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Authors: Veríssimo, Bruno Antônio, Auad, Alexander Machado, Silva, Sandra Elisa Barbosa, and Silva, Giani Batista da

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Biology and olfactory response of *Salpingogaster nigra* Schiner (Diptera: Syrphidae)

Bruno Antônio Veríssimo¹, Alexander Machado Auad^{2,*}, Sandra Elisa Barbosa Silva³, and Giani Batista da Silva⁴

Spittlebugs are sucking insects that cause decrease of forage grass quality, thereby constituting a serious problem for cattle rearing throughout tropical America (Valério & Nakano 1988). The most promising biological control agent of the spittlebug is the predator *Salpingogaster nigra* Schiner (Diptera: Syrphidae). This species lays its eggs in the foam produced by the spittlebug nymphs. Each larva feeds on a mean of 14 spittlebug nymphs (Guppy 1913; Paez et al. 1984). This predator has a short life cycle, with 2 to 3 generations during their prey's life cycle (Guagliumi 1970).

According to Parra (2002), the establishment of a program involving the use of natural enemies requires in-depth knowledge of the life history of the species involved so that the best performance may be obtained. However, it should be noted that even with all the biological and behavioral information of the developmental stages of the predator *S. nigra*, the host range has not been definitively determined, although the insect feeds on spittlebugs.

Here we report a study on the biology and olfactory response of *S. nigra* directed at the mass rearing of the predator. *Salpingogaster nigra* adults were field collected with an entomological net from Oct 2016 to Mar 2017. Then they were taken to the Entomology Laboratory in Juiz de Fora, Minas Gerais, Brazil, identified by sex, and paired. They were fed on flowers of *Parthenium hysterophorus* L. (Asteraceae) weeds and on *Emilia sonchifolia* (L.) DC (Asteraceae). Plants were replaced every 3 d. Honey drops (10% v/v) were placed on the cage walls. A pot with *Brachiaria decumbens* Stapf (Poaceae), infested with nymphs of the foam-producing prey *Mahanarva spectabilis* Distant (Hemiptera: Cercopidae) was placed in the cage as an egg-laying substrate. *Salpingogaster nigra* females mostly lay their eggs during the rainy season. Humidity was maintained by spraying water inside the cages 3 times per d.

Eighteen pairs of *S. nigra* were kept in 80 × 55 × 55 cm acrylic cages with the same food and egg-laying substrate mentioned previously. To have sufficient foam, nymphs of prey were added to the cages to maintain a density of 4 prey that were producing foam on each d. These insects were maintained in an acclimatized Fitotron-type chamber at 25 ± 2 °C, 70 ± 10% relative humidity (RH), and 12:12 h (L:D) photoperiod. *Salpingogaster nigra* eggs in the foam were counted daily. They were placed on petri dishes lined with filter paper and moistened with distilled water to keep them hydrated. Because the eggs were kept until they hatched, mean duration and viability of the predator eggs could be recorded.

Twenty-one larvae of *S. nigra* were placed in each 1 L pot (containing *B. decumbens* infested with *M. spectabilis* nymphs at the fourth or

fifth instar as food for the larvae) with a fine-bristle brush. This was replicated 10 times, hence the development and survival of 210 larvae was determined. Spittlebug nymphs were added daily so that food would not be lacking.

Forty-two larvae of *S. nigra* were obtained from pastures with *B. decumbens* so that the biological parameters of the pupa phase could be obtained. Thirty-three larvae pupated and were transferred to petri dishes lined with cotton and filter paper, and moistened with distilled water. Mean duration and survival of the pupa phase were assessed.

Olfactometry bioassays were performed at the Entomology Laboratory. Each arm of the Y-shaped glass olfactometer, with a continuous air flow of 1.0 L per min, was linked by silicone tubes to 2 glass chambers (42 cm H × 16 cm W) with the tested materials. Females and males from mating cages were separated approximately 2 h prior to the start of the bioassays. Olfactory responses of mated *S. nigra* females were then compared for the following odor combinations: (I) 3 spittlebug nymphs vs. air; (II) 3 spittlebug nymphs vs. foam of the spittlebug in the petri dish; (III) foam produced by the spittlebug in the petri dish vs. air. A bioassay also was conducted to test the bioassay apparatus without any odor in the olfactometer to confirm that the insects displayed positive anemotaxis in the arena when presented with clean air in both arms.

