

Antioxidant defence based on glutathione in grain aphid (*Sitobion avenae* (F.) and the bird cherry-oat aphid *Rhopalosiphum padi*: Responses to the host plant alteration

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ABSTRACT

We studied the effects of host plants on the level of glutathione (GSH) and GSH-based enzymes in tissues of *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.). We found that the transfer of aphids from winter wheat to the winter triticale caused significant decrease in GSH contents in their tissues. The activities of GSH-dependent enzymes - glutathione S-transferase (GST), Se-independent glutathione peroxidase (GST_{px}) and glutathione reductase (GR) were induced after altering the one's diet. The less susceptible triticale Witon cultivar affected the GSH content and GST_{px} activity in *S. avenae* more than the more susceptible triticale Tornado cultivar Witon cultivar. GST and GR activities in cereal aphids were similarly affected by the two studied triticale cultivars. Our results indicated that modulation of antioxidant mechanisms based on GSH plays major role in the interactions between the aphids and their host plants.

Key words: Cereal aphids, glutathione, glutathione reductase, glutathione transferase, oxidative stress

INTRODUCTION

Aphids are serious plant pests worldwide. In Poland, two cereal aphid species dominate on cereals: monophagous *Sitobion avenae* (F.) and oligophagous *Rhopalosiphum padi* (L.) (19). *R. padi* arrives first in the season, whereas, *S. avenae* usually arrives later, when plants are in the late tillering or stem-elongation stage (17,45). *S. avenae* and *R. padi* are vectors of BYDV (Barley yellow dwarf virus). The harmfulness of cereal aphids is associated with changes in the metabolism of host plants.

Phloem-feeding insects including the aphids, cause less injuries to plant foliage than chewing insects. Therefore, the sucking-piercing insects induce the same defence-signalling pathways that are activated by pathogens, such as fungi, viruses and bacteria (42). Plants respond to phloem-feeders attack with production of reactive oxygen species (ROS) (30). ROS, especially hydrogen peroxide (H₂O₂), plays multiple roles in plant resistance, they show direct toxicity to herbivores and act as signal molecules to induce the defense genes (7,21,46). The generation of ROS is consequence of biochemical reactions

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in aerobic organisms. Metabolism of various endogenous and exogenous compounds may also produce ROS. The balance between beneficial and harmful effects of ROS is very important, because accumulation of ROS caused damage may lead to aging and death. Among protective factor against ROS, is tripeptide glutathione (GSH), which is responsible for maintaining redox balance inside cells and may directly react with ROS (40). The rapid increase of ROS in plants may cause oxidative stress in herbivores. ROS react with biomolecules [proteins, lipids, DNA and RNA], damaging their structures. For herbivores, lipid peroxidation is very harmful as lipids are not only the components of membranes, but also have unique physiological functions juvenile hormones and sex-attractant pheromones (15).

To protect the herbivores from oxidative stress, glutathione and glutathione-dependent enzymes [glutathione transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR)] play important role. GSTs are a family of multifunctional enzymes involved in detoxification of many xenobiotics. They catalyse the conjugation of electrophilic compounds with the thiol group of GSH and resultant products are more water soluble than substrates (16). Among the substrates of GST, there are allelochemicals with pro-oxidant properties suitable for production of ROS. In mammalian tissues, the major enzyme reducing the H₂O₂ and organic peroxides is Se-dependent glutathione peroxidase (GPX). In this reaction glutathione (GSH) is transformed to oxidized form (GSSG). Glutathione reductase (GR) doesn't utilize ROS directly, but it interacts with GPX by converting GSSG to the reduced form (43). The herbivorous insects have very low level of GPX activity (2), which may be compensated by the presence of glutathione S-transferase with specific peroxidase activity (GST_{px}) targeting hydroperoxides, but not H₂O₂ (20). Weinhold *et al.* (44) noted the GST_{px} activity in 3-species of *Lepidoptera* [*Trichoplusia ni* (Hübner), *Papilio polyxenes* (F.) and *Spodoptera eridania* (Stoll)]. We have examined the activity of GST_{px} and GR in tissues of two species of cereal aphids: *S. avenae* and *R. padi* and the inhibitory effects of phenols on these enzymes (28). Our previous studies have focused mainly on the effect of plant pro-oxidants *in vitro* on glutathione-dependent defence mechanisms of aphids (26,28). Since the host plant may affect the antioxidant status of herbivores. This study was done to investigate the effects of diet alteration (from wheat to triticale) on antioxidant enzymes based on glutathione in cereal aphids.

