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FAT BODY MORPHOLOGY OF *THYRINTEINA ARNOBIA ARNOBIA* (LEPIDOPTERA: GEOMETRIDAE) LARVAE IN FUNCTION OF TWO ALIMENTARY SOURCES.

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ABSTRACT

Eucalyptus plantations in Brasil are affected periodically by several native herbivorous orthopterans, coleopteranss and lepidopterans. These insects feed on native Myrtaceae like guava or guayaba, *Psidium guajava*. Among the herbivorous insects which migrated from plantations of eucalyptus, *Psidium guajava*. (Lepidoptera, Geometridae) is the most damaging on guava crops in Brazil. Feeding with guava leaves a generation of caterpillars adapted to a diet of eucalyptus leaves, the larvae had a greater survival rate. The morphology of the fat body of *T. arnobia arnobia* larvae fed with leaves of *Eucalyptus cloesiana* and *Psidium guajava* is described. The fat body of 3rd and 6th larval stadia of *T. arnobia arnobia* fed with eucalyptus and guava, respectively, contained larger and homogeneous trophocytes. The survival rate of caterpillars fed with guava was 100%, and 50% for larvae fed with eucalyptus. Lipids and carbohydrates are present in large amounts in larvae of the 3rd and 6th stadia fed with eucalyptus and guava, respectively, with significant differences in cell size. Results are analyzed with respect the adaptation of these lepidopterans to different diets.
Keywords: Eucalyptus, fat body, herbivory, Lepidoptera, Myrtaceae, *Thyrinteina arnobia*.

RESUMEN

Las plantaciones de eucalipto en Brasil son afectadas periódicamente por varios ortópteros, coleópteros y lepidópteros herbívoros nativos. Estos insectos se alimentan de Myrtaceae nativas como la guava o guayaba, *Psidium guajava*. Entre las especies de insectos herbívoros que emigraron desde plantaciones de eucaliptos, *Thyrinteina arnobia arnobia* (Lepidoptera, Geometridae) es en Brasil el más dañino en cultivos de guava. Al alimentar con hojas de guava una generación de orugas adaptadas a una dieta de hojas de eucaliptos; las larvas tuvieron una mejor tasa de supervivencia. Se describe la morfología del cuerpo graso de larvas de *T. arnobia arnobia* alimentadas con hojas de *Eucalyptus cloesiana* y *Psidium guajava*. En el cuerpo graso del tercer y sexto estadíos larvarios de *T. arnobia arnobia* alimentados con eucalipto y guava, respectivamente, se encontraron trofocitos más grandes y homogéneos. La tasa de supervivencia de las orugas alimentadas con guava fue del 100% y de 50% para larvas alimentadas con eucalipto. Lípidos y carbohidratos están presentes en grandes cantidades en larvas de tercer y sexto estadíos alimentadas con eucalipto y guava, respectivamente, con diferencias significativas en el tamaño de las células. Los resultados se analizan en relación a la adaptación de estos lepidópteros a dietas diferentes.
Palabras clave: Cuerpo graso, *Eucalyptus cloesiana*, *Psidium guajava*, herbivoría, Lepidoptera, Myrtaceae, *Thyrinteina*.

INTRODUCTION

Native Myrtaceae in Brazil, such as guava tree (*Psidium guayava*), *Psidium littorale* and *Myrciaria jaboticaba* have an indigenous entomofauna that survives in these hosts without present population outbreak. But with the increment of eucalyptus plantation has been reported an increasing number of native Lepidoptera, coming of native Myrtaceae, to the eucalyptus, such as *Eupsodosoma aberrans* and *E. involuta* (Arctiidae), *Automeris* spp., *Eacles imperialis* and *Hylesia* spp. (Saturniidae), *Sabulodes caberata*, *Thyrinteina arnobia* and *Oxydia vesulia* (Geometridae) (Otero 1974 & Anjos et al. 1987). Zanuncio et al. (1990 a,

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b) stated that these insects live in host phylogenetically closed to the *Eucalyptus* and are able to cause considerable damages in reforestation areas, because they are in process of adaptation to the *Eucalyptus* spp. Ohmart (1990) have pointed out that more than 177 species of indigenous insects have been attacking eucalyptus plantations in Brazil.

