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

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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: *Acyrthosiphon 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), gibberellic 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 (\(\cdot O_2^-\)), hydrogen peroxide (\(H_2O_2\)) and hydroxyl radical (\(\cdot OH\)) (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 \(\beta\)-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 possess 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).

\[\text{CAT: } 2H_2O_2 \rightarrow 2H_2O + O_2\]
\[\text{APX: } ASA + H_2O_2 \rightarrow DHA + 2H_2O\]
\[\text{DHAR: } DHA + 2GSH \rightarrow AA + 2H_2O\]

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 Diefen, 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*. 

\[\text{DHA: } \text{Dehydroascorbate}\]
\[\text{DHAR: } \text{Dehydroascorbate reductase}\]
\[\text{APX: } \text{Ascorbate peroxidase}\]
\[\text{GSH: } \text{Glutathione}\]
\[\text{ASA: } \text{Ascorbic acid}\]
\[\text{SOD: } \text{Superoxide dismutase}\]
\[\text{CAT: } \text{Catalase}\]

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*Bulletin of Insectology* 64 (2): 153-158, 2011
ISSN 1721-8861
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). 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% H3PO4, 1.37 ml of 0.5% α,α′ – dipiridyl, and 0.28 ml of 1% FeCl3. The reaction mixture consisted of 0.75 ml of crude homogenate. ASA concentration in aphid tissues 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 H2O2 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 H2O2 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 H2O2. 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.

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.
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. pism 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 Golawska, 2007). The pea aphid has 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. pism genomic sequences showed no differences in the number of glutathione-S-transferases genes but cytochrome P-450 gene family was 40% larger in M. persicae than in A. pism. 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. pism) (Ramsey et al., 2010).

Since catalase is inefficient at removing low concentrations of hydrogen peroxide, alternative mechanisms 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., 1999; Mathews 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; Lukasik 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: F1.5 = 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: F1.5 = 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 α-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).

Golawska (unpublished data) proved that the most suitable host for A. pism 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.

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Aphid morphs</th>
<th>GSH</th>
<th>ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>apterae</td>
<td>17.50 ± 0.51b</td>
<td>880 ± 27a</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>11.76 ± 0.36c</td>
<td>850 ± 30</td>
</tr>
<tr>
<td>Broad bean</td>
<td>apterae</td>
<td>22.57 ± 0.42a</td>
<td>280 ± 12a</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>16.67 ± 0.39b</td>
<td>240 ± 19b</td>
</tr>
<tr>
<td>Vetch</td>
<td>apterae</td>
<td>11.11 ± 0.43c</td>
<td>280 ± 17c</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>8.11 ± 0.19a</td>
<td>440 ± 21b</td>
</tr>
</tbody>
</table>

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: F1.5 = 822.41, p < 0.001 for CAT; F1.5 = 16.26, p < 0.001 for APOX; F1.5 = 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 (Boisdouval) 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-Matařuga 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. dispers was studied. A comparison of antioxidant status of three species of Lepidoptera differing in their preference of host plants – 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. Lukasik (2009) noted the induction of CAT activity in migrants of Rhopalosiphum padi (L.) after transfer from primary (bird cherry) to secondary (triticale) 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 (Lukasik et al., 2009). This increase of APOX activity within cereal aphids under the influence of o-dihydroxynaphthols 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; Lukasik 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 H2O2 than apterae adults (Lukasik, 2007; Lukasik et al., 2009).

**Table 2. Activity of the CAT (μmol H2O2/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.**

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Aphid morphs</th>
<th>CAT</th>
<th>APOX</th>
<th>DHAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>apterae</td>
<td>7.61 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.38 ± 2.63&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>8.50 ± 0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.11 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>99.93 ± 5.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Broad bean</td>
<td>apterae</td>
<td>15.06 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>96.07 ± 3.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>16.67 ± 0.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.29 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>299.98 ± 15.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vetch</td>
<td>apterae</td>
<td>8.59 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.12 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.00 ± 2.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>nymph</td>
<td>15.83 ± 0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>127.47 ± 6.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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

**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 H2O2.
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Received December 12, 2010. Accepted June 6, 2011.