Insects were introduced individually in the common arm of the Y-tube, and each specimen was tested once to avoid pseudo-repetitions. The response was considered to be positive when the insect moved against the air current and reached the end of one of the Y-tube arms within 10 min. All olfactometry tests occurred between 2:00 PM and 4:00 PM. Fifty *S. nigra* females were tested for each treatment. Response data of were analyzed by BioEstat χ^2 test (Ayres et al. 2003). Insects that did not respond to the stimuli were excluded from statistical analysis.

The success biological control using lab-reared predators depends on production of healthy insects. This research revealed that *S. nigra* females maintained in the laboratory, and fed on honey and on *P. hysterophorus* and *E. sonchifolia* flowers, had a mean life span of 17.1 ± 1.6 d (SE) (11–30 d) and laid 2,209 eggs, with mean fecundity of 25.1 ± 6.1 (SE) eggs per female. According to Chambers (1988), syrphid flies require pollen and nectar for gametogenesis. Gomes & Lastra (2009) similarly maintained *S. nigra* in the laboratory and fed them on brown sugar and buds of *Echinochloa colona* (L.) Link (Poaceae), resulting in fecundity of 29 eggs per female.

In this study, we observed a mean egg development period of 2.7 ± 0.1 d in *S. nigra*, with a mean egg hatch rate of 64.0 ± 4.5%. Paez et

¹Federal University of Juiz de Fora, Department of Zoology, Juiz de Fora, Minas Gerais, Brazil, 36038-330; E-mail: bruno_averissimo@hotmail.com (B. A. V.)

²Embrapa Dairy Cattle Research Station, Entomology Laboratory, Juiz de Fora, Minas Gerais, Brazil, 36038-330; E-mail: alexander.auad@embrapa.br (A. M. A.)

³Federal University of Lavras, Department of Entomology, Lavras, Minas Gerais, Brazil, 37200-000; E-mail: sandraelisa.bio@gmail.com (S. E. B. S.)

⁴Center for Higher Education of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil, 36030-776, E-mail: gianibatistaa@outlook.com (G. B. S.)

*Corresponding author; E-mail: alexander.auad@embrapa.br

al. (1984) also reported a 2.7 d period for the embryonic development of *S. nigra* at room temperature. Guppy (1913) registered a mean developmental period of 2.5 d for eggs of the same species. The short embryonic period and high rate of egg hatch suggest easy rearing of the insect in the laboratory.

The mean larval period of *S. nigra* was 10.5 ± 0.4 d when fed on spittlebug nymphs. These results corroborate that obtained by Paez et al. (1984), who evaluated the larval duration of *S. nigra* and reported a mean period of 9.7 d. However, in our study the proportion surviving the larval stage was only $10.0 \pm 2.1\%$. This corroborates results by Paez et al. (1984), who maintained 115 *S. nigra* larvae in the laboratory, with only 4 completing the larval stage. The entomological literature fails to report the best conditions for maintaining larvae in the laboratory to obtain a high level of survival. The mean pupal duration was 10.7 ± 0.2 d and the proportion attaining the adult stage was $88.1 \pm 5.1\%$ in this study.

It should be noted that food provided to the predator was the same as was fed upon in the field (spittlebug). In mass production of this insect, different prey or an artificial diet would be required because spittlebug nymphs occur only during the rainy season. During the dry period of the year, spittlebugs are in diapause. Hence, for the breeding of *S. nigra* in the laboratory, it is necessary to conduct further research to find new prey or artificial diets.

Entomophagous insects have specialized sensory systems that allow them to use a variety of cues to find target organisms (Hatano et al. 2008). Visual cues play an important role in locating prey by different predators, such as *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the predation of aphids (Lambin et al. 1996). Besides visual cues, sound and chemical cues can be used to locate prey. In this study, we focused on the chemical cues used by the predator. *Salpingogaster nigra* females did not show a directional response (no preference for a given side independent of odor stimulus) when only clean air was presented through both arms ($\chi^2 = 0.381$; $df = 1$; $P = 0.537$). The predator did not display any preference for the odors of the foam produced by the nymph of the prey when compared to air (treatment I) ($\chi^2 = 0.32$; $df = 1$; $P = 0.572$). Also, there was no preference for nymphal odors when compared to the foam produced by the prey (treatment II) ($\chi^2 = 0.040$; $df = 1$; $P = 0.577$). On the other hand, when odor sources with spittlebugs were provided, there was a significant preference for odors from the nymphs compared to air (treatment III) ($\chi^2 = 13.52$; $df = 1$; $P = 0.001$) (Fig. 1). Shonouda (1996) and Sutherland et al. (2001) demonstrated that the honeydew excreted by the bugs is an attractive source for egg-laying in the adult stage of syrphids. This report on the behavior of *S. nigra* females showed that attraction was not due to the

foam excreted by the spittlebug but by the nymph, which most likely would function as a stimulus for egg-laying by the female predator.

