards were removed and the emerged parasitoids were counted.

To determine fertility of progeny produced, 50 females of *T. podisi* and *T. basalis* reared on *E. heros* eggs and 50 females of *T. basalis* reared on *N. viridula* eggs (control) were placed individually in glass tubes (8 x 2.5 cm). Honey was streaked on the inside walls of the tubes to provide food source. Two days after emergence, 20 *E. heros* eggs were offered to a female parasitoid for 6h. After this period the eggs were placed in petri dishes with adequate moisture and kept at laboratory environmental conditions. Emerging parasitoids were sexed.

Data Analysis. Data on parasitism, adult emergence and sex ratio, were subjected to analysis of variance. If the analysis of variance was significant at the $P < 0.05\%$ level, Tukey´s test was applied.

Results and Discussion

Methods of Exposure of *E. heros* Eggs to Parasitism. Egg masses of *E. heros* glued in upside position or random position on cardboard strips presented parasitism by *T. podisi* and *T. basalis* higher than 97.0%, similar to the control (99.7%) (Table 1). These results corroborate the high parasitism (99.7%) found by Corrêa-Ferreira & Moscardi (1994) when *N. viridula* eggs were exposed to *T. basalis*. Eggs of *E. heros* separated and glued in random position which were offered to *T. podisi* and *T. basalis* showed parasitism of 96.1% and 99.1% respectively. The parasitism by *T. podisi* in eggs separated and randomly exposed (96.1%) was similar to the parasitism found by other methods studied, but was significantly lower than in the control treatment (99.7%). The number of *E. heros* eggs separated and glued in random position and parasitized by *T. basalis* (99.1%) was statistically equal to the other treatments.

T. podisi parasitized egg masses in correct or random position on the cardboard resulted in 83.1% and 79.9% emergence rates, respectively. These treatments did not differ from the *T. basalis* emergence in *N. viridula* (control), which had the highest emergence rate (92.5%). However, emergence of *T. basalis* in *E. heros* egg masses in correct or random position (50.6% and 63.2%, respectively) was significantly lower than the control (92.5%), showing that the eggs of *E. heros* are moderately adequate for development of *T. basalis* (Table 1).

Eggs separated and parasitized in random position by *T. podisi* and *T. basalis* had the lowest emergence rates, 51.3% and 26.1%, respectively (Table 1). The separated eggs probably showed more water loss than egg masses (Capinera *et al.* 1981) revealing that single eggs are more susceptible to desiccation, which make them non-viable for development of the immature phases of the parasitoids. It is suggested that the single eggs naturally laid should not be used as hosts for multiplication of the parasitoids. These results agree with those of Dass & Parshad (1982), that observed significant reduction in the rates of parasitism and emergence of *Telenomus remus* Nixon in separated eggs of *Spodoptera litura* (Fabr.).

The average sex ratio in the progeny of *T. podisi* and *T. basalis* ranged from 0.7 to 0.8, suggesting that the change of host and the form of gluing the eggs on the cards did not interfere on this parameter (Table 1). Similar sex ratio was also observed in *T. podisi* emerged from *Podisus maculiventris* (Say) and *E. heros* eggs (Orr & Boethel 1990, Pacheco & Corrêa-Ferreira 1998) and *T. basalis* emerged from *N. viridula* eggs (Corrêa-Ferreira & Moscardi 1994).

Both *T. podisi* and *T. basalis* were capable of parasitizing egg masses of *E. heros* distributed at random, independently if egg masses were fixed upside, on lateral sides or upside down. The high acceptability of *E. heros* egg masses randomly exposed may be attributed to the size and shape of the egg masses. Usually, the egg masses of the brown stink bug, on average, seven eggs (Villas Bôas & Panizzi 1980), are arranged in small groups, so nearly all the eggs are located at

Table 1. Parasitism, adult emergence and sex ratio (mean ± SEM) of *Telenomus podisi* and *Trissolcus basalis* multiplied on *Euschistus heros* eggs exposed on different methods, compared to control.

