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Effect of host plants on antioxidant system of pea aphid *Acyrthosiphon pisum*

Iwona Łukasik[1], Sylwia Goławska[1], Agnieszka Wójcicka[1], Artur Goławski[2]
[1]*Department of Biochemistry and Molecular Biology, University of Natural Sciences and Humanities, Siedlce, Poland*
[2]*Department of Zoology, University of Natural Sciences and Humanities, Siedlce, Poland*

Abstract

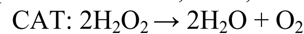
The effect of some species of family Fabaceae plants on the non-enzymatic and enzymatic antioxidants of the pea aphid, *Acyrthosiphon pisum* (Harris) has been studied. The highest concentration of ascorbate was noted for aphids fed on *Pisum sativum* L.. The content of another non-enzymatic antioxidant, GSH, was the highest within the tissues of the morphs fed on *Vicia faba* L.. The aphids reared on this host plant had 3-fold higher activity of the antioxidant enzymes than the ones fed on *Pisum sativum* L. and *Vicia sativa* L.. The influences of the host plants on antioxidant defence mechanisms within the pea aphid species are discussed.

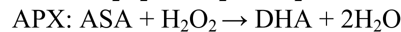
Key words: *Acyrtosiphon pisum*, Fabaceae, aphids, reactive oxygen species, oxidative stress, antioxidants.

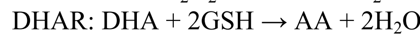
Introduction

In the natural environment, plants develop complex direct and indirect defence strategies against herbivores (Cory and Hoover, 2006). The basal defences are based on cell wall modification, antixenotic or antibiotic compounds and plant volatiles that repel aphids or attract their natural enemies (Goggin, 2007). Aphid feeding may trigger plant signalling pathways driven by jasmonic acid (JA), salicylic acid (SA), ethylene (ET), abscisic acid (ABA), gibberelic acid (GA), reactive oxygen species (ROS) or nitric oxide (NO) that induce the production of chemical defences (Smith and Boyko, 2007; Goggin, 2007; Walling, 2008). Aphids developed deceptive strategies to avoid or deter many plant defences. During feeding aphids introduce salivary effectors into the plant tissues to affect wound healing, defence-signalling pathways and volatile production (Walling, 2008).

One of the most rapid plant defence reactions to biotic stress is "oxygen burst" which constitutes the production of reactive oxygen species (ROS) including superoxide radical anion (O_2^{-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) (Kuźniak and Urbanek, 2000; Mittler, 2002). Among the generated ROS, a central role in plant defence responses is played by hydrogen peroxide. H_2O_2 exhibits direct toxicity toward herbivores, contributes to cell wall strengthening processes, triggers the hypersensitive responses (HR) and acting as signal molecule for the induction of defence genes (Kuźniak and Urbanek, 2000). Moloi and van der Westhuizen (2006) found that during infestation of wheat by Russian wheat aphid ROS, produced as a result of NADPH oxidase activation, act as signal for activation of defence enzymes – intercellular peroxidase and β-1,3-glucanase. Additionally, some of the plant pro-oxidant compounds upon photochemical or metabolic activation may also generate ROS (Barbehenn *et al*., 2003; Krishnan *et al*., 2007). In response to oxidative stress arising from host plants, phytophagous insects have evolved specific defence mechanisms to deal with ROS. Herbivores posses system of antioxidant enzymes composed of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), Se-independent glutathione peroxidase (GSTPX) and glutathione reductase (GR), as well as low-molecular-weight antioxidants such as glutathione (GSH) and ascorbic acid (ASA). Among them, two enzyme-catalyzed pathways for destroying toxic H_2O_2: catalase and antioxidant system consisting of ascorbate peroxidase, dehydroascorbate reductase, ascorbic acid and glutathione play an important role (Barbehenn *et al*., 2001; Summers and Felton, 1993).