Anjos et al. (1987) verified that when the population of *T. arnobia* in eucalyptus plants is high, they can feed on guava (*Psidium guajava)* and *Myrciaria jaboticaba* trees, which are native hosts of this species (Zanuncio & Lima 1975).

Holometabolous insects have more intense feeding period in the immature stage (Snodgrass 1935). Then, as the insects in the immature phase feed intensely, nutrients are partly consumed to supply the insect necessities and the remaining ones are stored in the fat body. The fat body is considered the main organ of the intermediary metabolism and of nutrient storage with its cells in intense biosynthetic activity producing proteins that can be stored or released for the haemolymph, as well as storing lipids and carbohydrate (Chapman 1998, Cruz-Landim 1983, Snodgrass 1935, Zhixiang & Haunerland 1991).

Holometabolus insect's larvae present fat body cells with great lipid droplets, glycogen accumulations and protein granules that are constantly stored and mobilized during the insect lifespan. However, at the end of the larval phase, the reserves are intensely metabolized and, in the early pupal phase these become available in order to sustain the individual and to supply energy for that transformation phase (Cruz-Landim 1983, Marx 1987). Structural analyses of trophocytes shown that they play a role in synthesis and storage of proteins, glycogen and lipids even so, it seems that most of these are absorbed directly from haemolymph and just stored (Locke & Collins 1966, Price 1973, Thomsen & Thomsen 1978, Wyatt 1980).

The purpose of this study was to verify the influence of diets constituted by leaves of *Eucalyptus cloesiana* or *Psidium guaj*ava (guava) in the morphology of the fat body cells of *Thyrinteina arnobia* larvae in order to test the hypothesis that this species is well adapted to the consumption of Eucalyptus.

MATERIAL AND METHODS

Adults of *T. arnobia* were obtained from eucalyptus plantations and maintained in the Department of Animal Biology of the Federal University of Viçosa, at 25 ± 2 °C and relative humidity 60 ± 10%. Couples were placed in plastic cups (500 ml) covered with nylon mesh. The offspring obtained of these couples were separated in two groups and, according to the treatment, daily fed with fresh leaves of eucalyptus (*Eucalyptus cloesiana*) or guava (*Psidium guajava*).

Caterpillar at 3rd and 6th instars was dissected in NaCl 125 mM and pieces of fat body transferred to 4% paraformaldehyde at phosphate buffer 0.1 M, pH 7.2. The samples were dehydrated in ethanol series, embedded in historesin (Leica), cut at 6-10μm thick and submitted to the following histochemical tests: PAS for carbohydrate, Nile Blue for neutral and acid lipids, Mercury bromophenol blue for total proteins and tuluidine blue. Measurements of trophocytes areas were obtained using the software Image-Pro Plus, version 4.0 (Medical Cybernetics, L.D., 1998).

RESULTS AND DISCUSSION

Fat body of *T. arnobia arnobia* in 3rd instar fed with eucalyptus presented cellular masses composed by compact, homogeneous and larger trophocytes, with many vacuoles and polymorph nuclei placed centrally (Fig. 1b). In the 3rd instar caterpillars fed with guava leaves, the fat body is poor developed with small trophocytes (Fig. 1a). On the other hard, 6th instar caterpillars fed with guava presented larger trophocytes (Table 1), with many small vacuoles when compared to those of caterpillars fed with eucalyptus, where these seem to be less numerous and arrayed in disorganized cellular masses, that is, trophocytes agglomerates (Figs. 1c, d).

Caterpillar survival, from the first instar to the adult phase, reared in guava leaves was about 100% while these fed on eucalyptus showed survival inferior to 50%. These results may be related with the absence of adaptation of *T. arnobia arnobia* to the new host, because eggs were collected in eucalyptus plantations and were not conditioned by several generations in this new host (guava). According to Berenbaum & Zangerl

(1996) when the adaptation of herbivores to a new host does not occur, they can supplant the existent toxicant chemical compositions in this new host in a first generation, even so, starting from the second generation and subsequent generations the insect can be affected by the chemical compositions. This hypothesis was corroborated by the higher survival rate of this generation of *T. arnobia arnobia* when fed with guava leaves in relation to the individuals fed with eucalyptus leaves. However, should be considered that guava is the natural host of *T. arnobia arnobia* and this species may be adapted to this diet.