	Parasitism[1] (%)		Emergence (%)		Sex ratio[2]	
Treatment	*T. podisi*	*T. basalis*	*T. podisi*	*T. basalis*	*T. podisi*	*T. basalis*
E. heros egg masses in correct position	97.3 ± 0.84 ab	98.7 ± 0.59 a	83.1 ± 3.43 a	50.6 ± 2.49 bc	0.8 ± 0.04 a	0.7 ± 0.06 a
E. heros egg masses at random	97.8± 1.13 ab	99.0 ± 0.45 a	79.9 ± 2.46 a	63.2 ± 2.63 b	0.8 ± 0.02 a	0.8 ± 0.04 a
E. *heros* eggs separated and glued at random	96.1 ± 1.30 b	99.1 ± 0.73 a	51.3 ± 9.22 b	26.1 ± 10.57 c	0.8 ±0.02 a	0.8 ±0.06 a
Control[3]	99.7 ± 0.31 a		92.5 ± 2.43 a		0.8 ± 0.03a	

Means followed by the same letter in each column, do not differ at the 5% level according to the Tukey test.
[1]Original dates were transformed on $\sqrt{x + 0.5}$ for analysis
[2]Sex ratio = female / (male + female)
[3]Control = *T. basalis* multiplied on *N. viridula* egg masses glued in correct position
n =10 strips of paper with 40 eggs/strip

the edge of the mass. This facilitates the insertion of the ovipositor in the lateral egg wall, at the tip of the operculum or even in the chorion joints of two neighboring eggs (Sales *et al.* 1978). Such arrangement of eggs in the mass also enables and favors the emergence of parasitoids from the eggs.

Thus in the biological control program for soybean stink bugs, *E. heros* egg masses randomly distributed can be successfully used as hosts for mass multiplication of *T. podisi* and *T. basalis* in the laboratory.

***E. heros* Egg Masses Randomly Positioned on Cardboard Cards.** An average (± SEM) of 161.9 ± 6.33 *E. heros* egg masses were randomly glued per cardboard, corresponding to a total of 1196.8 ± 27.50 eggs exposed to parasitism. Of these, a mean (± SEM) of 437.1 ± 15.52 (36.6%), 457.1 ± 22.29 (38.0%) and 302.6 ± 9.92 (25.4%) eggs were positioned upside, on the lateral sides and upside down, respectively. In the control, a mean of 20.1 ± 0.41 *N. viridula* egg masses were glued upside on each cardboard, corresponding to a total of 1365.9 ± 61.3 eggs, which were offered to *T. basalis* females.

Examination of *E. heros* eggs showed that about 28.7% (342.8 ± 30.6) of those eggs had lateral deformations. These deformed eggs were not suitable for parasitoid larval development and, therefore, they were excluded from calculation of the adult emergence.

The *E. heros* eggs were parasitized by *T. podisi* and *T. basalis* at the rate of 99.1% and 99.2%, respectively. These values were statistically equal between each other or the control (99.7%) (Table 2).

In general, adult emergence rates were close to 80% (Table 2), which is the value accepted by the soybean stink bug biological control program, using egg parasitoids. Sex ratio of *T. podisi* developed in *E. heros* eggs (0.8) did not differ from the sex ratio found for *T. basalis* developed in *N. viridula* eggs (control). However, *T. basalis* reared in *E. heros* eggs showed a significant reduction in its sex ratio, compared to the other treatments (Table 2). This reduction is probably due to the oviposition sequence followed by *T. basalis* females, and to the smaller size of *E. heros* egg masses compared to those of *N. viridula*. As females of *T. basalis* lay proportionally more male eggs early in the oviposition sequence (Strand 1988, Colazza & Wajnberg 1998), the decrease in the number of eggs offered resulted in a higher proportion of males in the offspring (Strand 1988, Weber *et al.* 1996, Colazza & Wajnberg 1998).