CAT: $2H_2O_2 \rightarrow 2H_2O + O_2$


APX: $ASA + H_2O_2 \rightarrow DHA + 2H_2O$


DHAR: $DHA + 2GSH \rightarrow AA + 2H_2O$

The pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera Aphididae) is becoming an increasingly important phytophagous on legumes due to the rising economic importance of these crops. The species is frequently used as a model piercing-sucking insect and recently its entire genome sequence has been published (The International Aphid Genomics Consortium, 2010). *A. pisum* is an oligophagous aphid species, one of the most serious pests of the Fabaceae plants: peas, alfalfas, vetchs, and red clovers (Cuperus *et al*., 1982; Lane and Walters, 1991). *A. pisum* is a vector of more than 30 viral diseases, including bean yellow mosaic virus, red clover vein mosaic virus and pea streak virus (Barnett and Diachun, 1986; Jones and Proudlove, 1991). Several reports have been published on the effect of different plant species and plant morphology on pea aphid development (Soroka and Mackay, 1990, 1991; Kaakeh and Dutcher, 1993; Sandström, 1994; Sandström and Pettersson, 1994) but there is a lack of studies relating to the role of oxidative stress in the process of adaptation of *A. pisum* to various host plants. Thus, the aim of the present paper was to investigate the effect of some Fabaceae plant species on antioxidant system removing hydrogen peroxide in *A. pisum*.

Materials and methods

Aphids

The insects came from the aphid stock cultures kept at the University of Natural Sciences and Humanities at Siedlce. The aphids were reared on pea seedlings, in an environmental chamber (21 °C, L16:D8 photoperiod, and 70% RH). Experiments were conducted on wingless females (apterae) and nymphs of the pea aphid.

Plants

Three species of Fabaceae were used in the experiments: *Pisum sativum* L. var. Tulipan, *Vicia faba* L. var Start and *Vicia sativa* L. var. Jaga. Seed samples were bought in Horticultural Plant Breeding Seed Production and Nursery in Ożarów Mazowiecki (administrative Warsaw, Poland). Seed samples were germinated in a climate chamber, which was kept at 21 ± 1 °C, L16:D8 photoperiod, and 70% RH. The seedlings were grown in plastic pots (10 x 10 cm, 5 seedlings per pot) filled with medium nutrient fine structure compost with sand. The soil was bought in ZPHU "Umex" from Łochów (administrative Warsaw, Poland). Nine-day-old seedlings of Fabaceae were infested each 20 aphids. After the aphids had fed on seedlings for seven days, they were collected and taken for assays of antioxidants. The experiment was conducted in four replicates.

Ascorbic acid assay

ASA concentration in aphid tissues was determined by adapting the procedure of (Omaye *et al*., 1979). 200 collected aphids were homogenized in a 67 mM potassium phosphate buffer (pH 6.5) containing 0.2 M EDTA and centrifuged at 3000 g for 15 min. Trichloroacetic acid (TCA) (1 ml of a 5% solution) was added to 0.5 ml of the supernatant, and the 1.5-ml volume was centrifuged at 12000 g for 20 min. ASA content was quantified after mixing 0.27 ml of the supernatant containing the aphid extract, 0.08 ml of 85% H_3PO_4, 1.37 ml of 0.5% α,α' – dipirydyl, and 0.28 ml of 1% $FeCl_3$. The reaction mixtures were incubated at 42 °C for 40 min, and absorbance at 525 nm was measured against a control containing 0.27 ml of 67 mM K-phosphate buffer (pH 6.5) instead of the aphid homogenate. ASA content was calculated from a calibration curve prepared with standards and was expressed in nmol per mg of protein.

Glutathione assay

Total glutathione concentration in aphid tissues was determined with an enzymatic recycling assay based on glutathione reductase (Griffith, 1980). Briefly, 200 collected aphids 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 rate of absorbance change to that of glutathione standards of known concentrations and was expressed as nmol per mg of protein.

Catalase assay

CAT activity was measured as described by Aebi (1984). 20 collected aphids were homogenized in 67 mM potassium phosphate buffer (pH 7) for 5 min at 0 °C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 g for 15 min. 0.5 ml of aphid extracts was added to 0.5 ml 30 mM H_2O_2 and disappearance of hydrogen peroxide was measured at 240 nm during 3 min at 30 s intervals. Activity of the catalase was expressed as μmol decomposed H_2O_2 per minute per mg protein.

