Acta Veterinaria (Beograd), Vol. 49. No. 2-3, 105-116, 1999.

UDK 619:612.394

ROYAL JELLY AS A FOOD ADDITIVE AND ITS POSSIBLE BIOSTIMULATING EFFECTS

VERA NENADOVIĆ, Z. PROLIĆ, JELICA LAZAREVIĆ, LARISA AL ARID AND JELISAVETA IVANOVIĆ

Institute for Biological Research "Siniša Stanković", 11000 Belgrade, Yugoslavia

(Received, 8. February 1999)

The experiment represents a contribution to knowledge about metabolic changes in insects reared on an artificial diet containing royal jelly.

The larvae of the cerambycid beetle Cerambyx cerdo, of average mass 0.458 g, were divided into four groups. One (AD) was placed under control conditions on a standard substrate, while two other groups (RJ_1 and RJ_2) (n=4) were given substrates with added royal jelly (RJ). Group RJ_1 was reared on the substrate with RJ till the end of the experiment, while group RJ_2 was returned to control conditions with the standard food substrate after 48 days on the substrate with RJ. Larvae from a natural population collected at the same locality and fed natural wood mass (NC) were also included in the experiment for comparison. The experiment lasted 151 days. The mass gain within each group was measured individually approximately every 7 days, when the food substrate was also changed. The larval mass gains were calculated relative to the initial values of the average weights of individual groups.

The data were analyzed by statistical methods. The results of the analysis showed a high degree of statistical confidence for the differences obtained. They are graphically presented.

After the period of 151 days, all three experimental groups and the group from the natural population were sacrificed.

The midgut protein content, proteolytic activity of midgut enzymes and amylase specific activity were determined biochemically.

It was concluded that the presence of royal jelly in the artificial diet for C. cerdo accelerates the development process, increases the number of moults and decreases the protease activity. The presence of royal jelly in the substrate does not significantly affect the specific activity of amylase.

Key words: Cerambyx cerdo, royal jelly, mass gain, digestive enzymes

INTRODUCTION

Xylophagous insects, to which the cerambycid beetle *Cerambyx cerdo* belongs, develop in the wood mass of different deciduous and coniferous trees. Regarding the trophic value, wood mass is characterized by a high content of carbohydrates (starch, cellulose) and lignin, while the percentage of nitrogen is quite low, in the range 0.001-0.1% of the dry mass (Mattson 1980). Since nitrogen content of an insect body ranges from 7% to 14% of the dry mass, the content of protein in the wood mass can be a limiting factor for the development of xylophagous insects (Scriber and Slansky 1981; Bernays and Chapman 1994). Besides quantity, great significance is attributed to the quality of the proteins, i. e. the profile of amino acids (Broadway and Duffey 1988), as well as the presence of secondary metabolites, phagostimulants and phagodeterrents (Barbosa and Krischik 1987), and the high tanin content in woody plants (Mattson 1980).

The specificity of wood mass is in its anatomical regionality: bark, subcortical region, sapwood, heartwood. These regions differ in hardness, as well as in the distribution of nutritive and non-nutritive components (Nikitin 1962; Mattson 1980). During their long coevolution with woody plants, xylophagous insects have developed a whole array of various strategies to maximize the use of the nutritive components of the wood mass, which is reflected in the increased amount of food consumed, extended periods of feeding, digestion and development, differentiation of the gut through morphological, metabolical and biochemical specialization, cannibalism, symbionates and body mass gain (Ivanović and Marinković 1970; Mattson 1980; Simpson and Simpson 1990; Terra and Ferreira 1994).

According to sparse literature data *C. cerdo* favorizes all species of the *Quercus* genus, but can also be found on beech, ash, and walnut trees in Russian regions(Rudnev 1957). In natural conditions the development lasts 3-4 years, with an unknown number of larval instars. The larvae develop at first in the subcortical region, subsequently progressing into sapwood and heartwood, making horizontal and vertical tunnels. The stem is degraded gradually, which enables a succession of other xylophagous species and fungi. The final outcome is a dried stem, with very little technically usable wood (Rudnev 1957).

In the laboratory, *C. cerdo* was reared from egg to adult on finely ground oak wood, mixed with carrot (1:1), at a temperature of 22.5^{0}C, which produced a shortening of the total development time by around one third compared to the development in natural conditions. Only about 30% of the hatched larvae completed development successfully, giving adults smaller than the ones in nature. The number of instars varied (from 3 to 9), as well as their duration. The development time did not depend on the number of moults (Marović 1973).

