Effect of Dithiocarbamate Fungicide Mancozeb on Development, Reproduction and Ultrastructure of Fat Body of *Agrotis segetum* Moths

Zbigniew Adamski[1, 2] *, Jolanta Krawiec[1], Ewa Markiewicz[1], Michał Bankiet[1], Eliza Rybska[3], Marlena Ratajczak[2], Małgorzata Glama[2], Laura Scrano[4], Sabino A. Bufo[4], Kazimierz Ziemnicki[1], Meltem Erdem[5], Ender Büyükgüzel[5]

[1] Electron and Confocal Microscope Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland
[2] Department of Animal Physiology and Developmental Biology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland
[3] The Faculty Laboratory for Teaching Biology and Natural Science. Faculty of Biology, Adam Mickiewicz University, Poznan, Poland
[4] Department of Agriculture, Forestry, and Environment, University of Basilicata, Potenza, Italy
[5] Department of Biology, Faculty of Arts and Science, Zonguldak Karaelmas University, Zonguldak, Turkey

Abstract

Sublethal effects of ethylene bis dithiocarbamate fungicide mancozeb on development and reproduction of turnip moth (*Agrotis segetum*) was investigated. Larvae were exposed to the fungicide in a diet. Exposure did not cause massive mortality of larvae. However, we observed various postponed sublethal effects. Larval development was longer than in control, also mortality of pupae and time of pupation was altered, ; exposure to mancozeb affected fecundity, too. Scanning electron microscopy revealed, that females had laid less eggsfewer eggs, and they showed various malformations, which may might affect hatching success. These changes are were also in tune with observations of the larval fat body ultrastructure (transmission electron microscopy). Fat body cells showed a range of malformations: envelopes of nuclei were invaginated and swollen. In cytoplasm, glycogen content was decreased, ER showed swelling and cytoplasm became more lucent. All these changes had been observed before the mortality of larvae was noticed. Therefore, we think that ultrastructural changes may be an important marker of stress caused by mancozeb in the environment.

Keywords: mancozeb, *Agrotis segetum*, moths, larval development, egg, electron microscopy

1. Introduction

Susceptibility to xenobiotics varies between organisms belonging to the same, as well as different species. Moreover, some species may show average higher susceptibility to the given pesticide, but be more resistant to the other, even if their biological activity is the same (compare: Adamski et al. 2003, Adamski 2007, Adamski et al. 2005a). Next, insects are exposed to various pesticides, which are used against other pests, like nematocides, fungicides or herbicides. They can affect non target species and cause variety of results, from mortality to sublethal effects. The latter ones are still not very well known. In case of low mortality, sublethal concentrations may lead to development of resistance by the surviving fraction of insects. On the other hand, exposure to sublethal concentrations may explain, how pesticides affect cells and tissues. Therefore, research on sublethal effects can be useful in explanation of pesticide resistance.

There is a wide range of sublethal effects, occurring on several levels, from the level of nucleic acids (Hreljac et al. 2008) through the level of proteins and enzymes (Soltani and Soltani-Mazouni 1992, Hyrsl et

*Corresponding author: ed@amu.edu.pl

al. 2008, Büyükgüzel 2009), ultrastructural, cellular and tissue level (Kajiura et al. 1993, Adamski et al. 2005a), to the level of whole organisms, their malphysiology, malbehaviour and morphological malformations (Yang and Du 2003, Wei et al. 2004, Zhou et al. 2005, Adamski 2007).

One of the most important aspects of sublethal concentrations of pesticides is their effect on reproduction. A variety of reproductive alteratnions were reported. Majority of them describes limited fecundity and fertility (Seth et al. 2004, Osorio et al. 2008, Pineda et al. 2009, Adamski et al. 2009a). The above mentioned effects are often correlated with abnormalities within reproductive organs, reported by various authors (Soltani and Soltani-Mazouni 1992, Seth et al. 2004, Khebbeb et al. 2008).

Malformed gonads may not produce proper egg/sperm. We observed malformations of eggshell of eggs laid by moths exposed to fenitrothion during larval stage (Fila et al. 2002, Adamski et al. 2005b). Decreased size of eggs exposed to tebufenozide was reported (Khebbeb et al. 2008, Yin et al. 2009). Such changes may cause lethality of developing embryo due to direct toxicity to embryos, improper development in smaller eggs, desiccation, parasitism or predation. Since that, reproductive effects reflect population stability and can cause serious effects in the next generations.

Sublethal effects caused by pesticides can be observed at various levels of biological organization. Moreover, they amalgamate and can lead to serious disturbances within exposed populations and communities. Yet, the knowledge of sublethal action of pesticides can be important in understanding of biological action of pesticides, insects' toleration to them, development of resistance. Our group focuses on research on the effect of sublethal concentrations of some pesticides on insects' ultrastructure, development and reproduction. Using microscopic methods we try to find explanation of some phenomena observed on the level of exposed populations. Next, the sublethal effects can be sometimes visible even if massive mortality is not observed within populations (Adamski et al. 2005a). Therefore, they may be used as bioindicators of stress.

