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Effect of phenolics from woody plants on activity of grain aphid oxidases

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Abstract: Changes in activity of the grain aphid peroxidase (Px) and polyphenol oxidase (PPO) towards phenolics isolated from leaves of black currant, sour cherry and walnut were examined. Slight increase in activity of peroxidase was found within insect tissues after 24 h of walnut extract treatment, whereas black currant and sour cherry strongly inhibited activity of this enzyme. Later on, the walnut extract reduced activity of the enzyme, finally about 30%. The other extracts showed slight changes in Px activity. The grain aphid's polyphenol oxidase was stimulated during the first 24 h of the experiment. Further treatment with the phenolics extracts reduced activity of the grain aphid polyphenol oxidase. Generally, phenolics isolated from the black currant and sour cherry were more effective in reducing activity of the aphid peroxidase, whereas phenolics from walnut reduced the polyphenol oxidase activity. Possible application of the phenolics isolated from the woody plants as modern biopesticides towards the grain aphid is discussed.

Keywords: polyphenol oxidase, peroxidase, grain aphid, black currant, sour cherry, walnut

INTRODUCTION

Aphids are important pests of various plants, because they penetrate through plant tissues to reach sieve elements. During ingestion of nutrient compounds from phloem sap, aphids inject salivary toxins and transmit virus diseases.

Phenolics are low molecular compounds ubiquitous in all tissues of plants and they are involved in diverse processes including resistance to biotic and

abiotic stresses [1] and some of them may be used as natural biopesticides [2-4]. On the other hand, herbivorous insects have developed physiological and biochemical strategies for neutralization of synthetic pesticides and natural plant allelochemicals. Oxigenation is one of the most frequent steps during biotransformation of different organic xenobiotics. Many of the redox reactions are catalyzed by oxidative enzymes, e.g. peroxidase and polyphenol oxidase [5].

Polyphenol oxidase (E.C. 1.14.18.1) acts as bifunctional enzyme of monophenol monooxigenase and *o*-diphenoloxidase. This enzyme catalyses hydroxylation of monophenols and oxidation of polyphenols to quinones and further to melanin pigments [6]. Peroxidase (E.C. 1.11.1.7) oxidizes phenols and other aromatic derivatives with help of hydrogen peroxide [7]. Miles [8] described polyphenol oxidase in the aphid saliva. Urbańska [9] showed also peroxidase activity in the cereal aphid tissues and suggested an interrelation between activity of the peroxidase and polyphenol oxidase. Both enzymes play fundamental role in oxidation of secondary substances to non-toxic products [10].

In this paper we report on influence of plant extracts from leaves of black currant, sour cherry and walnut on activity of grain aphid peroxidase and polyphenol oxidase.

MATERIAL AND METHODS

Insects

The green colour forms of the grain aphid (*Sitobion avenae* F.) used in the experiments were collected from a stock culture kept at the University of Podlasie in Siedlce. Parthenogenetic aphids were reared on aphid-susceptible seedlings of winter wheat (*Triticum aestivum* L.), cv. Tonacja, in a climatic chamber at 22 °C/16 °C (L/D) and photoperiod L16: D8 and their larvae were used in performed experiments.

Chemicals

Leaves of black currant, sour cherry and walnut were collected in July. One gram of dried plant material was homogenized with 50 ml of 80% methanol, and extracts were defatted with petroleum ether. After acidification with hydrochloric acid, phenolics were extracted with ethyl acetate. The organic solvent was evaporated to dryness under vacuum at 40 °C. Crude extracts were obtained by dissolving the residues in 10 ml of 80% methanol. Spraying mixtures (5% v/v; contained 4% of methyl alcohol) were prepared from the crude extracts after dilution with distilled water and addition of Tween 80 (50 µl).

Aphid treatment

The spraying tests were carried out in a climatic chamber at 16 h: 8 h photoperiod, temperature 22 ±2 °C (light phase) and 16 ±2 °C (dark phase) in 65 ±5% relative humidity. Fifty larvae (for each set) were caged on 25 seedlings of winter wheat cv. Tonacja, and after 30 min sprayed with 20 ml of the spraying mixtures prepared from the plant extracts. Control insects were prepared by spraying of larvae with water (containing 4% methanol without plant extracts). The treated aphids were collected 24, 48 and 168 h after application of the plant extracts and activity of peroxidase and polyphenol oxidase within their tissues was determined.

Oxidoreductases assays

Peroxidase was extracted from fifteen aphids (for each treatment) with 1.0 ml ice-cold 0.1 M Tris-HCl buffer pH 7.0, containing 1% polyvinylpyrrolidone (PVP) and 0.5% Triton X-100. The homogenate was centrifuged (10 000 g at 4 °C, for 20 min) and the supernatant was used for the enzyme activity assays. Activity of peroxidase was measured according to the method given by Hildebrand *et al*. [11] and Hori *et al*. [12] with slight modification. Reaction mixture contained: Tris-HCl buffer pH 7.0 (1.0 ml), 3% hydrogen peroxide (100 µl), 200 µM pyrogallol (100 µl), deionized water (700 µl) and the enzyme extract (100 µl). An increase in absorbance was measured at 430 nm after 10 min incubation at 30 °C. Px activity was expressed as $\Delta A \cdot 10\ min^{-1} \cdot mg\ of\ protein^{-1}$.

