TRYPSIN ACTIVITY IN THE MIDGUT OF GYPSY MOTH (*LYMANTRIA DISPAR* L.) LARVAE DURING THE INTERMOLT PERIOD.

Jelica Lazarević, Vesna Perić-Mataruga, Vera Nenadović, and **Milena Janković-Tomanić.** *Department of Insect Physiology and Biochemistry, Siniša Stanković Institute for Biological Research,* 11060 Belgrade, Serbia

The quantity and quality of proteins as well as their interaction with secondary metabolites in plants exert significant impact on the performance of phytophagous insects and may select for their specialization and host shift evolution (Felton, 1996). Locust-tree leaves represent a stressful food for the gypsy moth (*Lymantria dispar* L.), an extremely polyphagous insect, due to low protein and high allelochemical content. Our investigations of two host-associated gypsy moth populations from oak and locust-tree forests revealed local differentiation for many fitness, behavioral and physiological traits depending on the stage of development (Perić-Mataruga, 1997; Lazarević, 2000). In the present study, we attempted to determine if there are population and host-plant differences in the level and timing of trypsin activity during the fifth larval instar.

Gypsy moth larvae were hatched from egg masses sampled from two different populations referred to as *Quercus* (an oak forest at the Despotovačke šume locality) and *Robinia* (a locust forest at the Bačka Palanka locality) and fed on either oak (*Quercus cerris*) or locust-tree (*Robinia pseudoacacia*) leaves. They were reared at constant temperature of 23°C under conditions of L16:D8 light-dark regime and sacrificed on the first, third, fifth, and seventh day after molting into the fifth larval instar. Their midguts were removed by dissection in a cold 0.9% NaCl solution, homogenized individually in 10 mM Tris-HCl buffer (pH 7.2, 1:10 wet wt/vol) for 30 seconds, and centrifuged at 10,000 rpm using a Sorvall centrifuge for 10 minutes at 4°C. The supernatants were used as crude midgut extracts. Trypsin activities were determined using the chromogenic substrate BApNA (*N*-benzoyl-DL-arginine *p*-nitroanilide) at a final concentration of 1 mM in 0.1 M Tris-HCl (pH 8.0) at 25°C (Erlanger et al., 1961; Valaitis, 1995). The release of *p*-nitroanilide was continually monitored at 405 nm using a Shimadzu UV-160 spectrophotometer. Specific activity of trypsin (SAT) was expressed in U*mg proteins^{-1}. One enzyme unit corresponded to 1 μmol of p-nitroanilide liberated per minute, and protein concentration was estimated according to Lowry et al. (1951) using bovine serum albumin as the standard. Three-way ANOVA was applied on the log transformed values of trypsin activities to test the effects of three fixed factors (population - **Pop**, host plant – **H**, and time after molting into the fifth larval instar - **Day**) and their interactions on trypsin variation (Sokal and Rohlf, 1981). *Post hoc* comparisons among the groups were carried out by the LSD test.

On average, the highest trypsin activity was noticed on the third day of the intermolt period (significant Day effect, P<0.001, Fig. 1). It was higher in *Quercus* than *Robinia* larvae (the Pop effect was marginally significant, P<0.1) and in larvae fed on locust leaves compared to larvae fed on oak leaves (significant H effect, P<0.001). Trypsin responses to locust leaves depended on the time after molting into the fifth larval instar (significant H x Day effect, P<0.001). Significant differences between the host plants were detected only on the seventh day of the intermolt period in both the *Quercus* and the *Robinia* population (P<0.001, LSD test). In *Quercus* larvae, high trypsin activity was maintained from the first to the fifth day of the intermolt period regardless of the host plant. However, significant decrease of trypsin activity on the seventh day of the intermolt period was recorded only in larvae reared on oak leaves (P<0.001) while larvae reared on locust-tree leaves showed no change of trypsin activity from first to the seventh day (P>0.05). In *Robinia* larvae fed on oak leaves, changes of trypsin activity during the intermolt period were similar to changes in *Quercus* larvae fed on oak leaves (lack of significant changes from the first to fifth day and significant decrease on the seventh day). In *Robinia* larvae fed on locust-tree leaves, maximal trypsin activity, attained on the third day of the intermolt period, was significantly higher than on the first (P<0.01) and seventh day (P<0.05).