The *Morimus funereus* species, a member of the same family, has a much wider host range than *C. cerdo*. It attacks all deciduous and coniferous species. The development takes place in the subcortical region and lasts 3-4 years. The number of larval stages in natural conditions is also not known with this species. When this cerambycid beetle was reared on an artificial diet for *Drosophila* (Roberts 1986) at a constant temperature of 23^{0}C, a significant shorteining of the larval development time occurred. Already 6.5 months after hatching, the larvae reached the critical mass required for pupation. The number of larval instars was not constant here either, and ranged from 8 to 11 (Nenadović et al. 1989; Ivanović et al. 1989).

Investigations on the effects of quality and quantity of the artificial food mentioned on the development processes in *Morimus funereus* showed that significant changes in the general metabolism took place in larvae of this species. A significant increase in the proteolytic activity of the midgut was accompanied by body mass gain and indicated changes in protein metabolism. A significant enelargement of the fat body with a slight change of its glycogen content indicated that lipid metabolism was intensified. A parallel investigation by cytological methods of the state of activity of protocerebral neurosecretory neurons showed that the artificial diet applied to larvae of this species caused a significant increase in the number and activity of neurosecretory neurons, which could indicate possible intensification of the synthesis of metabolic and morphogenic neurohormones (Nenadović et al. 1989; Ivanović et al. 1989; 1991; Ivanović and Janković-Hladni 1991).

Various properties of plants affect the individual performance of a herbivorous insect through the influence on consumption and utilization of food (Slansky 1990). The quality and quantity of the food consumed affects the rate of insect development and the number of larval moults (House 1962; Dadd 1984; Stanić et al. 1989; Nenadović et al. 1989; Ivanović et al. 1989; 1991). Digestive enzymes have an important role in the survival of an insect under nutritive stress. The nutritive stress changes the activities of digestive enzymes, as well as their relative amounts (Applebaum 1985; Terra and Ferreira 1994), which can result in metabolic disbalance and decrease of larval mass (Klocke and Decrease Chan 1982; Broadway and Duffey 1986). Appropriate response of an insect to diet composition at the level of digestive enzymes is a prerequisite for its survival (Broadway 1995).

We are witness to an increasing occurrence of forest decline, especially oak forest. One of the many causes is insect attack, especially with the xylophagous species. With this aspect in mind, and given the number of xylophagous species - their development has been very poorly investigated, especially their digestion physiology.

The objective of the present paper was to investigate the effect of food richer in proteins than wood mass on the growth and development of *C. cerdo*, as well as on the activitty of digestive enzymes (protease and α-amylase). The effect of a standard substrate for *Drosophila* that contains about 10% of the protein zein was investigated, as well as the effect of enriching this substrate by adding lyophilized royal jelly.

MATERIAL AND METHODS

Incests and experimental groups

Cerambyx cerdo larvae were gathered at the beginning of November from oak stumps at the locality Mt. Fruška Gora. Larval mass ranged from 0.368 to 0.519 g (mean=0.458 g).

The larvae were divided into three experimental groups.

1. The control group of larvae (AD) was reared on a modified artificial diet for *Drosophila* (Roberts 1986) at a constant temperature of 23^{0}C, humidity of around 70%, in the absence of light, individually, in plastic boxes (50 cm^3 volume) for 151 days. Every 7 days they were moved to freshly prepared substrates. Each larva was given the same amount of food during the experiment (20 g). The diet composition was the following: corn grits 50 g; sugar 10 g; agar 4 g; nipagine (methyl p-oxybenzoate) 1 g, previously dissolved in 2 ml 96% ethanol; 400 ml water.

2. The second group of larvae (RJ_1) was reared under the same conditions as the control group, with one alteration: every 7 days 0.3 g of lyophilized royal jelly (RJ) per larva was added to the substrate for 151 days.

3. The third group of larvae (RJ_2) was reard under the same conditions as group RJ_1 until day 48, and then moved to the control diet until day 151.

In these three groups larval mass was measured at determined time intervals in order to monitor the mass gain. The number of moults was also recorded for each larva during the whole experiment.

