

# Life Cycle of *Glypheapomis spinosa* Campos & Grazia (Hemiptera: Pentatomidae): a New Pest of Rice in Brazil

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## Abstract

Nymph and adult biology of *Glypheapomis spinosa* Campos & Grazia (Hemiptera: Pentatomidae) was studied on rice plants under laboratory and greenhouse conditions. The full life cycle of *G. spinosa* lasted 50.1 day. Nymph development ranged from 2.7 days for the first instar up to 11.9 days for the fifth instar. The egg stage showed the highest per capita rate of mortality (0.16). Nearly 13% of all eggs were laid on the same day. The sex ratio was 0.5. The average hatching rate was 58.0%. Pre-oviposition, oviposition, and post-oviposition periods took 12.9, 34.4, and 6.2 days, respectively.

## Introduction

*Glypheapomis spinosa* Campos & Grazia (Hemiptera: Pentatomidae) is a new species of stink bug found on rice fields in the Brazilian central and northern states, commonly referred as the small black stem bug. Adults are usually black in coloration and are ventrally marked with rusty tracks, with humeral angles of the pronotum pointed (Campos & Grazia 1998). The biology and ecology of the *G. spinosa* and the factors affecting its population dynamics are largely unknown.

In preliminary studies, we observed that infestation and feeding might occur at any plant growth stage. Nymphs and adults feed on the base of stems by sucking on the plant sap. Because of its feeding, the region of the tissue around the injury collapses and a lesion is formed, and sap translocation is interrupted. Infestation at the tillering stage causes dead heart; leaves turn chlorotic or brown with reduction in tiller number as well as stunting and plant death in heavy infestations. Direct injuries to panicles were not observed, but if it occurs during the booting stage, white panicles with stained or empty spikelets may occur,

similarly to the damage inflicted by stem borers (Heinrichs & Miller 1991).

The feeding behavior and damage caused by *G. spinosa* are very similar to those imposed by the Malayan black bug, *Scotinophara coarctata* (Thunberg) (Hemiptera: Pentatomidae) (Morrill *et al* 1995) and *Tibraca limbativentris* Stal (Hemiptera: Pentatomidae) (Botton *et al* 1996, Ferreira *et al* 1997). Yield losses registered for the Malayan black bug ranged from 14.7% for resistant varieties to 23.0% for susceptible ones (Heinrichs *et al* 1987). For *T. limbativentris* yield losses ranged from 5% to 80%, depending on the plant development stage (Ferreira 1998). These yield losses are due to unfilled spikelets and to decreased tillers number. Even though there are no published studies on *G. spinosa* as a rice pest, this species can reduce plant stands and grain yield of individual plants. Consequently, a reduction in yield is expected. *Glypheapomis spinosa* occurs in upland rice, but it is found more commonly in larger populations in continued irrigated fields.

In this study, we first present data on nymph and adult biology of *G. spinosa* to fundament future works on pest management strategies for this species.

## Material and Methods

Studies were conducted under laboratory and greenhouse conditions at the “Centro Nacional de Pesquisa de Arroz e Feijão–Embrapa,” Santo Antônio de Goiás, Goiás, Brazil (16° 28′S; 49°17′W; 823 m asl). Adults and egg masses were obtained from the first generation of a greenhouse colony kept in nylon screen cages. Each cage held 10–20 male/female pairs. Egg masses were daily removed from the plants to avoid egg parasitization. A colony of this stink bug was established with insects collected from rice fields during the summer, in Formoso do Araguaia, Tocantins, Brazil (11°48′S; 49°32′W) and Lagoa da Confusão, Tocantins, Brazil (10°01′S; 49°58′W). Relative humidity and temperature were recorded every hour by using a hygrothermograph data logger (Reed 8829™). The environmental data collected were monthly recovered and a simple arithmetic average was calculated. Irrigation was provided through water plates placed under the pots. Previous observations before the establishment of the experiments ensured similar survivorship as well as the determination of analogous life cycle.