Trophocytes shape and size vary proportionally with the morphologic stage of the insect, but mainly with the nutritional state of them (Chapman, 1998). In addition, trophocytes play important role in the vitellogenin synthesis, excretion and detoxification processes (Cruz-Landim 1983).

Mercury Bromophenol blue tests showed positive reaction for both studied instars and alimentary sources (Figs. 1d, 2a). The proteins granules are more evident in the trophocytes of 3rd instar *T. Arnobia arnobia* larvae fed with eucalyptus (Fig. 2b) and in the 6th instar larvae fed with guava leaves (Table 1; Fig. 1d). In Lepidoptera, different granules of proteins are described; some are specific containing several types of proteins (Jones et al. 1988).

Nile blue tests for neutral and acids lipids showed that caterpillars of 3rd instar feed with eucalyptus presented stronger positive reaction than those found for 6th instar caterpillars fed with this same alimentary source, while 6th instar caterpillars fed with guava showed stronger positive reaction (Table 1). Similar results were found with PAS tests (Table 1).

Amount of stored lipids varies depending on the developmental and nutritional states of the insect. In the fat body of 6th instar caterpillars fed with guava was found higher rate of neutral than acid lipids. Neutral lipids were placed in vacuoles scattered by the whole cytoplasm, while acid lipids are peripherally. The lipids storage increases during the active feeding period (larval stage) and decline when the insect stop feeding (pupal stage) (Downer, 1985). The amount of lipids accumulated in the fat body can largely exceed those absorbed from food due to the additional lipids synthesized from carbohydrate, being suggested that trophocytes have similar activity to vertebrates liver (Dower, 1985; Steele, 1985).

Carbohydrate deposits were centrally located in the trophocytes cytoplasm of the caterpillars fed with eucalyptus and in areas outlining the trophocytes plasma membrane for caterpillars fed with guava (Figs. 2c, d).

Results showed that in the early developmental stages, caterpillars fed with eucalyptus leaves presented larger nutrients accumulation in the trophocytes. However, with the advance of larval development there is a decrease in the accumulation of nutrients, while in caterpillars fed with guava leaves occurs the inverse, that is, as advances of insect development there is a gain of nutrients that are stored in the trophocytes. Perhaps this explains the mortality of almost 50% of the caterpillars fed with eucalyptus leaves.

Therefore, can be suggested that in spite of, both

Table 1. Histochemical tests and averages areas of fat body cells of *Thyrinteina arnobia arnobia* (Lepidoptera: Geometridae) larvae (3rd and 6th), fed with different diets at 25°C.

	Eucalyptus		Guava	
Larval instar	3rd	6th	3rd	6th
Area (mm²)	211.2± 47.1	139 ± 22.6	133.2 ± 661	1006.8 ± 370.9
Protein	++	++	++	++
Lipid	+++	+	+	+++
Carbohydrates	+++	+	+	+++

+ Weak positive reaction. ++ Positive reaction. +++ Strong positive reaction.

alimentary sources are phylogenetically close, *T. arnobia arnobia* seems not completely adapted to the eucalyptus use as alimentary source, at least in their first generation.

