

BIOGENIC AMINES IN PROTOCEREBRAL A2 NEUROSECRETORY NEURONS OF *LYMANTRIA DISPAR* L. (LEPIDOPTERA: LYMANTRIIDAE) – RESPONSE TO TROPHIC STRESS

VESNA PERIĆ - MATARUGA^{1*}, MARIJA MRDAKOVIĆ¹, MILENA VLAHOVIĆ¹, LARISA ILIJIN¹,
MILENA JANKOVIĆ TOMANIĆ¹, D. MIRČIĆ² and VERA NENADOVIĆ¹

¹ Department of Insect Physiology and Biochemistry, Institute for Biological Research “Siniša Stanković”,
University of Belgrade, 11060 Belgrade, Serbia

² State University of Novi Pazar, Department of Biochemical and Medical Sciences, 36300 Novi Pazar, Serbia

Abstract - The number, morphometric parameters and amount of aminergic neurosecretory product of protocerebral A2 neurosecretory neurons were investigated in the fifth instar of *Lymantria dispar* caterpillars, following a suitable or unsuitable trophic regime. Caterpillars originated from two populations (*Quercus rubra* or *Robinia pseudoacacia* forest) and were differently adapted to trophic stress, i.e. feeding on locust tree leaves – unsuitable host plant. The number of neurosecretory neurons was higher in the caterpillars originated from *Robinia* population than in *Quercus* population, regardless of feeding. A2 neurosecretory neurons, nuclei and their nucleoli were larger in caterpillars fed with unsuitable leaves in both populations. There was more aminergic product in the A2 neurosecretory neurons of the caterpillars fed with unsuitable leaves independently of population origin.

Key words: Neurosecretory neurons, biogenic amines, physiological adaptations; trophic stress.

UDC 595.786:577.1

INTRODUCTION

The gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) is a gradogenic pest species which defoliates forests and fruit trees in temperate areas and is a serious pest throughout Eurasia (Elkinton and Liebhold, 1990). It is well-studied ecologically (Rossiter 1991; Stockoff 1991) but its neuroendocrinology is poorly understood, especially the role of neurosecretory neuron plasticity in the adaptation of *L. dispar* to novel host-plants (Perić Mataruga et al., 2001; 2004).

The neuroendocrine system quickly reacts to environmental changes, including trophic stress (Ivanović and Janković-Hladni 1991; Perić Mataruga

2001, 2004). Its activity determines the content and interrelation of hormones and the metabolic activity of insect which affects metamorphosis, fitness components, reproduction and consequently population density (Nijhout 1994, Hirashima 2000; Perić Mataruga 2007). In order to understand how polyphagous phytophagous species change their host range, it is necessary to study adaptations at the level of the neuroendocrine system.

Biogenic amines control the energy metabolism in insects and regulate the secretion of the main morphometric and metabolic hormones – juvenile hormones and ecdysteroids (Evans, 1985). Such hormones are also part of the mechanisms of endocrine stress reactions (Rauchenbach et al., 1993; Hirashima et al., 2000). Besides the specific neuropeptides that

have prothoracicotropic, allatotropic and allatostatic effects (Simonet et al., 2000), the metabolism of juvenile hormone and ecdysteroids may be regulated by biogenic amines (octopamine and dopamine) whose effect on metamorphosis and reproduction have been established in various insect species (Hirashima et al., 1999; Granger et al., 1996; Gruntenko et al., 2004).

Stress increases energy demands and allocates resources to energy metabolism. Biogenic amines are assumed to be responsible for the stressogenic changes in the energy metabolism of insects (Wilson and Rounds, 1992). It has been shown that stressful conditions (physical/chemical stressors, injury etc.) induce an increase in the content of the biogenic amines (octopamine and dopamine) in insects. Scientists have confirmed that octopamine in insects can function as a neurohormone controlling carbohydrate and lipid metabolism as the primary response to the effect of stressors (Davenport and Evans 1994).