MATERIAL AND METHODS

Aphids: The insects came from the aphid stock cultures kept in our University of Natural Sciences and Humanities, Siedlce. The aphids were reared on seedlings of winter wheat cv. Tonacja, in an environmental chamber (21 °C, L16:D8 photoperiod, and 70% r.h.). Experiments were conducted on wingless females (*apterae*) of the grain aphid and the bird cherry-oat aphid.

Plants: Two cultivars of winter triticale (*Triticum secale* Wittm.) characterized by different degree of susceptibility to cereal aphids were chosen for experiments: Tornado (more susceptible) and Witon (less susceptible). Plants were cultivated in a climate chamber [21 ± 1°C and 70% relative humidity (HR) 16 h photoperiod]. The seedlings were grown in

plastic pots (10 x 10 cm, 8 seedlings per pot) filled with medium nutrient fine structure compost with sand.

Infestation procedure: Nine-day-old seedlings of triticale were infested each with 20 aphids obtained from wheat. After feeding the aphids for 24, 48, 72 and 96 h on triticale seedlings, they were collected and taken for assays of glutathione and glutathione-dependent antioxidant enzymes. Aphids collected from the winter wheat at time of transfer were used as control. The experiment was conducted in four replicates.

Bioassays: Total glutathione concentration in aphid tissues was determined with an enzymatic recycling assay based on glutathione reductase (18). Briefly, 200 aphids collected from several pots were homogenized in 50 mM phosphate buffer pH 7.8 containing 0.2 M EDTA and centrifuged at 3000 *g* for 15 min. The obtained supernatants were recentrifuged after addition of 50% TCA. Content of glutathione was determined after mixing 0.5 mL of the aphid extracts, 0.3 mL of 0.3 mM NADPH, 0.1 mL of 6 mM DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)] and 0.1 mL of GR (50 units/mL). The absorbance at 412 nm was recorded after 5 min at room temperature. The GSH content within the aphid homogenates was determined by comparing the change in rate of absorbance to that of glutathione standards of known concentrations and was expressed as nmol per mg of protein.

Activity of glutathione S-transferase was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as per Leszczyński and Dixon (23). One hundred aphids, collected from several pots, were homogenized in 0.2M Na-phosphate buffer pH 6.5 and centrifuged at 3000 *g* for 15 min. The obtained supernatants were used for assay of GST. The reaction mixture contained 1 ml of crude supernatant of aphids and 0.05 ml of 50 mM reduced glutathione (GSH). After incubation for 5 min at 25°C, 0.025 ml of 40 mM CDNB was added into the mixture and absorbance at 340 nm was recorded for 5 min. Activity of GST was expressed as nmol CDNB conjugated/min/mg protein, using extinction coefficient of 9.6 mM⁻¹ x cm⁻¹ for S-(2,4-dinitrophenyl)glutathione.

GST_{px} was determined as per Ahmad and Pardini (3). One hundred aphids, collected from several pots, were homogenized in 50 mM phosphate buffer pH 7.0 containing 1 mM EDTA and centrifuged at 3000 *g* for 15 min. The obtained supernatants were used for assay of GST_{px}. The reaction mixture consisted of 0.4 ml crude homogenate of aphids, 0.1 ml of 1 mM GSH, 0.2 ml of 1mM NADPH, 0.1 ml of GR solution (5U/ml), and 0.2 ml of 1.2 mM cumene hydroperoxide solution. The reaction was started by the addition of cumene hydroperoxide and then oxidation of NADPH was recorded at 340 nm for 3 min., in comparison to the control containing 0.4 ml of 50 mM K-phosphate buffer pH 7.0 with 1mM EDTA instead of aphid homogenate. GST_{px} was determined by comparing the change in the rate of absorbance and expressed as nmol oxidized NADPH/min/mg protein.

