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Development, Survival and Reproduction of *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae) with Salt and Amino Acids Solutions Supplementary Diet

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ABSTRACT

This study presents the effect of a supplementary diet with amino acids and sodium chloride solutions in addition to prey on the development, survival and reproduction of the predator Podisus nigrispinus *(Heteroptera, Pentatomidae). Both solutions showed deleterious effects on nymph survival, adult weight, female longevity, number of egg masses, eggs per female, eggs per egg mass and nymphs per female besides egg viability of* P. nigrispinus *when compared with diet with water and prey. When compared with plant supplements in the diet the use of amino acids and salt solutions for mass rearing of* P. nigrispinus *was inferior.*

Key word: Nutrition, zoophytophagous, insecta, biological control

INTRODUCTION

Predatory Asopinae of the genus *Podisus* have been registered in many ecosystems, especially, associated with Lepidoptera in eucalyptus plantations (Zanuncio et al., 1994). *Podisus nigrispinus* (Dallas, 1851) (Heteroptera: Pentatomidae) is an important biological control agent found in South and Central America (Thomas, 1992) mostly in the Neotropical area (De Clercq, 2000) in many cultures (Michel, 1994; Medeiros et al., 2000). Studies on Asopinae have been made mainly with natural and alternative prey to achieve the maximum development and reproduction of these bugs (Zanuncio et al., 1996). The availability and quality of prey may influence the life history of these predators (Valicente and O´Neil, 1993; De Clercq et al., 1998; Mourão et al., 2003), but it can also feed on plants without damaging them (Naranjo and Stimac, 1985, 1987; Ruberson et al., 1986; De Clercq and Degheele, 1992; Lemos et al., 2001; Evangelista Jr. et al., 2004). This feeding behavior allows classifying these species as zoophytophagous (Coll and Guershon, 2002) because feeding on plant and prey may improve biological characteristics of natural enemies (Lemos et al., 2001; Oliveira et al., 2002). However, it is not clear if these predators obtain only water or also nutrients from plants (O´Neil and Wiedenmann, 1990; De Clercq and Degheele, 1992; Armer et al. 1998). There are at least three hypotheses to explain the

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zoophytophagy in these insects: (1) equivalence - plants can supply nutrients obtained from prey when these are lacking; (2) facilitation - vegetable material supplies essential components to aid predation and (3) independence - vegetable material supplies essential nutrients not present in prey (Lalonde et al., 1999; Eubanks and Denno, 2000; Gillespie and McGregor, 2000; Sinia et al., 2004).

The objective of this work was to study the effect of amino acids (commonly found in plants) and sodium chloride (NaCl) on survival, development and reproduction of the predator *P. nigrispinus*.

MATERIAL AND METHODS

The experiment was conducted at 25 ± 5ºC, 70 ± 5% RH and photophase of 12 hours. Seventy-five first instars nymphs of *P. nigrispinus* were placed in groups of five per Petri dish (9 x 1.2 cm). A total of 25 nymphs received one of the following solutions: 125 mM NaCl solution (treatment 1); a complex of amino acids based on the Grace's culture for insects (Table 1) (treatment 2) and distilled water (treatment 3), as the control. Each solution was offered in a moistened cotton ball inside the Petri dishes and changed every 48 hours. The nymphs of *P. nigrispinus* received two *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae every other day from the second instar. The duration and survival of each instar and the weight of adults in the day of their emergence were obtained. Males and females *P. nigrispinus* were separated during three days to reach sexual maturity (Zanuncio et al., 1992). Ten pairs of this predator were used per diet and confined in Petri dishes where they received daily two *T. molitor* pupae and the same solutions given to the nymphs, which originated them. Males of *P. nigrispinus* that did not mate up to 48 h were substituted. The preoviposition, oviposition and post-oviposition periods, number of egg masses, eggs/female, eggs/egg mass, nymphs per female, eggs and nymphs, egg viability and longevity of *P. nigrispinus* females were evaluated.

Table 1 - Amino acids supplied for nymphs and adults of *Podisus nigrispinus* (Heteroptera, Pentatomidae) at 25 ± 5ºC, 70 ± 5% of relative humidity and 12 hours photophase.

Component	g/l
Calcium chloride	1.0
Magnesium chloride	1.06
Magnesium sulfate	1.35
Potassium chloride	2.24
Sodium monobasic phosphate	0.87
Alanine	0.20
Arginine	0.70
Aspartic acid	0.35
Asparagine	0.35
Cysteine	0.025
Glutamic acid	0.60
Glutamine	0.60
Glycine	0.65
Histidine	2.50
Isoleucine	0.05
Lisine	0.62
Phenylalanine	0.15
Proline	0.35
Serine	1.10
Treonine	0.175
Tryptophan	0.10
Tyrosine	0.07
Valine	0.10

The results were submitted to the Lavene test to verify the homogeneity of variance and submitted to the analysis of variance and the Tukey test at 5% probability. Dataset on egg viability, post-oviposition period and number of nymphs were

submitted to the Kruskal-Wallis test, because they did not have homogeneous variance.