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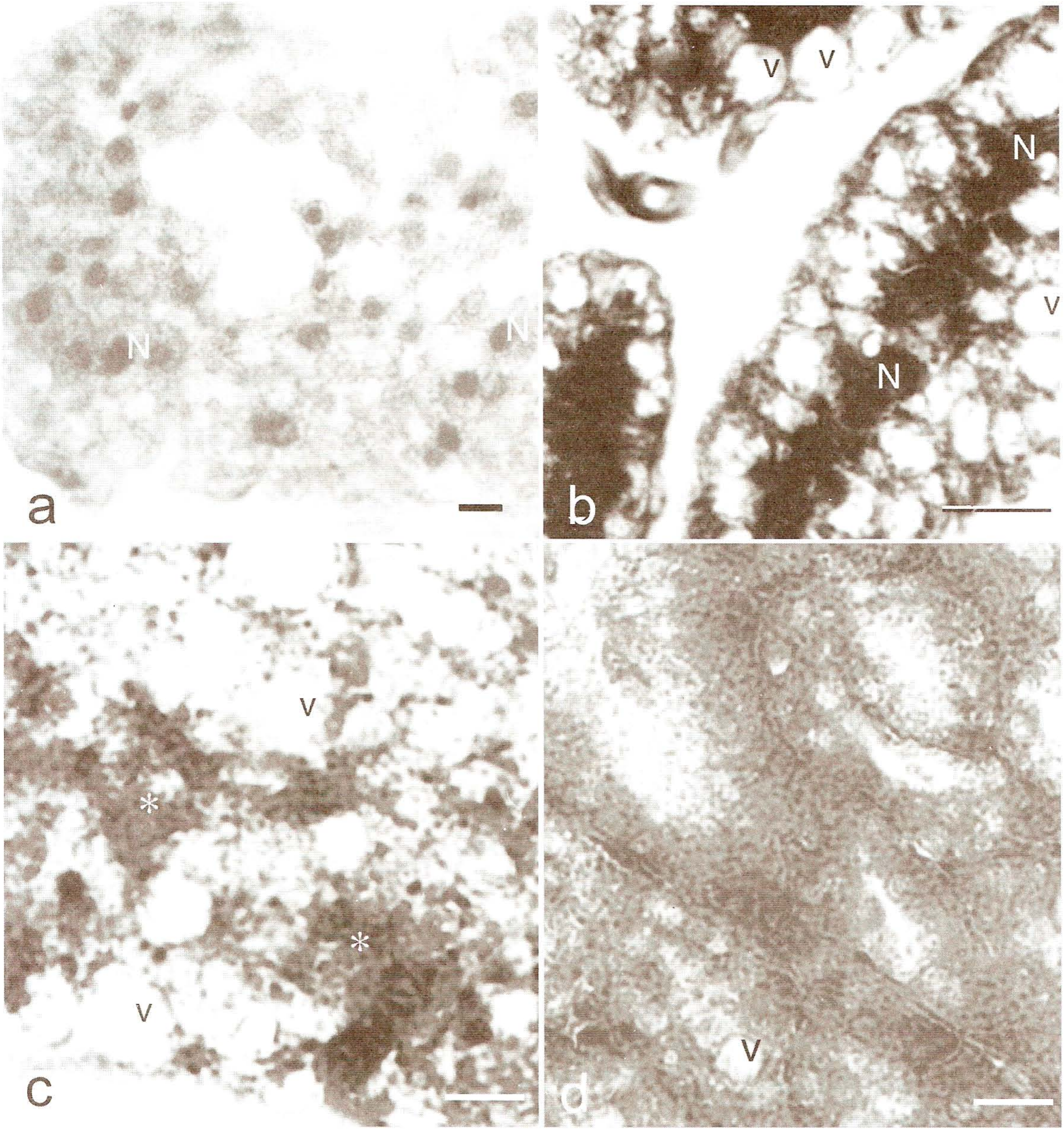

Figure 1. Fat body of *Thyrinteina arnobia arnobia* caterpillar. a) third instar larvae fed with guava leaves showing trophocytes with large vacuoles (v). Toluidine blue stained. Bar = 15 mm. b) Third instar larvae fed with eucalyptus leaves showing small fat body areas (cg). m – muscle. Toluidine blue stained. Bar = 15 mm. c) Sixth instar larvae fed with eucalyptus leaves showing large trophocytes with many vacuoles. N – nucleus. Bromophenol blue stained. Bar = 15 mm. d) Sixth instar larvae fed with guava leaves showing trophocytes in an irregular array. N – nucleus. Bromophenol blue stained. Bar = 15 mm.