The activity of GR was determined with method of Racker (35). One hundred aphids, collected from several pots were homogenized in 50 mM phosphate buffer pH 7.6 and centrifuged at 3000 *g* for 15 min. The obtained supernatants were used for assay of GR. Firstly, oxidation of NADPH was measured in the reaction mixture containing 0.1 ml of 50 mM phosphate buffer pH 7.6, 0.1 ml of 1 mM NADPH, 0.1 ml of 0.1% BSA, and 0.3 ml of 2% GSSG at 340 nm for 3 min. Then 0.5 ml of crude homogenate of aphids was

added and NADPH oxidation was recorded. GR activity was calculated by subtracting the earlier rate from the latter one and expressed as nmol oxidized NADPH/min/ mg protein.

The protein content in the studied aphid supernatants was determined using the method given by Bradford (13).

Statistical analysis: All data are reported as means \pm SD, $n = 4$, where each replication represents one independent aphid homogenate. Data were subjected to a one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range test.

RESULTS AND DISCUSSION

The high concentration of GSH protects the biomolecules, including proteins, DNA and RNA. The synthesis of a high amount of tripeptide GSH seems to be more economic than synthesis of longer peptide (9,32). In our experiments the higher GSH concentration was recorded in females of *S. avenae* as occurred on winter wheat than on winter triticale. The exposure of *S. avenae* adults to Witon cv. decreased the GSH in their tissues. The highest depletion of GSH was noted after 48 h and 72 h of experiment. Then the GSH level rose and reached 77% of GSH content in control aphids. The transfer of *S. avenae* to Tornado cv. less affected the GSH concentration than transfer to Witon cv. (Fig. 1). After transfer of *R. padi* females to Witon cv. the content of GSH in their tissues decreased, but the decrease was lower than in *S. avenae*. The decrease of GSH was independent on time of feeding on Witon cv. The same trend was observed for insects transferred to Tornado cv. The both studied cultivars similarly affected the GSH content in *R. padi* (Fig. 1). Similar results were obtained by Łukasik (26), where GSH concentration decreased after the exposition of aphids to *o*-dihydroxyphenols with pro-oxidant properties. Barbehenn *et al.* (12) stated that the concentration of antioxidants in the midgut fluids of herbivores depend on the efficiency of its extraction from leaf tissues. The author noted that two species of caterpillars: tannin-tolerant *Orgyia leucostigma* (Smith) and the tannin-sensitive *Malacosoma disstria* (Hübner) contain very high level of GSH, when they are fed on the spring foliage of *Populus tremuloides*. In this time *P. tremuloides* had the highest content of GSH through the vegetation season. It was found that the transferring of *Lymantria dispar* (L.) population which originated from oak forest to the locust leaf diet caused increase of GSH (33). Additionally, the oak leaf didn't contain alkaloids and had a low content of flavonoids. so GSH might increase as response to the plant allelochemicals generated ROS (6). On other hand, the lack of changes in GSH and GSSG content by dietary juglone was found in larvae *Actias luna* (L.) and *Callosamia promethea* (Drury) (40). The consumption of wheat leaves treated with tannic acid didn't affect the concentration of GSH in the midgut of grasshoppers *Melanolus sanguinipes* (F.) and *Aulocara ellioti* (Thomas) (11). Thus changes in GSH content in herbivores may be due to GSH status of their host plants and intensity of ingestion. The absorption of GSH from plant food could be made difficult when the epithelium of midgut was damaged by plant allelochemicals. The decrease of GSH in cereal aphids after alteration of food type could be related with reduced availability of exogenous GSH or with the higher level of pro-