4. The fourth group of larvae (NC) consisted of individuals gathered at the end of March from the same habitat as the experimental larvae.

Biochemical methods

Homogenate preparation

After sacrifice, the larvae were dissected on ice and midguts were removed, weighed and homogenized in an appropriate volume of cold distilled water (10 mg midgut/ml) with a teflon pestle. The homogenates were kept at -20^{0}C until used.

Enzyme assays

The total proteolytic activity was determined according to Kunitz (1947), α-amylase activity according to Bernfeld (1955), and proteins according to Lowry et al. (1951). Protease activity was expressed as OD280/OD750/h, and amylase activity as OD550/OD750/h.

Statistical methods

Mean values and standard errors were determined for all the para- meters investigated, while comparisons between the groups were made by analysis of variance and Scheefe's multiple range test (Sokal and Rohlf, 1981).

RESULTS

Larval growth and development

The data obtained in the present experiment showed that the presence of royal jelly, as a high energy and active substance, allowed a greater mass gain in insects reared on the substrate to which lyophilized royal jelly was added (Fig. 1). In contrast to the control group (AD), that exhibited an average mass gain of 72% on the artificial diet for *Drosophila*, the group that fed on the same substrate with a royal jelly supplement had an average mass gain of 100% in the same period. In larvae of the RJ_2 group that were moved to the *Drosophila* substrate after 48 days on the royal jelly diet, similar dynamics in mass gain were observed as in group RJ_1 up to day 48, after which it was similar to group AD. The comparison carrid out between the groups by analysis of variance and the multiple range test (Scheefe's test) showed the existence of significant differences between all experimental groups (P<0.001).

The number of moults was monitored in parallel with the body mass gain, and the results are shown in Fig. 2. It can be observed that the average number of moults was highest in group RJ_1 (2,00), in group RJ_2 it was 1.50, while in the control groupit was 1.33.

Midgut mass and midgut protein content

Since midgut mass is correlated to larval mass, fresh midgut mass was expressed as a percentage of the larval body mass (Fig. 3). The mass of the midgut with its contents can be taken as a measure of midgut filling. The analysis of variance did not show any significant differences between the group.

Protein content of the midgut (Fig. 4) did not show statistically significant differences between the groups either.

Digestive enzymes

When larvae were moved from natural conditions to the standard substrate an increase in the specific proteolytic activity was observed, while the addition of royal jelly reduced protease activity in such a way that it approached the value of the natural control (Fig. 5). This level of acivity was maintained in the group that was moved onto the standard substrate after 48 days.

Adding royal jelly to the substrate had no significant effect on the specific activity of amylase (Fig. 6). The activity was neither changed in group RJ_1 nor in group RJ_2, with respect to larvae fed the standard *Drosophila* diet. Significant differences could only be observed between the group of larvae from natural conditions and larvae fed artificial diets. Larvae from natural conditions had a significantly higher specific amylase activity.

DISCUSSION

The general nutritional requirements of insects and other organisms have been reviewed in several papers (House 1962; Dadd 1985; Raubenheimer and Simpson 1997).

Little is known about the effects of the quantity and quality of proteins on reproductive parameters and parameters concerning the development of insects that feed on the bark and wood mass of various deciduous and coniferous trees (Mattson 1980). Food quantity, and especially its qualitative composition (protein enrichment), are key factors that affect the development time and the number of *moults* in *M. funereus* larvae (Nenadović et al. 1989; Stanić et al. 1989; Ivanović et al. 1989; 1991). The results of the present experiment on *C. cerdo* larvae also showed a significant effect of food quality on larval growth and development (Fig. 1, Fig. 2). Both protein quality and quantity in the diet affected the development time, adult body mass and egg

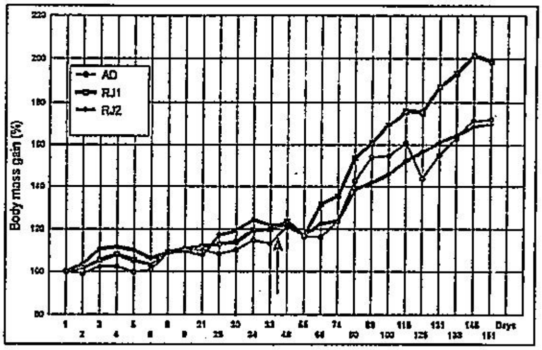

Figure 1. Body mass gain of *Cerambyx cerdo* larvae in three experimental groups expressed as a percentage of the initial body mass. AD - the control group reared on the *Drosophila* food substrate; RJ_1 - the group of larvae from the substrate with royal jelly (RJ) added during the whole experiment; RJ_2 -the group of larvae from the substrate with royal jelly added until day 48, after which they were returned to control feeding conditions.