Although pesticides may have various chemical structure, affect various target molecules within cells and tissues (like various channels within nerve cells), they often cause similar effects within nontarget tissues and organs, leading to similar physiological, morphological and anatomical effects, like above mentioned decreased fecundity and fertility, postponed development (Dorcas et al. 1992, Sadeghi et al. 2008, Jenson et al. 2009).

In one of the previous works we described serious malformations of *Spodoptera exigua* moths exposed to mancozeb. Although the insecticide did not cause high mortality, it caused serious sublethal effects: altered antioxidant enzyme, decreased fat body weight, malformed imagoes (Adamski and Ziemnicki 2004). Therefore, we were interested in checking, if sublethal effects can be observed also in case of other species. We stated a hypothesis, that even the fungicide will not cause massive mortality, but can lead to serious effects that may alter insects' reproduction, decrease population headcount and vigour of insects. We addressed ourselves some specific questions:

1) What is susceptibility of *A. segetum* larvae to mancozeb?

2) Does exposure during larval stage result in alterations/malformations during the latter stages?

3) Is there any correlation between ultrastructural, developmental and reproductive changes?

4) Can sublethal effects be treated as important action for non-target species?

To verify the above mentioned hypothesis and to answer these questions, we decided to test the effect of mancozeb on ultrastructure, developmental and reproductive parameters of *Agrotis segetum* Den et Schiff. (Lepidoptera: Noctuidae). This paper reports the observed changes at the subcellular, organismal and population level.

2. Material and Methods

2.1 Exposure of Larvae to Pesticides

The experiments were carried out on turnip moth (*Agrotis segetum*) Den et Schiff. (Lepidoptera: Noctuidae) larvae. They were maintained in chambers at 25°C with a 16:8, L:D photoperiod. Four groups of L3 larvae (30 individuals in each) were reared as previously described for *Spodoptera exigua* (Adamski et. al. 2005b), on a semi-synthetic nutrient (David et. al. 1975) with addition of mancozeb added to the liquid nutrient, before it became solid. Groups varied in tested concentration of the fungicide. We created three groups which got the

pesticide in various final concentrations: 1, 0.1 and 0.01% (called M1, M2 and M3, respectively) and the control group. Each larva was kept separately in a flask and received a portion of a nutrient in the form of a pill cut from the nourishment in desired concentration. The larvae were exposed until pupation. Larval mortality was checked each day. We did not measured the weight of larvae. After four days of exposure, some larvae were taken for microscopic observations of fat body (transmission electron microscopy). Pupae were collected and their gender was determined. Pairs of insects were kept separately in 100 mL phials. After they had reached imago stage they laid eggs. Number of eggs was counted and eggs were measured, using scanning electron microscope.

2.2 Chemicals

Mancozeb (ethylene bis dithiocarbamate fungicide) was bought as a commercial product (Dithane M-45) produced by Dow AgroSciences as a powder with 80% of the active substance (mancozeb). The powder is dissolved in water. Mancozeb is used for control of a wide range of fungal pathogens : *Phytophthora sp.*, *Alternaria sp.*, *Cercospora sp.*, *Botrytis sp*. and many other, to protect various crops: cotton, apples, potatoes, tomatoes, corn, cereal grains, sunflowers and the others. It shows also acaricidal activity (Auger et al. 2004, Miles and Kemmitt 2005). At the physiological level, mancozeb alters lipid metabolism (Pesticide Properties DataBase) and is also known as endocrine and reproductive disruptor (Baligar and Kalliwel 2001, Extoxnet).

2.3 TEM Peparation

After four days of exposure, some insect, taken by chance, were collected and sectioned. Samples of fat body were was isolated from larvae, fixed with 2% glutaraldehyde in 0.175 M cacodylate buffer, postfixed with 1% osmium tetroxide, dehydrated and finally embedded in Spurr resin. Ultrathin sections were obtained using a Leica ultramicrotome, stained with uranyl acetate and lead citrate and observed under the JEOL 1200EX II JEM TEM.

2.4 SEM Preparation

Eggs were measured and photographed with a Zeiss Evo 40 Scanning Electron Microscope. They were fixed in 1% glutaraldehyde in 0.175 m cacodylic buffer (pH 7.4). Then they were rinsed with the same buffer, and mounted on stubs with double sticky tabs. The eggs were then coated with gold in a Balzers SPC 050 ion coater and were observed in a

