

## **SUPEROXIDE ANION GENERATION IN LARVAE AND PUPAE OF THE EUROPEAN CORN BORER *OSTRINIA NUBILALIS* HUBN.**

G. GRUBOR-LAJSIĆ, A. JOVANOVIĆ-GALOVIĆ, K. TASKI AND G. VUJOVIĆ

Institute of Biology, Faculty of Sciences, University of Novi Sad,  
Trg D. Obradovića 2, YU-21000 Novi Sad

Superoxide anion production was found in both larvae (diapausing and nondiapausing) and pupae of European corn borer *Ostrinia nubilalis*. It was higher in pupae than in larvae probably due to pronounced processes of histolize and histogeneze in pupae and to free radicals mediating in pupae cuticle formation. The levels of superoxide production were higher in diapausing than in non-diapausing larvae pointing out a distinguished character of diapause metabolism.

KEY WORDS: superoxide, *Ostrinia nubilalis*, larvae and pupae.

Reactive oxygen species (ROS) such as superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $\cdot OH$ ) are generated in all aerobic organisms during metabolic processes such as photosynthesis and respiration or from redox-active small molecules in diets and environmental pollutants. Since the ROS are responsible for oxidative stress including membrane lipid peroxidation and the oxidation of proteins and DNA, a complex antioxidant protection and repair system is essential for surviving (CADENAS, 1995).

Although the antioxidant system of insects is well examined the production and possible role of superoxide anion in insects metabolism have been intensively studied (AHMAD & PARDINI, 1990; NIVSARKAR *et al.*, 1992). After the reports suggesting superoxide production in the homogenate of an insect whole body or a certain tissue, the same system was detected in the haemolymph of few lepidopteran species (ARAKAWA, 1995). In addition, *Pseudaletia* superoxide generative system was further characterized showing presence of at least two components, i.e. low and high

molecular weight factors. It was hypothesized that the low molecular weight factor is a substrate(s) discharging electrons while high molecular weight factor is an enzyme(s) to mediate the electron transfer to  $O_2$  forming  $O_2^{\cdot -}$ .

The present report demonstrates that superoxide anion production occurs in larvae and pupae of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Noctuidae) discussing possible implication on insect metabolism.

Non-diapausing and diapausing 5<sup>th</sup> instar larvae and pupae of *Ostrinia nubilalis* were collected from maize plants in the fields in Vojvodina. Larvae and pupae were homogenized (20% w/v) on ice in 50 mmol/L phosphate buffer with 0.05% phenylthiourea pH 7.0. Homogenates were centrifuged at 15 000 g for 15 min at 4°C and supernatant was used as the source of superoxide anions. The presence of  $O_2^{\cdot -}$  in homogenate of larvae and pupae was measured by the reduction of cytochrome c by the method of NAPPI *et al.* (1995). Protein concentrations were determined by the method of LOWRY *et al.* (1951), using bovine serum as a standard.

The present study focused on the  $O_2^{\cdot -}$  production in different stages of European corn borer *Ostrinia nubilalis*. We found that superoxide anion generation is higher in pupae (from diapausing and non-diapausing larvae) than in larvae (diapausing and non-diapausing) Fig. 1., possibly due to more pronounced processes of histolysis and histogenesis in pupae than in larvae and to free radicals mediating in pupae formation cuticle. The levels of superoxide anion production were surprisingly high in diapausing (non-feeding stages and low metabolic rate) comparing to non-diapausing larvae (feeding stages) showing pointed out independence of this production from diet. In addition, our previous results showing the presence of a more pronounced lipoprotein band in diapausing *O. nubilalis* larvae polyacrylamid gel electrophoresis analysis when compared with non-diapausing sample (GRUBOR-LAJSIĆ, 1992) and the most recent finding on lipophorin as high molecular weight factor of superoxide anion production in *Bombyx mori* haemolymph (ARAKAWA *et al.*, 1996) suggest that, possibly, the same system is operating in *Ostrinia*.

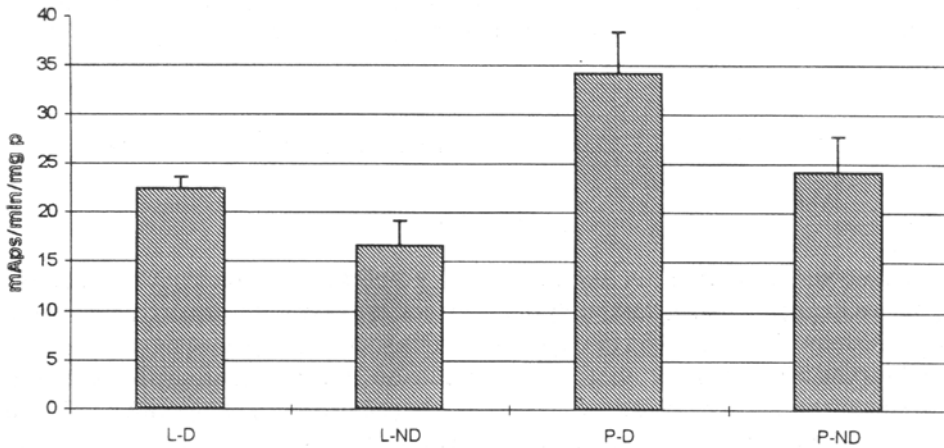


Fig. 1. Superoxide anion production in homogenates of larvae and pupae of the European corn borer, *Ostrinia nubilalis* measured by the cytochrome c reduction at 550 nm. [L - larvae, P - pupae, D - diapausing, ND - non diapausing]

### ACKNOWLEDGMENTS

This study was supported by the Research Science Funds of Serbia, Grant No. 036.

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НАСТАЈАЊЕ СУПЕРОКСИД АНЈОНА У ЛАРВАМА И ЛУТКАМА  
ЕВРОПСКОГ КУКУРУЗНОГ ПЛАМЕНЦА *OSTRINIA NUBILALIS* HUBN.

Г. ГРУБОР-ЛАЈСИЋ, А. ЈОВАНОВИЋ-ГАЛОВИЋ, К. ТАСКИ И Г. ВУЈОВИЋ

## И з в о д

Продукција супероксид анјона ( $O_2^{\cdot -}$ ) је утврђена код гусеница (дијапаузирајућих и недијапаузирајућих) и лутки Европског кукурузног пламенца *Ostrinia nubilalis* Hubn. Значајно већа продукција је добијена код лутки у односу на гусенице. Могуће објашњење је постојање наглашених процеса хистолизе и хистогенезе код лутки као и формирање кутикуле у чију синтезу су укључени слободно радикалски процеси. У односу на дијапаузу већа продукција супероксид анјона је добијена код гусеница у дијапаузи. Овај, обзиром на низак ниво метаболичких процесња у дијапаузи, изненађујући резултат указује на могућу повезаност продукције реактивних кисеоничних врста са специфичним метаболизмом током дијапаузе. Већа продукција супероксид анјона код дијапаузирајућих гусеница и наши претходни резултати о наглашеној липопротеинској фракцији у њиховој хемолимфи указује на могућност да је липофорин укључен у продукцију супероксид анјона.

Received October 17, 1997

Accepted December 8, 1997