The response of biogenic amines to unfavorable conditions was shown to be non-specific and it arose under the action of stressors of different origin: high population density, stressful temperatures (high/low), mechanical or chemical stimuli, immobilization conditions (Raushenbach et al., 1993; Hirashima et al., 1999). The system for biogenic amine metabolism responded to stressors by an increase in the content of the amine (Raushenbach et al., 1993), and by a decrease in the activity of the enzymes of amine synthesis – Tyr-hydroxylase and Tyr-decarboxylase, (Raushenbach et al., 1995)

The aim of our study was to determine the population differences in the response to an unsuitable host plant (locust tree) *Robinia pseudoacacia* on the level of number and morphometric parameters of the protocerebral A2 neurosecretory neurons in the 5th instar caterpillars of the gypsy moth, *Lymantria dispar* (Linnaeus 1758). The caterpillars originated from populations differently adapted to trophic stress (locust tree or oak forest). Optimal gypsy moth habitats are oak forests, while gypsy moth populations are only rarely found in locust tree forests.

MATERIALS AND METHODS

Experimental animals

The egg masses of *Lymantria dispar* originated from an oak forest – *Quercus rubra* (Sremački Rit) and locust tree forest *Robinia pseudoacacia* (locality Bagremara – Bačka Palanka). The caterpillars were reared in 200 cm³ plastic containers at a temperature of 23°C and a 16 h light : 8 h day regimen; they were fed fresh food daily (oak or locust tree leaves). After hatching, the caterpillars were distributed into the following experimental groups (n=15). OO-oak forest origin, fed on oak leaves from hatching; OL- oak forest origin, fed on locust tree leaves from hatching; LO-locust tree forest origin, fed on oak tree leaves from hatching; LL- locust tree forest origin, fed on locust tree leaves from hatching. The caterpillars were sacrificed three days after molting to the 5th instar.

Histological technique and A2 neurosecretory neurons

The effects of the host-plant on the activity of the neurosecretory neurons were detected in the caterpillars on the third day after molting to the 5th instar. After the caterpillars were sacrificed, their brain complexes were dissected quickly in ice-cold insect ringer solution and immersed in a fixative (ethanol 42.5 ml, acetic acid 2.5 ml, formaldehyde 37% 5 ml) for 1-2 h. After rinsing and dehydration, the brain complexes were embedded in paraffin wax (MERCK 59°C). Serial 3.5 µm thick cross-sections of the brain were stained by Schmid (1989) for the detection of monoamines (biogenic amines).

Based on their morphological characteristics, the dorsomedial neurosecretory neurons were divided into A1, A1' and A2 groups. The groups can be distinguished by their size, staining affinities, size of neurosecretory granules and by their protocerebral location (Raab, 1982). The average diameter of the A2 neurosecretory neurons was approximately 21.94 µm. The activity of this type of neuron was estimated by the size of the neurosecretory neurons, their nu-

clei and nucleoli, expressed as the mean value of the smallest and the largest diameter (µm).

Each neurosecretory neuron was observed in all of the sections in which it appeared, and the number of A2 neurons was noted for all animals. All the histological parameters were analyzed with a QWIN image analysis tool kit linked to a Leica DMLB light microscope (Leica, Cambridge, UK). The density of the stained neurosecretory product in the perikaryon of the A2 neurosecretory neurons was estimated by National Institute of Health (NIH) software Image J 1.42q (NIH, Bethesda, Maryland, USA).

Data analysis was carried out by two-way analysis of variance (ANOVA), and multiple range tests – LSD (Sokal and Rohlf, 1981).

RESULTS

Number of A2 neurosecretory neurons

There are differences in the number of A2 neurosecretory neurons depending on the population origin of *Lymantria dispar* caterpillars (Fig. 1). This was confirmed by two-way ANOVA that showed significant population effects for the number of A2 neurosecretory neurons (Tab. 1). The number of A2 neurosecretory neurons was higher in the groups originating from locust tree forest (LO and LL) independently of regime feeding (oak or locust tree leaves), than in oak forest (OO and OL), Fig. 1 and 6. There are no differences in the number of A2 neurosecretory neurons in the groups of caterpillars originating from the oak forest (OO and OL) and fed oak or locust tree leaves (Fig. 1).