Ascorbate peroxidase assay

APX activity was determined according to Asada (1984). 100 collected aphids were homogenized in 67 mM potassium phosphate buffer (pH 7) for 5 min at 0 °C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 g for 15 min. The reaction mixture consisted of 0.75 ml of crude homogenate of aphids and 0.25 ml of 67 mM potassium phosphate buffer (pH 7) containing 2.5 mM ascorbic acid and 0.2 ml of 30 mM H_2O_2. The decrease in absorbance at 290 nm was monitored for 5 min using a spectrophotometer. Boiled samples served as controls. APX activity was expressed as μmol ascorbate oxidized/min/mg protein, using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Dehydroascorbate reductase assay

DHAR activity was determined by adapting the procedure of Asada (1984). 100 collected aphids were homogenized in 67 mM potassium phosphate buffer (pH 7) for 5 min at 0 °C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 g for 15 min. The reaction mixture consisted of 0.6 ml of crude homogenate of aphids, 0.1 ml of 5 mM GSH, 0.1 ml of 0.1 mM EDTA and 0.2 ml of 0.06 mM DHA in 67 mM potassium phosphate buffer pH 7. The enzyme activity was determined by the formation of ASA at 265 nm. The increase in absorbance at 265 nm was monitored for 6 min against the blank contained boiled homogenates. DHAR activity was calculated as nmol ASA/min/mg protein.

Protein assay

The protein content in the studied aphid supernatants was determined using the method given by Bradford (1976).

Statistics

All data are reported as means ± 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 Tukey's multiple-range test.

Results and discussion

Removing toxic hydrogen peroxide is very important for herbivorous insects because they lack a Se-dependent glutathione peroxidase, which is a main antioxidant enzyme for neutralizing hydrogen peroxide in mammalian tissues (Ahmad *et al*., 1987). The genome of *A. pisum* lacks the genes encoded selenocysteine (The International Aphid Genomics Consortium, 2010). However, it may be compensated for the presence of the specific peroxidase activity of glutathione transferase (GSTPX), which catalyzes the reduction of wide range of hydroperoxides (Łukasik and Goławska, 2007). The pea aphid have a least 18 genes encoding glutathione-S-transferases (GST) that may detoxify stress agents, including ROS (Gerardo *et al.,* 2010; Ramsey *et al.,* 2010). A comparison of *Myzus persicae* (Sulzer) cDNA and *A. pisum* genomic sequences showed no differences in the number of glutathione-S-transferase genes but cytochrome P-450 gene family was 40% larger in *M. persicae* than in *A. pisum*. It is partially consistent with hypothesis that the different host ranges of the two aphids caused that generalist (*M. persicae*) requires a greater number of detoxification enzymes than a specialist (*A. pisum*) (Ramsey *et al*., 2010).

Since catalase is inefficient at removing low concentrations of hydrogen peroxide, alternative mechanism in the form of an ascorbate-recycling system (ASA, APX, GSH and DHAR) seems to be very important in protection of herbivores against oxidative stress. Hitherto antioxidant system for removing hydrogen peroxide has been detected in Lepidoptera larvae and other leaf-chewing insects (Barbehenn *et al*., 2001; Mathews *et al*., 1997; Wang *et al*., 2001; Krishnan and Kodrik, 2006) as well as in sucking-piercing insects, including aphids (Figueroa *et al*., 1999; Loayza-Muro *et al*., 2000; Lukasik, 2007). The obtained results showed the pea aphid had less efficient antioxidant mechanisms than the cereal aphids with regard to lower level of CAT and APX (Lukasik, 2007; Łukasik *et al*., 2009). It may be associated with the presence of specific compounds in the cereals (e.g. hydroxamic acids) that affected antioxidant system (Figueroa *et al*., 1999).

