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Resumo

O setor florestal brasileiro tem grande importância na economia e na sustentabilidade do país. Com a grande expansão da monocultura, e muitas vezes a proximidade das plantações agrícolas e florestais favorece a migração ou o estabelecimento de pragas. A *Helicoverpa armigera* é uma praga extremamente polifágica que ocorre principalmente em culturas agrícolas e que foi encontrada casualmente se alimentando de plantações de eucalipto na região de Chapadão do Sul, Mato Grosso do Sul, Brasil. Neste estudo, os parâmetros de consumo, tamanho e longevidade das lagartas foram medidos. O delineamento experimental foi inteiramente casualizado com cinco tratamentos (clones I 144; 08; Urocam VM01; AEC 1528 e dieta artificial, sendo esta uma testemunha) e seis repetições de 10 larvas de lagarta.

Os clones foram cultivados em casa de vegetação para produzir folhas isentas de produtos químicos para proteção de plantas e com bom estado nutricional das quais removeram as folhas e forneceram às lagartas. Não houve diferença estatística entre os clones testados em nenhum dos parâmetros avaliados. Somente a dieta artificial apresentou diferença estatística em relação ao tamanho. As lagartas alimentadas com folhas dos clones não completaram seu desenvolvimento, sugerindo que o efeito da antibiose interfere na biologia de *H. armigera*, não atingindo o status de praga.

Palavras-chave: Antibiose; Polifágico; Lagarta.

Abstract

The Brazilian forestry sector has great importance in the economy and the sustainability of the country. With the great expansion of monoculture, and many times the proximity of agricultural and forestry plantations favors the migration or establishment of pests. The *Helicoverpa armigera* is a pest extremely polyphagous that occurs mainly in agricultural crops, and that casually was found feeding on eucalyptus plantations in the region of Chapadão do Sul, State of Mato Grosso do Sul, Brazil. In this study the parameters of consumption, size, and longevity of caterpillars were measured. The experimental design was completely randomized with five treatments (clones I 144; 08; Urocam VM01; AEC 1528 and artificial diet, this being a witness) and six replicates of 10 caterpillar larvae. The clones were grown in greenhouse conditions to produce leaves free of chemical plant protection products and with good nutritional status from which they have removed the sheets and supplied to the caterpillars. There was no statistical difference between the clones tested in none of the evaluated parameters. Only the artificial diet showed statistical difference concerning the size. The caterpillars fed on leaves of the clones have not completed their development, suggesting that the effect of antibiosis interfering on the biology of *H. armigera* not reaching status of pest.

Keywords: Antibiosis; Polyphagous; Caterpillar.

Resumen

El sector forestal brasileño tiene una gran importancia en la economía y la sostenibilidad del país. Con la gran expansión del monocultivo, y muchas veces la proximidad de las plantaciones agrícolas y forestales favorece la migración o el establecimiento de plagas. La *Helicoverpa armigera* es una plaga extremadamente polífaga que se produce principalmente en cultivos agrícolas, y que se encontró casualmente alimentándose de plantaciones de

eucalipto en la región de Chapadão do Sul, estado de Mato Grosso do Sul, Brasil. En este estudio se midieron los parámetros de consumo, tamaño y longevidad de las orugas. El diseño experimental fue completamente al azar con cinco tratamientos (clones I 144; 08; Urocam VM01; AEC 1528 y dieta artificial, esto es un testigo) y seis réplicas de 10 larvas de oruga. Los clones se cultivaron en condiciones de invernadero para producir hojas libres de productos químicos para la protección de las plantas y con un buen estado nutricional del cual se retiraron las hojas y se suministraron a las orugas. No hubo diferencia estadística entre los clones probados en ninguno de los parámetros evaluados. Solo la dieta artificial mostró diferencia estadística con respecto al tamaño. Las orugas alimentadas con hojas de los clones no han completado su desarrollo, lo que sugiere que el efecto de la antibiosis que interfiere en la biología de *H. armigera* no alcanza el estado de plaga.

