Larval density of Melolonthidae (Coleoptera) in succession cropping systems in Chapadão do Sul, MS, Brazil

Densidade larval de Melolonthidae (Coleoptera) em sistema de sucessão de culturas em Chapadão do Sul, MS, Brasil

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ABSTRACT
The order Coleoptera of the family Melolonthidae comprises a large group of species, whose larvae can feed on roots of crops and cause damage thereof. This paper aims to investigate the larval density of Melolonthidae in succession cropping systems in the municipality of Chapadão do Sul, state of MatoGrosso do Sul, Brazil. The studies were conducted from March 2016 to May 2017. Samples of larvae were collected from soybean-maize and soybean-cotton succession cropping systems. The sampled larvae were reared until adult stage at the entomology laboratory. In the soybean-maize succession cropping system, the highest larval densities were observed in the conventional maize crop, whereas in the soybean and maize crops with Bt protein, Melolonthidae larvae were not found. In the soybean-cotton succession cropping system, the highest larval densities were observed in the non-Bt soybean crop, and in the Bt cotton crops these larvae were not found.

Keywords: Glycine max, Gossipyumhirsutum, Bt protein, Scarabaeoidea, Zea mays.

RESUMO
A ordem Coleoptera da família Melolonthidae compreende um grande grupo de espécies, cujas larvas podem alimentar raízes de culturas e causar danos. Este trabalho tem como objetivo investigar a densidade larval de Melolonthidae em sistemas de cultivo de sucessão no município de Chapadão do Sul, Mato Grosso do Sul, Brasil. Os estudos foram realizados de março de 2016 a maio de 2017. Foram coletadas amostras de larvas de sistemas de cultivo de soja e milho-soja. As larvas amostradas foram criadas até o estágio adulto no laboratório de entomologia. No sistema de cultivo de soja e milho, as maiores densidades larvais foram observadas na cultura de milho convencional, enquanto que nas culturas de soja e milho com proteína Bt, as larvas de Melolonthidae não foram encontradas. No sistema de cultivo de soja e algodão, as maiores densidades larvais foram observadas na cultura de soja não Bt, e nas culturas de algodão Bt essas larvas não foram encontradas.

Palavras-chave: Glycinemax, Gossipyumhirsutum, proteína Bt, Scarabaeoidea, Zea mays.
1 INTRODUCTION

The order Coleoptera of the family Melolonthidae (Cherman e Morón, 2014) comprises a large group of species, with different sizes and colors, whose diet include fruits, flowers, leaves, roots or organic matter (Ratcliffe, 2003; Oliveira e Ávila, 2011; Rodrigues et al., 2016).

In Brazil, the occurrence of 1,008 species of Melolonthidae (Morón, 2004) has been reported. Some of these species may be important crop pests, mainly because of the damage caused by their larvae feeding on plant roots.

In the state of Paraná, Brazil, since 1985, larvae of Phyllophagacuyabana (Moser) have been observed causing damage to soybean crops. In the state of Rio Grande do Sul, Brazil, larvae of Dilobderusabderus (Sturm) were described as important crop pests (Silva e Costa, 2002).

In the state of MatoGrosso do Sul, Brazil, the findings by Ávila e Pípolo (1992) demonstrated damages caused by Melolonthidae larvae to wheat crops. In sugarcane crops, Coutinho et al. (2011) reported damage caused by larvae of Liogenys fusca Blanchard, Cyclocephala verticallis Burmeister, Cyclocephala forstery Endrodi and Anomonyx sp. In maize crops, larvae of Liogenys saturalis Blanchard were described as important pests (Santos e Ávila, 2009), while in soybean crops the related pests were C. forstery larvae (Santos e Ávila, 2007).

In the state of Goiás, Brazil, larvae of Phyllophagacapillata (Blanchard) were found in soybean crops (Oliveira et al., 2007a), while larvae of Aegopsisbolboceridus (Thomson) were observed in vegetable crops (Oliveira et al., 2008).

For the control of Melolonthidae larvae, chemical products are usually used, which can decrease population densities (Santos et al., 2008). However, some succession cropping systems can also aid in decreasing larval density in the field (Rodrigues et al., 2011).

Due to the importance of Melolonthidae as pests for various crops, studies have been conducted to investigate the effect of succession cropping on larval density in the municipality of Chapadão do Sul, state of MatoGrosso de Sul, Brazil.