SEM. Diameter of eggs as well as diameter of of micropylar rosettae and length and width of cell of rosettae were measured. Cells were selected at random, always on 12th, 3rd, 6th and 9th hour (Fig. 1).
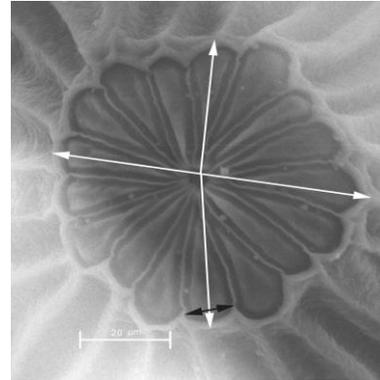
Figure 1. Aeropyle of *A. segetum* eggs. Four cells, located at 12th, 3rd, 6th and 9th hour were always taken for measuring of whole aeropylum (two-sided arrow), cell length (white arrows) and width (black, two sided arrow).
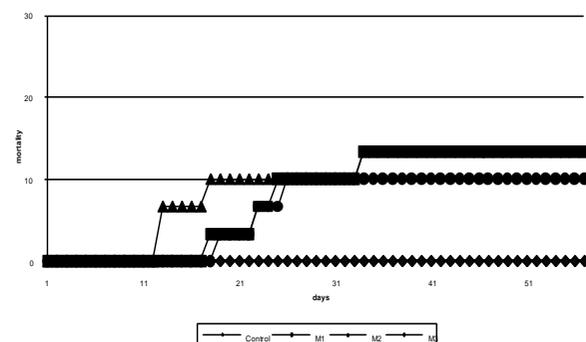
2.5 Statistics

Presented data, are expressed as mean values ± SE. Results were analyzed by Student's t-test, and p-values < 0.05 were considered to be statistically significant.

3. Results

3.1 Exposure of A. segetum Larvae to Mancozeb – Effect on Mortality and Duration of Larval Development

Mancozeb did not cause significant mortality of larvae. The final mortality varied between 10 (M1) and 13 % (both M2 and M3) (Fig. 2).

Figure 2. Mortality of larvae of *A. segetum* exposed to mancozeb. M1, M2,M3 - groups of larvae exposed to 1%, 0.1% and 0.01% of mancozeb within diet. Data are means ± SD; * - values statistically significant at p < 0.05.

The first dead larvae appeared after approx. two - three weeks of exposure and after the next few days mortality leveled off at the low, above mentioned level. The effect of three concentrations was not linear – the first dead larvae were observed within M2 groups, and the higher and lower concentration effected in mortality after 5-6 days later, respectively.

Although mortality was low, larval development was significantly longer within all groups exposed to mancozeb (Fig. 3).
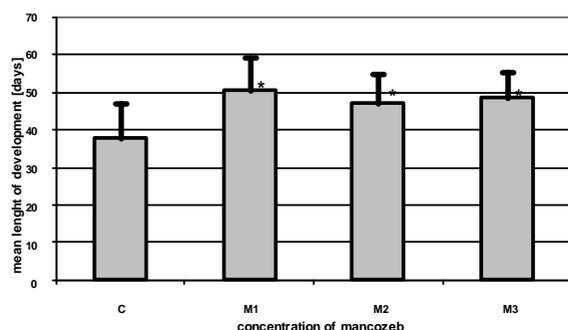
Figure 3. Length of larval development of *A. segetum* larvae exposed to mancozeb. M1, M2 and M3 – groups of larvae exposed to three concentrations of mancozeb. Data are means ± SD. * - difference statistically significant at p < 0.05.

Mancozeb affected pupation, too. The percentage of pupae was much lower in exposed groups, than in control. We could observe almost 40% drop of pupae in M1 group. M2 and M3 caused the same, 11% decrease in percentage of obtained pupae. All three mancozeb-exposed groups showed postponed early pupation: from 8 to 13 days (i. e. from 25 to 42 percent) (Table 1). However, this effect was visible only in case of the lower percentage of pupae. Later on, the exposed population showed faster pupation, than the control ones and the time for obtaining almost all pupae present within these populations was shorter, than within control.

3.2 Effect of Mancozeb on Moths' Fecundity

Insects exposed to mancozeb laid less eggs than the control ones. Interestingly, only those exposed lowest concentration laid statistically significant different number of eggs (Table 2). Higher concentrations caused clear, but statistically not important decrease of fecundity.

3.3 Effect of Mancozeb on Eggs - Scanning Electron Microscopy

Eggs of *A. segetum* are spherical in shape, little bit flattened at the base, leaf attaching, side. They have about 0.6 mm in diameter. At the top of the egg one can find rosettae-like strurcture - micropylar region -

Table 1. Effect of mancozeb on pupation.

	C	M1	M2	M3
Concentration of mancozeb	0	1%	0.1%	0.01%
% of obtained pupae	100	62.96	88.89	88.89
First pupa	(100)	141.9	125.8	138.7
25% of pupae	(100)	131.6	118.4	121.1
50% of pupae	(100)	78.7	74.5	76.6
95% of pupae	(100)	87.7	94.7	100

Values for exposed groups described as percent of control (100%).

Whereas this term was about 38 days within control group, the same term differed significantly: from nine to twelve days longer in tested groups than in the control one. Again, effect was not linear – M2 had value most similar to the control but there were no statistical differences between results observed for the three exposed groups.

where sperm enters the egg. Down to the equator the ridges of sculpture contain numerous aeropyle, which are necessary for proper gaseous exchange (Fig. 4). The characteristic sculpture effects from the composition of follicle cells, that produce chorion. Therefore, to simplify, we decided to call elements of rosettae as "cells".