The results of this study demonstrate that Fabaceae plants clearly affected the content of non-enzymatic antioxidants and antioxidant enzymes in the pea aphid. The concentration of GSH and ASA within studied aphid morphs varied depending on the host plant on which they were reared (table 1). The highest GSH level was recorded for morphs that fed on a broad bean (ANOVA: $F_{1.5} = 701.03$, $p < 0.001$). The aphids reared on this host plant had 2-fold higher concentration of GSH in comparison with insects fed on vetch. In contrast, the content of another non-enzymatic antioxidant, ASA, was the highest in tissues of morphs reared on pea (ANOVA: $F_{1.5} = 737.56$, $p < 0.001$). The wingless females that fed on vetch and broad bean had similar ASA concentration whereas the nymphs showed 2-fold higher level of this antioxidant during feeding on vetch. Because the level of low-molecular-weight antioxidants is one of the biochemical markers of oxidative stress, differences in ASA and GSH content within aphid tissues point to diversity of defence mechanisms of host plants against the aphids. Petrić-Mataruga *et al*. (1997) suggest that regulation of the level of GSH in herbivores may be one of the rapid forms of adaptive responses against nutritive and oxidative stress. Previously, it has been reported that the alteration of the diet caused changes in the GSH amount in the midgut of *Lymantria dispar* (L.) (Petrić-Mataruga *et al*., 1997). The concentration of ASA within gut tissues of *Trichoplusia ni* (Hubner) and *Depressaria pastinacella* (Duponchel) varied in proportion to levels of ASA in the diets (Timmermann *et al.,* 1999). Our previous studies demonstrated the depletion of GSH and ASA in the cereal aphids upon treatment of plant *o*-dihydroxyphenols (Łukasik, 2006; Łukasik *et al.,* 2009). Thus pro-oxidants status of host plants and their qualitative composition may affect level of non-enzymatic antioxidants within aphid tissues.

Among the studied morphs, GSH concentrations were higher for apterae females in comparison to nymphs fed on all studied host plants. A different pattern was recorded for ascorbate, where the studied morphs reared on pea and broad bean had similar ASA content whereas nymphs fed on vetch contained significantly higher level of ASA than apterae. Krishnan *et al*. (2007, 2009) noted a lower level of ROS and a higher antioxidant potential in adults of *Leptinotarsa decemlineata* (Say) than in the larval stage. Different results were obtained for morphs of cereal aphids, in which the ASA and GSH concentrations were higher within nymph tissues than apterae ones (Łukasik, 2006; Łukasik *et al*., 2009).

Goławska (unpublished data) proved that the most suitable host for *A. pisum* is pea plant. The aphids fed on pea showed higher fecundity and survival than those feeding on broad bean, alfalfa or clover. This may be conditioned by a very high level of ASA within tissues of aphids fed on the pea. ASA is an essential nutrient for insects that is

Table 1. Content of the GSH and ASA (nmol/mg protein) within tissues of the pea aphid fed on Fabaceae plants.

Host plant	Aphid morphs	GSH	ASA
Pea	apterae	17.50 ± 0.51^b	880 ± 27^a
	nymph	11.76 ± 0.36^c	850 ± 30^a
Broad bean	apterae	22.57 ± 0.42^a	280 ± 12^c
	nymph	16.67 ± 0.39^b	240 ± 19^c
Vetch	apterae	11.11 ± 0.43^c	280 ± 17^c
	nymph	8.11 ± 0.19^d	440 ± 21^b

Data are presented as mean ± SD; n = 4.
Values in columns not followed by the same letter are significantly different (ANOVA: $p < 0.001$).

Table 2. Activity of the CAT (μmol H_2O_2/min/mg protein), APOX (μmol ASA oxidized/min/mg protein) and DHAR (nmol ASA/min/mg protein) within tissues of the pea aphid fed on Fabaceae plants.

