Acta Histochemica 116 (2014) 771–780

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Effects of neem oil (*Azadirachta indica* A. Juss) on the replacement of the midgut epithelium in the lacewing *Ceraeochrysa claveri* during larval-pupal metamorphosis

Elton Luiz Scudeler[a,*], Carlos Roberto Padovani[b], Daniela Carvalho dos Santos[a,c]

[a] *Laboratory of Insects, Department of Morphology, Bioscience Institute, UNESP – São Paulo State University, Botucatu, SP 18618-970, Brazil*
[b] *Department of Biostatistics, Bioscience Institute, UNESP – São Paulo State University, Botucatu, SP 18618-970, Brazil*
[c] *Electron Microscopy Center, Bioscience Institute, UNESP – São Paulo State University, Botucatu, SP 18618-970, Brazil*

ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 8 January 2014
Accepted 9 January 2014

Keywords:
Apoptosis
Autophagy
Cellular injury
Digestive system
Lacewing
Necrosis

ABSTRACT

Larvae of the lacewing *Ceraeochrysa claveri* were fed on eggs of *Diatraeasaccharalis* treated with neem oil at concentrations of 0.5%, 1% and 2% throughout the larval period. Pupae obtained from treated larvae were used in the study at five days after the completion of cocoon spinning to investigate the effects of neem oil on the replacement of the midgut epithelium during the larval-pupal transition. We observed that the old larval epithelium was shed into the midgut lumen and transformed into the yellow body. Old cells from the yellow body were destroyed by apoptosis and autophagy and were not affected by neem oil. However, neem oil did affect the new pupal epithelium. Cells from treated pupae showed cellular injuries such as a loss of microvilli, cytoplasmic vacuolization, an increase of glycogen stores, deformation of the rough endoplasmic reticulum and dilation of the perinuclear space. Additionally, the neem oil treatment resulted in the release of cytoplasmic protrusions, rupture of the plasma membrane and leakage of cellular debris into the midgut lumen, characteristics of cell death by necrosis. The results indicate that neem oil ingestion affects the replacement of midgut epithelium, causing cytotoxic effects that can alter the organism's physiology due to extensive cellular injuries.

© 2014 Elsevier GmbH. All rights reserved.

Introduction

The lacewing *Ceraeochrysa claveri* (Navás, 1911) (Neuroptera: Chrysopidae) is a holometabolous insect and is considered to be an important polyphagous predator in neotropical agroecosystems. It has long been recognized as a biological control agent for a broad range of arthropod pests such as aphids, thrips, mites, whiteflies, eggs and small larvae of lepidopterans (Principi and Canard, 1984; Albuquerque et al., 2001; De Freitas and Penny, 2001; Pappas et al., 2011).

The midgut occupies most of the body cavity in lacewing larva and it is the organ that carries out digestive and absorptive functions. The midgut epithelium of lacewing is composed of: (1) columnar cells involved in the secretion, digestion and absorption processes; (2) endocrine cells, which are responsible for endocrine functions and for coordinating digestive and metabolic processes; (3) regenerative cells, which are responsible for midgut growth and replacement of epithelium (Baldwin and Hakim, 1991; Billingsley and Lehane, 1996; Sehnal and Zitnan, 1996; Marana et al., 1997; Gül et al., 2001; Hakim et al., 2001, 2010; Levy et al., 2004; Corley and Lavine, 2006; Rost-Roszkowska et al., 2008; Fialho et al., 2009; Scudeler and Santos, 2013; Teixeira et al., 2013).

During larval-pupal metamorphosis, the larval midgut epithelium is replaced by a new epithelium. The discharge of the larval epithelium to the midgut lumen forms an amorphous mass named the yellow body. The old larval epithelium is eliminated by cell death, through the combined actions of apoptosis and autophagy. These processes play important roles during renewal of larval organs, homeostatic maintenance of organism development and in recycling molecules from degenerating cells (Komuves et al., 1985; Hakim et al., 2001, 2010; Uwo et al., 2002; Tettamanti et al., 2007a, 2007b, 2011; Goncu and Parlak, 2008; Kourtis and Tavernarakis, 2009; Malagoli et al., 2010; Rost-Roszkowska et al., 2010; Franzetti et al., 2012).