**Table 2.** Effect of mancozeb on fecundity of *A. segetum*.

	Cross between			
	Cont.♀ x cont.♂	M1♀ x M1♂	M2♀ x M2♂	M3♀ x M3♂
Number of pairs	10	7	13	5
Mean no of eggs ± SD	125.7 ± 77.53	51.71 ± 77.41	53.08 ± 84.21	12.4* ± 17.04

Data are means ± SD; * - values statistically significant at $p < 0.05$.

Eggs of insects exposed to mancozeb revealed some malformations: diminution (Fig. 5a) and crackings (Fig. 5b) within the exochorion layer. The changes were observed within aeropylar and micropylar region (Fig. 5d). The intensity of malformations seem to be concentration-dependent (Compare Fig. 5b and 5c).

4. Discussion

Organisms meet various xenobiotics in their environment. Some of them are designed to fight particular species as pests, but very often these organisms are non-target species. In this case, they may be killed by the poisonous substances or they
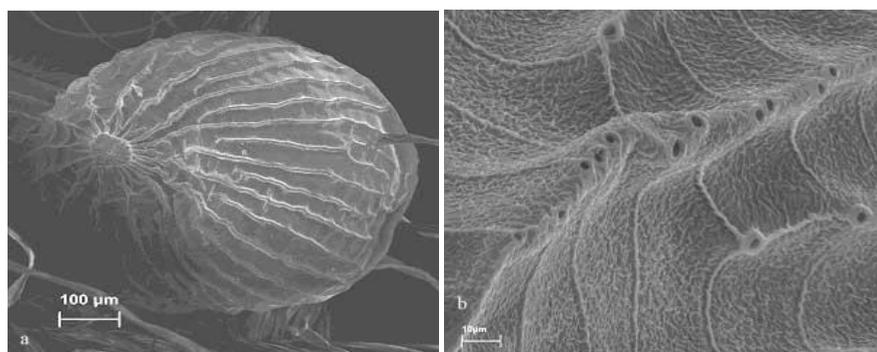
Figure 4. Egg of *A. segetum* is spherical in shape (a) with micropylar region at the top (a) and numerous aeropyle (b).

3.4 Effect of Mancozeb on Fat Body Tissue – Transmission Electron Microscopy

Typical trophocytes of fat body contain large lipid granules, centrally located nucleus, which is surrounded by cytoplasm reach in mitochondria (Fig. 6). These cells play very important role in storage of fats, proteins and carbohydrates.

Cells of organisms exposed to mancozeb revealed various alterations (Fig. 7). Nuclear endoplasmic reticulum was swollen, glycogen granules were less frequent. Cytoplasm was much more electron lucent, than in the control group. Also cell-to-cell adhesion was disturbed, we could observe large intercellular spaces. These changes seemed to be concentration-dependent.

can survive. However, the chemicals to which they were exposed to may affect their biochemical pathways, alter physiological balance, cause malformations and malfunctions and affect behaviour. This kind of effects may often be unnoticed, due to their subtle nature and sometimes postponed appearance of effects. Pesticides, the only xenobiotics that are spread intentionally by humans in the environment, may very often lead to sublethal poisoning of nontarget species. Therefore, these effects should be carefully studied.

Mancozeb is a fungicide, widely used in Europe. Therefore, many species may be exposed to this chemical. We wanted to check, if non target species, can be seriously endangered by this fungicide. Since it is used against fungi, it is probably non-lethal to the majority of individuals within exposed species.
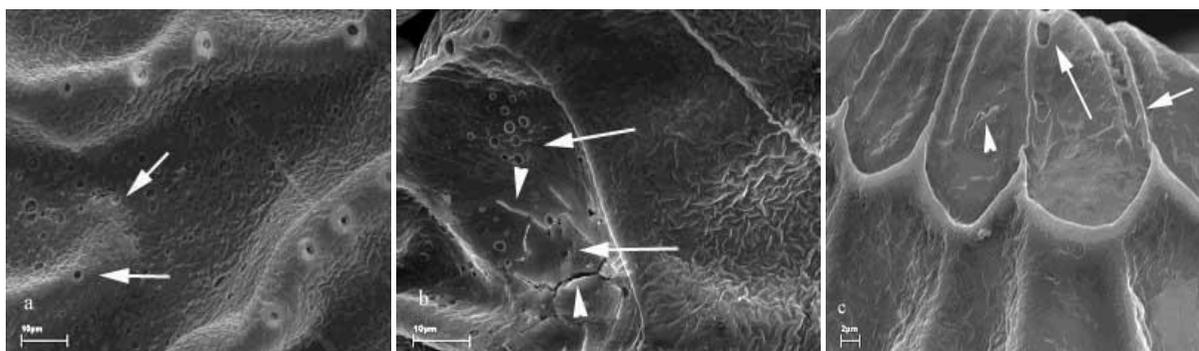
Figure 5. Malformations of eggs laid by insects exposed to mancozeb during their larval development. Note diminutions of the exochorion (a, b, c, arrows), crackings of the chorion (b, c arrowhead). a-b) aeropylar region, c) micropylar

Indeed, *A. segetum* larvae did not show high mortality. 10-15% mortality could be unnoticed in the environment. However, even such "low" mortality may be crucial for the exposed population. Next, gentle sublethal changes may be used as bioindicators of the general exposure of the community to any chemical.