Polyphenol oxidase activity was determined using the method described earlier by Mils [13] and Laurema *et al*. [14]. Aphids (15 individuals) were homogenized in 0.1 M ice-cold Tris-HCl buffer pH 7.4 (1.0 ml), containing 1% PVP and 0.5% Triton X-100. After centrifugation (12 000 g for 30 min, at 4 °C), supernatant was used for determination of the polyphenol oxidase activity. Enzyme extract (250 µl) was mixed with 500 µl of the substrate solution (5 mM catechol in Tris-HCl buffer pH 7.4) and 250 µl of Tris-HCl buffer pH 7.4. Activity of the polyphenol oxidase was expressed as $\Delta A_{460} \cdot 1\ h^{-1} \cdot mg\ of\ protein^{-1}$.

Protein assay

Total protein content in the enzyme extracts was determined by the Bradford's method [15], using acidic solution of Coomassie Brilliant Blue G-250 (Bio-Rad Laboratories GmbH, Munich, Germany) and bovine serum albumin (Sigma, St. Louis, Mo, USA) as standard. Absorbance of the reaction mixture was measured at 595 nm.

Statistical analysis

All assays were replicated three times. Differences in activity of the studied enzymes after application of the plant extracts were subjected to ANOVA and estimated using Tuckey's post-hoc test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Changes in activity of the peroxidase and polyphenol oxidase after phenolics extracts application was found within the grain aphid tissues. The highest activity of Px was observed after 24 h exposure of the aphid to extract from walnut (Figure 1a). However, the same extract in the next hours of the experiment showed a slight decrease in activity of the aphid enzyme. Black currant and sour cherry strongly inhibited the aphid peroxidase activity after 24 h (Figure 1a), and later on the black currant extract caused weaker inhibition of the enzyme (Figure 1b and 1c). Activity of the grain aphid polyphenol oxidase 24 h after treatment was stimulated and later on reduced by all studied extracts (Figure 2a). The strongest stimulation of the PPO activity was showed by the black currant extract and the weakest by sour cherry. During the next days the strongest reduction of the enzyme activity was observed in the case of application of the walnut extract. The sour cherry extract reduced PPO activity of the grain aphid only about 10-15%.

Plant phenolics are major allelochemicals in host plant resistance to aphids, because they are toxic natural bioinsecticides [16-18]. Chrzanowski [3] described antibiotic properties of caffeic, ferulic and *p*-coumaric acids in relation to *S. avenae*. Antimicrobial properties of water extracts from walnut were reported by Oliveira *et al*. [19]. Stampar *et al*. [20] found phenolic compounds and Mahoney *et al*. [21] proved also occurrence of juglone (phenolic naphtoquinone) within the walnut tissues.

Herbivorous insects possess various physiological traits that enable them to exploit chemical substances that are potentially damaging to their cellular processes. These studies have shown that the studied oxidoreductases play an important role in insect counter-adaptation to plant secondary metabolites. Oxidation by both salivary and gut oxidases appears to be a basic mechanism of detoxication of plant phenolics by the aphids [23-25]. Phytophagous *Homoptera* and *Hemiptera* secreted catechol oxidase and peroxidase in the saliva and injected into host plant tissues [26]. Miles and Oertli [27] found that herbivorous insects could run the redox system during their feeding on phenols-rich diets and/or host plants.

