

## EFFECTS OF SPINOSAD ON VITELLOGENIN EXPRESSION IN THE CADMIUM-SELECTED BEET ARMYWORM STRAIN

A. Plachetka, Maria Augustyniak, M. Tarnawska, A. Kafel, A. Babczyńska

*University of Silesia, Department of Animal Physiology and Ecotoxicology, Katowice, Poland*

[maria.augustyniak@us.edu.pl](mailto:maria.augustyniak@us.edu.pl)

**Keywords:** *Spodoptera exigua*; cadmium; vitellogenin, spinosad, reproduction

### Introduction

In the organisms exposed to strong stressors, including metals or pesticides, reproduction processes may be repressed. The impairment of reproduction may be indicated by a lower number of eggs or reduction in hatching success. The changes in vitellogenin synthesis (Vg), precursors of egg yolk proteins, may be a crucial symptom of this impairment.

Results presented here contribute to a larger project led on *Spodoptera exigua* laboratory strain, bred for over ten years in permanent contact with sublethal Cd concentration ( $44 \mu\text{g Cd}\cdot\text{g}^{-1}$  dry weight of food) (Kafel et al, 2012a,b). We have previously concluded that in spite of lower mRNA content in insects from cadmium strain, some compensation must occur at the stage of translation. Therefore, the aim of this study was to check if a long lasting contact with cadmium may prepare the organism to additional acute stressor (spinosad) referring to Vg expression in insects from both strains.

### Methods

Fifth-instar larvae from control and cadmium strains were exposed to spinosad (0.02 mg/L of food). Newly eclosed females (time 0) and individuals after 12, 18 and 24 hours post emergence were dissected and fat body was isolated. After tissue preparation and/or purification the following parameters were studied:

Vg gene expression (qRT-PCR). Based on known insect RNA sequences of actin (Act) and vitellogenin (Vg) genes, primers were designed for these genes. In this study, internal and external (from plasmid) Act gene was used as a reference gene. For analyses of gene expression, Relative Quantification method with calibrator normalized and efficiency correction was used (Peng et al., 2012). As calibrator mix RNA (transcribed to cDNA) collected from 10 individuals of control group immediately after adult eclosion, was used. The target gene expression was calculated by using  $\Delta\Delta\text{Cp}$  algorithm.

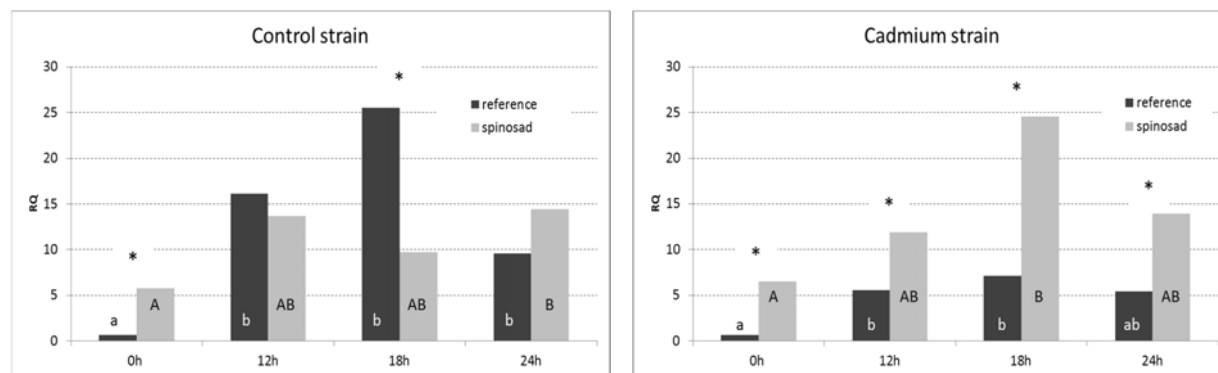
Protein profile and Vg detection (Western blotting, WB) in the fat body at appropriate time points with SDS polyacrylamide gel electrophoresis were checked (Sorge et al., 2000). The gels were stained with 0.1% Coomassie Brilliant Blue R-250. After protein profile analysis, WB was performed by using antibodies from rabbits immunized with peptides synthesized based on protein sequences in NCBI for *Spodoptera litura*.

Immunohistochemical Vg detection and localization in the females' abdomen were performed using the same antibodies as in WB.

### Results

In insects exposed to spinosad, the changes in Vg expression in both strains were found. In general, Vg mRNA synthesis commenced earlier than in not exposed females (Fig. 1). Even at time 0 Vg expression is significantly higher in females from both strains in comparison with the control insects. In the insects from Cd strain, exposed to spinosad Vg expression in each time point is significantly higher than in the untreated insects. Unlike the Cd strain, in the females from the control strain spinosad did not cause any increase in

Vg expression in time points t12 and t48. Surprisingly, Vg expression decrease significantly in females from the control strain in t18 time point. As a rule, usually in 18h since the eclosion, Vg expression has increased, what is strongly correlated with female readiness for mating. This periodicity has been observed in not exposed groups in both strains as well as in Cd strain exposed to spinosad.



**Figure 1.** Vitellogenin expression (median) in females' fat body of *S. exigua* from control and cadmium strain, exposed in an acute test to spinosad. Stars denote significant differences for particular time period (0, 12, 18 or 24 hours after eclosion) between reference and spinosad-treated groups. The same letters indicate homogenous time-groups within strain and within reference or treated insects separately (Kruskal-Wallis test,  $p < 0.05$ ).

These results are correlated with the amount of synthesized vitellogenin, measured by means of WB. Also, immunohistochemical assays of female abdomens revealed the presence of these proteins in fat body and ovaries.

## Conclusion

Acute pesticide application, caused time-dependent changes in the Vg expression profile in *S. exigua* from both strains. Most likely, especially in the insects of the Cd strain, a strong and fast mobilization of energy reserves takes place. The energy is necessary for fast synthesis of spare materials, deposited in the eggs. The results let us think that long lasting contact with cadmium can cause a selection of individuals that are able to cope with the pesticide and reproduce efficiently. It is possible, however, that the exposure to an additional strong stressor could cause the acceleration but also shortening of reproduction period in females from the Cd line. This takes place in a trade-off with female longevity.

This project was supported by National Science Centre, Poland (decision DEC-2013/09/B/NZ9/01754)

## References

- Kafel, A., Nowak, A., Bembek, J., Szczygieł, J., Nakonieczny, M., Świergosz-Kowalewska, R. (2012a) The localisation of HSP70 and oxidative stress indices in heads of *Spodoptera exigua* larvae in a cadmium-exposed population. *Ecotox Environ Safe* 78, 22–27.
- Kafel, A., Zawisza-Raszka, A., Szulińska, E. (2012b) Effects of multigenerational cadmium exposure of insects (*Spodoptera exigua* larvae) on anti-oxidant response in haemolymph and developmental parameters. *Environ Pollut* 162, 8–14.
- Sorge, D., Nauen, R., Range, S., Hoffmann, K.H. (2000) Regulation of vitellogenesis in the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J Insect Physiol* 46, 969–976.
- Peng, R., Zhai, Y., Ding, H., Di, T., Zhang, T., Li, B., Shen, W., Wei, Z. (2012) Analysis of reference gene expression for real-time PCR based on relative quantitation and dual spike-in strategy in the silkworm *Bombyx mori*. *Acta Biochim Biophys Sin* 44, 614–22.