One of the first sublethal effects we could observe was increased length of larval development. All exposed groups showed longer mean larval development. Moreover, this effect was not concentration-dependent. Previously, we reported the same effect caused by the other pesticide – fenitrothion, an organophosphate insecticide – which affected development of *Spodoptera exigua* moths (Adamski et al. 2009a). Also that effect was not concentration-dependent. Therefore, this effect seems to be universal. As we previously proposed, the main idea of such a strategy is to "wait" till/if the environment become clean and not to undergo pupal ecdysis, which is one of the critical moments, when insects are susceptible to xenobioticx (Adamski and Ziemnicki 2004, Adamski and Ghiradella in press). This was also proved by the decreased pupation reported in this study. We noticed delayed appearance of pupation in all groups. The first quarter of pupa appeared significantly later, than in control. However, later on, the next pupae were noticed earlier than in the control group. This may be due to selective pressure of mancozeb: weaker larvae died and did not enter pupation. The stronger individuals survived the stress and when they did not feed (before ecdysis) they could detoxify the fungicide and pupate. Control contained whole range of individuals – from very susceptible, to very resistant ones. Therefore, the weaker insects pupate later than the selected, strong ones in exposed groups. This hypothesis is supported by concentration-dependent final effect (i.e. 95% of pupae).

Table 3. Size of *A. segetum* eggs and their particular regions.

| Concentration of mancozeb	Diameter of eggs [μm]	Diameter of rosettae [μm]	Mean lenght of "cell" of rosettae [μm]	Mean width of "cell" of rosettae [μm]
Control	611.17±45.43	65.42 ± 6.92	33.77 ± 4.87	13.54 ± 2.30
M1	600.35±51.63	69.49 ± 7.88	32.88 ± 4.28	10.87 ± 1.98
M2	608.64±70.41	66.52 ± 5.61	30.62 ± 4.28	12.08 ± 2.01
M3	591.88±78.19	58.74 ± 6.67	29.37 ± 6.67	10.88 ± 0.08

Eggs were laid by females fertilized by the males from the same group (Control ♀ x control ♂, M1 ♀ x M1 ♂, M2 ♀ x M2, M3 ♀ x M3 ♂). No statistically significant differences were observed.
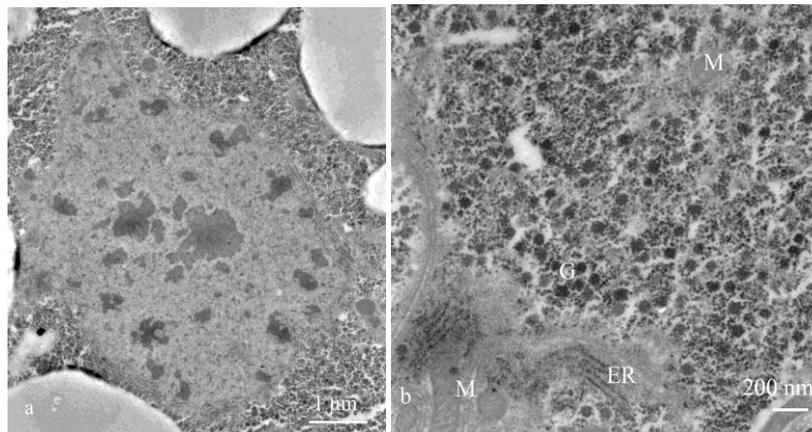
Figure 6. Control fat body cells of *A. segetum*. Note central nucleus with surrounding cytoplasm and lipid droplets (a). Perinuclear cytoplasm is reach in mitochondria, endoplasmic reticulum, as well as numerous glycogen granules (b). (L) - lipid droplets, M – mitochondria, N – nucleus, ER – endoplasmic reticulum, G – glycogen granules.