### Nymphal development

On October 24, 2008, 30 egg masses (totaling 375 eggs) were removed from rice plants and transferred to Petri dishes (9×1.5 cm) lined with filter paper moistened with distilled water in the laboratory (25±2°C; 75±0.60% RH; 13h photophase), and daily inspected to determine egg hatching. After hatching, nymphs remained clumped close to eggshells until they molted to the second instar, when they disperse and start feeding. At that time, it was difficult to determine the precise number without disturbing the nymphs and inducing unnatural mortality. The same day the nymphs molted to second instar, all nymphs from the same egg mass were transferred to rice plants, cv. Jaçanã, grown in plastic pots (25 cm diameter and 20 cm height). Nymphs were removed from each Petri dish and transferred to potted plants using a tiny brush. To prevent insects from escape the plants, pots with rice plants were covered with a nylon screen cage and randomly placed on a bench. Pots with plants and nymphs were kept in the greenhouse until the nymphs completed their immature development.

We followed the development of each individual nymph throughout the adult stage by daily visual inspections and counting. Any nymph dropped to the ground during evaluations was returned to the plant. During evaluations, all stems and leaves of each plant in the pot were inspected. Any dead or missing individuals were recorded. The developmental time for each stage was calculated, and the number of surviving individuals was recorded daily. At

adult emergence, the sex of all individuals was determined by the morphology of their external genitalia (Campos & Grazia 1998). Survival was calculated as the difference between the number of individuals alive in two consecutive stages. The sex ratio was determined by dividing the number of emerged females by the total number of emerged adults.

### Adult biological parameters

Adult survivorship and female fecundity of *G. spinosa* were determined in a greenhouse with 14h photophase, adults emerged on November 25, were separated as individual 25 couples (one male to one female) and held in a potted rice plant as previously described for feeding and reproduction. Plants were checked every day to assess adult longevity and egg laying. The laid eggs by each couple were transferred to Petri dishes, kept in the laboratory, and daily checked for assessment of the incubation period, female fecundity, and fertility. In addition, the pre-oviposition, oviposition, and post-oviposition periods were also calculated.

Measures of central tendency and variability of biological variables were performed using SAS (SAS Institute Inc. 2006). Means were compared with a t test when necessary ( $\alpha=0.05$ ), and all graphics were generated with SigmaPlot 10.0 (Systat Software, Inc. 2006).

## Results

### Nymphal development

*Glypompomis spinosa* required 32.1 days to develop from first instar to adult (Table 1) when reared at 26.6±0.2°C and 74.6±0.7% RH (Fig 1). Fourth and fifth instars were the longest stages of nymph development, representing 58% of the total period required to adulthood. During the first 3 days after hatching, nymphs remained adjacent to one

Table 1 Instar-specific duration (mean ± SE) for *Glypompomis spinosa* kept under greenhouse conditions (26.6±0.2°C; 74.6±0.7% RH; 13h photophase).

Stage	Stadium	Duration (days)	Range (days)
Egg <sup>a</sup>		5.0±0.1	0–10
	First instar	2.7±0.2	1–5
	Second instar	5.8±0.3	3–12
Nymph	Third instar	4.8±0.2	2–13
	Fourth instar	6.8±0.2	5–10
	Fifth instar	11.9±0.8	8–25

<sup>a</sup> Results are based on 375 eggs.