Morphometric parameters of A2 neurosecretory neurons

The size of the A2 neurosecretory neurons was significantly larger in the groups of caterpillars originating from both populations (oak and locust tree forest) fed with locust tree leaves (Figs.1 and 6). This was shown by the two-way ANOVA which confirmed significant population effects for the number of A2

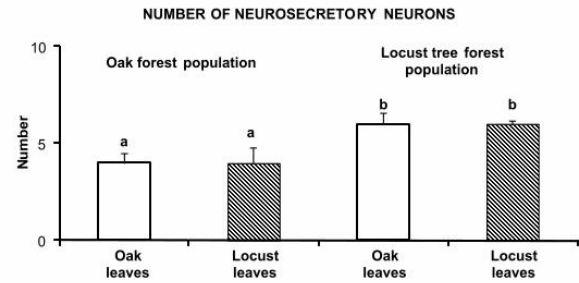


Fig.1. The number of protocerebral A2 neurosecretory neurons. Different letters (a, b) indicate statistical differences between experimental groups.

Table 1. A two-way ANOVA of the number and A2 neurons cytological parameters of 5th instar larvae of *Lymantria dispar*. The population origin (P) and host plant (H) are fixed factors. The mean squares (MS) were multiplied by 1000.

Parameters	Source of variations				Error
	Population origin (P)	Host Plant (H)	P x H		
Number of A2 neurons	Df	1	1	1	62
	MS	62,20	0,33	0,034	5,97
	F	56,08***	0,27	0,04	
Size of A2 neurons	Df	1	1	1	62
	MS	2,92	35,20	0,12	2,88
	F	1,01	12,22***	0,04	
Size of nuclei	Df	1	1	1	62
	MS	12,79	20,76	0,59	5,05
	F	2,53	4,11*	0,12	
Size of nucleoli	Df	1	1	1	62
	MS	29,28	0,98	84,18	8,89
	F	3,29+	0,11	9,47***	

* P<0.05; *** P<0.001

neurosecretory neurons (Tab. 1).

The sizes of the nuclei differ between the experimental groups depending on their feeding regime (Figs. 2 and 6, Table 1). Two-way ANOVA showed this effect of the host plant (suitable or unsuitable) to the change in A2 neurosecretory neuron size (Tab. 1). The nuclei were larger in caterpillars from the experimental groups which were fed an unsuitable host plant, *Robinia pseudoacacia* (OL and LL) than

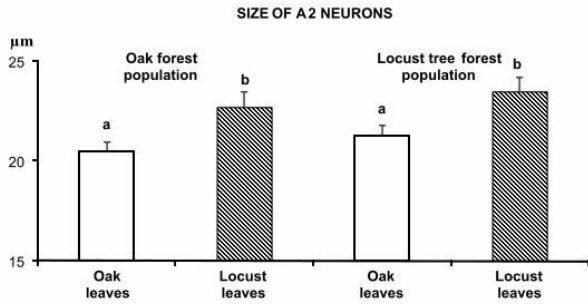


Fig. 2. The size of protocerebral A2 neurosecretory neurons. Different letters (a, b) indicate statistical differences between experimental groups.

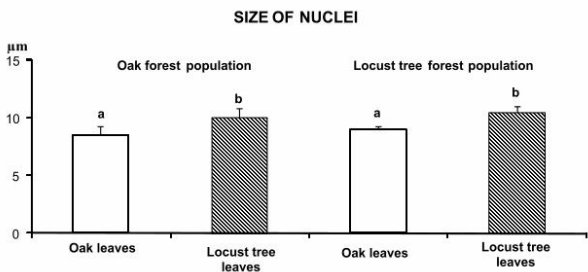


Fig. 3. The size of the nuclei in protocerebral A2 neurosecretory neurons. Different letters (a, b) indicate statistical differences between experimental groups.