2 MATERIALS AND METHODS

The studies were carried out at a Santa Olinda farm owned by Mr. Claudio Bragante and at a Catléia farm owned by Mr. Josué Corso Neto, both located in the municipality of Chapadão do Sul, state of Mato Grosso de Sul, Brazil, at Km 6, BR 360 (Figure 1). The climate of the region, according to the Köppen classification, is tropical wet and dry (AW), with well-defined seasons, rainy in the summer and dry in the winter, with average annual precipitation of 1,733 mm and local altitude of 798 m.
Santa Olinda farm has an area of 600 hectares, and larvae were sampled in a 155.7-hectare plot, called Plot 1. Catléia farm has an area of 7000 hectares, and the larvae were sampled in a 268.9-hectare plot, called Plot 2 (Figure 1).

Santa Olinda farm in February 2016, the NS70 (conventional) maize cultivar was sown. From July to September 2016, the area remained fallow. In October 2016, the 5G8015IPRO (with Bt protein, cry1Ac) soybean cultivar was sown. In February 2017, the NS90 PRO (with Bt protein, cry1A.105 + cry 2Ab2) maize cultivar was sown. Catléia farm in February 2016, the FM 975 WS (with Bt protein, cry1Ac + cry1F) cotton cultivar was sown. This area remained fallow from August to October 2016. In November 2016, the NA 5909RR (without Bt protein) soybean cultivar was sown. In February 2017, the FM 975 WS (with Bt protein, cry1Ac + cry1F) cotton cultivar was sown.

In order to obtain Melolonthidae larvae, the crops were sampled every 15 days in each experimental area. The larvae were collected by excavating the soil along the crop rows. Each sample was represented by a trench with 25 cm x 25 cm x 30 cm (width, length and depth), making a total of 51 trenches per sampling day for each crop (Pardo-Locarno et al., 2005), randomly chosen in the plot (Figure 2). The soil samples were sieved and the larvae were collected and taken to the entomology laboratory of the State University of Mato Grosso do Sul (UEMS), in Cassilândia, state of
MatoGrosso do Sul, Brazil, and reared until adult stage. With this method, the 3m² area was examined for larvae every 15 days, so it was possible to determine larval density per square meter.

![Figure 2. Samples collected from the crops to obtain Melolonthidae larvae.](image)

In the entomology laboratory, the larvae were coded and individualized in 500 mL plastic containers, evaluated every three days, and reared until adult stage (Figure 3). In the plastic containers, about 2/3 of their volume was filled with soil where a seedling of *Brachiariabrizantha* Stapf was placed in order to provide roots to feed the larvae, until they reached the pre-pupal stage; the seedlings were replaced biweekly, when necessary.

![Figure 3. Rearing containers with soil, *Brachiaria decumbens* seedlings and Melolonthidae larvae.](image)
The data on larval counts were transformed into $\sqrt{x + 1}$, submitted to analysis of variance, and later the means were compared using the Tukey test ($P < 0.5$).

**3 RESULTS AND DISCUSSION**

In the soybean-maize succession cropping system, larvae were found in the maize crop, with densities of 0.3 to 5.0 larvae/m$^2$, and the highest density was observed in May (Table 1).

The maize plants where the larvae were found had curved stems, as there was morphological alteration resulting from the damages caused by the larvae (Figure 4).

Conventional maize crop has proven to be a good host for this group of pest insects. The data obtained in the present experiment are similar to those observed by Rodrigues et al. (2011), who found that succession cropping systems of conventional maize favored the development of Melolonthidae larvae.

![Figure 4. Morphological alteration in the stems of maize plants due to the action of Melolonthidae](image)

During the fallow period, from July to September, larvae were still found in the field (Table 1). In both soybean and maize samples with Bt protein, larvae were not found in the field (Table 1).

The highest larval densities were observed in the conventional maize cultivar, with significant differences between the larval densities sampled in the fallow period, in the Bt soybean crop and in
Soybean and maize crops have been described as good hosts for some species of Melolonthidae (Garcia et al., 2003; Oliveira et al., 2007; Rodrigues et al., 2011; Rodrigues e Pereira, 2014), however, in the present experiment, when the plants expressed Bt proteins, no larvae were found.

In the soybean-cotton succession cropping system, in the Bt cotton samples, in 2016 and 2017, Melolonthidae larvae were not found (Table 1). Likewise, in the fallow period samples, no larvae were evident (Table 1). Samples collected in the soybean culture without Bt protein, in November and December, were not collected, however, in January and February the larvae were found in this culture (Table 1).

