STRESS MARKERS IN THE BEET ARMYWORM FROM CONTROL AND CADMIUM STARINS EXPOSED TO ADDITIONAL STRESSORS

Monika Tarnawska, A. Kafel, A. Babczyńska, A. Zawisza-Raszka, A. Płachetka, B. Adamek, M. Augustyniak

University of Silesia, Department of Animal Physiology and Ecotoxicology, Katowice, Poland
monika.tarnawska@us.edu.pl

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Introduction

Long lasting exposure of animals to particular stressor may lead to the selection of individuals which cope better with this, or perhaps also other, stressors. Insects laboratory selected for many generations under a specific stress factor can serve as a good, simplified model which can explain processes existing in the environment. This phenomenon is of particular and practical importance in case of insect species usually recognized as dangerous, highly expansive crop pests, e.g. *Spodoptera exigua* (Zheng et al., 2000). Acquisition of resistance toward specific toxin may serve as "new equipment" toward another, for example pesticide.

The study was conducted on *S. exigua* laboratory strain, bred for over ten years in permanent contact with sublethal Cd concentration (44 μg $Cd \cdot g^{-1}$ dry weigh of food). We may suppose that insects from this strain can react to additional stressing factors in a different way than control ones (Kafel et al., 2012a, b).

In order to check if long-lasting, multigenerational contact with cadmium influences the ability of *S. exigua* to cope with additional stress factors we assessed the level of 4 sensitive stress markers (heat shock proteins, ATP concentration, ADP/ATP ratio and DNA damage) in the insects from the cadmium and control lines and exposed in an acute test to heat shock or pesticide.

Methods

IVth *S. exigua* instar from both lines were subjected to different temperature conditions: 20°C, 25°C 30°C (groups t20, t25 and t30, respectively) or spinosad: 0.2, 0.02 i 0.002 mg/kg dry weight of the diet (groups s1, s2, s3, respectively). HSP70 level, DNA damage, ATP concentration and ADP/ATP ratio were measured in fifth-instar larvae according to the following procedures:

HSP content was determined by indirect ELISA protocol (Pyza et al.,1997; Chavez-Crooker et al., 2003), using primary (Monoclonal Anti-Heat Shock Protein 70 produced in mouse) and secondary (Anti-Mouse IgG – Alkaline Phosphatase antibody produced in goat) antibody (Sigma-Aldrich).

For ATP concentration and ADP/ATP ratio BioVision ApoSENSORTM Cell Viability Assay Kit and ApoSENSORTM Assay Kit were used, according to the manufacturer's protocol.

DNA damage was measured by the Comet assay according to the method described by Bilbao et al. (2002) with slight modifications implemented by Augustyniak et al. (2006). Analysis was done with image analysis system Komet 5.5 (Kinetic Imaging, Liverpool, UK).

Results

The analyses of stress parameters revealed differences between control and cadmium strains. In the insects form Cd strain a strong increase in HSP70 synthesis in response to spinosad was found (Fig. 1), whereas

the level of DNA damage was significantly higher only in s2 group in comparison with the remaining groups. ADP/ATP ratio was similar to the one of the reference group, however, a subtle increase of this marker with spinosad concentration was noticed.

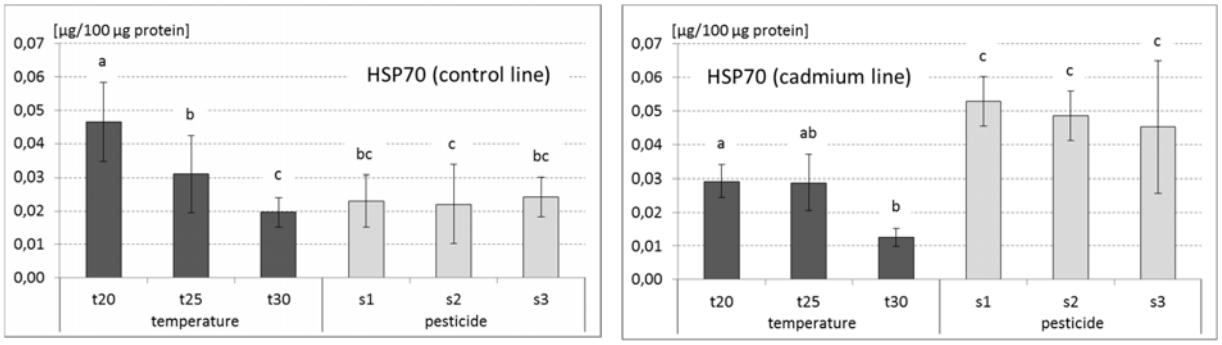

Figure 1. HSP70 (mean ± SD) in 5th instar of *S. exigua* from control and cadmium strain, and exposed in an acute test to different temperatures or pesticide (spinosad). The same letters indicate homogenous groups (ANOVA, Tukey test; $p<0.05$).

The insects from the control group did not show a distinct increase in HSP70 level after spinosad application, although the temperature of 20 °C stimulated their synthesis (Fig. 1) and enhanced the level of DNA damage. Higher ATP concentration in the control insects was detected only after the exposure to the highest spinosad concentration. In all the spinosad-exposed groups ADP/ATP ratio was high, what may suggest that apoptotic and/or necrotic changes take place.

Conclusion

Long-lasting, multigenerational contact with cadmium caused several changes that enable the insect to survive under a chronic stress, preparing the organism to the contact with an additional, new stressor. This increases the probability of population survivorship and, at the same time, decreases the efficiency of plant protection efforts.

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