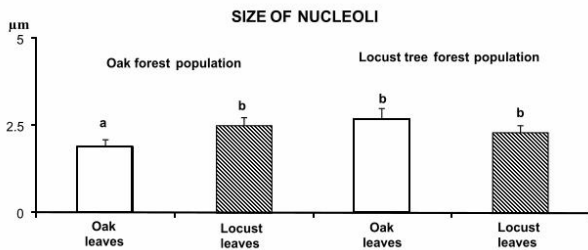


Fig. 4. The size of the nucleoli in protocerebral A2 neurosecretory neurons. Different letters (a, b) indicate statistical differences between experimental groups.

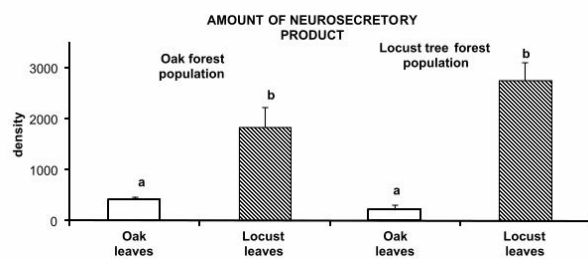


Fig. 5. The amount of aminergic neurosecretory product in protocerebral A2 neurosecretory neurons. Different letters (a, b) indicate statistical differences between experimental groups.

in those fed with a suitable host plant, *Quercus rubra* (OO and LO).

It is interesting that nucleoli sizes were high, and they are clearly visible in all experimental groups of caterpillars, except the group originating from oak forest and fed with oak leaves – OO, (Figs. 3. and 6). This indicates different responses to trophic stress in caterpillars from differently adapted populations. A significant “population x host plant” interaction is recorded for this cytological parameter, revealing the different neuroendocrine susceptibility of caterpillars originating from the two populations (Tab 1).

The cytoplasm of A2 neurosecretory neurons contained a larger quantity of fine-grained neurosecretory material in the caterpillars which were fed the unsuitable host plant, *Robinia pseudoacacia*, independently of their population origin, than the caterpillars in the groups fed the suitable host plant (Figs. 5 and 6).

DISCUSSION

The *Lymantria dispar* L. is an extremely polyphagous insect. It avoids leaves of *Robinia pseudoacacia* as food (Janković 1958) because of the content of large quantities of insect defense allelochemicals (alkaloids, flavonoids and tannins) and also it has a high buffering capacity as well as a low quantity of protein and free sugar compared to oak leaves. *Quercus* species are the principal and optimal host plants of the gypsy moth.

The population of *Lymantria dispar* in the locust tree forest, near Bačka Palanka (used in experiments) has survived for more than 60 years. In this forest defoliation never occurs, i.e. the dynamics of population size oscillates in a milder form than in oak forest (Sidor and Jodal, 1983).

The unfavorable effect of locust tree leaves on gypsy moth performance could be explained by low nutrient content and the presence of allelochemicals (Montgomery 1986; Barbosa and Krischik 1987). A locust leaf diet leads to a decrease in survival rate,

