# LACK OF MACRONUTRIENTS IN *Eucalyptus urophylla* S.T. Blake (Myrtaceae) SEEDLINGS AFFECTS FEED AND DEVELOPMENT OF *Podisus nigrispinus* (Hemiptera: Pentatomidae)

DEFICIÊNCIA DE MACRONUTRIENTES EM MUDAS DE Eucalyptus urophylla S.T. Blake (Myrtaceae) AFETAM A ALIMENTAÇÃO E O DESENVOLVIMENTO DE Podisus nigrispinus (Hemiptera: Pentatomidae)

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**ABSTRACT:** Several studies have reported the importance of phytophagy in the history of life from predator stinkbugs. However, knowledge of the nutrients acquired by these insects, from plant materials, is scarce. The phytophagy studies of zoophytophagous predators benefit biological control programs, making them more effective, since the best resources may be offered to these insects, increasing the potential for predation and, thus obtaining, success in pest control. Thus, this study investigated the development and zoophytophagy of predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) in eucalyptus seedlings, deficient in macronutrients. The experiment was conducted in a greenhouse and the treatments consisted of individual nitrogen omission, phosphorus, potassium, calcium, magnesium and sulfur nutrient solution, where the eucalyptus seedlings were being conducted. The pupae of *Tenebrio molitor* L. (Coleoptera: Teneobridae) were offered *ad libitum*. The results of this experiment demonstrated the importance of nutrition in plants for the development and predation of *P. nigrispinus*. The plants deficient in nitrogen and magnesium reduce the predation efficiency of this zoophytophagous. The omission of calcium, magnesium and sulfur adversely affected the development of this insect predator, extending its immature stages. The proper nutritional balance of the plants is essential for the success in biological control programs that use zoophytophagous predators.

**KEYWORDS:** Biological Control. Plant Nutrition. Zoophytophagy.

#### INTRODUCTION

Predator stinkbugs belonging to the subfamily Asopinae (Hemiptera: Pentatomidae) are an alternative for the management of agricultural and forest pests (PEREIRA et al., 2010). These insects occur naturally in different crops and feed on a variety of insects (PIRES et al., 2015).

Podisus nigrispinus (DALLAS, 1851) is an important predator of this subfamily, feeding on larvae and adults of more than 30 species of Lepidoptera defoliators (RODRIGUES et al., 2008; ZANUNCIO et al., 2014). Brazilian forestry companies have been using *P. nigrispinus* and other Asopinae predators on the management of defoliating caterpillars on eucalyptus plantations to reduce the use of pesticides (DE BORTOLI et al., 2011; HOLTZ et al., 2011; PIRES et al., 2015).

These bugs exhibit five larval stages until become adults. They have carnivorous feeding habits, but may use their prey host plants as a supplement in their diet (COELHO et al., 2009;

EVANGELISTA JÚNIOR et al., 2004). The phytophagy may promote the maintenance of these natural enemies in the field, regardless of the presence of the pest (HOLTZ et al., 2011). These insects reduce their nymphal period, increase their weight as adults, longevity, and improve the predation efficiency and performance in reproduction by feeding on plant materials (ARMER et al., 1998; ASSIS JÚNIOR et al., 1998; EVANGELISTA et al., 2004; HOLTZ et al., 2011; LEMOS et al., 2010; ZANUNCIO et al., 2000).

Studies of phytophagy on predator stinkbugs have shown the importance of plant origin food in their lives (EUBANKS; DENNO, 1999), but the knowledge about the role of nutrients that are acquired on plants and prey to these insects is scarce. It is important to know these nutrients and their importance for the development and behavior of predators to make most effective biological control programs (COELHO et al., 2009).

The objective of this study was to assess the effect of the omission of the macronutrients:

nitrogen, phosphorus, potassium, calcium, magnesium and sulfur, in eucalyptus seedlings in the development time and predation rate of the predator stinkbug *P. nigrispinus*.

#### MATERIAL AND METHODS

The experiment was conducted in a greenhouse at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), in Diamantina, State of Minas Gerais, Brazil (18°15 'S, 43°36' W, 1,400 m above sea level). The temperature and average relative humidity at the greenhouse were 26.6 °C and 46%, respectively.