The observed decreased fecundity is a frequent effect of pesticides (Seth et al. 2004, Wang et al. 2005, Pineda et al. 2007, Adamski et al. 2009b, Pineda et al. 2009). It can result from two different phenomena. First, xenobiotics often affect physiological processes, including mating behaviour (Biddinger and Hull 1999, Seth et al. 2004), egg formation and egg laying (Fila et al. 2002, Adamski et al. 2009c). Next, the egg laying may be postponed due to contamination of environment. Females do not lay eggs in the improper environment. If the concentration of a given xenobiotic had reduced in time, the next generation would live in better environment. Since the development of insects may be quite fast, postponed egg laying reduces interspecyfic comptetition, too. The physiological malfunctions are rather clear in the reported case. We saw numerous malformations of the eggshell. They were of the same type we could observe in case of fenitrothion (Fila et al. 2002, Adamski et al. 2005b, Adamski et al. 2009b). Probably they are due to malfunctions of follicle cells, what was described previously for fenitrothion (Adamski et al. 2009c). The observed diminutions and crackings may not lead to direct mortality of embryos but in the environment the improper eggshell can lead to increased predation or parasitism or disturb internal environment of the egg.
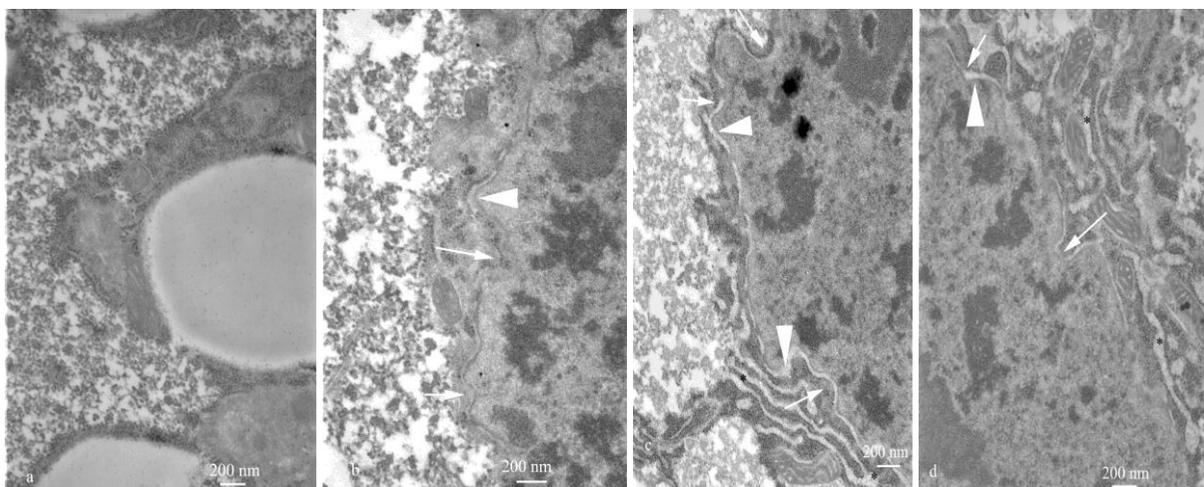
Figure 7. Malformations of fat body cells of larvae exposed to mancozeb. Note electron lucent cytoplasm (a, b), invaginations and swelling of nuclear envelope (b - d, arrows and arrowheads, respectively), few granules of glycogen (a-d), swelling of ER (c, d, asterisks).

However, the stress did not affect the size of eggs. Probably, the females prefer to produce less eggs, but of the highest possible quality. This observation differs from the one observed by Yin and co-workers (2009), who observed decreased size and number of eggs laid by *Plutella xylostella* moths exposed to spinosad. It is hard to compare the two research, which focus on two different chemicals. Spinosad is an insecticide that causes hyperactivity of nervous system and is used against insects as target species. Perhaps, the slight, insignificant decrease of the egg size is due to weak, sublethal effect. However, we cannot test much higher concentrations of mancozeb – they would by much over environmental practice.

Observations in TEM showed, that mancozeb causes multilevel alterations, within various tissues and systems. Fat body is one of the most important structures of insects. They store fats, proteins and carbohydrates. With this tissue insect may starve for some time and thus limit amount of poisons ingested by larvae. Next, fats are backbones for insects' hormones. Therefore, disturbances within this tissue may affect whole body metabolism and physiology, including observed inhibited molting and altered development. The observed malformations are similar to those caused by fenitrothion and carbaryl (carbamate insecticide) in *Spodoptera exigua* and *Tenebrio molitor* fat body (Mehlhorn et al. 1999, Adamski et al. 2005a, c, Adamski 2007). Therefore, they seem to be rather universal, caused by chemical imbalance within cells, not direct action of pesticides on target tissues and cells. TEM observations revealed various malformations of fat body cells. Changes of nucleus must have caused disturbances in cellular metabolism. Loss of glycogen granules suggest that insects starved. That let them to limit the amount of digested toxicant and survive the stress. However, starvation might have led to other physiological problems, which affected growth and development. The above mentioned changes are similar to those reported by Sakr et al. (2005) for mice exposed to mancozeb. These authors reported irregularities of nuclear structure, that led to apoptosis, loss of glycogen, dilated ER. Such changes obviously slow down activity of cells. Therefore, activity of fat body may be decreased. If decreased weight of fat body, reported for *S. exigua* (Adamski and Ziemnicki 2004), is an universal phenomenon, the activity of fat body and its effect on insect's development would be drastically decreased. It is worthy to stress, that these changes were observed after four days of exposure. Ultrastructural changes may slowly lead to other changes, which affect development and reproduction. An important aspect of observed ultrastructural changes is the day of these observations. At fourth day of exposure the mortality within all groups equaled zero. Since that, microscopic observations may be used as bioindicators of stress.

Answering the questions raised in the first chapter, we have to point out on the low susceptibility of *A. segetum* to mancozeb as a lethal xenobiotic. However, as non-target species, these insects are exposed to significant stress, which causes a variety of sublethal effects. These effects seem to be correlated, result as consequences of the other ones. Ultrastructural malformations cause disturbances of development and later, of reproduction. Mancozeb can cause delayed sublethal effects and lead to malformations and/or malfunctions at later stages of development, lead to reduced reproduction. In consequence, they make exposed population weaker. To sum up, we have to emphasize the need of careful and detailed studies of xenobiotics both before introducing these substances to the environment and during their application.