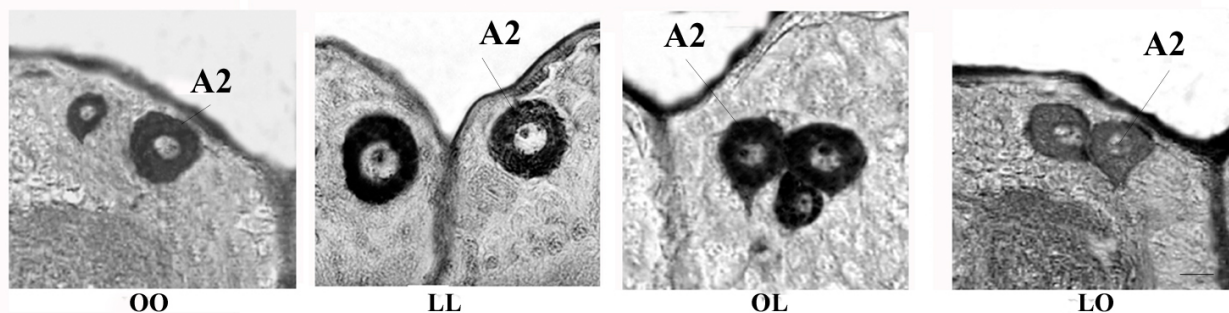


Figure 6. Brain transverse cross-section of *Lymantria dispar* 5th instar caterpillars. Protocerebral A2 neurosecretory neurons are marked. OO – oak forest origin, fed on oak leaves from hatching; OL – oak forest origin, fed on locust tree leaves from hatching; LO – locust tree forest origin, fed on oak tree leaves from hatching; LL – locust tree forest origin, fed on locust tree leaves from hatching. The bar represents 10 μ m.

prolonged duration of development and weight loss of the pupae (Perić et al., 1994; Lazarević 1994). An increased consumption rate in response to low nutritional content of food is a characteristic of the gypsy moth (Stockhoff 1991; Lindroth et al., 1997; Erelli et al., 1998) and many other insects (Felton 1996; Woods 1999). Decrease in food quality could be compensated by increase of food ingestion.

There are data about the important role of biogenic amines (DL- octopamine) in insect feeding, i.e. the hyperphagic effect and receptor hyperresponsiveness. The behavioral effects of biogenic amines can be summarized as a positive modulation of feeding behavior which promotes the initiation of feeding and prolongs its duration (Long and Murdock, 1983; Stoffolano et al., 2007). It is necessary for the caterpillars which were fed locust tree leaves as unsuitable food with low nutritional quality. The decrease in efficiency of food utilization could be compensated for by increased food ingestion.

Nutritive-deficient food and the presence of defense allelochemicals in locust tree leaves needs physiological adaptations of the *Lymantria dispar* from the *Robinia* population for detoxification (Terriere, 1984). However, the allelochemicals present in locust tree leaves cause oxidative stress (Ahmad and Pardini, 1990). Our previous investigations showed that a locust tree leaf diet correlates with the high

activity of antioxidative defense enzymes and glutathione amount (Perić Mataruga et al., 1997). The constitutive activity of these enzymes was higher in the midgut tissue of the caterpillars originating from the locust tree population in comparison to the oak forest population independently of the presence of trophic stress (Perić Mataruga et al., 1997). It could be part of the physiological adaptations of the locust tree forest population that live long in conditions of trophic deficiency and presence of toxins. The constitutive number of A2 neurosecretory neurons with aminergic synthesis was higher in the protocerebra of the *Lymantria dispar* caterpillars from the locust tree forest population than those from the oak tree forest (Fig. 1).

Biogenic amines have an indirect influence on the antioxidative protection of prooxidative effects of plant allelochemicals through the regulation of 20-hydroxyecdysone content. Octopamine, one of the widespread biogenic amines, is known to increase 20-hydroxyecdysone content (Gruntenko et al., 2003, 2005, 2007).

20-hydroxyecdysone restrained lipid peroxidation and the formation of protein carbonyls, ameliorated changes in microsomal membrane fluidity, enhanced the level of reduced glutathione, and up-regulated the activity of glutamyl transpeptidase. At the organismic level, 20-hydroxyecdysone curtailed

three detrimental effects caused by paraquat injection: the disappearance of a blood protein, the suppression of fecundity and egg hatchability, and the shortening of adult life-span. The data showed that 20-hydroxyecdysone provided a systemic antioxidant protection but the significance of endogenous ecdysteroids in the management of oxidative stress remains to be shown (Krishnan et al., 2007).

Thus, a more than fifty-year adaptation of the gypsy moth population to the locust forest must have resulted in changes in the neuroendocrine strategy in reaction to trophic stress conditions. The differences observed between the two populations (adapted to varying degrees of stress) in activity and number of aminergic A2 protocerebral neurosecretory neurons (Table 1 and Fig. 6) showed specific adaptation mechanisms to a diet of an unfavorable host plant.

Plastic responses of the neuroendocrine system (protocerebral neurosecretory neurons i.e. neurohormones and hormones) to the chemical variability of host plants are estimated to be a physiological basis of fitness plasticity in phytophagous insects living in changing and stressful environments.