These studies demonstrate that chemical cues may be involved in host finding by the predator as it searches for nymphs. Our results show that although *S. nigra* adults, eggs, and pupae may be kept in the laboratory, mass breeding of the predator is not feasible due to low larval viability of larvae. Further studies are required to minimize such low viability and make possible mass rearing in the laboratory.

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Summary

Salpingogaster nigra is a promising species for biological control of the spittlebug *Mahanarva spectabilis* Distant (Hemiptera: Cercopidae). This study determined the biology of the immature stage, as well as aspects of *S. nigra* adult behavior. Female fertility, egg viability, and the duration and viability of the larval and pupal stages were evaluated. Behavioral aspects were studied by the olfactory response of mated females, with a Y-type olfactometer. We assessed predator response to spittlebug nymphs vs. clean air; spittlebug nymphs vs. spittlebug foam, and spittlebug foam vs. clean air. Female fecundity reached 25.1 ± 6.1 (SE) eggs per female per d, and egg viability was $64.0\% \pm 4.5\%$. Duration and viability of the larval period were 10.5 ± 0.4 d and $10.0 \pm 2.1\%$, respectively. Duration of the pupal stage was 10.7 ± 0.2 d, and viability was $88.1\% \pm 5.1\%$. Significant preference was observed for odors from nymphs in contrast to clean air. Results show that although *S. nigra* adults, eggs, and pupae may be kept in the laboratory, the mass breeding of the predator presently is not feasible due to low larval viability.

Key Words: Brachiaria; pasture; predator

Sumario

A espécie *Salpingogaster nigra* é considerada promissora no controle biológico das cigarrinhas das pastagens. Assim, o objetivo deste trabalho foi determinar a biologia da fase imatura, bem como aspectos comportamentais de *S. nigra*. Avaliou-se a fertilidade das fêmeas, viabilidade dos ovos, duração e viabilidade do período larval e pupal. Os aspectos comportamentais foram estudados por meio da resposta olfativa de fêmeas acasaladas, em olfatômetro do tipo Y. Avaliou-se a resposta do predador aos odores: ninfas de cigarrinha vs. ar limpo; ninfas de cigarrinha vs. espuma da cigarrinha e, espuma da cigarrinha vs. ar limpo. A fertilidade das fêmeas foi de $25,1 \pm 6,1$ ovos/dia, e a viabilidade dos ovos foi de $64,0\% \pm 4,5\%$. A duração e viabilidade do período larval foi de $10,5 \pm 0,4$ dias e $10,0 \pm 2,1\%$, respectivamente. Na fase de pupa verificou-se uma duração de $10,7 \pm 0,2$ dias e viabilidade de $88,1\% \pm 5,1\%$. Observou-se preferência significativa de *S. nigra* pelos odores provenientes das ninfas em contraste com o ar limpo. Dessa forma, conclui-se que, embora os adultos, ovos e pupas de *S. nigra* podem ser mantidos em laboratório, a criação massal desse predador é inviável devido à baixa viabilidade larval.

Palavras Chave: Braquiária; pastagem; predador

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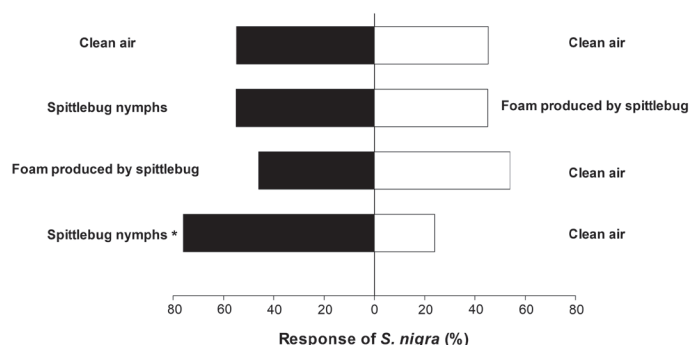


Fig. 1. Olfactory response of predator *Salpingogaster nigra* in an olfactometer, with different treatments: spittlebug nymphs vs. air; foam produced by spittlebug vs. air; spittlebug nymphs vs. foam produced by spittlebug, and air vs. air. An asterisk (*) means significant difference using χ^2 test at $P < 0.05$.

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