size in insects (Horie and Watanabe 1983). The nutritional quality of a protein is a function of its amino acid composition. Nutritionally unbalanced protein imposes a metabolic load (Karowe and Martin 1989). In the amino acid composition of zein, a characteristic protein in the artifical diet for *Drosophila,* one of the essential amino acids is missing - Lys. The dominant ones are Leu, Ala, Glu (Broadway and Dyffey 1986). Protease hyperproduction under the conditions of increased protein concentration in food means consumption of amino acids for enzyme synthesis, which reduces the poll of amino acids that can be used for growth and development. It was established in our experiment

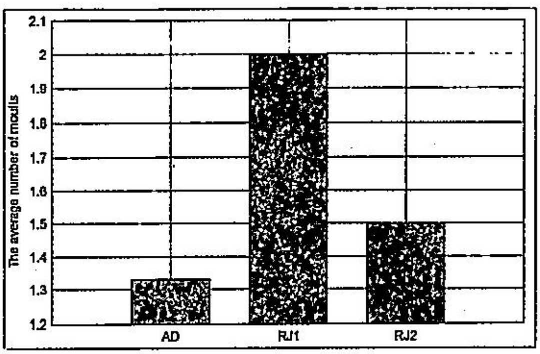

Figure 2. The average number of moults of *Cerambyx cerdo* larvae in three experimental groups during the 151 day observation period. The other labels are as in Figure 1.

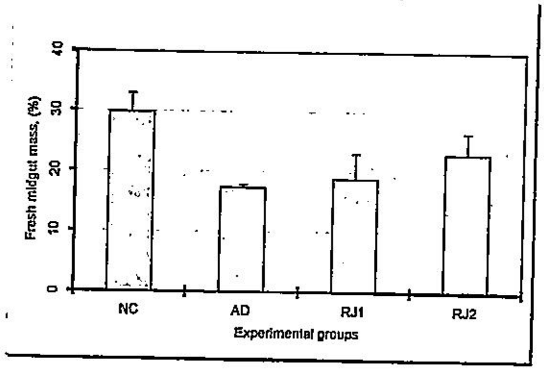

Figure 3. Fresh midgut mass expressed as a percentage of the body mass of *Cerambyx cerdo* larvae reared on various food substrates (AD, RJ_1, and RJ_2 - the labels are as in Figure 1) and of larvae from natural conditions (NC).

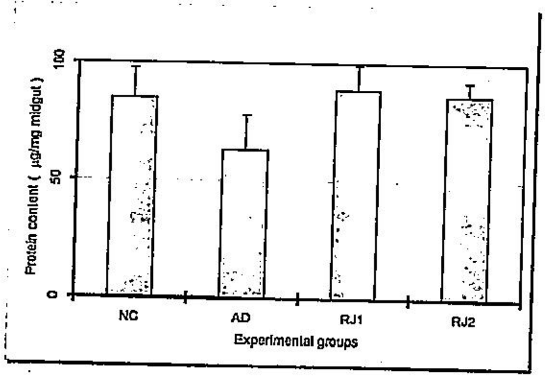

Figure 4. Midgut protein content of *Cerambyx cerdo* larvae in relation to the food substrate (labels as in Figure 3).

that *C. cerdo* larvae reared on the protein rich artificial diet (group AD) had greater protease acivity than the larvae from natural conditions (NC), (Fig. 5). There are no data about the effect of this artificial substrate on the development of *C. cerdo* larvae, although it is known that rearing these larvae in laboratory conditions on a mixture of carrot and oak wood mass yields smaller adults