Seedlings of Eucalyptus urophylla S. T. Blake (Myrtaceae) with 45 days provided by the Company Aperam BioEnergia (Minas Gerais, Brazil) were used. The plants had similar shoot and root system, with height and average diameter of  $34.4 \pm 1.2$  cm and  $0.19 \pm 0.4$  cm, respectively. The roots were rinsed in water and kept in deionized water for 15 days, to reduce the fertilization excess of clonal nursery. The seedlings were placed in the center of circular polystyrene dishes (2 cm thick) and placed in plastic pots with 4.5 L of nutrient solution. The experiment was conducted in hydroponic system with aeration by compressed air and nutrient solution (CLARK, 1975), prepared with pure chemical reagents: ammonium nitrate, phosphoric acid, potassium chloride, anhydrous calcium chloride, magnesium chloride, sodium sulfate, boric acid, copper chloride, iron chloride, manganese chloride, zinc chloride, sodium molybdate, which were replenished weekly. The seedlings were acclimated in hydroponic condition with the concentrations used in the nutrient solution gradually increased every week (25, 50 and 75%) until it reaches a concentration of 100% (CARVALHO NETO et al., 2011). The pH was adjusted to 5.5 on each concentration change interval. The determination of the levels of macronutrients was performed (MALAVOLTA et al., 1997).

Eggs of *P. nigrispinus* were obtained from a rearing at Biological Control Laboratory of UFVJM,  $(25 \pm 2^{\circ} \text{ C}, 70 \pm 10\% \text{ relative humidity}$  and photoperiod of 12 hours). One hundred eggs were placed in Petri dishes with moistened cotton attached to the lid and kept in a room with the same conditions. A total of 70 nymphs hatched from the eggs, and one day old, were randomly chosen and transferred to seven Petri dishes, in groups of 10, which were fed only with distilled water, until they reach the second instar. Two *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae were placed in

each Petri dishes containing nymphs of this stage, when they start predation (MENEZES et al., 2014). The pupae of *T. molitor* were reared feeding on wheat bran and pieces of chayote.

Five of the ten nymphs of the predator were chosen randomly when they reached the third instar and were transferred for organza bags (30 x 15 cm) wrapped around eucalyptus seedlings inside the greenhouse.

The influence of the absence of each macronutrient in development time and predation rate of *P. nigrispinus* on *T. molitor* pupae was evaluated. We used a completely randomized design, with seven treatments and five repetitions (N = 35). Each replication was a seedling of eucalyptus containing five nymphs. The treatments were T1 = nitrogen omission (N); T2 = phosphorus omission (P), T3 = potassium omission (K); T4 = calcium omission (Ca); T5 = magnesium omission (Mg); T6 = sulfur omission (S); T7 = control (CON) with macro and micronutrients of the nutrient solution of Clark (1975).

The first symptoms of nutritional deficiency in the eucalyptus seedlings were observed with 60 days after the beginning of the experiment. At this time, the predator third instar nymphs were placed on the seedlings. The development of the nymphs was daily assessed until they reach adulthood.

Three *T. molitor* pupae, one day old, were offered to each group of five nymphs on eucalyptus seedlings to assess the food intake of the predator. The pupae were weighed on analytical balance and placed in the organza bags, with the predator. Another weighing was made after 72 hours to obtain the amount (g) of food ingested by *P. nigrispinus*. The average of three consumption evaluations of *T. molitor* pupae was performed for each stage of the predator. Five samples with three pupae were weighed and kept on the same conditions to determine the amount of water lost by the prey during 72 hours. After this time the pupae were weighed again and the average water lost subtracted from food consumption data.

Development data were submitted to analysis of variance and then to Dunnett test at 5% probability, compared to the control treatment. Data on predation were subjected to analysis of variance and then to Scott & Knott test at 5%.

Nutritional analysis of pupae used for feeding *P. nigrispinus* was performed to identify the nutrients present in this prey. Five samples of *T. molitor* were used, containing three pupae, with average weight of  $60 \pm 4$  mg each, which were dried in an oven at  $65^{\circ}$ C until reach constant weight (about 48 hours).

The determination of nitrogen on the samples was performed by sulfuric digestion, with Kjeldahl analytical procedure (MALAVOLTA et al., 1997). For the other nutrients, it was used an energy dispersive x-ray fluorescence spectrometer.

#### **RESULTS**

#### Lack of macronutrients in eucalyptus seedlings and food consumption by Podisus nigrispinus

The nitrogen and magnesium omission in the nutrient solution reduced the predator feeding in pupae of T. molitor (Table 1). The average total consumption of pupae, for each group of five predatory stinkbugs, was 100mg on the control and, 38mg and 72mg for omission of N and Mg, respectively. The omission of P, K, Ca, S did not affect the feeding of the nymphs and the average total consumption ranged from 96mg to 106mg. A progressive increase in predation rate was observed in these treatments.