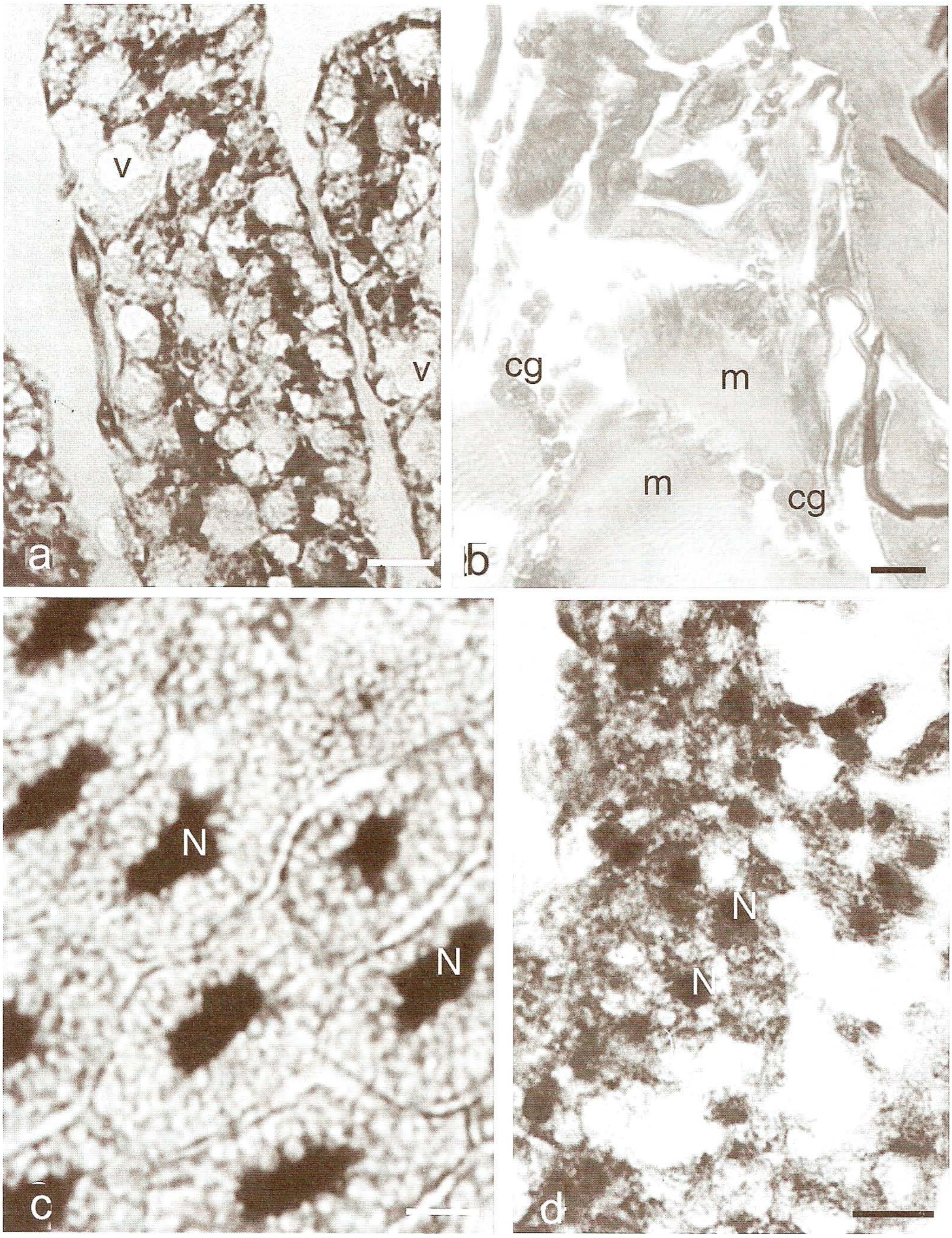

Figure 2. Fat body of *Thyrinteina arnobia arnobia* caterpillar. a) Third instar larvae fed with eucalyptus stained with bromophenol blue showing positive reaction. . N – nucleus. Bar = 15 mm. b) Third instar larvae fed with guava leaves stained with bromophenol blue showing positive reaction. . N – nucleus. Bar = 15 mm. c) Third instar larvae fed with eucalyptus leaves stained with PAS showing positive reaction (*) among negatively stained vacuoles (v). Bar = 15 mm. d) Sixth instar larvae fed with guava leaves stained with PAS showing strong positive reaction outlining the trophocytes plasma membrane. v – vacuole. Bar = 15 mm.