Palabras clave: Antibiosis; Polífago; Oruga.

1. Introduction

Planting forests is of great importance not only concerning economy and employment generation but also on sustainability, biodiversity protection, water and soil resources, and CO₂ sequestration. Brazil stood at 9th largest pulp producer in the world with 2.9 million hectares of Eucalyptus destined for this branch and expected to reach 12 million hectares cultivated with this culture until 2030 (Martinez, Kings, Saints & Winter, 2018). With the increase of plantations, monocultures, and the quest for homogeneous forests, often with the genetic material coming within the same planting favor the attack of pest insects, mainly due to the abundance of food (Panizzi & Parra, 2009).

On the other hand, the eucalyptus plants contain a high concentration of secondary compounds as tannin, phenols, and essential oils that can develop the role of defense against the attack of herbivores (Angels, Saints & Zanuncio, 1986; Berry, Picanço & Zanuncio, 1998). When the resistance is chemical nature and adversely affects the insect biology, without which no interference in their behavior of supply or oviposition, it can be said that the plant is resistant by antibiosis to certain insect (Busoli et al., 2015).

The defoliating caterpillars (Lepidoptera) are among the major pests of Eucalyptus in Brazil (Zanuncio et al., 1992). The earworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a pest that attacks various crops, causing a considerable loss throughout the world (Sharma & Kumar, 2005), it is highly polyphagous (ITF et al., 2018). Feed on leaves

and stems of plants, causing direct damage and indirect in more than 100 species of wild and cultivated plants of 45 families (ITF, 1989; There & Choudhury, 2009).

This exotic pest was observed in Brazil in the year of 2013, where he recorded reports of severe economic damage in different crops, mainly agricultural interest crops (Czepak et al., 2013). Successive attacks were reported in several states and crops in the same year (Avila et al., 2013), which reinforced the hypothesis of potential migration of long-range transport by air masses, usually these moths (Tay et al., 2013).

The *H. armigera* monitoring in high production agricultural areas with cotton, soybeans, corn, tomatoes, beans, oranges, coffee, eucalyptus and Pinus crops in the Cerrado region was prioritized in 23 mesoregions of the states of Bahia, Goiás, Mato Grosso do Sul, Minas Gerais, Paraná, Rondônia, São Paulo and Tocantins (Pessoa et al., 2016). In the year of 2014 was found in *H. armigera* caterpillars feeding on leaves of *Eucalyptus grandis* plantations in the city of Chapadão do Sul, MS, Brazil (Muchalak et al., 2017). After this fact and due to the high degree of polyphagia (or "hyperphagia"), it is necessary for the study of this insect on the crop of eucalyptus, noting its potential for development and the possibility of achieving the status of pest.

2. Material and Methods

2.1 Obtaining insects and creation of maintenance

The insects were collected in the field at the stage of caterpillar and taken to the laboratory, being fed with artificial diet modified Greene et al. (1976) and recommended by Parra (2001) until the pupal stage. The pupae were sexed and separated in couples being wrapped in PVC cages with 20 cm and 10 cm in diameter, internally lined with paper that served as a substrate for oviposition. This unit was closed at its upper end with a fabric of the type "VOILE", and on the other end was supported in Styrofoam plate covered by bond paper. For the adults as diet was offered a solution containing sterile distilled water, honey, and yeast in equal proportions. Daily maintenance was performed these cages for removal of eggs, which were placed in Petri dishes (30 cm diameter) and wrapped in BOD at 25 ± 1 °C, $70\pm 10\%$ relative humidity and 12 h photoperiod.

After the outbreak of the caterpillars were individualized in plastic pots with capacity for 100 mL and offered artificial diet modified (*Ibidem*, 1976). The caterpillars became

subsequently pupae and adults. For installation of the experiment were used caterpillars of the second generation of *H. armigera*, from the creation in the laboratory.