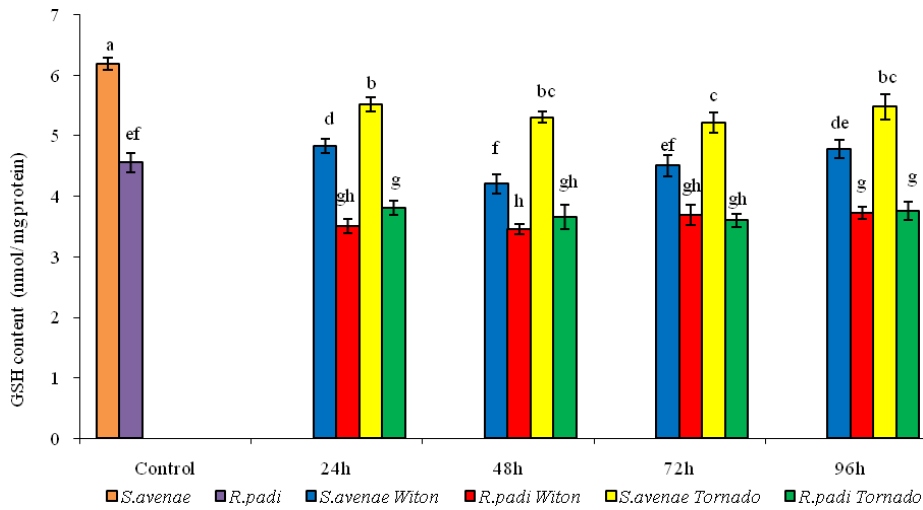


Figure 1. Changes in the content of glutathione (GSH) (nmol/mg protein) in *apterae* morphs of *S. avenae* and *R. padi* after transferring on to winter triticale (control - aphids on the winter triticale Tonacja cv.). Values not followed by the same letter are significantly different at level $p \leq 0.05$ (Duncan's test).

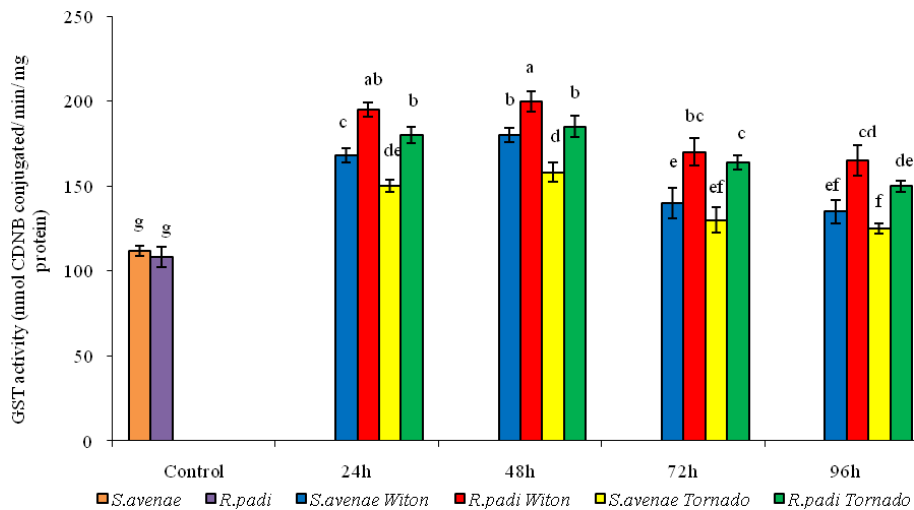


Figure 2. Changes in the GST activity (nmol CDNB conjugated/min/mg protein) within *apterae* morphs of *S. avenae* and *R. padi* after transferring on to winter triticale (control - aphids on the winter triticale Tonacja cv.). Values not followed by the same letter are significantly different at level $p \leq 0.05$ (Duncan's test).

oxidant allelochemicals in tissues of new host plants. Petrić-Mataruga *et al.* (33) suggest that the regulation of the content of GSH in herbivores is a one of the rapid forms of adaptive responses to nutritive stress.