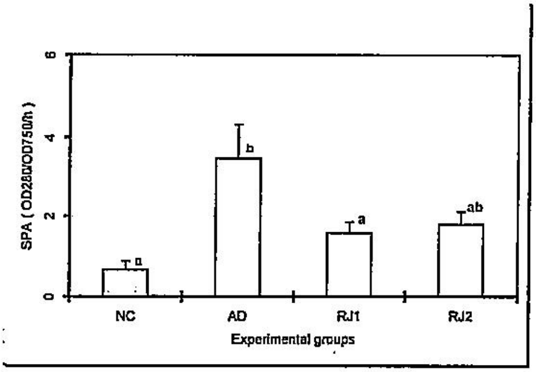

Figure 5. Specific midgut protease activity (SPA) of *Cerambyx cerdo* larvae of the four experimental groups. The bars labelled by different letters (a, b) are significantly different (Scheefe's multiple range test, P<0.05). Other labels are as in Figure 3.

and shortens the preadult development time (Marović 1973). *M. funereus* adults reared on the Drosophila substrate during larval development are also smaller than the adults from natural conditions (unpublished data). One possible explanation could be protease hyperproduction as was also observed in *M. funereus* larvae (Ivanović et al. 1989; 1991). Adding royal jelly of the substrate means adding amino acids Lys, His, Arg, Pro etc. (a total of 18 amino acids, out of which 8 are essential ones), which can also explain the greater mass gain (Fig. 1), together with the increased number of moults, i. e. faster development (Fig. 2) of *C. cerdo* larvae that were fed royal jelly. Protease hyperproduction on an unsuitable diet (cat leaves) is also described in the grasshopper *Melanoplus sanguinipes* (Hinks et al. 1991). Adding amino acids to the diet

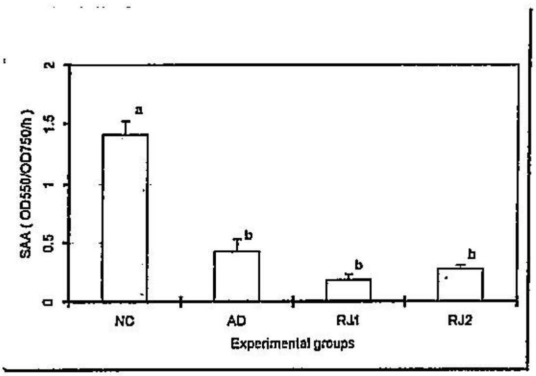

Figure 6. Specific amylolytic activity (SAA) of the midgut of *Cerambyx cerdo* larvae of the four experimental groups. The bars labelled by different letters (a, b) are significantly different (Scheefe's multiple range test, P<0.05). Other labels are as in Figure 3.

through yeast reduces trypsin activity in *Ceratitis capitata,* with no effect on amylase (Lemos et al. 1992), which agrees with our data (Fig. 5, Fig. 6). This is especially interesting, since *C. capitata,* as like, *C. cerdo*, lives on a substrate rich in carbohydrates and poor in proteins (decaying plant material and feces of herbivorae). In contrast to this, adding yeast to corn meal significantly increased the activity of amylase in *Tribolium confusum* (Woll et al. 1986).

It is known that diet composition (Christopher and Mathavan 1985; Baker 1988) and especially sugar content, affect the activity of amylase in insects (Milanović and Anđelković 1993). In the present experiment significant differences were observed between larvae from natural conditions and larvae reared on various artificial substrates (Fig. 6). Thus, the presence of additional amounts of sugar in the royal jelly did not change amylase activity. The different compositions of the natural substrate and the standard diet for *Drosophila* could account for the differences that occurred in amylase activity, given that the high starch content in the artificial diet (more than 6%) caused a drop in amylase activity in the xylophagous cerambycid beetle *M. funereus* (Ivanović 1970). Enzyme synthesis and secretion is controlled by humoral factors (Applebaum 1985). Humoral factors can also be affected by increased food consumption, namely an increase in nutrient inake (Muraleedharan and Prabhu 1981), in such a way that humoral mechanisms and the secretagogue mechanism (stimulative action of food ingredients) are not mutually exclusive in the regulation of synthesis and secretion of digestive enzymes. The data obtained with *C. cerdo* fresh midgut mass (Fig. 3) expressed as a percentage of the body mass, did not show significant differences. This indirectly indicates that the diet did not affect food consumption. The weighed protein contents of the midgut (gut wall proteins and proteins of undigested food in the gut lumen) did not show any statistical differences (Fig. 4).