Host plant	Aphid morphs	CAT	APOX	DHAR
Pea	apterae	7.61 ± 0.30[e]	0.08 ± 0.02[c]	25.38 ± 2.63[d]
	nymph	8.50 ± 0.25[d]	0.11 ± 0.03[c]	99.93 ± 5.33[c]
Broad bean	apterae	15.06 ± 0.21[b]	0.22 ± 0.05[ab]	96.07 ± 3.28[c]
	nymph	16.67 ± 0.40[a]	0.29 ± 0.07[a]	299.98 ± 15.27[a]
Vetch	apterae	8.59 ± 0.16[d]	0.12 ± 0.03[c]	40.00 ± 2.29[d]
	nymph	15.83 ± 0.35[c]	0.16 ± 0.02[bc]	127.47 ± 6.31[b]

Data are presented as mean ± SD; n = 4.
Values in columns not followed by the same letter are significantly different (ANOVA: $p < 0.001$).

involved in many metabolic processes and required for normal growth, reproduction and development.

The highest level of studied antioxidant enzymes was noted in morphs reared on broad bean (ANOVA: $F_{1.5}$ = 822.41, $p < 0.001$ for CAT; $F_{1.5}$ = 16.26, $p < 0.001$ for APX; $F_{1.5}$ = 719.70, $p < 0.001$ for DHAR). In comparison to broad bean, vetch and pea reduced aphid enzyme activities. The aphids fed on these host plants had 2- or 3-fold lower levels of the studied enzymes than insects originating from broad bean. Comparing the other host plants, the induction of enzyme activities was shown to be greater in aphids fed on vetch than in those fed on pea (table 2). Krishnan and Kodrik (2006) observed a significant up-regulation of CAT and APX activity in *Spodoptera littoralis* (Boisduval) fed on plant diet in comparison to semi-artificial diet. Thus insects are able to adapt quickly to increased oxidative stress arising from plant pro-oxidant allelochemicals by regulation of antioxidant enzyme activities (Ahmad and Pardini, 1990; Krishnan and Kodrik, 2006; Lukasik, 2007). Petrić-Mataruga *et al.* (1997) observed induction or inhibition of antioxidant enzymes when the effect of host plant of different population origins on the antioxidant defence of *L. dispar* was studied. A comparison of antioxidant status of three species of Lepidoptera differing in their preference of host plant – *T. ni*, *Spodoptera eridania* (Stoll), *Papilio polyxenes* F. pointed towards a correlation of antioxidant defence with natural feeding habits of these insects and their susceptibility to pro-oxidant plant compounds in the diet (Ahmad and Pardini, 1990). The high antioxidant enzyme activities within tissues of aphids fed on broad bean suggest that this host plant may possess allelochemical compounds acting as inducers of antioxidant system. Figueroa *et al.* (1999) reported the twofold increase of CAT activity in the grain aphid *Sitobion avenae* (F.) fed on DIMBOA-containing artificial diets with respect to control diets without DIMBOA. This contrasts with the results obtained by Loayza-Muro *et al.* (2000) that indicate no significant effect of wheat cultivars differing in the hydroxamic acid concentrations on CAT activity within the grain aphid. Łukasik (2009) noted the induction of CAT activity in migrants of *Rhopalosiphum padi* (L.) after transfer from primary (bird cherry) to secondary (triticales) host plants, but the level of activity was closely associated with feeding duration on the secondary host. Krishnan and Sehnal (2006) noted that the ingestion of tannic acid significantly increased the activity of CAT and APOX in the midgut of *S. littoralis* larvae. These results are in accordance with our studies in which exposure of cereal aphids to plant pro-oxidants clearly induced the activity of APOX (Łukasik *et al.*, 2009). This increase of APOX activity within cereal aphids under the influence of *o*-dihydroxyphenols may compensate for the inhibition of catalase by these compounds (Lukasik, 2007). However, studies of the ascorbate-recycling system in the midgut of caterpillars *Malacosoma disstria* (Hubner) and *Orgyia leucostigma* (Smith) showed that APOX and CAT activities were reduced by ingestion of tannic acid (Barbehenn *et al.*, 2001). Thus the induction/inhibition of antioxidant system might depend on insect species and on biochemical properties of enzymes. For example, quercetin, a pro-oxidant flavonoid, caused an increase of superoxide dismutase (SOD) activity within cereal aphid tissues, but significantly suppressed the CAT and the glutathione reductase (GR) activity (Lukasik, 2007; Łukasik and Goławska, 2007).