The females of *S. avenae* and *R. padi* present on winter wheat had the same GST activity. The highest induction of GST was observed after 48 h of experiment. The prolonged aphid feeding on triticale (72 and 96 h) decreased the GST activity, but it was still higher than in control aphids. Similar trend was observed for females of *R. padi*, although triticale cultivars affected GST activity stronger in tissues of oligophagous species than monophagous ones (Fig. 2). The activity and distribution of GST have been extensively studied in many insects species, including aphids (8, 23, 24, 31). GST is involved in direct conjugation of GSH with many plant allelochemicals including coumarins, indole alkaloids, flavonoids, phenols and hydroxamic acids (2, 22, 23, 25, 31, 39). The transfer of aphids from wheat to winter caused the induction of GST activity. It is known that different plant xenobiotics can induce or inhibit GST activity in insects (8, 22, 23, 31, 36, 41). Dietary xanthotoxin and harmine significantly induced GST activity in larvae *T. ni*, but dietary phenolics inhibited it (22). GST activity in *S. avenae* was stimulated by catechol, gramine and L-ornithine-HCl (47). Arora *et al.* (8) noted the induction of GST activity in mustard aphid *Lipaphis erysimi* (Kalt.) nymphs treated by coumarin. In our study we observed the highest induction of GST activity at the beginning of experiment. Results presented by Łukasik (27) showed that short-term feeding of *R. padi* adults derived from the bird cherry on the spring triticale induced GST in their tissues. The results obtained by Leszczyński *et al.* (24) showed a higher activity of GST in the grain aphid and the bird cherry-oat aphid fed on resistance varieties of cereals, which contained high concentration of allelochemicals. GST activity in the grain aphid *S. avenae* was positively correlated with the total content of DIMBOA (cereal hydroxamic acid) in its host seedlings (23). However, DIMBOA-aglucone incorporated into artificial diets significantly reduced GST activity in *S. avenae* (23). On other hand, phenolics compounds *in vitro* reduced the GST activity in females of cereal aphids – *S. avenae* and *R. padi* (26). Thus, the induction of GST in aphids observed in our experiment might result from the action of other allelochemicals which occurred in the tested triticale cultivars. It has been showed that the level of GST activity is affected by the concentration and nature of plant compounds (14).

The females of *R. padi* were characterized by higher activity of GST_{px} than *S. avenae* ones. GST_{px} activity in *S. avenae* was affected by the two test triticale cultivars. In Witon cv., the induction of GST_{px} was independent of time of feeding. The aphids transferred to Tornado cv. had similar GST_{px} activity during all test periods. The greater induction of GST_{px} in *S. avenae* was noted for insects fed on Witon cv., after 48 and 72 h (Fig. 3). The transfer of *R. padi* to Witon cv. increased the GST_{px} activity. In the beginning of experiment, the induction of GST_{px} was lowest, then activity of GST_{px} rose up and remained at stable level till the end of experiment. In Tornado cv., the short time of feeding didn't affect the GST_{px} activity. The induction of enzyme in females of *R. padi* was observed 72 h after feeding on Tornado cv. Both studied cultivars similarly affected the activity of GST_{px} in adults of *R. padi*, except the 48 h, when aphids fed on Witon cv. had the higher GST_{px} activity. The adults of *S. avenae* had higher activity of GR in comparison to *R. padi*. The activity of GR in *S. avenae* was induced 48 h after feeding on

