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Effect of plant *o*-dihydroxyphenols and quinone on generation of reactive oxygen species within the grain aphid tissues

Iwona ŁUKASIK and Sylwia GOŁAWSKA

Department of Biochemistry and Molecular Biology, University of Podlasie, 12 B. Prusa St., 08-110 Siedlce, Poland, e-mail: iluk_71@tlen.pl

Abstract: Effect of some dietary *o*-dihydroxyphenols and quinones on hydrogen peroxide (H_2O_2) concentration within tissues of the grain aphid *Sitobion avenae* (F.) (*Homoptera*, *Aphididae*) has been studied. Among the studied aphid morphs the highest level of H_2O_2 total was noted for winged adults (*alatae*) and the lowest for *larvae*. The aphids exposed to the dietary pro-oxidative *o*-dihydroxyphenols demonstrated significantly higher concentration of hydrogen peroxide than the control ones. Among the studied compounds, caffeic acid showed the strongest effect on the H_2O_2 level within the aphid tissues. The highest concentration (0.1%) of this phenolic acid caused above 2-fold increase in the content of this radical within the grain aphid tissues. The significance of these results for understanding the toxicity of phenols to cereal aphids is discussed.

Keywords*:* *Rhopalosiphum padi*, *Sitobion avenae*, hydrogen peroxide, pro-oxidants

INTRODUCTION

The grain aphid *Sitobion avenae* (F.) is one of the main pests of cereals across Europe. Chemical control of the cereal aphids is pretty difficult and cause undesirable pollution of plants and environment. Thus ecologically friendly methods to control these pests were developed. One of them is natural plant resistance which may be used in integrated pest management. Phenolic compounds and quinones present in cereals are plant secondary metabolites which can act as natural insecticides. They have strong effect on feeding behavior, fecundity and survival causing reduction of the aphid population [1-3]. The *o*-dihydroxyphenols and quinones may be metabolically activated to a very

reactive semiquinone radicals, which in turn react with molecular oxygen to generate superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) [4]. It may evoke the cascade of other reactive oxygen species and results in alterations within structures of macromolecules such as DNA, protein and lipids. For phytophagous insects, lipid peroxidation is especially harmful, since lipids are not only cell membrane components, but also play other specific physiological functions as juvenile hormones or pheromones [5].

The previous work conducted on *Lepidoptera* larvae pointed out ability of the phenolic compounds to generate reactive oxygen species witin insect bodies [6-8]. Thus the aim of the present study was investigation of pro-oxidant properties of the *o*-dihydroxyphenols and quinone towards the grain aphid *Sitobion avenae* (F.) by assay of hydrogen peroxide generation within its tissues.

MATERIAL AND METHODS

Aphids

Experiments were conducted on various morphs of the grain aphid such as wingless adults (*apterae*),winged adults (*alatae*) and *larvae*. The insects came from the aphid stock cultures kept on winter wheat cv. Tonacja at the University of Podlasie in Siedlce.

Effect of phenolic compounds on H_2O_2 content

The influence of *o*-dihydroxyphenols and quinone on the concentration of hydrogen peroxide within aphid bodies was determined by placement starved *apterae* morphs on agarose-sucrose gels (1.25% agarose-30% sucrose) containing the tested chemicals at three concentrations, 0.001%, 0.01% and 0.1% and put on test. The following compounds were tested: (1) derivatives of benzene (catechol, pyrogallol); (2) derivatives of *trans*-cinnamic acid (caffeic acid, chlorogenic acid); (3) flavonoids (quercetin, catechin); (4) quinone (juglone). After 12 hours of the gels probing, the aphids were collected and changes in the content of H_2O_2 were assayed, in comparison to control aphids that were placed on agarose-sucrose gels without the tested chemicals.

Preparation of aphid homogenates

The collected aphids were homogenized in 50 mM K-phosphate buffer pH 7.0. The obtained homogenates were filtered through two layers of cheesecloth and centrifuged at 3000 *x g* for 15 min. The pellets were discarded, the supernatants were used to assay of hydrogen peroxide content.

Hydrogen peroxide assay

The concentration of hydrogen peroxide was determined according to Green & Hill [9], based on the reaction of 4-aminoantipyrine and phenol with H_2O_2, catalysed by peroxidase. Thereby the coloured product (chinonimin) was formed that was determined spectrophotometrically. The reaction mixture was consisted of 1 ml of reagent contained 4 mM of 4-aminoantipyrine, 24 mM of phenol, 0.4 U/ml of peroxidase dissolved in 0.1 M phosphate-buffer pH 7.0 and 0.3 ml of aphid homogenates. After addition of homogenates the reaction mixture was incubated at 30 °C for 10 min and the absorbance was measured at 510 nm against blank contained 0.3 ml of distilled water instead of the aphid homogenate. The hydrogen peroxide content was calculated from a calibration curve prepared for this standard and expressed in nmol per mg of protein.

Protein assays

The protein content in the studied aphid supernatants was determined using the method given by Bradford [10].

RESULTS AND DISCUSSION

The conducted experiments showed clear differences in the content of the hydrogen peroxide within homogenates of the studied aphid morphs. The highest H_2O_2 concentration was recorded for the winged adults (*alatae*) of grain aphid which had 2-fold higher content of this radical in comparison to *larvae* (Figure 1). It might result from a high rate of oxygen consumption during flying and associated with its increase in production of reactive oxygen species [11]. Moreover, migrants are expansive forms of the aphids that colonise new host plants thus probably they more often can penetrate through cell walls of peripheral plant tissues where the pro-oxidant compounds are located.