The intake of neem oil and its components have direct actions on the midgut morphology of different insect pest species (Nasiruddin and Mordue (Luntz), 1993; Nogueira et al., 1997; Lucantoni et al., 2006; Ndione et al., 2007; Correia et al., 2009; Roel et al., 2010) and on their natural enemies, such as lacewings (Scudeler and Santos, 2013). Neem oil is a natural insecticide obtained from neem seeds

* Corresponding author at: Laboratório de Insetos, Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, SP, CEP: 18618-970, Brazil.
E-mail addresses: eltonscudeler@hotmail.com (E.L. Scudeler), daniela@ibb.unesp.br (D.C.d. Santos).

0065-1281/$ – see front matter © 2014 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2014.01.008

(*Azadirachta indica*) (Meliaceae) and has long been thought to be a promising insect control agent. The main component of neem oil is azadirachtin. It can act in different ways against insects such as being an ovicide; a repellant; an antifeedant; and by interference in growth, molting, oviposition and mortality (Rembold, 1989; Schmutterer, 1990; Mordue (Luntz) and Blackwell, 1993; Mordue (Luntz) et al., 1998; Mordue (Luntz) and Nisbet, 2000; Morgan, 2009; da Silva et al., 2013).

The non-target and selectivity effects of neem oil and its compounds on natural insect pest enemies have been recently questioned in studies with lacewings (Qi et al., 2001; Ahmad et al., 2003; Medina et al., 2003; Aggarwal and Brar, 2006; Cordeiro et al., 2010; Scudeler and Santos, 2013; Scudeler et al., 2013). Therefore, we conducted a series of assessments using morphological, immunohistochemical and ultrastructural approaches to evaluate the effects of neem oil ingestion on the replacement of the midgut epithelium in *C. claveri* during the larval-pupal transition. The structure and functions of new pupal epithelium were unchanged and are important for maintaining pupal homeostasis during development of the adult phenotype.

Materials and methods

Insect rearing

Eggs of *C. claveri* were obtained from the rearing stock in the Laboratory of Insects in the Department of Morphology at the Bioscience Institute at UNESP, Botucatu, Brazil. Just after egg hatching, larvae were placed individually in polyethylene cups (2 cm height × 6 cm diameter). The insect rearing was maintained in a climate controlled chamber under conditions of 25 ± 1 °C with a relative humidity of 70 ± 10% and a photoperiod of 12:12 (L:D).

Treatments

Emulsifiable Neem oil (commercial formulation Natuneem®) (Natural Rural Ind. e Com. de Produtos Orgânicos e Biológicos Ltda, Araraquara-SP, Brazil) (organic product, certified by BCS ÖKO – Garantie, Doc. Natur – 9009/09.95/7331-BR), a pure cold-pressed neem oil extracted from neem seeds containing 1500 ppm of azadirachtin A as the active ingredient, was diluted in distilled water. Three experimental concentrations of 0.5, 1 and 2% (v/v) were used and prepared fresh daily starting from the stock commercial formulation of Natuneem®.

Fresh egg clusters recently deposited by females of *D.saccharalis* (Lepidoptera: Crambidae) were collected and dipped once in the neem oil solution in a volume of 50 mL for 5 s and air-dried at room temperature for 1 h. For control group, egg clusters were dipped in distilled water (Scudeler and Santos, 2013).