Increased release of digestive enzymes in many insects is related to feeding. For insects that feed continuously (including Lepidoptera larvae), enzyme activity is minimal at the time of molting and rises to a maximum when the insect starts to feed (Chapman, 1998). Our previous studies on *L. dispar* reported prolonged duration of larval development and larval instars (a longer period of feeding) in gypsy moths fed on a locust leaf diet and artificial diets supplemented with allelochemicals characteristic of locust leaves. *Robinia* larvae were shown to be less sensitive to stressful food (Perić, 1990; Perić-Mataruga and Lazarević, 2001). Hormonal changes related to delayed molting in locust-fed larvae, could explain time-specific changes in trypsin activity (Fig. 1). However, many data argue against a direct effect of hormones on enzyme sectretion. According to Lehane et al. (1996), the influence is rather a consequence of changes in food consumption.

Biosynthesis of trypsin-like enzymes in the midgut of Lepidoptera during development is modulated, among other
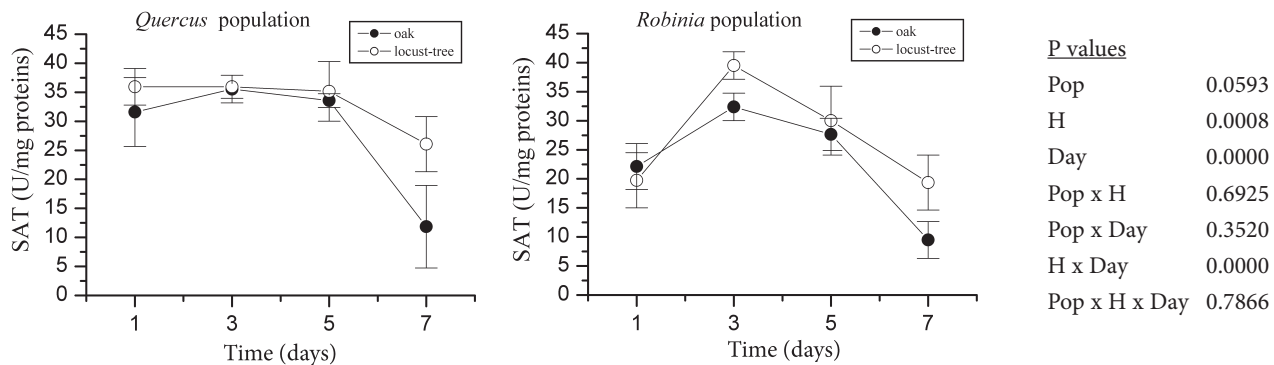
Fig. 1. Changes in the specific activity of trypsin (SAT) in fifth-instar gypsy moth larvae during the intermolt period depending on population origin and host plant. P values represent the significance of main (Pop – population origin, H – host plant, Day - time after molting into the fifth-instar) and interactive effects from three-way ANOVA.

factors, by a hemolymph circulating trypsin modulating oostatic factor or TMOF. Trypsin activity decline before the molt into the next larval instar is related to TMOF increase at the end of the larval instar and at the end of larval development in general (Nauen et al., 2001). *In vitro* incubation of *L. dispar* prothoracic glands with *Aedes aegypti* TMOF revealed that this decapeptide, in the presence of brain extract, also modulates ecdysteroid production. High concentrations of TMOF stimulate ecdysteroid biosynthesis in larvae of *L. dispar* (Gelman and Borovsky, 2000).

