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Antioxidative responses in females and males of the spider *Xerolycosa nemoralis* (Lycosidae) exposed to natural and anthropogenic stressors

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ABSTRACT

The aim of this study was to assess the intensity of enzymatic antioxidative parameters [i.e., superoxide dismutase (SOD), catalase (CAT), and the glutathione peroxidases each selene dependent, GPOX or selene independent, including GSTPx, glutathione S-transferase, and GST] and non-enzymatic antioxidative parameters [i.e., glutathione total (GSH-t), the heat shock proteins of Hsp70, and metallothioneins (Mt)] in the midgut glands of female and male wolf spiders *Xerolycosa nemoralis* (Lycosidae) exposed to natural stressors (i.e., heat shock and starvation) and anthropogenic stressors (i.e., the organophosphorous pesticide dimethoate) under laboratory conditions. The spiders were collected from two differentially polluted sites both localized in southern Poland: Olkusz, which is heavily polluted with metals, and Pilica, the reference site. In response to the stressing factors, increases in Hsp70 levels, in the concentrations of total glutathione and in the activity levels of glutathione-dependent enzymes (GPOX, GSTPx, and GST) were found in the midgut glands of males. In the females, high levels of activity of CAT and SOD were revealed, as well as an increased percentage of Mt-positive cells. Preexposed females, in comparison to the individuals from the reference site, responded with increased SOD activity, irrespective of the stressing factor. In contrast, the changes in the antioxidative parameters in the midgut glands of male *X. nemoralis* seem to reflect a short-term reaction to the applied stressors and do not confirm the effects of long-term selection in a polluted environment.

1. Introduction

Organisms in their environments are subject to various stressors. These stressing factors, depending on their intensity and duration, can modify the physiological processes of organisms and, under extreme conditions, can decrease their ability to survive. The stressors can be either natural, e.g., too high or too low temperatures, humidity, food availability, and interactions with other species, or anthropogenic, e.g., chemicals. All of these elements contribute to the term "environmental stress", which is understood as a set of stressing factors or as the response of an organism to the stressing factors (van Straalen, 2003).

Stressors may enhance the rates of the endogenic production of reactive oxygen species. Prooxidative responses were found to be typical for anthropogenic stressors, such as metals (Kang, 1997; Lagadic, 1999; Pulido and Parrish, 2003), pesticides (Masoud et al., 2003), polycyclic aromatic hydrocarbons (PAHs) (Cruz-Rodriguez and Chu, 2002), and polychlorinated biphenyls (PCBs) (Rodriguez-Ariza et al., 2003), as well as natural factors, such as extreme thermal conditions (Gorman et al., 1999) or food deficiency and starvation (Pascual et al., 2003; Morales et al., 2004).

The tolerance of organisms to stressing factors is possible because of defensive mechanisms that are aimed at the maintenance of internal homeostasis. The systems of antioxidative defense in vertebrates and invertebrates include, among others, superoxide dismutase (SOD) and catalase (CAT), as well as glutathione (GSH) and enzymes connected with the metabolism of tripeptides, including the glutathione peroxidases: selene dependent, GPOX and selene independent (GSTPx; glutathione S-transferase, GST) (Ahmad, 1995; van Bladeren, 2000). Stress proteins, including heat shock proteins (Hsp) and metallothioneins (Mt), can also play an antioxidative role, which was revealed in numerous animal groups. The induction of these proteins in response to specific stressors has been documented in animals both in laboratory studies (Berntssen et al., 2001; Chabicovsky et al., 2004) and field studies (Köhler et al., 1999a,b; Carpenter and Hofmann, 2000; Hamer et al., 2004).

Relatively scarce information about the antioxidative defensive reactions of spiders has been collected. The majority of the data have been obtained from studies on individuals collected directly from the field. According to these studies, the antioxidative reactions of these predators to metal pollution, similar to other enzymatic detoxifying reactions, are species specific (Wilczek et al., 2003, 2004; Wilczek, 2005; Wilczek et al., 2008). Funnel web spiders *Agelena labyrinthica* (Agelenidae) from areas heavily polluted with zinc and lead were characterized by high

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levels of GPOX and GSTPx and by a high concentration of glutathione in their tissues. Under the same conditions, actively hunting spiders, such as *Pardosa lugubris* (Lycosidae), had lower concentrations of total glutathione and lower activity levels of the glutathione peroxidases than the web-building spiders (Wilczek et al., 2004). In addition, the results of studies on another lycosid spider, *Pardosa palustris,* suggested that high concentrations of Zn, Pb and Cd in the bodies of these spiders were correlated with a high CAT activity. In comparison, money spiders, *Linyphia triangularis* (Linyphiidae), had enhanced antioxidative reactions that were associated with the activity of SOD and CAT and low metal concentrations (Wilczek and Migula, 1996).

In response to the controlled actions of various stressing factors, it has been demonstrated that the exposure of *P. lugubris* (Lycosidae) to heat shock or to organophosphate pesticide resulted in low frequencies of apoptotic and necrotic changes in its midgut glands. In contrast, in *A. labyrinthica* (Agelenidae), the number of cells that overcame apoptosis or necrosis increased under the same experimental conditions. Significantly higher numbers of cells reacting positively with anti-metallothionein and anti-Hsp antibodies, as well as high activity levels of superoxide dismutase and catalase in the midgut glands of actively hunting spiders, indicated that high temperatures and the pesticide may contribute to the development of tolerance to environmental stressors in individuals of the analyzed spider species (Wilczek, 2005). In turn, the exposure of *A. labyrinthica* to cadmium and copper under laboratory conditions demonstrated that the level of Mt increased significantly mainly in females that were exposed to both metals, irrespective of the degree of pollution at their origin site, which indicates a defensive role for these proteins. In general, the Mt level was positively correlated with the Cd and Cu concentrations in the spider body (Babczyńska et al., 2011).