Table 1. Podisus nigrispinus (Dallas, 1851) (Hemiptera: Pentatomidae) feeding on Tenebrio molitor L. (Coleoptera: Tenebrionidae) pupae in Eucalyptus urophylla (Myrtaceae) seedlings cultivated with the omission of macronutrients.

| Treatment   | Average consumption (mg) of <i>Tenebrio molitor</i> pupae for each immature stage of <i>Podisus nigrispinus</i> |   |           |   |          |   |         |   |  |  |
|-------------|---|---|-----------|---|----------|---|---------|---|--|--|
|             |   |   |           |   |          |   |         |   |  |  |
|             | Instar III  |   | Instar IV |   | Instar V |   | Average |   |  |  |
| Control     | 0,031   | a | 0,104     | a | 0,165    | a | 0,100   | a |  |  |
| N Omission  | 0,035   | a | 0,034     | b | 0,044    | b | 0,038   | b |  |  |
| P Omission  | 0,038   | a | 0,108     | a | 0,159    | a | 0,102   | a |  |  |
| K Omission  | 0,061   | a | 0,129     | a | 0,129    | a | 0,106   | a |  |  |
| Ca Omission | 0,037   | a | 0,101     | a | 0,149    | a | 0,096   | a |  |  |
| Mg Omission | 0,041   | a | 0,096     | a | 0,077    | b | 0,072   | b |  |  |
| S Omission  | 0,036   | a | 0,097     | a | 0,174    | a | 0,102   | a |  |  |
| CV (%)      | 48,04   |   | 28,75     |   | 26,94    |   | 30,01   |   |  |  |

Means followed by the same letter in the column do not differ between each variable evaluated by the Scott & Knott test at 5%.

There was no positive correlation between advance in the development and increased predation rate for all instars changes in treatments where the Mg and N were omitted, but the insects reached adulthood. The consumption of prey in the fourth instar was lower for the nitrogen omission treatment when compared to other treatments. In the fifth instar it was observed that with the omission of nitrogen and magnesium the prey consumption was lower when compared to other treatments.

#### Lack of macronutrients in eucalyptus seedlings and the development of Podisus nigrispinus

The nymphs of the predator in the third instar in plants lacking of calcium, took 2.6 days more to change to the next stage, compared to the control (Figure 1 and Table 2). In the fourth stage, it was found that treatments lacking on N, P and S increased nymphal period on 2.93; 3.73 and 3.02 days, respectively, compared to control. The development of the fifth stage, the treatments that were lacking P, Mg and S, increased by 0.49; 4.25 and 3.62 days, respectively, compared to the control.

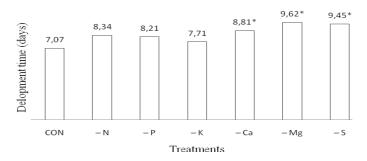


Figure 1. Average time (days) of development in stages III, IV and V of *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) on eucalyptus leaves in different fertilization treatments: CON (control); -N (N omission); -P (P omission); -K (K omission); -ca (Ca omission); Mg (Mg omission) and S (S omission). \* p <0.05 compared to control treatment by Dunnett's test.

Lack of macronutrients... VIEIRA, E. R. D. et al.

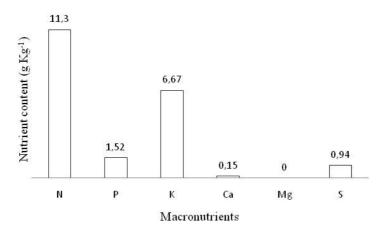
**Table 2.** Stages development time (days) of *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) in *Eucalyptus urophylla* (Myrtaceae) leaves, cultivated with the omission of macronutrients.

| Stage | Control | N     | P     | K    | Ca     | Mg     | S      |
|-------|---------|-------|-------|------|--------|--------|--------|
| III   | 7,76    | 9,20  | 6,80  | 8,40 | 10,36* | 9,44   | 8,92   |
| IV    | 5,94    | 8,87* | 9,67* | 5,49 | 8,59   | 8,36   | 8,96*  |
| V     | 7,51    | 4,99  | 8,00* | 9,26 | 6,60   | 11,76* | 11,13* |

<sup>\*</sup> p <0.05 compared to control treatment by Dunnett's test.

### Nutritional analysis of *T. molitor* used to feed *P. nigrispinus*

All macronutrients were found in *T. molitor* pupae, except magnesium (Figure 2).