Among the studied aphid morphs the highest activities for CAT and DHAR were found for nymphs. In case of the DHAR, nymphs had nearly 3-fold higher activity than wingless apterae fed on the studied host plants. Different results were obtained for the APX, where apterae as well as nymphs reared on all tested Fabaceae plants had comparable enzyme activities (table 2). These results may point out more effective antioxidant mechanisms that protect nymphs from damages caused by oxidative stress. It was evidenced by results of our earlier studies related to antioxidant mechanisms in cereal aphids in which nymphs had higher levels of enzymes targeting H_2O_2 than apterae adults (Lukasik, 2007; Łukasik *et al.*, 2009).

Conclusions

It was evidenced by results presented here that host plants clearly affected the antioxidant defence mechanisms in the pea aphid tissues. The pea seems to be the most suitable host to *A. pisum* since aphids reared on this plant had the lowest level of antioxidant enzymes and the highest content of the main non-enzymatic antioxidant - ascorbate. This is probably conditioned by a lower level of specific plant metabolites that may promote the oxidative stress within herbivores tissues. The larval stages of *A. pisum* seem to posses more effective antioxidant mechanisms than wingless females with regard to higher activity of enzymes removing H_2O_2.

References

AEBI H., 1984.- Catalase in vitro.- *Methods in Enzymology*, 105: 121-126.

AHMAD S., PARDINI R. S., 1990. - Mechanisms for regulating oxygen toxicity in phytophagous insects.- *Free Radicals in Biology and Medicine*, 8: 401-413.

AHMAD S., PRITSOS C. A., BOWEN S. M., KIRKLAND K. E., BLOMQUIST G. J., PARDINI R. S., 1987.- Activities of enzymes that detoxify superoxide anion and related oxyradicals in *Trichoplusia ni*.- *Archives of Insect Biochemistry and Physiology*, 7: 85-96.

ASADA K., 1984.- Chloroplasts: formation of active oxygen species and its scavenging.- *Methods in Enzymology*, 105: 422-429.

BARBEHENN R. V., BUMGARNER S. L., ROOSEN E. F., MARTIN M. M., 2001.- Antioxidant defenses in caterpillars: role of the ascorbate-recycling system in the midgut lumen.- *Journal of Insect Physiology*, 47: 349-357.

BARBEHENN R. V., POOPAT U., SPENCER B., 2003.- Semiquinone and ascorbyl radicals in the gut fluids of caterpillars measured with EPR spectrometry.- *Insect Biochemistry and Molecular Biology*, 33: 125-130.

BARNETT O. W., DIACHUN S., 1986.- Virus diseases of clovers: etiology and crop losses, pp. 625-675. In: *Viruses infecting forage legumes* (EDWARDSON J. R.,CHRISTIE G., Eds).- Florida Agricultural Experiment Station, Gainesville, USA.

BRADFORD M. M., 1976.- A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.- *Analytical Biochemistry*, 72: 248-254.

CORY J. S., HOOVER K., 2006.- Plant-mediated effects in insect-pathogen interactions.- *Trends in Ecology and Evolution*, 21 (5): 278-286.

CUPERUS C. W., RADCLIFFE E. B., BARNES D. K., MARTEN G. C., 1982.- Economic injury levels and economic thresholds for pea aphid, *Acyrthosiphon pisum* (Harris) on alfalfa.- *Crop Science*, 1: 453-463.

FIGUEROA C. C., KOENIG C., ARAYA C., SANTOS M. J., NIEMEYER H. M., 1999.- Effect of DIMBOA, a hydroxamic acid from cereals, on peroxisomal and mitochondrial enzymes from aphids: evidence fort he presence of peroxisomes in aphids.- *Journal of Chemical Ecology*, 25: 2465-2475.