RESULTS

The duration of the first, second and fourth instar was similar with both solutions supplied, but the third and fifth instars of *P. nigrispinus* were affected by the diet (Table 2). Nymphs of this predator had longer duration of the third instar with NaCl solution than with water. Fifth instar was shorter with water and longer with the amino acid solution (Table 2). The duration of the nymph stage of *P. nigrispinus* was longer with the NaCl and amino acid solutions than with water (Table 2).
Nymphs of *P. nigrispinus* survived longer during the third instar longer with water while those with NaCl solution presented lower survival in the fourth instar. Nymphs of this predator showed longer survival during the nymph stage with water (Table 2). Males and females *P. nigrispinus* were lighter with saline and amino acid solutions than with water (Fig. 1).
The pre-oviposition and pos-oviposition periods of *P. nigrispinus* were similar between treatments. However, the oviposition period and the number of egg masses presented higher values for females of this preditor with water and NaCl solution (Table 3). The numbers of eggs, eggs per egg mass and nymphs besides the viability of eggs and the longevity of *P. nigrispinus* females had higher values with water than with saline or amino acid solutions.

Table 2 - Duration and survival during each instar and of the nymph stage (means ± standard error) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed on *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and with NaCl 125 mM (NaCl) or amino acid (AA) solutions or distilled water at 25 ± 5ºC, 70 ± 5% relative humidity and photophase of 12 hours.

	Duration of the instars (days)*					
Treatments	**I**	**II**	**III**	**IV**	**V**	**Nymph stage**
NaCl solution	2.0 ± 0.1a	4.1 ± 0.6a	4.7 ± 0.5a	4.6 ± 1.1a	6.7 ± 0.4b	22.1 ± 1.6a
AA solution	2.0 ± 0.1a	3.8 ± 0.3a	4.6 ± 0.5ab	4.5 ± 0.1a	7.3 ± 0.5a	22.3 ± 1.1a
Distilled water	1.9 ± 0.1a	3.9 ± 0.5a	4.2 ± 0.2b	4.2 ± 0.3a	5.8 ± 0.3c	20.1 ± 0.9b
	Survival (%)*					
	I	**II**	**III**	**IV**	**V**	**Nymph stage**
NaCl solution		98.0a	81.50b	75.33b	92.16a	58.0b
AA solution		100.0a	92.0ab	95.50a	89.50a	76.0ab
Distilled water		96.0a	100.0a	91.50a	100.0a	88.0a

*Means followed by same letter, in the columns, do not differ by the test of Tukey ($p < 0.05$).

Table 3 - Parameters (means ± standard desviation) of the adult stage of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed on *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and solutions of NaCl 125 mM (NaCl) and amino acids (AA) or distilled water at 25 ± 5ºC, 70 ± 5% relative humidity and 12 hours photophase.

Parameters *	**NaCl solution**	**AA solution**	**Distilled water**
Preoviposition (days) * *	5.4 ±1.4a	4.4 ± 3.4a	3.7 ± 1.1a
Post-oviposition (days) *	6.1 ± 4.1a	3.0 ± 3.6a	2.9 ± 2.3a
Oviposition (days) *	23.7 ± 7.5a	7.4 ± 7.9b	34.6 ± 18.5a
Number of egg masses *	10.0 ± 3.7a	3.3 ± 3.1b	11.1 ± 6.2a
Eggs/female*	244.7 ± 126.9ab	77.4 ± 82.1b	386.8 ± 219.9a
Eggs/egg mass *	22.8 ± 5.6ab	15.54 ± 12.9b	33.8 ± 9.2a
Number of nymphs * *	161.6 ± 86.1ab	47.0 ± 5.9b	289.7 ± 157.2a
Egg viability (%) * *	60.0 ± 10.0ab	30.0 ± 4.0b	80.0 ± 10.0a
Female longevity (days) *	29.8 ± 13.9ab	19.6 ± 9.9b	43.2 ±19.5a

*Means followed by the same letter, in the line, do not differ by the test of Tukey ($p < 0.05$). **Means followed by the same letter, in the line, do not differ by the test of KrusKal-Wallis ($p < 0.05$).

DISCUSSION

The duration of the nymph stage of Pentatomidae predators varies with diets such as found for the longer duration of the third, fourth and fifth instars of *P. nigrispinus* with saline and amino acid solutions. This seemed to be the most critic instars for the development of Pentatomidae predators which was also found for *P. nigrispinus* with different diets (Zanuncio et al., 1996) and *Supputius cincticeps* (Stal, 1860) (Heteroptera: Pentatomidae) exposed to environmental stresses (Zanuncio et al., 2003).