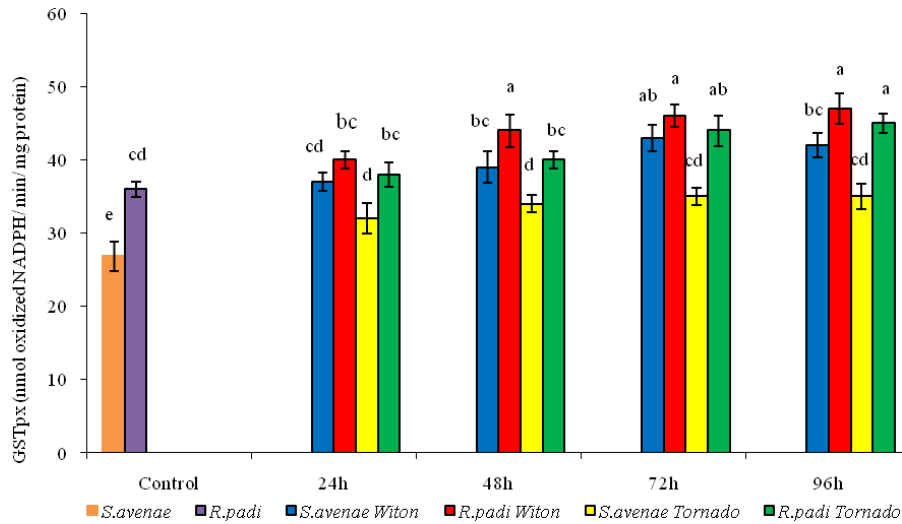


Figure 3. Changes in the activity of Se-independent glutathione peroxidase (GST_{px}) (nmol oxidized NADPH/min/mg protein) within *apterae* morphs of *S. avenae* and *R. padi* after transferring onto winter triticale (control - aphids on the winter triticale Tonacja cv.). Values not followed by the same letter are significantly different at level $p \leq 0.05$ (Duncan's test).

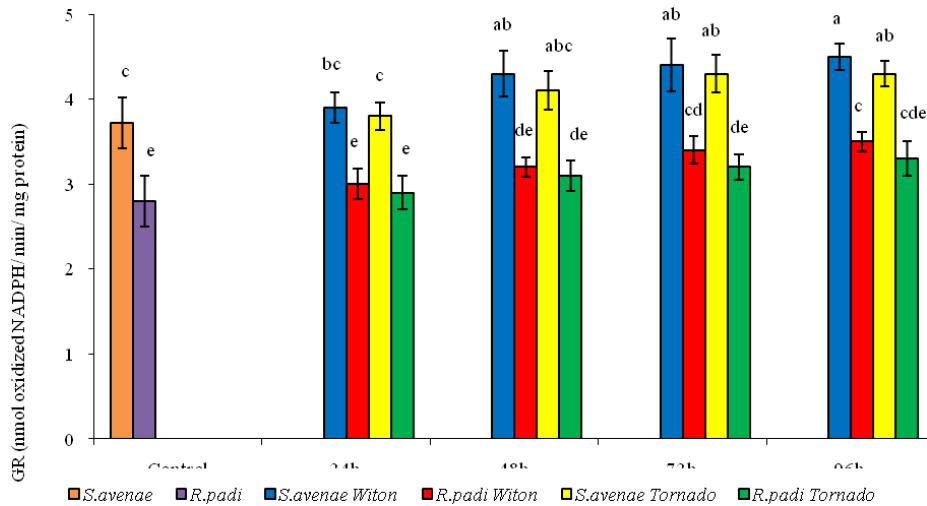


Figure 4. Changes in the activity of glutathione reductase (GR) (nmol oxidized NADPH/min/mg protein) within *apterae* morphs of *S. avenae* and *R. padi* after transferring onto winter triticale (control - aphids on the winter triticale Tonacja cv.). Values not followed by the same letter are significantly different at level $p \leq 0.05$ (Duncan's test).