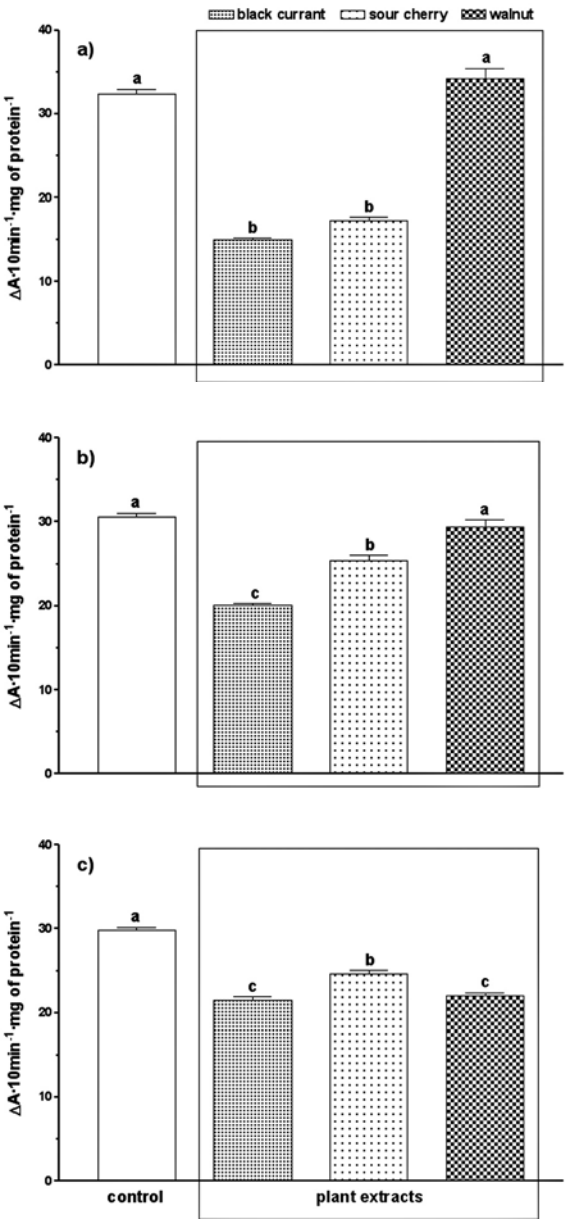

Figure 1. Activity of peroxidase within the grain aphid affected by woody plant extracts, a) after 24 h; b) after 48 h; c) after 168 h. The same letters at bars show no statistically significant differences at 5% level.

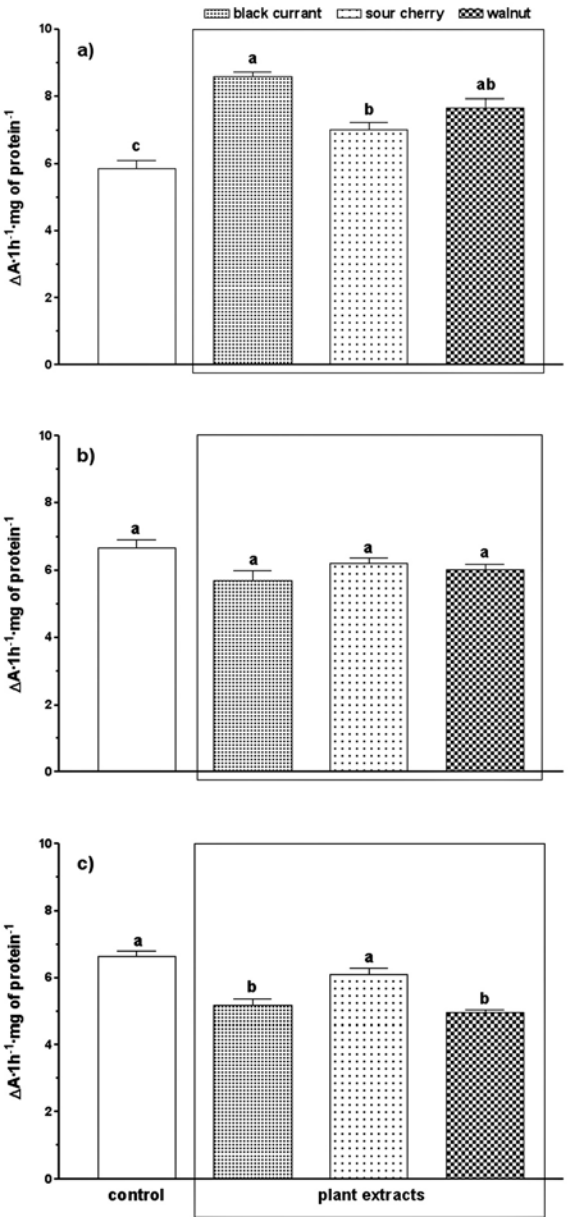

Figure 2. Activity of the polyphenol oxidase within tissues of *S. avenae* after treatment with plant extracts, a) after 24 h; b) after 48 h; c) after 168 h. The same letters at bars show no statistically significant differences at 5% level.

Presented data have shown that application of the phenolic extracts affected activity of both of the analyzed oxidoreductases. It was also shown that the aphid peroxidase and polyphenol oxidase functions were complementary. It was evident especially during the first 24 h of the experiment, when activity of the peroxidase decreased whereas activity of the polyphenol oxidase increased. These observations confirmed an earlier statement by Urbańska *et al*. [9] that Px and PPO within aphid saliva were complement to one another. Thus, the oxidation of the phenolics by the aphid oxidases protects it from toxiferous effect of plant allelochemicals. A similar role of these enzymes was earlier reported for other aphid species [28, 29]. It was shown that rose aphid and spotted alfalfa aphid neutralized phenolics and converted them into phagostimulative derivatives with use of the peroxidase and polyphenol oxidase. During the performed experiments, the activity of the polyphenol oxidase generally increased after the first 24 h. Such rapid defense reaction was also observed in the case of the grain aphid reaction towards gramine [30].

The obtained results suggest that the grain aphid peroxidase and polyphenol oxidase participated in detoxication of plant phenolics. The defensive reaction was especially visible during the first 24 hours after treatment with the phenolic extracts. It is also clear that the grain aphid peroxidase and polyphenol oxidase act as complementary oxidases in neutralizing the toxic effect of the plant phenolics.

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