The duration of the instars of *P. nigrispinus* was longer with NaCl or amino acid solutions than that reported for this predator fed on different prey in the presence or absence of plants (Zamperline et al., 1992; Lemos et al., 2001, 2003; Evangelista Jr. et al., 2003). This showed that the development of predators could be affected by the type and quality of prey and the dietary supplement to them (Strohmeyer et al., 1998). However, the use of amino acids or mineral salts with prey was not enough to simulate the effect of plants on nymph development of *P. nigrispinus*.

The harmful effect of the saline and amino acid solutions on nymph survival, adult weight, female longevity, numbers of egg masses, eggs/female, eggs/egg mass and nymphs besides the egg viability of *P. nigrispinus* was not expected. This indicate that the nutrients obtained from plants by Pentatomidae predators were unknown. On the other hand, the benefits of feeding on plants to predators has been demonstrated (O'Neil and Wiedenmann, 1990; De Clercq and Degheele, 1992; Cohen, 1996; Naranjo and Gibson, 1996; Armer et al., 1998; Coll, 1998) suggesting that the nutrients supplied by them to these predators were more complex than the solutions tested.

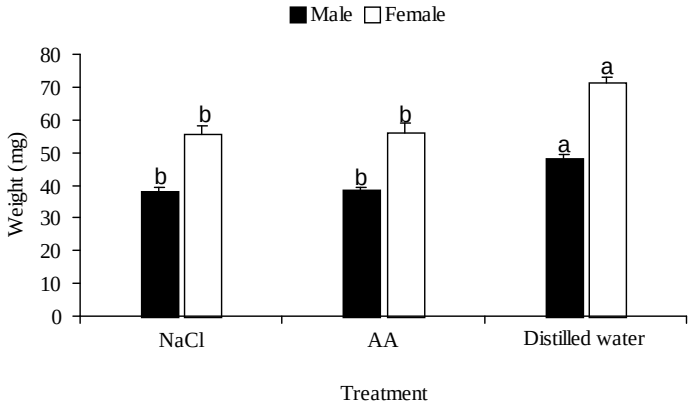

Figure 1- Weight of *Podisus nigrispinus* (Heteroptera: Pentatomidae) adults with *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and solutions of NaCl 125 mM (NaCl) or amino acids (AA) or distilled water at 25 ± 5ºC, 70 ± 5% of relative humidity and 12 hours photophase. Columns followed by the same letter, do not differ by the test of Tukey ($p < 0.05$).

The osmolarity of diets is important for the development of parasitoids, because the osmotic pressure can exceed the benefits of their nutrients (Thompson, 1983). The amino acid solution had osmolarity between 390-400 mOsm/l, which was similar to that of the haemolymph and tissues of most insects (Chapman, 1998). Therefore, the osmotic pressure of the diets did not seem to be responsible for the harmful effect on *P. nigrispinus*.

The results support the facilitation hypothesis when the vegetable material supplies essential components to aid predation (Gillespie and McGregor, 2000). The zoophytophagy facilitates the predation by supplying water for the extra-oral digestion of the prey (Sinia et al., 2004). This might occur for *P. nigrispinus* because individuals of this predator receiving water and prey had better development and reproduction than those with NaCl or amino acid solutions. However, the

independence hypothesis (Gillespie and McGregor, 2000) should not be discarded. In this case the vegetable material supplied essential nutrients not available on the animal tissues such as methyl esters of amino acids which regulated the absorption of these compounds in the midgut of caterpillars (Giordana et al., 2002).

The evolution of the feeding habit of Hemiptera suggested that their ancestral were, probably, similar to the Auchenorrhyncha adapted to suck xylem and phloem (Silva et al., 2004) with low quantity of proteins and free amino acids (Terra et al., 1996). This rejected the equivalence hypothesis (Gillespie and McGregor, 2000) that the vegetable material supplied enough nutrients to substitute those obtained by predators from animal tissue, at least, for *P. nigrispinus*. Therefore it could be recommended to use plants in rearing this predator in laboratory instead of amino acid or NaCl solutions.

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RESUMO

O presente estudo mostra o efeito da suplementação alimentar com soluções de aminoácidos e salina (NaCl) no desenvolvimento, sobrevivência e reprodução de *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae). Ambas soluções causaram efeito deletério na sobrevivência ninfal, peso dos adultos, longevidade das fêmeas e nos números de posturas, de ovos/fêmea, de ovos/postura e de ninfas, bem como na viabilidade dos ovos de *P. nigrispinus* quando comparado com estes insetos que além de presa receberam água. Estes resultados são discutidos em comparação com o efeito positivo que a suplementação alimentar com plantas tem sido relatada para esses predadores e sugerem que o uso de plantas é melhor que a substituição por solução de aminoácidos em sistemas de criação em laboratório desses predadores.

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