Witon cv. and 72 h after feeding on Tornado cv. The level of induction was stable until the end of experiment and was similar for both studied cultivars. In *R. padi*, initial feeding (24 h) on triticale didn't affect the activity of GR. However, the prolonged feeding (72 and 96 h) increased the GR activity, but only in insects fed on Witon cv. (Fig. 4). Se-dependent GPX, the main enzyme removing H₂O₂ in mammals, was not evident in cereal aphid species. *Lepidoptera* larvae (*T. ni*, *P. polyxenes*, *S. eridania*) have very low activity of GPX (3, 34). The lack of Se-dependent GPX might lead to two evolutionary adaptations – one adaptation is wide subcellular distribution of CAT in herbivores and another is the elaboration of GST_{px} activity (1). The high activity of GST_{px} may substitute for GPX in removing of organic hydroperoxides. Since the reactions catalyzed by GST_{px} produce GSSG, GST_{px} and GR seem to be a pair of complementary enzymes in protection against lipid peroxidation. The activities of GST_{px} and GR were noted for *Lepidoptera* (20, 33, 34, 43), *Orthoptera* (10) and aphids as well (8, 28). The results of our earlier studies showed that GST_{px} and GR activities in aphids were comparable with those reported for *Lepidoptera* larvae. In our experiment oligophagous species *R. padi* had a higher level of GST_{px} activity than monophagous species *S. avenae*, but opposite tendency was noted for GR. Thus GR activity is determined not only by the level of GST_{px} activity, but other metabolic processes and non-enzymatic oxidation of glutathione (34). The induction or inhibition of GST_{px} and GR seems to be depended on the nature and biochemical properties of allelochemicals. Namely, Arora et al. (8) observed a highly significant increase of GR activity in *L. erysimi* larvae treated by coumarin, but GPX activity showed a suppression under treatment with this compound. The activities of GST_{px} and GR were induced by plant phototoxin, xanthotoxin, but flavonoid quercetin caused inhibition of both enzymes in lepidopteran larvae – *T. ni*, *P. polyxenes* and *S. eridania* (4,5). GST_{px} activity was also reduced in grasshoppers *A. ellioti* and *M. sanguinipes* by ingested phenols (10). The inhibitory action of plant *o*-dihydroxyphenols (quercetin, caffeic acid and chlorogenic acid) on GST_{px} and GR activities was observed for cereal aphids as well (28). Quercetin may inhibit NADPH-utilizing enzymes, including GR, by competing with NADPH for the active site (5). Thus, the induction of GST_{px} and GR in cereal aphids after alteration of the type of diet might be associated with action of other plant allelochemicals than phenols. Another likely mechanisms of this induction could result from „the oxidative burst” in plants infested by aphids. Krishnan and Kodrik (20) noted a significant up-regulation of GST_{px} activity in larvae of *Spodoptera littoralis* (Boisduval) fed on the plant diets (potato plants) in comparison to the semi-artificial diets. Petrić-Mataruga et al. (33) observed the induction of GST_{px} in *L. dispar* derived from oak (favorable host plant) after transfer to locust (unfavorable host plant). Thus the antioxidant mechanisms associated with GSH seem to be dependent on the presence of specific pro-oxidant allelochemicals in the diet.

Two studied cultivars similarly affected the level of antioxidant mechanisms based on GSH. However, the less susceptible Witon cv. affected GSH content and GST_{px} activity in *S. avenae* more than Tornado cv. Opposite results were obtained by Leszczyński et al. (24), where higher activity of GST was observed in *S. avenae* and *R. padi* fed on a moderately resistant wheat variety than on a susceptible variety. However, in our previous study we observed stronger generation of ROS in the cereal aphids fed on the less susceptible Witon cv. (29). The increase of ROS is one of the biochemical markers of oxidative stress, so the aphids fed on Witon cv. were more subjected on negative

consequences of ROS action. The cereal aphids performed on Witon cv. were characterized by a longer prereproductive period, higher mean time of generation development and lower values of daily fecundity in comparison to the insects occurred on Tornado cv. (37, 38). Electrical penetration graphs (EPG) recordings showed that cereal aphids fed on Witon cv. seedlings were characterized by longer duration of no probing, total pathways and shorter time of salivation into sieve element and phloem sap ingestion that aphids fed on Tornado cv. (37).

The sucking-piercing insects respond to the alteration of the diet through biochemical adaptations, which include changes in antioxidant defence. The high concentration of ROS generated in new infested plants is toxic to aphids. Thus the host plant alteration can stimulate mechanisms scavenging ROS among those important role plays GSH and GSH-dependent enzymes. The induction of GST and GST_{px} in cereal aphids in response to change of diet type altered the level of intracellular GSH causing its depletion. The increase of GR activity during diet alteration prevents accumulation of GSSG and allows maintenance redox balance in cells. The decrease in the amount of GSH, as well as the increase of activities of GSH-dependent enzymes may be related to the changes in pro-oxidative status of host plants. Further studies are needed to explain the role of specific plant compounds in induction of oxidative stress in cereal aphids.

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