5. References

Adamski, Z. 2007. Exposure to carbaryl leads to ultrastructural changes and alters activity of antioxidant enzymes in *Spodoptera exigua* (Lepidoptera: Noctuidae). *Inv. Biol.,* 126: 191–201.

Adamski, Z. Machalska, K., Chorostkowska, K., Niewadzi, M., Ziemnicki, K., Hirsch, HVB. 2009b. Effects of sublethal concentrations of fenitrothion on beet armyworm (Lepidoptera: Noctuidae) development and reproduction. *Pestic. Biochem. Physiol.*, 94: 73–78.

Adamski, Z., Banaszkiewicz, M., Ziemnicki, K. 2005a. Ultrastructural alterations induced by fenitrothion on fat body cells and midgut cells of *Tenebrio molitor* L. (Insecta, Coleoptera) larvae. *J. Biol. Res.*, 3: 15-22.

Adamski, Z., Fila, K., Maćkowiak, J., Ziemnicki, K. 2005c. Fenitrothion-induced cell malformations in vitro in *Spodoptera exigua* cell line UCR-Se-1. *Biol. Lett.*, 42: 41-47.

Adamski, Z., Ghiradella, H. Sublethal Effects of Pesticides: Their Impairment of Biology and Physiology of Exposed Moths and their Unexposed Progeny. In: Cauterruccio L. [ed], Moths: Types, Ecological Significance and Control Methods. Nova Science Publishers, Inc., New York (in press).

Adamski, Z., Korolczuk, D., Purgiel, M., Grewling, A., Ratajczak, M., Bankiet, M., Ziemnicki, K. 2009c. Effect of fenitrothion on *Spodoptera exigua* larval development and ultrastructure of follicle cells. *Biologia*, 64: 197—202.

Adamski, Z., Niewadzi, M., Ziemnicki, K. 2005b. Inheritance of chorionic malformations and insecticide resistance by *Spodoptera exigua*. *J. Appl. Entomol.,* 129: 526 – 533.

Article I.   Adamski, Z., Ziemnicki, K. 2004. Side-effects of mancozeb on *Spodoptera exigua* (Hübn.) larvae. *J. Appl. Entomol.,* 128: 212–217.

Adamski, Z., Ziemnicki, K., Fila, K., Žikić, R., Štajn, A. 2003. Effects of long-term exposure to fenitrothion on *Spodoptera exigua* and *Tenebrio molitor* larval development and antioxidant enzyme activity. *Biol. Lett.,* 40: 43-52.

Adamski, Z., Ziemnicki, K., Marciniak, P., Halamunda, J., Nawrocka, M., Lelario, F., Scrano, L., Bufo, S. 2009a. Effect of Various Xenobiotics on Hatching Success of *Spodoptera exigua* Eggs as Compared to a Natural Plant Extract. *J. Toxicol. Env. Heal. A.,* 72: 1132–1134.

Auger, P., Kreiter, S., Mattioda, H., Duriatti, A. 2004. Side effects of mancozeb on Typhlodromus pyri (Acari: Phytoseiidae) in vineyards: results of multi-year field trials and a laboratory study. *Exp. Appl. Acarol.,* 33: 203 – 213.

Baligar, PN., Kalliwel, BB. 2001. Induction of gonadal toxicity to female rats after chronic exposure to mancozeb. *Ind. Health*, 39: 235–243.

Biddinger, DJ., Hull, LA. 1999. Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: Tortricidae). *J. Econ. Entomol.,* 92: 314-324.

Büyükgüzel, E. 2009. Evidence of oxidative and antioxidative responses by *Galleria mellonella* larvae to malathion. *J. Econ. Entomol.,* 102: 152-159**.**

David, W., Ellaby, S., Taylor, G. 1975. Rearing *Spodoptera exempta* on semi-synthetic diets and on growing maize. *Entomol. Exp. Appl.,* 19: 226–236.

Dorcas, B., Polar, L., Tag, ED., Mahmoud, H., Eross, K., Wing, KD. 1992. Developmental disturbances in different insect orders caused by an edy steroid agonise, RH 5849. *J. Econ. Entomol.,* 85: 2107-2112.

Extoxnet 1996.

http://extoxnet.orst.edu/pips/pyrethri.htm

Fila, K., Adamski, Z., Ziemnicki, K. 2002. Exposure to Fenitrothion causes malfunctions of *Spodoptera exigua* Hübn. (*Lepidoptera: Noctuidae*) eggs. *J. Appl. Entomol.,* 126: 114-118.

Hreljac, I., Zajc, I., Lah, T., Filipic, M. 2008. Effects of model organophosphorous pesticides on DNA damage and proliferation of HepG2 cells. *Environ. Mol. Mutagen.,* 49: 360-367.