Besides amino acids, royal jelly contains sugars, fats, and vitamins (Janković 1977). Many insects require the dietary presence of pantothenic acid, vitamin B, ascorbic acid, etc, for growth and development (House 1962). The presence of vitamin B12 is irrelevant for some insects, since symbiotic microorganisms in their gut lumen synthesize this vitamin (Slansky and Rodriguez 1987). Ascorbic acid provides virus protection in some insects (Lindroth et al. 1991). In any case, the aciton of vitamins should not be excluded from the positive effect of royal jelly on the growth and development of *C. cerdo* larvae (Fig. 1, Fig. 2).

Royal jelly, as a direct bee product, finds increasing applications in human and animal diets, and became the subject of very broad interests and numerous research studies (Janković 1977). Research into diet quality is still very motivated by the increasing pollution of the living environment, including food, as well as by the need to elucidate the mechanisms involved in the reversible and irreversible damage to DNA from contamination or food of suboptimal nutritive value. Since the metabolic activity of an organism depends on its physiological state and factors of the external environment, food quality can be of crucial significance for the maintenance of metabolic homeostasis under stress conditions.

Our results indicate a positive effect of royal jelly on the mass gain, development time and the number of moults. To interpret these effects additional investigations are necessary on a larger number of metabolic parameters, with a larger number of individuals, and the complete development cycle of *C.cerdo.*

Acknowledgments

This work was supported by the Ministry of Science and Technology of the Republic of Serbia under Project no. 03E13. We extend our thanks to ALTAMED company from Belgrade who provided lyophilized royal jelly for the purposes of this work.

REFERENCES

1. *Applebaum, S.W.* 1985. Biochemistry of digestion. In Comprehensive Insect Physiology, Biochemistry and Pharmacology. Vol. 7, pp. 219-311. Ed. by G. A. Kerkut and L.I. Gilbert. Pergamon, Oxford.

2. *Barbosa, P. and Krischik, V. A.* 1987. Influence of alkaloids on feeding preference of eastern deciduous forest trees by the gypsy moth Lymantria dispar L. Am. Nat. 130, 53-69.

3. *Bernaysm E.A. and Chapman, R. F.* Host-Plant Selection by Phytophagous Insects, pp. 18-25. Chapman and Hall.

4. *Bernfeld, P.* 1956. Amylase, alpha and beta. In Methods in Enzymology. Vol. 1,pp. 1949-1958. Ed. by P. S. Colowick and O. N. Kaplan. Academic Press, New York.

5. *Broadway, R. M.* 1995. Are insects resistant to plant proteinase inhibitors? J. Insect Physiol. 41, 107-116.

6. *Broadway, R. M. and Duffey, S.S.* 1986. Plant proteinase inhibitors: mmechanism of action and effect on the growth and digestive physiology of larval Heliothis zea and Spodoptera exigua. J. Insect Physiol. 32, 827-833.

7. *Broadway, R. M. and Duffey, S.S.* 1988. The effects of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors. J. Insect Physiol. 34, 1111-1117.

8. *Christopher, M.S.M. and Mathavan, S.* 1985. Regulation of digestive enzyme activity in the larvae of Catopsilia crocale (Lepidoptera). J. Insect Physiol. 31, 217-221.

9. *Dadd, R. H.* 1985. Nutrition: Organisms. In Coprehensive Insect Pharmacology. Vol. 4, pp. 313-390. Ed. by G.A. Kerkut and L.I. Gilbert. Pergamon, Oxford.

10. *Hinks, C. F., Cheeseman, M.T.,Erlandson, M. A., Olfert, O. and Westcott, N. D.* 1991. The effects of kochia, wheat and oats on digestive proteinases and the protein economy of adult grasshoppers, Melanoplus sanguinipes, J.Insect Physiol. 37, 417-430.

11. *Horie, Y. and Watanabe, K.* 1983. Effect of variouskinds of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in the silk worm Bombyx mori. J. Insect Physiol. 29, 187-199.

12. *House, H. L.* 1962. Insect nutrition. Ann.Rev. Biochem. 31, 653-672.

13. *Ivanović, J.* 1970. Basis of the oligophagy of the species Morimus funereus. Arch. Biol. Sci. 20, 65-69.

14. *Ivanović, J. and Janković-Hladni, M.* 1991. Horones and Metabolism in Insect Stress, pp. 1-178. CRC Press, Boca Raton, FL.