Table 1. Density of Melolonthidae larvae sampled in soybean-maize succession cropping systems on Santa Olinda farm and cotton-soybean on Catéléia farm, Chapadão do Sul, MS, Brazil, from March 2016 to May 2017.

| Location      | Crop                  | Larval density/m² | Location      | Crop                  | Larval density/m² |
|---------------|-----------------------|-------------------|---------------|-----------------------|-------------------|
| Santa Olinda  | Conventional maize NS70 | 2.0               | Catéléia      | Cotton FM 975 WS (Bt cry1Ac + cry1F) | 0.0               |
| Farm          | Fallow Soybean 5G8015IPRO (Bt cry1Ac) | 1.5               | Farm          | Fallow Conventional soybean (NS5909 RR) | 0.0               |
|               |                       | 5.0               |               |                       | 0.0               |
|               |                       | 0.33              |               |                       | 0.0               |
|               |                       | 0.16              |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 2.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |
|               |                       | 0.0               |               |                       | 0.0               |

In the soybean-cotton succession cropping system, in the Bt cotton samples, in 2016 and 2017, Melolonthidae larvae were not found (Table 1). Likewise, in the fallow period samples, no larvae were evident (Table 1). Samples collected in the soybean culture without Bt protein, in November and December, were not collected, however, in January and February the larvae were found in this culture (Table 1).

In this crop, a number of reefs were observed in the field, in which there was a smaller number of plants, smaller size of plants and smaller number of pods per plant (Figures 5 and 6). When digging under the plants, several larvae of Melolonthidae were obtained.
Figure 5. Decrease in the density of soybean plants in the crop rows due to the action of Melolonthidae larvae.

Figure 6. Soybean plants with smaller size and smaller number of pods (left) due to the presence of Melolonthidae larvae in their roots.
The larval densities obtained in Bt cotton crops and in the fallow period were significantly lower than the densities sampled in the non-Bt soybean crop (Table 2).

Studies have shown the effect of cotton crops on populations of Melolonthidae larvae (Rodrigues et al., 2011; Rodrigues e Pereira, 2014), as well as on their non-preference for oviposition by adults (Oliveira et al., 2007b). For such a crop, Macedo et al. (2007) reported that there are numerous aldehydes and terpenes, such as gossypol, heliocides and hemigossipolone, which confer resistance to caterpillars of various lepidopteran species. Although no information is known about the action of these compounds on Melolonthidae larvae, they may have an effect on the larvae.

Table 2. Mean density of Melolonthidae larvae sampled in soybean-maize succession cropping systems on Santa Olinda farm and cotton-soybean on Catléia farm, Chapadão do Sul, MS, Brazil, from March 2016 to May 2017.

| Crop                  | Larval density in soybean-maize succession | Crop                  | Larval density in soybean-cotton succession |
|-----------------------|-------------------------------------------|-----------------------|---------------------------------------------|
| Conventional Maize    | 1,71a¹                                  | Bt Cotton             | 1,0b                                       |
| Fallow                | 1,12b                                   | Fallow                | 1,0b                                       |
| Soybean Bt            | 1,0b                                     | Conventional Soybean  | 1,2a                                       |
| Maize Bt              | 1,0b                                     | Bt Cotton             | 1,0b                                       |

¹Means followed by the same letter with the same column do not differ among each other by the Tukey test at the 0.05 significance level. Data transformed into $\sqrt{x} + 1,0$.

In addition to the presence of secondary components in cotton plants, cry1Ac + cry1F Bt proteins are present in the cultivars used in this study, and it is possible that these proteins are having an effect on the Melolonthidae larvae in the field, preventing them from developing.

In the two succession cropping systems studied, when there was no Bt protein, Melolonthidae larvae were found in the field, and with Bt protein such larvae were not found, indicating insecticidal activity on this group of pest insects.

In Brazil, extensive areas are cultivated with plants genetically modified with Bt proteins, which very likely may be helping to reduce the population density of Melolonthidae in the field. No studies have been conducted in Brazil about the action of Bt plants on the native species of this group of pests. However, studies conducted by researchers in other countries have demonstrated the insecticidal activity of Bt proteins on Melolonthidae larvae (Suzuki et al., 1993; Asano et al., 2003; Shu et al., 2007).

In the fields where the experiments were conducted, the larvae collected and reared in the laboratory generated adults of *Cyclocephalamelanocephala* (Fabricius), *L. suturalis*, *Anomalatestaceipennis* Blanchard and *Leucothyreusalvarengai* Frey. Of these, only the larvae of *C. melanocephala* are not considered crop pests, while the others have already been described as pests in...
several regions, associated with crops of economic importance in Brazil (Rodrigues et al., 2008; Ávila eSantos, 2009; Santos e Ávila, 2009; Pereira et al., 2013).

As observed so far, the succession cropping systems of plants with proteins Bt did not allow the development of Melolonthidae larvae, therefore minimizing potential damages to the crops.

4 CONCLUSIONS

Regarding the soybean-maize and the soybean-cotton succession cropping systems, the maize crop with Bt protein Cry1A.105 + Cry2Ab2, the soybean crop with the Bt protein Cry1Ac, and the cotton crop with Bt protein Cry1ac + Cry1F had an effect on the densities of Melolonthidae larvae in the field.

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