

MIDGUT PROTEINASE ACTIVITIES OF *CERAMBYX CERDO* (COLEOPTERA, CERAMBYCIDAE) LARVAE FED ON DIFFERENT DIETS

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Total proteolytic and leucyl-aminopeptidase activities were analyzed in the midgut of *Cerambyx cerdo* larvae fed on three different diets. The highest activities were found in the larvae fed on artificial diet.

KEY WORDS: Cerambycidae, *Cerambyx cerdo*, proteinase activity, leucyl-aminopeptidase activity

One of the important means of using artificial diets in examinations of xylophagous insect, to which cerambycid beetle *Cerambyx cerdo* belongs, is a contribution to knowledge about metabolic changes that then occurs. Metabolic response of some Cerambycidae to different diets (NENADOVIĆ *et al.*, 1999; IVANOVIĆ *et al.*, 2002) and temperatures (NENADOVIĆ *et al.*, 1994) have been studied. Understandings of these metabolic changes, especially on the proteinases level, could lead to some explanations concerning development and survival (NENADOVIĆ *et al.*, 1989), and dispersion, having in mind low abundance of proteins in wood. It is also very important from the ecological and economical point of view because one of the many causes of forest decline is insect attack.

In this work, larvae from the environment collected in December (NC), larvae fed on modified artificial diet (AD) for *Drosophila* (ROBERTS, 1986) and larvae fed on same artificial diet with royal jelly (RJ) were used. Experimental conditions were as described (NENADOVIĆ *et al.*, 1999) with one alteration: RJ group

was reared for a month.

Midguts were dissected out, weighed and homogenized with a pre-chilled mortar and pestle in 4. vol. (g/mL) of ice-cold, 50 mM Tris buffer, pH 7,5 with the addition of quartz sand. After the centrifugation resulting supernatants were treated with an equal volume of carbon tetrachloride for lipid removal, followed by centrifugation (Božić *et al.*, 2003). In each extract protein concentration was determined (BRADFORD, 1976).

Total proteolytic activity was assayed by using 1% casein dissolved in 50 mM carbonate buffer pH 10,5. Reaction mixtures contained 10 μ L of crude midgut extracts and 1 mL casein solution in buffer. The reaction was terminated after 2 h at 37°C by adding 0,5 mL 15% TCA. The content of free amino groups in the supernatants was determined using TNBS method as was described (Božić *et al.*, 2003). Data are the means of triplicate measurement. Standard errors were within 5% of the means.

Leucyl-aminopeptidase activity was determined using specific chromogenic substrate LpNA (LEE & ANSTEE, 1995). Reaction mixtures contained 5 μ L of crude midgut extracts in 0,5 mL of 50 mM Tris pH 8 and 1 mM substrates dissolved in DMF. After 10 min at 30°C enzymatic reactions were terminated by adding 0,1 mL 30% acetic acid. The concentration of the resulting p-nitroaniline was estimated by measuring the absorbance at 410 nm (ERLANGER *et al.*, 1961). Result are shown in Figure 1.

Food quality and quantity, especially protein enrichment are key factors for growth and development of *C.cerdo* larvae, as well as for digestive proteinase ac-

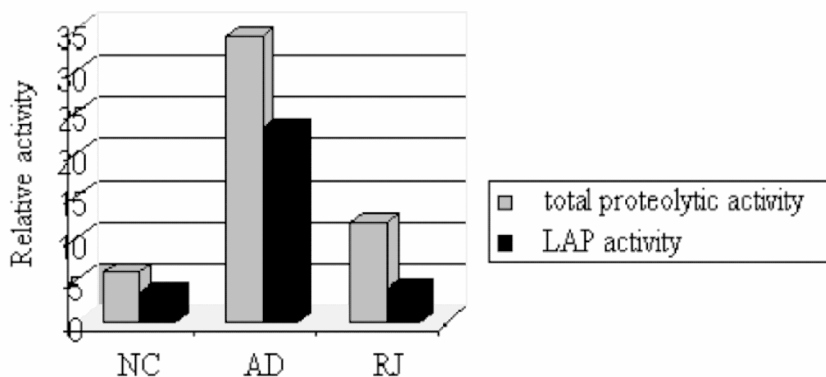


Fig. 1. Midgut proteinase activities of *Cerambyx cerdo* (Coleoptera, Cerambycidae) larvae fed on different diets.

tivities. The higher proteinase activities obtained for AD and RJ can be explained from the point of adding new proteins and amino acids to diet, and the differences in activities between AD and RJ is probably due to differences in amino acids content of artificial diet and royal jelly and content in general (NENADOVIĆ *et al.*, 1999). This is probably the reason for higher LAP activities for AD and RJ, too. Higher LAP activity in AD comparing to NC have the similar manner as for *Mormus funereus*, a member of the same family (VUJČIĆ *et al.*, 1998), which make this enzyme a potential marker for monitoring different metabolic parameters in Cerambycidae.

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ПРОТЕИНАЗНЕ АКТИВНОСТИ ЛАРВИ *CERAMBYX CERDO* (COLEOPTERA, CERAMBYCIDAE) ХРАЊЕНИХ РАЗЛИЧИТИМ СУПСТРАТИМА

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У средњем цреву ларви *Cerambyx cerdo* храњених са три различита супстрата анализирана је укупна протеолитичка и леуцил-аминопептидазна активност. Највећа активност нађена је код ларви храњених вештачким супстратом.

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