Acknowledgments - This study was supported by the Serbian Ministry of Education and Science (grant No.173027).

REFERENCES

- Ahmad, S. and R.S. Pardini (1988). Evidence for the presence of glutathione peroxidase activity toward an organic hydroperoxide in larvae of the cabbage looper moth, *Trichoplusia ni*. *Insect. Biochem.* **18**, 861-866.
- Barbosa, P. and V.A. Krischik (1987). Influence of alkaloids in feeding preference of eastern deciduous forest trees by the gypsy moth *Lymantria dispar*. *Am. Nat.* **130**, 53-69.
- Davenport, A.P., and P.D. Evans (1994). Stress induced changes in the octopamine levels of insect hemolymph. *Insect Biochem.* **14**,135.
- Elkinton, J.S., and A.M. Liebhold (1990). population dynamics of gypsy moth in North America. *Annu. Rev. Entomol.* **35**, 571-596.
- Erelli, M.C., Ayers M.P. and G.K. Eaton (1998). Altitudinal patterns in host suitability for forest insects. *Oecologia*, **117**, 133-142.
- Evans, P.D. (1985). Octopamine. In: Kerkut, G.A. and L.I. Gilbert, (Eds.). *Comprehensive insect physiology, biochemistry and pharmacology*. Pergamon Press - New York, pp. 499-538.
- Felton, G.W. (1996). Nutritive quality of plant protein; Sources of variation and insect herbivore responses. *Arch. Insect Biochem. Physiol.* **32**,107-130.
- Granger, N.A., Sturgis, S.L., Ebersohl, R., Geng, C., and T.C. Sparks (1996). Dopaminergic control of *corpora allata* activity in the larval tobacco hornworm, *Manduca sexta*. *Arch. Insect Biochem. Physiol.* **32**, 449-466.
- Gruntenko, N.E., Chentsova, N.A., Andreenkova, E.V., Karpova, E.K., Glazko, G.V., Monastiriotti, M. and I.Yu. Rauschenbach (2004). The effect of mutations altering biogenic amine metabolism in *Drosophila* on viability and the response to heat stress. *Arch. Insect Biochem. Physiol.* **55**, 55-67.
- Hirashima, A., Hirokado, S., Ohta, H., Suetsugu, E., Sakaguichi, M., Kuwano, E., Taniguchi, E. and M. Eto (1999). Titres of biogenic amines and ecdysteroids: effect of octopamine on the production of ecdysteroids in the silkworm *Bombyx mori*. *J. Insect Physiol.* **45**, 843-851.
- Hirashima, A., Sukhanova, M.Jh. and I.Yu. Rauschenbach (2000). Biogenic amines in *Drosophila virilis* under stress conditions. *Biosci. Biotechnol. Biochem.* **64**, 2625-2630.
- Ivanović J. and M. Janković-Hladni (1991). Hormones and Metabolism in Insect Stress: CRC Press, Boca Raton / USA.
- Janković, Lj. Prilog poznavanju biljaka hraniteljki gubara u toku poslednje gradacije (1953-1967). *Zaštita bilja*. **36**, 58-64.
- Krisnan, N., Vecera, J., Kodrik, D. and F. Sehna (2007). 20-Hydroxyecdysone prevents oxidative stress damage in adult *Pyrrohocoris apterus*. *Arch. Insect Biochem. Physiol.* **65**(3),114-24.
- Lindroth, R.L., Klein, K.A., Hemming, J.D.C. and A.M. Feuker (1997).Variation in temperature and dietary nitrogen affect performance of the gypsy moth (*Lymantria dispar* L.). *Physiol. Entomol.* **22**, 55-64.
- Long T.F. and L.L. Murdock (1983). Stimulation of blowfly feeding behavior by octopaminergic drugs. *Proc. Natl. Acad. Sci. USA*, **80** (13), 4159-4163.
- Montgomery, M.E.(1986). Gypsy moth host plant relationships and population dynamics. Pp. 743-745. Proceeding 18th IUFRO World Congress. Div. 2., Vol.II, Forest Plants and Forest Protection, Ljubljana, Yu.
- Nijhout, H.F. (1994). Insect hormones. Princteon: Princteon University Press, New Jersey.
- Perić Mataruga, V. (1997). The activities of the antioxidative defence enzymes and protocerebral neurosecretory neurons under the trophic stress in *Lymantria dispar* L. caterpillars