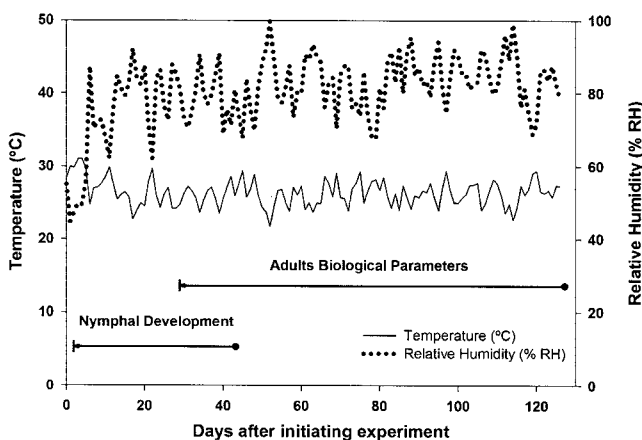


Fig 1 Temperature and relative humidity registered during nymph and adult biological experiments.

another on their eggshells until they molted to the second instar, when they started to feed and disperse. The nymphs did not disperse widely and remain preferentially on the same plant. All the surviving nymphs progressed through five molts, and 35.2% of them developed to the adult stage (Table 2). Mortality was highest in the egg stage, lowest in the first, and slowly increased until the fifth instar (Table 2). Among the 132 individuals reared from eggs, 65 were males and 67 females (sex ratio of 0.5).

*Adult biological parameters*

Females needed at least 9 days to start laying eggs, remaining reproductively active for up to 90 days (Table 3) under the rearing conditions on this study (26.3±6.7°C; 82.3±22.8% RH) (Fig 1). The post-oviposition period was short as some females died immediately after the last oviposition. Females lived as long as males (*t* test=1.16; *df*=45; *P*=0.25) (Table 3).

Table 2 Number of survivors (absolute and relative values), mortality (percent) and log transformation number of individuals alive in each class (*n<sub>x</sub>*) of *Glypheidomyia spinosa* reared on potted rice plants under controlled conditions in nymph experiment (26.6±0.2°C; 74.6±0.7% RH; 13h photophase).

Life class (x)	Live individuals ( <i>n<sub>x</sub></i> ) <sup>a</sup>	Relative survival (%)	Mortality into class (%)	Log ( <i>n<sub>x</sub></i> ) - Log ( <i>n<sub>x+1</sub></i> )
Egg	12.5	100	31.4	0.16
First instar	8.5	68.6	5.2	0.02
Second instar	8.1	65.0	6.1	0.03
Third instar	7.6	61.0	8.5	0.04
Fourth instar	6.9	55.8	15.4	0.07
Fifth instar	5.9	47.2	25.4	0.13
Adult	4.4	35.2	-	-

<sup>a</sup> Mean number of eggs out of 30 masses.

Table 3 Biological variables for adults of *Glypheidomyia spinosa* kept under greenhouse conditions (26.3±6.7°C; 82.3±22.8% RH; 14h photophase).

Variable	Mean±SE (days)	Coefficient of variation (%)	Range (days)
Pre-oviposition	12.9±0.4	19.3	9-19
Oviposition	34.4±4.4	65.0	3-90
Post-oviposition	6.2±1.8	146.4	0-34
Female longevity	53.5±4.0	37.5	24-106
Male longevity	61.7±5.9	45.4	20-129

Oviposition by the same female tended to occur sporadically, independently of its age (Table 4). Females laid 13.8% of egg masses in a single day, and the bouts were shorter than 3 days in 80.3% of the consecutive oviposition (Table 4). Additionally, the first egg masses laid were larger and eggs had a higher hatchability than those egg masses laid later during the female reproductive period. Only 7% of the egg masses were laid separately, and 48% had fewer than ten eggs, ranging from one to 34 eggs per mass, with a mode of eight eggs and average of 9.0±0.2 eggs (Fig 2). Mean egg viability was 58.0±1.9%, but a 100% hatchability was observed in only 22.3% of the egg masses. Nearly one fourth of all the egg masses produced had no viable eggs at all. Peak oviposition was observed for 20-day-old females and steadily declined until female death (Fig 3). At the age of 60 days, half of the females had already died, but 80%

Table 4 Frequency distribution of egg masses between consecutive ovipositions by female *Glypheidomyia spinosa* on potted rice plants under controlled conditions (26.3±6.7°C; 82.3±22.8% RH; 14h photophase).