Newly hatched larvae were selected randomly and divided into four experimental groups ($n = 15$ per group). The groups were tested under the same environmental conditions as described for rearing. In the control group, larvae were fed *ad libitum* on *D. saccharalis* eggs treated with water. In the treated groups (0.5, 1 and 2%), larvae were fed *ad libitum* on eggs treated with neem oil throughout the larval period until pupation. After cocoon spinning, specimens remained in the same polyethylene cups under the same controlled conditions for the entire experiment. Each experiment consisted of three replicates per concentration and controls. Pupae were used five days after the completion of cocoon spinning. In this period, the old larval epithelium is shed into the lumen to produce the yellow body, which occurs jointly with the active formation and differentiation of new pupal epithelium. For each morphological study, at least 12 pupae ($n \geq 3$ from each experimental group) were processed and examined.

Light microscopy

Morphological and histochemical analysis

Pupae were quickly cryoanesthetized, and their guts were dissected in insect saline solution (0.1 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4). The insects were cut dorsally, and the midguts were removed and fixed in a 2.5% glutaraldehyde and 4% paraformaldehyde solution in 0.1 M phosphate buffer (pH 7.3) for 24 h.

The specimens were the dehydrated in an ethanol series (50%, 70%, 100%), and embedded in glycol methacrylate (Leica HistoResin Embedding Kit, Leica Biosystems, Wetzlar, Germany) according to the manufacturerís instructions. Sections (3 μm) were cut with a Leica RM 2045 microtome and stained with hematoxylin–eosin (Pearse, 1972) for morphological studies. To establish the histochemical analysis for neutral polysaccharides such as glycogen stores, the sections were stained with Periodic acid-Schiff (PAS) reagent (Pearse, 1972) and counterstained with hematoxylin. To determine the negative control for this staining, identical section was incubated with salivary α-amylase at 37 °C for 4 h and then stained with (PAS). Glycogen deposits are removed as a result of treating the sections with amylase (adapted from Bancroft and Gamble, 2008). The sections were analyzed and photographed using a Zeiss Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany).

TUNEL assay

DNA fragmentation is one of first changes in apoptotic cells, which was detected by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) assay. To compare the incidence of apoptotic cell death in the midgut between different treatments by DNA fragmentation labeling, the midguts were isolated from pupae and fixed in 10% formalin in 0.1 M phosphate buffered saline (pH 7.2) for 24 h. After fixation, the specimens were dehydrated in a series of ethanol, cleared in xylene and embedded in Paraplast® (Leica Biosystems, Richmond, IL, USA). Sections (5 μm) were cut with a Leica RM 2045 microtome and mounted on silanized slides. Briefly, dewaxed and rehydrated tissue sections were rinsed in tris-buffered saline (TBS, 20 mM Tris, pH 7.6, 140 mM NaCl) and permeabilized by incubation in Proteinase K (2 μg/ml in 10 mM Tris pH 8) at room temperature for 30 min. Further procedures were conducted in accordance with the manufacturerís instructions (TdT-FragEL™ DNA Fragmentation Detection Kit, Calbiochem®, Merck KGaA, Darmstadt, Germany). 3,3í-Diaminobenzidine (DAB) substrate was used for chromagen development. Sections were counterstained with methyl green (0.3% aqueous solution) and mounted under glass coverslips. The negative control was prepared according to the Kit protocol (TdT-FragEL™ DNA Fragmentation Detection Kit, Calbiochem®, Merck KGaA, Darmstadt, Germany), where sections were incubated with TdT labeling reaction mix but without TdT enzyme. Three pupae were used in each treatment group, and three sections were randomly selected and imaged at a 200× magnification per insect. The sum of the TUNEL positive signals for each treatment and the sequence percentages were determined. The positivity percentages for each treatment were compared using the Goodman test for contrast between binomial proportions and a significance level of 5% (Goodman, 1964).

Electron microscopy

Scanning electron microscopy (SEM)

Pupae of *C. claveri* were briefly cryoanesthetized, and the midguts were dissected in insect saline solution (0.1 M NaCl, 0.1 M Na_2HPO_4 and 0.1 M KH_2PO_4) and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 48 h at room temperature. Next, the midguts were washed with distilled water and postfixed

Download English Version:

https://daneshyari.com/en/article/1923478

Download Persian Version:

https://daneshyari.com/article/1923478

Daneshyari.com