Other investigators have described a modulating effect of secretions from medial neurosecretory neurons or MNSC (Muraleedharan and Prabhu, 1981; Ivanović et al., 1989), known to be the site of allatotropin synthesis in Lepidoptera (Bogus and Scheller, 1994). MNSC regulate the level of juvenile hormone (JH) which stimulates food consumption and secretion of digestive enzymes. Relatively high JH activity has been recorded during early development of the last larval instar of the gypsy moth, after which it gradually declined and a similar pattern has been shown for body weight and frass production (Tanaka et al., 1989). The results of our investigations revealed an increased number and activity of MNSC (Perić-Mataruga and Lazarević, 2004), a high relative consumption rate (Lazarević and Perić-Mataruga, 2001; Lazarević et al., 2002), and increased trypsin activity (present results, Fig. 1) in *Robinia* gypsy moth larvae.

In conclusion, 40 generations of adaptation from a locust forest did not affect either time-specific regulation of trypsin activity (insignificant Pop x Day interaction) or trypsin plasticity in response to locust leaves (insignificant Pop x H interaction). Increase of trypsin activity in *Robinia* larvae (marginally significant Pop effect) can be considered an adaptive response to low-protein, tannin-rich food.

References: - Bogus, M. I., and K. Scheller (1994). *Naturwissenschaften* **81**, 87-89. - Chapman, R. F., (1998). *The Insects, Structure and Function*, 38-58. University Press, Cambridge. - Erlanger, B. F., Kokowsky, N., and W. Cohen, (1961). *Arch. Biochem. Biophys.* **9**, 355-357. - Felton, G. W. (1996). *Arch. Insect Biochem. Physiol.* **32**, 107-130. - Gelman D. B., and D. Borovsky (2000). *Arch. Insect Biochem. Physiol.* **45**, 60-68. - Ivanović, J., Janković-Hladni, M., Stanić, V., Nenadović, V. M. and Frušić (1989). *Comp. Biochem. Physiol.* **94A**, 167-171. - Jongsma, M. A., Peters, J., Stiekema, W. J., and D. Bosch (1996). *Insect Biochem. Mol. Biol.* **26**, 185-193. - Lazarević, J. (2000). Ph. D. Thesis, Faculty of Biology, University of Belgrade, 1-166. - Lazarević, J., and V. Perić-Mataruga (2001). *Ekologija (Belgrade)* **36**, 39-45. - Lazarević, J., Perić-Mataruga, V., Stojković, B., and N. Tucić (2002). *Entomol. Exp. Appl.* **102**, 75-86. - Lehane, M. J., Muller, H. M., and A. Crisanti (1996). *Biology of the Insect Midgut* (Eds. M. J. Lehane, and P. F. Billingsley), 195-205, Chapman & Hall, London. - Lowry, O. H., Rosebrough, A. L., Farr, A. L., and R. J. Randal (1951). *J. Biol. Chem.* **193**, 265-275. - Muraleedharan, D., and V. K. K. Prabhu (1981). *Physiol. Entomol.* **6**, 183-189. - Nauen, R., Sorge, D., Sterner, A., and D. Borovsky (2001) *Arch. Insect Biochem. Physiol.* **47**, 169-180. - Perić, V. (1990). M. Sc. Thesis, Faculty of Biology, University of Belgrade, 1-98. - Perić-Mataruga, V. (1997). Ph. D. Thesis, Faculty of Biology, University of Belgrade, 1-219. - Perić-Mataruga, V., and J. Lazarević (2001). *Pesticides (Belgrade)* **16**, 259-274. - Perić-Mataruga, V., and J. Lazarević, J. (2004). *Entomol. Gener.* **27**, 15-24. - Sokal, R. R., and F. J. Rohlf (1981). *Biometry*. Freeman, San Francisco, 1-857. - Tanaka, S., Chang, M. T., Denlinger, D. L., and Y. A. I. Abdel-Aal (1989). *J. Insect Physiol.* **35**, 897-905. - Valaitis, A. P. (1995). *Insect Biochem. Mol. Biol.* **25**, 139-149..