GERARDO N. M., ALTINCICEK B., ANSELME C., ATAMIAN H., BARRIBEAU S. M., DE VOS M., DUNCAN E. J., EVANS J. D., GABALDÓN T., GHANIM M., HEDDI A., KALOSHIAN I., LATORRE A., MOYA A., NAKABACHI A., PARKER B.J., PÉREZ-BROCAL V., PIGNATELLI M., RAHBÉ Y., RAMSEY J. S., SPRAGG C. J., TAMAMES J., TAMARIT D., TAMBORINDEGUY C., VINCENT-MONEGAT C., VILCINSKAS A., 2010.- Immunity and other defenses in pea aphid, *Acyrtosiphon pisum*.- *Genome Biology*, 11: R21.

GOGGIN F. L., 2007.- Plant-aphid interactions: molecular and ecological perspectives.- *Current Opinion in Plant Biology*, 10: 399-408.

GRIFFITH O. W., 1980.- Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine.- *Analytical Biochemistry*, 106: 207-212.

JONES R. A. C., PROUDLOVE W., 1991.- Further studies on cucumber mosaic virus infection of narrow-leafed lupin (*Lupinus angustifolius*): seed-borne infection, aphid transmission, spread and effects on grain yield.- *Annals of Applied Bioloy*, 118: 319-329.

KAAKEH W., DUTCHER J. D., 1993.- Rates of increase and probing behaviour of *Acyrthosiphon pisum* (Homoptera: Aphididae) on preferred and nonpreferred host cover crops.- *Environmental Entomology*, 22: 1016-1021.

KRISHNAN N., KODRIK D., 2006.- Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): are they enhanced to protect gut tissues during oxidative stress?- *Journal of Insect Physiology*, 52: 11-20.

KRISHNAN N., SEHNAL F., 2006.- Compartmentalization of oxidative stress and antioxidant defense in the larval gut of *Spodoptera littoralis*.- *Archives of Insect Biochemistry and Physiology*, 63: 1-10.

KRISHNAN N., KODRIK D., TURANLI F., SEHNAL F., 2007.- Stagespecific distribution of oxidative radicals and antioxidant enzymes in the midgut of *Leptinotarsa decemlineata*.- *Journal of Insect Physiology*, 53: 67-74.

KRISHNAN N., KODRIK D., KŁUDKIEWICZ B., SEHNAL F., 2009.- Glutathione-ascorbic acid redox cycle and thioredoxin reductase activity in the digestive tract of *Leptinotarsa decemlineata* (Say).- *Insect Biochemistry and Molecular Biology*, 39: 180-188.

KUŹNIAK E., URBANEK H., 2000.- The involvement of hydrogen peroxide in plant responses to stresses.- *Acta Physiologiae Plantarum*, 22: 195-203.

LANE A., WALTERS K. F. A., 1991.- Effect of pea aphid (*Acyrthosiphon pisum*) on the yield of combining peas.- *Aspects of Applied Biology*, 27: 363-368.

LOAYZA-MURO R., FIGUEROA C. C., NIEMEYER H. M., 2000.- Effect of two wheat cultivars differing in hydroxamic acid concentration on detoxification metabolism in the aphid *Sitobion avenae*.- *Journal of Chemical Ecology*, 26: 2725-2736.

ŁUKASIK I., 2006.- Effect of *o*-dihydroxyphenols on antioxidant defence mechanisms of cereal aphids associated with glutathione.- *Pestycydy/Pesticides*, 3-4: 67-73.

LUKASIK I., 2007.- Changes in activity of superoxide dismutase and catalase within cereal aphids in response to plant *o*-dihydroxyphenols.- *Journal of Applied Entomology*, 131: 209-214.

ŁUKASIK I., 2009.- Effect of host alternation on the activity of adaptive enzymes of the bird cherry-oat aphid *Rhopalosiphum padi* (L.).- *Journal of Pest Science*, 82: 203-209.

ŁUKASIK I., GOŁAWSKA S., 2007.- Activity of Se-independant glutathione peroxidase and glutathione reductase within cereal aphid tissues.- *Biological Letters*, 4: 31-39.

ŁUKASIK I., GOŁAWSKA S., WÓJCICKA A., 2009.- Antioxidant defense mechanisms of cereal aphids based on ascorbate and ascorbate peroxidase.- *Biologia*, 64: 994-998.