Interval between consecutive egg masses (days) <sup>a</sup>	Number of egg masses	Percentage (%)	Cumulative frequency (%)
0	52	13.8	13.8
1	130	34.5	48.4
2	84	22.3	70.7
3	36	9.5	80.3
4	28	7.4	87.7
5	16	4.2	92.0
6	11	2.9	94.9
7	3	0.8	95.7
8	10	2.6	98.4
11	1	0.2	98.6
12	1	0.2	98.9
13	1	0.2	99.2
14	2	0.5	99.7
17	1	0.2	100.0

<sup>a</sup> Ovipositional intervals were calculated by the number of days between pairwise of consecutive ovipositions laid by the same female during life cycle.

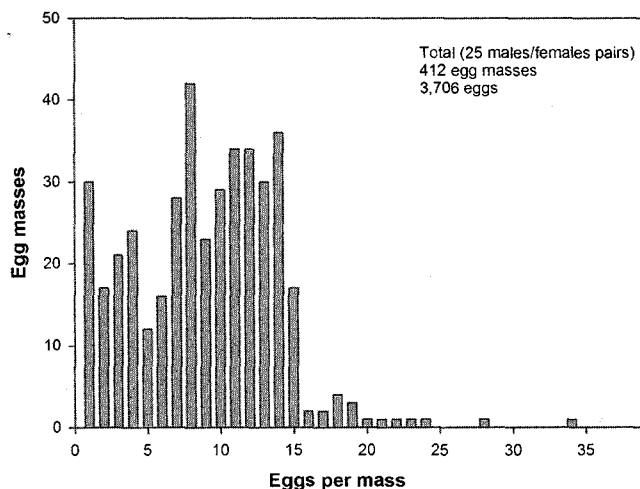


Fig 2 Frequency distribution of the number of eggs per mass of *Glypheapomis spinosa* based on 25 male/female pairs on potted rice plants under controlled conditions ( $26.3 \pm 6.7^\circ\text{C}$ ;  $82.3 \pm 22.8\%$  RH; 14h photophase).

of the total number of eggs produced had already been laid (Fig 3). Egg production decreased with female age (Fig 3).

## Discussion

Environmental conditions are determinant for insect development and survivorship of nymphs and adults (Hirose et al 2006, Manrique et al 2011, Silva et al 2011). In our study, there was no significant variability in temperature and relative humidity during the experiments (Fig 1), and the embryonic development took as long as that of the rice stalk stink bug *T. limbativentris* reared under similar conditions (Botton et al 1996) and was very close to the period of development observed for five other species of stink bugs (Ehler 2000).

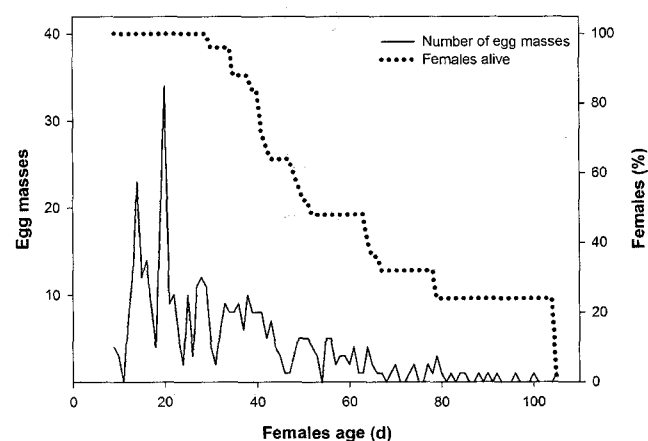


Fig 3 Egg mass production by 25 females of *Glypheapomis spinosa* during their life cycle under controlled conditions ( $26.3 \pm 6.7^\circ\text{C}$ ;  $82.3 \pm 22.8\%$  RH; 14h photophase).