2.2 Eucalyptus clones

In 5-L plastic pots, seedlings of *Eucalyptus clones* were cropping (Table 1).

Table 1 - The genetic material of *Eucalyptus* clones used in bioassays.

Clone	Genetic Material
I 144	<i>E. urophylla</i>
08	<i>E. urophylla</i> X <i>E. grandis</i>
Urocam VM01	<i>E. urophylla</i> X <i>E. camaldulensis</i>
AEC 1528	<i>E. grandis</i> x <i>E. urophylla</i>

Source: Authors.

Pots were filled with commercial substrate, composed of 60% of pine bark, 15% of vermiculite, and 25% of humus, more soil collected in regions of soils classified as Latossolo Vermelho in the ratio 1:1. The soil was fertilized with NPK (Nitrogen, Phosphorus and Potassium) fertilizer (10-10-10) being applied in pots after 30 days of planting in quantity proportional to the content of the soil in each pot.

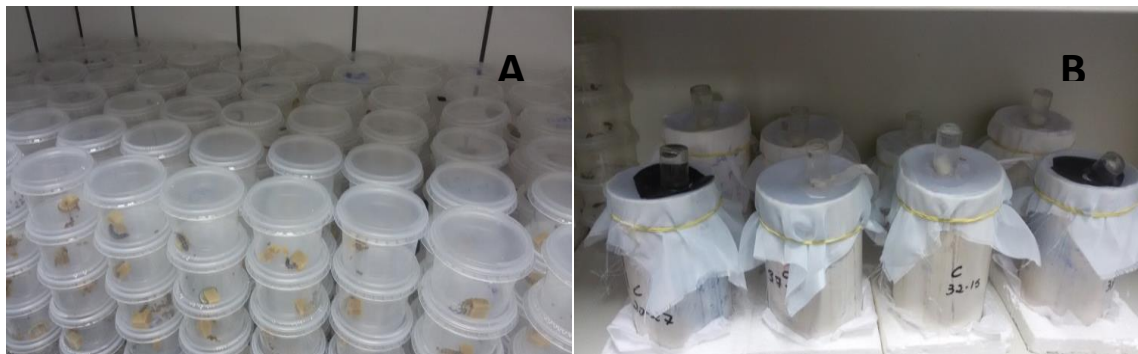
The pots remained in the greenhouse until they reach a height of approximately 2.5 meters after pruning was performed by removing the apical meristem promoting sprouting lateral and reduction in the size of the plant. The plants were grown to produce leaves free of chemical plant protection products and with good nutritional status to be used in bioassays of comparative biology of *H. armigera*.

2.3 Bioassay of comparative biology of *Helicoverpa armigera* in *Eucalyptus*

The study of the biology of *H. armigera* was performed by comparing the development of the insect in four different clones (Table 1) and on an artificial diet. The experimental design was completely randomized with five treatments and six replicates of 10 caterpillar larvae that were kept individualized to prevent cannibalism. As a witness, we used an artificial diet modified Greene et al. (1976).

Neonate larvae were individualized in plastic pots (Figure 1), being fed with leaves from the clones (Table 1) and artificial diet. Before the supply, all the leaves were disinfected in alcohol 70% and double rinse in sterile distilled water. The maintenance was performed every two days where the sheets were changed to ensure that the quality of the food was maintained, being measured leaf area before and after the delivery with the help of equipment Infrared Gas Analyzer (IRGA).

Figure 1. A- individualized caterpillars; B- *helicoverpa armigera* adults in cages.



Source: Authors.

All the bioassay was conducted in a BOD chamber at $25\pm 1^{\circ}\text{C}$, $70\pm 10\%$ relative humidity, and 12 h photoperiod. In addition to the area consumed, it was evaluated the biological parameters duration of larval stage, leaf consumption, and size of caterpillars, which were measured every two days with the aid of a millimeter sheet (Muchalak et al., 2017).