MATHEWS M. C., SUMMERS C. B., FELTON G. W., 1997.- Ascorbate peroxidase: a novel antioxidant enzyme in insects.- *Archives of Insect Biochemistry and Physiology*, 34: 57-68.

MITTLER R., 2002.- Oxidative stress, antioxidants and stress tolerance.- *Trends in Plant Science*, 7: 405-410.

MOLOI M. J., VAN DER WESTHUIZEN A. J., 2006.- The reactive oxygen species are involved in resistance responses of wheat to the Russian wheat aphid.- *Journal of Plant Physiology*, 163: 1118-1125.

OMAYE S. T., TURNBULL J. D., SAUBERLICH H. E., 1979.- Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids.- *Methods in Enzymology*, 62: 3-11.

PETRIĆ-MATARUGA W., BLAGOJEVIĆ D., SPASIĆ M.B., IVANOVIĆ J., JANKOVIĆ-HLADNI M., 1997.- Effect of host plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins.- *Journal of Insect Physiology*, 43: 101-106.

RAMSEY J. S., RIDER D. S., WALSH T. K., DE VOS M., GORDON K. H. J., PONNALA L., MACMIL S. L., ROE B. A., JANDER G., 2010.- Comparative analysis of detoxification enzymes in *Acyrtosiphon pisum* and *Myzus persicae*.- *Insect Molecular Biology*, 19: 155-164.

SANDSTRÖM J., 1994.- High variation in host adaptation among clones of the pea aphid, *Acyrthosiphon pisum* on peas, *Pisum sativum*.- *Entomologia Experimentalis at Applicata*, 71: 245-256.

SANDSTRÖM J., PETTERSSON J., 1994.- Amino acid composition of phloem sap and the relation to intraspecific variation in pea aphid (*Acyrthosiphon pisum*) performance.- *Journal of Insect Physiology*, 40: 947-955.

SMITH C. M., BOYKO E. V., 2007.- The molecular bases of plant resistance and defense responses to aphid feeding: current status.- *Entomologia Experimentalis et Applicata*, 122: 1-16.

SOROKA J. J., MACKAY P. A., 1990.- Growth of pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae), populations on caged plants of six cultivars of field peas and the effects of pea aphids on harvest components of caged field peas.- *The Canadian Entomologist*, 122: 1193-1199.

SOROKA J. J., MACKAY P. A., 1991.- Antibiosis and antixenosis to pea aphid (Homoptera: Aphididae) in cultivars of field peas.- *Journal of Economic Entomology*, 84: 1951-1956.

SUMMERS C. B., FELTON G. W., 1993.- Antioxidant role of dehydroascorbic acid reductase in insects.- *Biochimica at Biophysica Acta*, 1156: 235-238.

THE INTERNATIONAL APHID GENOMICS CONSORTIUM, 2010.- Genome sequence of the pea aphid *Acyrtosiphon pisum*.- *PloS Biology*, 8: e1000313.

TIMMERMANN S. E., ZANGERL A. R., BERENBAUM M. R., 1999.- Ascorbic and uric acid responses to xanthotoxin ingestion in a generalist and a specialist caterpillar.- *Archives of Insect Biochemistry and Physiology*, 42: 26-36.

WALLING L. L., 2008.- Avoiding effective defenses: strategies employed by phloem-feeding insects.- *Plant Physiology*, 146: 859-866.

WANG Y., OBERLEY L. W., MURHAMMER D. W., 2001.- Evidence of oxidative stress following the viral infection of two lepidopteran cell lines.- *Free Radicals in Biology and Medicine*, 31: 1448-1455.

Authors' addresses: Iwona ŁUKASIK (corresponding author, e-mail: iluk_71@tlen.pl), Sylwia GOŁAWSKA, Agnieszka WÓJCICKA, Department of Biochemistry and Molecular Biology, University of Natural Sciences and Humanities, ul. B. Prusa 12, 08-110 Siedlce, Poland; Artur GOŁAWSKI, Department of Zoology, University of Natural Sciences and Humanities, ul. B. Prusa 12, 08-110 Siedlce, Poland.

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