Adults were observed for long periods under soil clods, fallen leaves, and in the middle of stems close to the roots. We believe that *G. spinosa* is restricted to warm areas and its habit to hide in the soil may contribute to reduce its exposure to predators, parasites, and insecticides. The absence of diapause in this species also supports this hypothesis. Thus, adults and older nymphs are able to withstand adverse conditions during the hottest periods of the day. However, eggs and newly hatched nymphs are more likely to be affected by variations in temperature and relative humidity because of their limited mobility. Therefore, its cryptic behavior may be a strategy to reduce water loss due to transpiration and to avoid high temperature exposure.

Feeding occurred preferably in the intern stems. Adults feed from the rice stem with their heads pointing downwards, similar to what has been reported to other species of pentatomids such as *Edessa meditabunda* (F.) on soybean and sunflower plants (Panizzi & Machado-Neto 1992). Egg hatching, nymphal molt, and adult emergence occurred through several hours of the day. A lack of diurnal synchrony in stage transition was previously observed for another pentatomid, *T. limbativentris* (Botton et al 1996). Females laid their eggs in double rows beneath upper leaves, preferably.

First instars of *G. spinosa* remained aggregated on the eggshells until molting to the second instar, when they started to disperse and feed. This behavior is common for most pentatomids (Simmons & Yeargan 1988). Aggregation in pentatomids can reduce the duration of nymph and adult development (Panizzi et al 2005, Nielsen et al 2008) and may be required for symbiont acquisition (Prado & Almeida 2009).

The highest mortality was observed in the egg stage (Table 2), which was due to desiccation (Dzerefos et al 2009) and egg sterility. Thus, in our experiments, the possibility of egg parasitism was excluded. Furthermore, we transformed the data using log scale to allow comparisons among other species. This transformation makes it easier to visualize per capita rates of change in population rather than absolute numerical changes (Krebs 1978).

*Glypheapomis spinosa*, *S. coarctata*, and *T. limbativentris* may be the only stink bugs feeding on stems of rice plants, while others will usually feed on the reproductive tissues. The nymph developmental time of *G. spinosa* (32.1 days) was similar to that of *T. limbativentris* (30.6 days) (Botton et al 1996), and much shorter than the one of *S. coarctata* (54–66 days) (Batay-an et al 2007). Nevertheless, the food source, temperature, relative humidity, and photoperiod may influence the developmental time and mortality of nymphs and adults (Ehler 2000, Chocorosqui & Panizzi 2003, Niva & Takeda 2003, Hirose et al 2006). *Glypheapomis spinosa* has an average fecundity of 148.2 eggs/female

(Fig 2), with an asymmetric ( $G_1=0.46$ ) distribution of eggs/mass, with a mean of nine eggs/mass and a mode of eight eggs/mass (Fig 2). Because of this asymmetry, Matesco *et al* (2009) argued that in Pentatomidae, the eggs/mass are better estimated by the mode than by the average number. Fecundity was inversely proportional to the age of females, although there were females laying eggs at the end of the oviposition period. Nevertheless, the viability of the eggs laid at this stage was lower than those eggs laid in the beginning of the oviposition period.

Considering the pre-oviposition time (12.9 days) and development from egg to adult (37.2 days), the duration of one generation was about 50 days. Therefore, at least two generations may take place in a single growing season because many rice varieties take a minimum of 110 days to develop from seeding to harvest. In the field, infestations by *G. spinosa* begin 30 days after rice seeding when adults move from refuges to rice fields.

Our data suggest that this species may have multiple generations/year, with potential to become a frequent rice pest in most of the tropical production areas. To gain a better understanding of this species and its role in rice ecosystems, it is necessary to understand its bionomics. From our greenhouse study, we can conclude that in the absence of natural biological mortality factors (e.g., predators or parasites), one female can add more than 50 adults to the next generation in less than 60 days. The developmental data presented herein provide a basis for predicting the phenology of *G. spinosa* populations on rice fields. However, ecological and biological studies are required to determine survivorship and reproductive rates of *G. spinosa* in the field.

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