The data were subjected to analysis of variance, and the averages were compared by the Scott-Knott test ($p = 0.05$) and processed in $(x)^{0.5}$.

3. Results and Discussion

Only the caterpillars fed with artificial diet completed the larval stage (Table 2). All clones tested interfered in larval survival, forming a pattern in the results; the lower consumption resulted in lower longevity and body size (Table 2).

Lemos et al. (1999) to evaluate the non-preference of food and the influence of leaf age of *Eucalyptus* spp. for *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae), noted that the leaves of *E. grandis* were more consumed than *E. citriodora* by caterpillars of *T. arnobia*.

Oliveira et al. (1984) studying the behavior of some species of eucalyptus in the laboratory, found that *T. arnobia* showed high preference for *E. saligna* and *E. grandis*, and low preference for *E. camaldulensis*.

Table 2 - Values of total leaf consumption (cm²), the average size of caterpillars (cm) and the duration of the larval stage (days) of *H. armigera* (temperature of 25±1 °C, relative humidity of 70±10% and photoperiod of 12 h). Chapadão do Sul, MS, Brazil. 2018.

Treatment	Consumption (cm²)	Average size of caterpillars (cm)	Duration of the larval stage (days)	Survival (%)
08	3.29 ± 1.29 a	0.47 ± 0.23 b	6.66 ± 3.81 a	0.0
Urocam VM 01	3.96 ± 1.00 a	0.51 ± 0.22 b	7.46 ± 2.88 a	0.0
AEC 1528	3.74 ± 1.52 a	0.51 ± 0.23 b	7.60 ± 2.82 a	0.0
I 144	3.76 ± 2.40 a	0.53 ± 0.27 b	5.86 ± 2.09 a	0.0
Artificial Diet	_____	1.29 ± 0.70 a	10.56 ± 1,781 a	80.0
CV (%)	25.21	19.29	47	_____

Averages followed by the same letter in the column did not differ significantly by the Scott-Knott test (p < 0.05). Source: Authors.

The performance verified for the caterpillars fed with the clones suggests the presence of some factor that confers resistance. When the resistance is chemical nature and adversely affects the biology of the insect, without which no interference in their behavior of supply or oviposition, it can be said that the plant is resistant by antibiosis to the certain insect. The main purpose of this type of plant defense on the biological parameters of insects is the mortality of immature stage, lower growth and weight, deformations, and increase the life cycle of the insect (BUSOLI et al., 2015). These effects observed in the present study; there was a supply for part of the caterpillars; however, these died before completing the cycle, which can then affirm that occurred the effect of antibiosis.

The eucalypt plants contain a high concentration of secondary compounds as tannin, phenols, and essential oils that can develop the role of defense against the attack of herbivores (ANJOS et al., 1986; BERRY et al., 1998). Secondary compounds can affect the nutritional quality and digestibility of plants by herbivores due to reduced availability of proteins (JÁREMO et al., 1999).

In studies conducted by Pereira (2010), who found a high content of 1.8-cineole (Eucalipitol) in species of *E. camaldulensis* and *E. urophylla* being these values of 66.2 and 65.4% of the total composition, respectively. Some studies of characterization of the essential oil from leaves of guava trees demonstrated that this presents in its constitution important potential insecticide compounds, such as the 1.8-cineole, d-limonene, and pinene (Carnation et al., 1981; Cuellar et al., 1984).

Pilon et al. (2006) verified that caterpillars fed with diet containing protease inhibitors had reduced development concerning the caterpillars fed on the same diet without the inhibitor.

4. Final Considerations

There was interference on the biology of *H. armigera* fed with leaves of clones of *Eucalyptus* spp. tested.

No clone of *Eucalyptus* spp. tested has allowed the development of *H. armigera* occurring effect of antibiosis.

Under the conditions and for the clones of *Eucalyptus* spp. tested the *H. armigera* has no potential to achieve the status of pest.

Aknowlegments

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