15. *Ivanović, J., Janković-Hladni, M., Stanić, V., Nenadović, V. and Milanović, M.* 1978. Midgut of coleopteran larvae, the possible target organ for the action of neurohormones. In Neurosecretion and Neuroendocrine Activity; Evolution, Structure and Function pp. 373-376. Ed. by W. Bargmann, A. Oksche, A. Polenov and B. Scharrer. Springer-Verlag, Berlin.

16. *Ivanović, J. and Marinković, D.* 1970. Individual variability of the pH optimum of amylase in larval populations of the insec species Morimus funereus L. Genetika 2, 105-111.

17. *Ivanović, J., Janković-Hladni, M., Stanić, V., Nenadović, V. and Frušić, M. 1989. The role of neurosecretion and metabolism in development of an oligophagous feeding habitin Morimus funereus larvae (Col., Cerambycidae). Comp. Biochem. Physiol.94A, 167-171.*

18. *Ivanović, J., Stanić, V., Rauschenbach, M., Janković-Hladni, M., Nenadović, V. and Budker, V.* 1991. Influence of nugrition substrate on neuro-endocrine system in the ontogenesis of cerambycid, Morimus funereus(Insecta). Zh. Obc. Biol. 52, 205-213. (in Russian)

19. *Janković, A.* 1977. The Bee's Products: Food and Medicine. Kosmos, Beograd (in Serbian).

20. *Karrowe, D. N. and Martin,M. M.* 1989. The effects of quantity and quality of diet nitrogen on the growth,efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar Spodoptera eridania larvae (Lepidoptera: Noctuidae).J. InsectPhysiol. 35, 699-708.

21. *Klocke, J. A. and Chan, B. G.* 1982. Effects of cotton condensed tannin on feeding and digestion in the cotton pest, Heliothis zea. J. Insect Physiol .28, 911-915.

22. *Kunitz, M. 1947. Crystalline soybean trypsin inhibitor - II. General properties. J. Gen.Physiol. 30,* 291-310.

23. *Lemos, F. J., Zucoloto, F. S. and Terra, W. R.* 1992. Enzymologicaland excretory adaptations ofCeratitiscapitata(Diptera: Tephritidae) larvae to high protein and high salt diets. Comp. Biochem. Physiol. 102A, 776-779.

24. *Lindroth, R. L., Barman, M. A. and Weisbrod., A. V.* 1991. Nutrient deficiencies and the gypsy moth, Lymantria dispar. Effect on larval performance and detoxication enzyme activities.J. Insect Physiol.37, 45-52.

25. *Lowry, O. H., Rosebrough, N. J., Farr, A. L. andRandall, R. J.* 1951. Protein measurement with the Folin phenolreagent. J. Biol.Chem. 193, 265-275.

26. *Marović, R. 1973. The development of Cerambyx cerdo L. (Col., Cerambycidae)* under lagoratory conditions. Acta Entomol. Jugoslav , 9, 1-2.(in Serbian)

27. *Mattson, Jr. W. J.* 1980. Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 11, 119-161.

28. *Milanović, M. and Anđelković, M.* 1993. Biochemical and genetic diversity of alpha-amylase in Drosophila, Arch. Biol. Sci. 45, 63-82.

29. *Murelledharan, D. andPrabhu, V. K. K.* 1981. Hormonal influence of feeding and digestion in plant bug Dysdercus cingulatus and caterpillar Hyblaeapuera. Physiol. Entomol. 6, 183-189.

30. *Nenadović,V., Janković-Hladni, M. and Ivanović, J.* 1989. The effect of food substrate on the neuroendocryne system and metabolism of Morimus funereus (Insecta, Coleoptera).Gen. Comp. Endocrinol.74, 211-212.

31. *Nikitin, N. I.* 1962. Khimia Drevesiny i Tsellulozy, pp.376-391. Izd. Akad. Nauk SSSR, Moskva.

32. *Raubenheimer, D. and Simpson, S. J.* 1997. Integrative models of nutrient balancing: Application to insects and vertebrates. Nutr. Res.Rev.10, 151-179.

33. *Roberts, D. B.* 1986.Drosophila: A Practical Approach, pp. 15-19. IRL Press, Oxford.

34. *Rudnev, D. F.* 1957. Bolshoi Dubovi Usach v Lesah Sovetskova Sousa. Izd. Akad.Nauk Ukraine, Kiev.