In the present study, we concentrated on assessing the intensity of enzymatic and non-enzymatic parameters that indicate the efficiency of antioxidative defenses in the midgut glands of female and male wolf spiders *Xerolycosa nemoralis* (Lycosidae) exposed to natural stressors (i.e., heat shock and starvation) and anthropogenic stressors (i.e., the organophosphorous pesticide dimethoate) under laboratory conditions. *X. nemoralis* is a palearctic species that is widespread in northern Europe. The individuals of this species inhabit the edges of limestone deposits, heatlands, grasslands, woodlands and clearings. These spiders do not build webs; instead, they actively search for their potential prey. They hunt based on the vibrations of the ground that are caused by the prey's movement. They eat mainly the larvae of Diptera, Collembola and, more rarely, Coleoptera and Hymenoptera (Locket and Millidge, 1951; Varol et al., 2006).

The stressing factors were chosen such that they reflected the potential dangers the spiders could be exposed to in their natural habitats. The obtained results provide a strong basis for establishing the possible differences in the sensitivities of female and male wolf spiders to specific stressors. Comparisons among the levels of the analyzed parameters in individuals from areas differentially polluted with heavy metals enabled us to assess if exposure to pollutants changes the types and degrees of antioxidative reactions of actively hunting *X. nemoralis* in response to sudden contact with additional stressing factors.

2. Materials and methods

2.1. Sites

The spiders were collected from two meadow sites in southern Poland. The reference, unpolluted site was in the community of Pilica (50°28′ N,19°39′ E, Silesian Voivodship), far from major industrial plants. The other site, heavily polluted with metals, was located in the western part of Kraków–Częstochowa Upland, near Olkusz town (50°17′ N, 19°34′ E). The analyses of metal contents in the humus layer revealed that the concentration of copper in the polluted site was 4 times higher than in the same layer of the reference site. In addition, the concentration of lead was 20 times higher, that of zinc was 70 times higher, and that of cadmium was 100 times higher (Stone et al., 2001, 2002). The high levels of these elements in the upper layers of the soil result both from natural ore-bearing dolomites of high metal concentrations and from human activity (e.g., the mine and smelter plant Bolesław, which are located nearby).

2.2. Spiders

The investigations were conducted on adult males and females of the non-web building spider *X. nemoralis* (Lycosidae) (Westring, 1861). Adults (♀4.5–7.5 mm, ♂4.5–6.0 mm) appeared toward the end of May and in June. Female and male spiders were hand-caught and placed directly into glass tubes. In the laboratory, the spiders were kept in plastic containers in rearing chambers with a standard photoperiod (14L:10D), temperature (L:25 °C, D:15 °C) and humidity (70% ± 10%). Every day, the spiders were fed with the larvae of *Acheta domesticus* (at the II and III larval stages) and adult *Drosophila melanogaster*. The spiders from each experimental group were also given drinking water.

2.3. Experimental groups

In the laboratory, the spiders were exposed to natural stressing factors (T — heat shock, S — starvation) and to anthropogenic stressing factors (D — the organophosphorous pesticide dimethoate), acting separately or in combinations of the two (e.g., T + D — temperature and dimethoate). The thermal conditions were selected based on the data presented by Pulz (1987) and Schmalhofer (1999), as well as on personal experience (Wilczek, 2005). Applying the values of the critical maximal temperature (CT_{max}), namely the highest temperature at which an animal is still able to move, the spiders from T group were exposed to 45 °C for 5 min daily for 5 days.

The spiders from group D were exposed daily to a solution of dimethoate that was administered by spraying on the prosoma. Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) is a moderately toxic compound in the EPA toxicity class II and is a General Use Pesticide (GUP) (WHO, 1989). As an organothiophosphorus cholinesterase inhibitor, it is still used against a wide range of insects and mites. The pesticide is a commercially formulated product formed with 400 g of active ingredient (Bi_R58; Bayer) diluted in distilled water to provide the required dose in 1 μL of solution. The dose per day per specimen was 0.16 μg of active substance in 1 μL of solution, which is a sublethal dose for the analyzed spiders (Toft and Jensen, 1998; Wilczek, 2005). Considering the duration of the toxic activity of dimethoate (up to 2–3 weeks after the field application), the application of the pesticide was repeated five times under the assumption that, under natural conditions, chronic contact with such substances cannot be avoided. Because the active ingredient of dimethoate is water soluble, the individuals from the control group were treated with 1 μL of water dropped on an upper side of the prosoma once a day for 5 days.

The spiders from the T + D group were exposed to a combination of heat shock and dimethoate. Each time, the sequence of application was the same: heat shock followed by the pesticide.

Spiders from the S-group were not fed for 14 days, but, as with the spiders from the remaining experimental group, they had unlimited access to drinking water. The duration of starvation was chosen randomly with the assumption that its length could be possible under natural conditions, especially if the hunting activity of these predators is determined by weather conditions.

During the experiment, the mortality of spiders both in the control group and in the groups exposed to the stressors was 5% on average.

2.4. Cytometric and spectrophotometric analyses

Twenty-four hours after the last exposure to the respective stressing factors, the individuals from the T, D and T + D groups, as well as the