Validation of Egg Parasitoids Multiplication Methodology on *E. heros* Egg Masses. Parasitism of 98.0% for *T. podisi* and 99.7% for *T. basalis* were found in the validation study on *E. heros* eggs. These values were statistically equal between each other and to the 99.8% parasitism by *T. basalis* in *N. viridula* eggs (control) (Table 3).

T. podisi and *T. basalis* emerged successfully from egg masses of *E. heros* randomly distributed on cardboard cards tied on soybean plants. The emergence rates were higher than 78% (Table 3) and close to the emergence rate of 80%, which is considered acceptable in parasitoid multiplication for field releases (Corrêa Ferreira 2002) Similarly, as in the previous study, a mean of 26.3% eggs offered had lateral deformation, and were non-viable for parasitoid multiplication and, therefore, were not considered in the emergence rate calculation.

No differences were detected in courtship behavior of *T. podisi* and *T. basalis* emerged from egg masses of *E. heros*, comparatively to *T. basalis* emerged from egg masses of *N. viridula*. Males were the first parasitoids to emerge from stink bug eggs. They remained on the egg masses waiting for female emergence to copulate, which occurred immediately after female emergence, a behavior already described to *Telenomus heliothidis* Ash. (Strand 1988) and *T. basalis* (Colazza & Wajnberg 1998). After mating, the females remained on the soybean plant leaves, flying to search for hosts and start the new life cycle.

The average (±SEM) sex ratios in the offsprings of *T. podisi* (0.8 ± 0.02) and *T. basalis* (0.9 ± 0.01) emerged from *E. heros* eggs were similar to that exhibited by the offspring of *T. basalis* emerged from *N. viridula* eggs (0.9 ± 0.02). Of the fifty female samples, only one *T. podisi* female, (2%) which multiplied on *E. heros* eggs, had its subsequent generation composed only of males, indicating no copulation after emergence.

The results indicate that the methodology developed allows easy and efficient multiplication of a great number of parasitoids to be released in the field and can be very useful in biological control programs of soybean stink bugs. The lateral deformation observed on *E. heros* egg is

Table 2. Parasitism, adult emergence and sex ratio (Mean ± SEM) of *Telenomus podisi* and *Trissolcus basalis* multiplied on *E. heros* egg masses glued at random on cardboard cards, compared to control.

Treatment	Parasitism (%)	Emergence (%)	Sex ratio[1]
T. podisi on *E. heros* egg masses	99.1 ± 0.35 a	87.9 ± 2.23 b	0.8 ± 0.01 a
T. basalis on *E. heros* egg masses	99.2 ± 0.18 a	80.4 ± 2.34 c	0.7 ± 0.02 b
Control[2]	99.7 ± 0.14 a	95.8 ± 0.36 a	0.9 ± 0.01 a

Means followed by the same letter do not differ at the 5% level according to the Tukey test.
[1]Sex ratio = female / (male + female)
[2]Control = *T. basalis* multiplied on *N. viridula* egg masses glued in correct position
n = 5 cardboard card with ca. 1200 eggs/cardboard

Table 3. Parasitism (mean[1] ± SEM) of *Telenomus podisi* and *Trissolcus basalis* multiplied on *E. heros* egg masses glued at random on cardboard cards and emergence on soybean plants, compared to control.

Treatment	Parasitism (%)	Emergence (%)
T. podisi on *E. heros* egg masses	98.0 ± 0.31 a	86.5 ± 2.23 b
T. basalis on *E. heros* egg masses	99.7 ± 0.13 a	78.2 ± 3.21 c
Control[2]	99.8 ± 0.11 a	93.9 ± 2.93 a

[1]Means followed by the same letter do not differ at the 5% level according to the Tukey test.
[2]Control = *T. basalis* multiplied on *N. viridula* egg masses glued in correct position
n = 4 cardboard card with ca. 1060 eggs/cardboard

probably related to the storage temperature used (5ºC) which may not have completely blocked eggs metabolism reducing host suitability for the parasitoids (Gautam 1987). Further researches on the cause of lateral deformation and on the storage techniques of *E. heros* eggs are necessary to improve production of the egg parasitoids.

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