- originated from different population. PhD Thesis, Faculty of Natural and Mathematical Sciences, University of Belgrade, Serbia.
- Perić Mataruga, V. and J. Lazarević (2004). Plasticity of medial neurosecretory neurons in response to nutritive stress in the gypsy moth *Lymantria dispar* (Lepidoptera: Lymantriidae). *Entomol. Gener.* **27**(1), 15-24.
- Perić Mataruga, V., Blagojević, D., Spasić, M.B., Ivanović, J. and M. Janković Hladni (1997). Effect of the host plant on the antioxidative defense in the midgut of *Lymantria dispar* L. caterpillars of different population origins. *J. Insect Physiol.* **43**, 101-106.
- Perić Mataruga, V., Lazarević, J. and V. Nenadović (2001). A possible role for the dorsolateral protocerebral neurosecretory neurons in the trophic adaptations of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Eur. J. Entomol.* **98**, 257-264.
- Perić, V., Ivanović, J. and M. Janković-Hladni (1994). The effect of host plant on survival and development of the gypsy moth caterpillars *Lymantria dispar* L. originating from different populations. *Bull. Acad. Sci. Serb.* **35**, 126-140.
- Raab, M. (1982). Insect neurohormones. *Plenum Press, New York and London*.
- Rauschenbach, I.Yu., Serova, L.I., Timochina, I.S., Chentsova, N.A. and L.V. Shumnaja (1993). Analysis of differences in dopamine content between two lines of *Drosophila virilis* in response to heat stress. *J. Insect Physiol.* **39**, 761-767.
- Rauschenbach, I.Yu., Shumnaja, L.V., Khlebodarova, T.M., Chentsova, N.A. and L.G. Greenback (1995). Role of phenol oxidases and tyrosine hydroxylase in control of dopamine content in *Drosophila virilis* under normal conditions and heat stress. *J. Insect Physiol.* **41**, 279-286.
- Rossiter, M.C. (1991). Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. *Funct. Ecol.* **5**, 386-393.
- Shmid, A. (1989). How to use Heidenhains AZAN staining in insects. *Neuroscience.* **101**, 35-38.
- Sidor, C., and I. Jodal (1983). Rezultati ispitivanja zdravstvenog stanja gubara *Porthetria dispar* L. u bagremovoj šumi "Bagremara" kod Bačke Palanke. *Zaštita bilja*, **34**, 445-455.
- Simonet, G., Poels, J., Claeys, I., Van Loy, T., Franssens, V., De Loof, A. and J. Vanden Broeck (2004). Neuroendocrinological and molecular aspects of insect reproduction. *J. Neuroendocrinol.* **16**, 649-659.
- Sokal, R.R. and F.J. Rohlf (1981). *Biometry*. W.H. Freedman and Co. San Francisco.
- Stockhoff, B.A. (1991). Starvation resistance of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae): Trade-offs among growth, body size, and survival. *Oecologia* **88**, 422-429.
- Stoffolano J.G., Lim M. A. and K.E. Downer (2007). Clonidine, octopaminergic receptor agonist, reduces protein feeding in blow fly *Phormia regina* (Meigen). *J. Insect Physiol.* **53**(12), 1293-1299.
- Terriere L.C. (1984). Induction of detoxification enzymes in insects. *Ann. Rev. Entomol.* **29**, 71-88.
- Wilson, M.H. and H.D. Roundus (1992). Stress induced changes in glucose levels in cockroach hemolymph. *Comp. Biochem. Physiol.* **64**, 161.
- Woods, H.A. (1999). Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low-or high-protein food during early instars. *Physiol. Biochem. Zool.* **72**, 445-454.