Hyršl, P., Büyükgüzel, E., Büyükgüzel, K. 2008. Boric acid-induced effects on protein profiles of *Galleria mellonella* hemolymph and fat body. *Acta Biol. Hung.,* 59: 281-288.

Jenson, EA., Arthur FH., Nechols JR. 2009. Efficacy of methoprene applied at different temperatures and rates on surface substrates to control eggs and fifth instars of *Plodia interpunctella*. *J. Econ. Entomol.,* 102: 1992-2002.

Kajiura Z., Nakagaki, M., Takei, R. 1993. Spermiogenesis of the testes of juvenile hormone-treated silkworm larvae, *Bombyx mori. Comp. Biochem. Physiol. A,* 106: 495-499.

Khebbeb, MEH., Gaouaoui, R., Bendjeddou, F. 2008. Tebufenozide effects on the reproductive potentials of the mediterranean flour moth, *Ephestia kuehniella. Afr. J. Biotechnol.,* 7: 1166-1170.

Mehlhorn, H., Mencke, N., Hansen, O. 1999. Effects of imidacloprid on adult and larval stages of the flea *Ctenocephalides felis* after in vivo and in vitro application: a light- and electron-microscopy study. *Parasitol. Res.,* 85: 625–637.

Miles, M, Kemmitt, G. 2005. Field studies to determine mancozeb based spray programmes with minimal impact on predatory mites in European vine cultivation. *Commun. Agric. Appl. Biol. Sci.,* 4: 559-567.

Osorio, A., Martínez, AM., Schneider, MI., Díaz, O., Corrales, JL., Avilés, MC., Smagghe, G., Pineda, S. 2008. Monitoring of beet armyworm resistance to spinosad and methoxyfenozide in Mexico. *Pest Manag. Sci.* 64: 1001-1007.

Pesticide Properties DataBase 2009. http://sitem.herts.ac.uk/aeru/projects/ppdb/index.htm.

Pineda, S., Martínez, AM., Figueroa, JI., Schneider, MI., Del Estal, P., Viñuela, E., Benjamín Gómez, B., Smagghe, G., Budia, F. 2009. Influence of Azadirachtin and Methoxyfenozide on Life Parameters of *Spodoptera littoralis* Lepidoptera: Noctuidae). *J. Econ. Entomol.,* 102: 1490-1496.

Pineda, S., Schneider, MI., Smagghe, G., Martínez, AM., Del Estal, P., Viñuela, E., Valle, J., Budia, F. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad. *J. Econ. Entomol.,* 100: 773-780.

Sadeghi A., Smagghe, G., Broeders, S., Hernalsteens, JP., De Greve, H., Peumans, WJ., Van Damme, EJ. 2008. Ectopically expressed leaf and bulb lectins from garlic (*Allium sativum* L.) protect transgenic tobacco plants against cotton leafworm (*Spodoptera littoralis*). *Transgenic Res.,* 17: 9-18.

Sakr, SA., Mahran, HA., Abo-Elyazid, SM. 2005. Effect of DBB on mancozeb fungicide induced ultrastructural and biochemical changes in the liver of albino mice. *Proc. 9th Conf. Environ. Sci. Tech.,* pp. 809-816.

Seth, RK., Kaur, JJ., Rao, DK., Reynolds, SE. 2004. Effects of larval exposure to sublethal concentrations of the ecdysteroid agonists RH-5849 and tebufenozide (RH-5992) on male reproductive physiology in *Spodoptera litura*. *J. Insect Physiol.,* 50: 505-517.

Soltani, N., Soltani-Mazouni, N. 1992. Diflubenzuron and oogenesis in the codling moth, *Cydia pomonella* (L.). *J. Pestic. Sci.,* 34: 257–261.

Wang, AH., Wu, JC., Yu, YS., Liu, JL., Yue, JF., Wang, MY. 2005 Selective insecticide-induced stimulation on fecundity and biochemical changes in *Tryporyza incertulas* (Lepidoptera: Pyralidae). *J. Econ. Entomol.,* 98: 1144-1149.

Wei, H., Huang, Y., Du, J. 2004. Sex pheromones and reproductive behavior of *Spodoptera litura* (Fabricius) moths reared from larvae treated with four insecticides. *J. Chem. Ecol.,* 30: 1457-1466.

Yang, Z., Du, JW. 2003. Effects of Sublethal Deltamethrin on the Chemical Communication System and PBAN Activity of Asian Corn Borer, *Ostrinia furnacalis* (Güenee). *J. Chem. Ecol.,* 29: 1611-1619.

Yin, XH., Wu, QJ., Li, XF., Zhang, YJ., Xu, BY. 2009. Demographic changes in multigeneration *Plutella xylostella* (Lepidoptera: Plutellidae) after exposure to sublethal concentrations of spinosad. *J. Econ. Entomol.,* 102: 357-365.

Zhou, H., Du, J., Huang, Y. 2005. Effects of Sublethal Doses of Malathion on Responses to Sex Pheromones by Male Asian Corn Borer Moths, *Ostrinia furnacalis* (Guenée). *J. Chem. Ecol.,* 31: 1645-1656.