35. *Scriber, J. M. and Slansky, Jr. F.* 1981. The nutritional ecology of immature insects. Ann. Rev. Entomol. 26, 183-211.

36. *Simpson, S. J. and Simpson, C. L.* 1990. The mechanismms of nutritional compensation by phytophagousinsects. In Insect-Plant Interactions. Vol.2, pp. 111-160. Ed.by E. A. Bernays, CRC Press, Boca Raton, Fl.,

37. *Slansky, F. Jr.* 1990. Insect nutritional ecology as a basis for studying host plant resistance. Florida Entomol. 73, 359-378.

38. *Slansky, Jr. F. and Rodriguez, J. G.* 1987. Nutritional ecology of insects, mites, spiders and related invertebrates: An overview. In Nutritional Ecology of Insects, Mites and Spiders. pp. 1-69. Ed.by F. Jr Slansky and J. G. Rodriguez. John Wiley, New York.

39. *Sokal, R. R. and Rohlf, F. J.* 1981. Biometry.Freeman, San Francisco.

40. *Stanić, V., Janković-Hladni, M., Ivanović, J. and Nenadović, V.* 1989. Joint effects of temperature, food quality and season on the development of cerambycid Morimus funereus under laboratory conditions. Entomol. Exp. Appl. 51, 261-267.

41. *Terra, W. R.and Ferreira, C.* 1994. Comparative total activities of digestive enzymes in different gut regions of the house cricket Acheta domesticus L. (Orthoptera: Gryllidae). Ann Entomol. Soc. Am. 87, 886-890.

42. *Woll, D., Namir, Z. andBergersom, O.* 1986. Dietary regulation of amylase acivity levels in flour beetles (Coleoptera: Tenebrionidae): (Tribolium). Ann. Entomol. Soc. Am. 79, 407-413.

KORIŠĆENJE MLEČA KAO DODATAK HRANI I NJEGOV MOGUĆI BIOSTIMULATIVNI EFEKAT

VERA NENADOVIĆ, Z. PROLIĆ, JELICA LAZAREVIĆ, LARISA AL ARID, JELISAVETA IVANOVIĆ

SADRŽAJ

Ispitivan je uticaj veštačke hrane za Drosophila i istog supstrata kome je dodavan matičin mleč na razviće velike hrastove strižibube Cerambyx cerdo. Korišćeni supstrati se znatno razlikuju od drvne mase kao prirodnog supstrata, naročito u pogledu kvaliteta i kvantiteta proteina i amino kiselina.

Larve strižibube *C. cerdo* prosečne težine 0.458g podeljene su u četiri grupe od kojih je jedna AD postavljena u kontrolne uslove na standardnu podlogu, dve druge grupe RJ_1 i RJ_2 na podlogu kojoj je dodat matični mleč (MM). Grupa RJ_1 je do kraja ogleda odgajana na podlozi sa MM, a grupa RJ_2 je posle 48 dana ishrane obogaćene MM vraćena na kontrolne uslove i do kraja ogleda imala standardnu ishranu. Za poređenje sa laboratorijski gajenim larvama u ogled su uključene i larve iz prirodne populacije sa istog lokaliteta NC. Ogled je trajao 151 dan. Priraštaj biomase u okviru svake grupe je meren individualno u određenim vremenskim intervalima kada je menjana i podloga-hrana. Procenat porasta mase tela larvi obračunat je u odnosu na početnu vrednost prosečne težine eksperimentalnih grupa.

Posle perioda od 151 dana sve tri ogledne grupe su žrtvovane zajedno sa grupama larvi iz prirodne populacije koja se hranila drvnom masom. Biohemijska analiza se odnosila na sadržaj proteina u srednjem crevu, proteiolitičku aktivnost enzima srednjeg creva i specifičnu aktivnost amilaze.

Podaci su prikazani grafički i obrađeni statističkim metodama, pri čemu rezultati analize pokazuju visok stepen statističke pouzdanosti za dobijene razlike.

Iz podataka se zaključuje da prisustvo mleča u veštačkoj ishrani larvi *C. cerdo* utiče na ubrzavanje procesa razvića, povećanja broja presvlačenja i smanjenja aktivnosti proteze u odnosu na kontrolnu grupu. Prisustvo mleča u podlozi ne utiče značajno na specifičnu aktivnost amilaze.