**Figure 2.** Macronutrients in *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae.

#### **DISCUSSION**

The need of *P. nigrispinus* to feed on plants was observed. Plants provide nutrients that are essential to these insects and are not available in their preys (HAMDI et al., 2013; VANKOSKY et al., 2015; ZANUNCIO et al., 2008).

It is not known the exact role the Mg plays in insect nutrition (CLARK, 2012). However, as shown here, it was observed that the lack of Mg in plants decreases the feed rate of the predators (CLANCY; KING, 1993).

It is clear the need of nitrogen from the plant food source for phytophagous insects due to the central role this element plays in metabolic processes, cell structure and genetic code (PIZZAMIGLIO, 1991).

The zoophytophagous *Dicyphus hesperus* Knight (Hemiptera: Miridae) feed more on prey that have high nitrogen concentrations in comparison to ones with low concentrations (VANKOSKY et al., 2015). In this work, *P. nigrispinus* decreased his predation, compared to control treatment, even preys having large amounts of nitrogen. This may be due the fact of zoophytophagous needs of the

water obtained through plant food source to perform the predation (GILLESPIE, 2000; McGREGOR, 2000). Thus, the lack of nitrogen in plants discourages the phytophagy and as a consequence the insects got less water, compromising in obtaining prey. The longest development time of P. nigrispinus due the lack of sulfur may be associated with the synthesis of juvenile hormone, essential for the molt. This nutrient is a structural component of acetyl-CoA, which gives rise to Acetoacetil CoA, precursor of key enzymes to the synthesis of juvenile hormone (KLOWNDEN, 2007). It is possible that the lack of S in plants changed the synthesis of juvenile hormone, delaying the molting in the predator nymphs, making them staying longer in the fourth instar.

Lack of Ca also delayed the development of the predator, probably due the fact that it is required for the production of ecdysone, another hormone involved in molting. The lack of calcium prevents the action of the HPTT hormone when there is an influx of Ca<sup>2+</sup> into cells of prothoracic gland (KLOWNDEN, 2007). Thus, the cAMP (cyclic adenosine monophosphate) concentration will not elevate in the absence of these ions. The cAMP

activates protein kinase and the consequence of the lack of Ca is the deficiency of the production and release of ecdysone (MATTSON; SPAZIANI, 1986; RUPPERT et al., 2005).

The extension of nymphal stage time due the lack of those nutrients is not interesting for biological control programs (MENEZES et al, 2014). These programs search for insects that reaching the reproductive phase quickly. This study showed that nutritional balance of the host plant is essential for good development and predation rate of *P. nigrispinus*.

#### **CONCLUSIONS**

The omission of macronutrients may be a disadvantage to *P. nigrispinus*.

A proper nutrient balance in plants is important for biological control programs and eucalyptus plantations should be kept in a good nutritional state in order to promote these predators.

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**RESUMO:** Diversos estudos têm relatado a importância da fitofagia na história de vida dos percevejos predadores. Porém, não se conhece quais são os nutrientes adquiridos por estes insetos nos materiais vegetais. Sabe-se que a fitofagia realizada pelos predadores zoofitófagos beneficia os programas de controle biológico, tornando-os mais eficazes, e que as plantas oferecem recursos que poderão aumentar o potencial de predação, garantindo o sucesso no controle de pragas. Deste modo, este trabalho investigou o consumo alimentar e desenvolvimento do percevejo *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), sob mudas de eucalipto, submetidas à solução nutritiva com omissão de macronutrientes. O experimento foi realizado em casa de vegetação e os tratamentos consistiram na omissão individual de nitrogênio, fósforo, potássio, cálcio, magnésio e enxofre da solução nutritiva, onde as mudas de eucalipto se desenvolveram. Pupas de *Tenebrio molitor* L. (Coleoptera: Teneobridae), foram oferecidas *ad libitum*. Os resultados deste experimento comprovaram a importância da complementação nutricional em plantas para o consumo alimentar e desenvolvimento do *P. nigrispinus*. A alimentação nas plantas em que o nitrogênio e magnésio foram omitidos diminuiu o consumo de biomassa animal das presas deste zoofitófago. E a omissão de cálcio, magnésio e enxofre afetou, negativamente, o desenvolvimento deste percevejo predador, prolongando seu estádio imaturo. O balanço nutricional adequado das plantas é fundamental para obter sucesso em programas de controle biológico em que se utilizam predadores zoofitófagos.

PALAVRAS-CHAVE: Controle Biológico. Nutrição de plantas. Zoofitófagos.

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Lack of macronutrients... VIEIRA, E. R. D. et al.

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