The aphids exposed to all tested dietary plant allelochemicals demonstrated significantly higher level of hydrogen peroxide than the control insects. The increase of H_2O_2 within aphid tissues depended on the concentration of the applied chemicals. The highest accumulation of this radical was observed for insects probed into the gels containing tested compounds at concentration of 0.1% Among studied pro-oxidants caffeic acid showed the strongest effect on the H_2O_2 content within tissues of the studied aphids. The caffeic acid was more effective than chlorogenic one and at the higher doses (0.01% and 0.1%) caused above 2-fold increase in content of the hydrogen peroxide within cereal aphid tissues (Figure 2). The similar effect evoked juglone but only the highest concentration (Figure 5). In the case

of tested flavonoids, the influence of catechin on H_2O_2 level was associated with applied concentrations while the quercetin effect was concentration-independent. At the lowest concentration (0.001%) quercetin stronger affected hydrogen peroxide content but the catechin caused higher accumulation of this oxygen radical at higher doses (Figure 3). Among benzene derivatives, catechol more strongly affected hydrogen peroxide generation within the grain aphid tissues than pyrogallol, especially at higher concentrations (Figure 4).

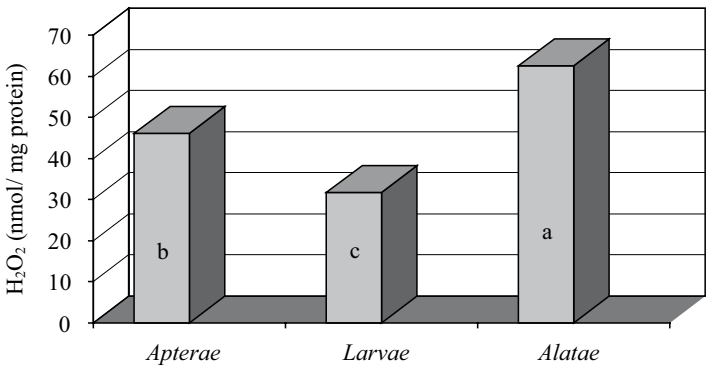

Figure 1. The hydrogen peroxide content within tissues of the grain aphid morphs. Values not followed by the same letter are significantly different at level $p \leq 0.01$ (Duncan's test).

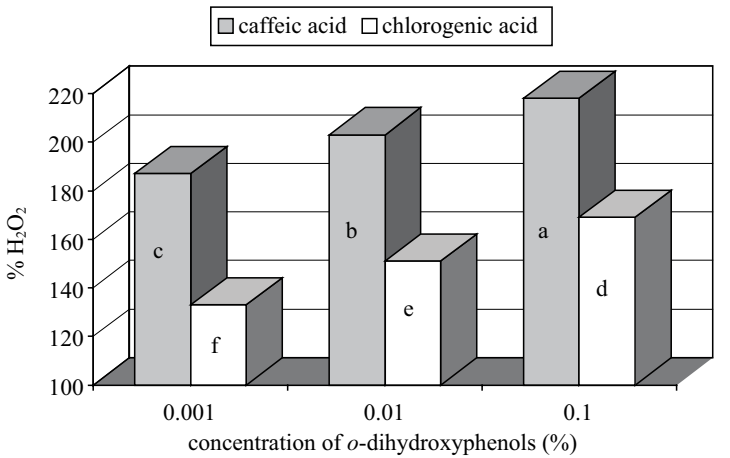

Figure 2. Effect of *trans*-cinnamic acid derivatives on content of the hydrogen peroxide content within tissues of the grain aphid (control without tested phenols = 100%). Values not followed by the same letter are significantly different at level $p \leq 0.01$ (Duncan's test).

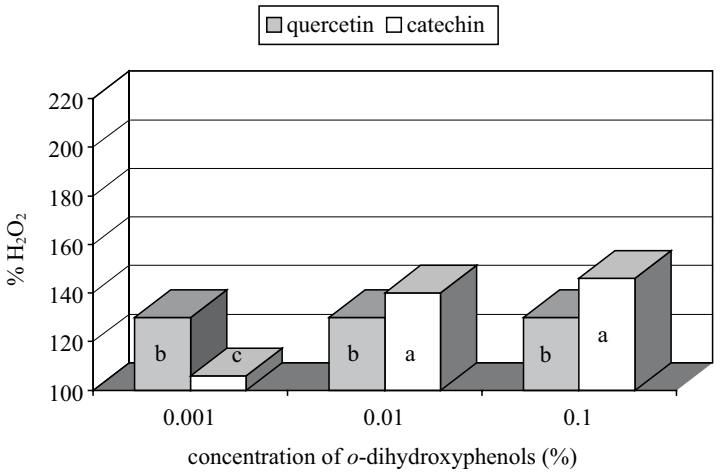

Figure 3. Effect of flavonoids on content of the hydrogen peroxide content within tissues of the grain aphid (control without tested phenols = 100%). Values not followed by the same letter are significantly different at level $p \leq 0.01$ (Duncan's test).

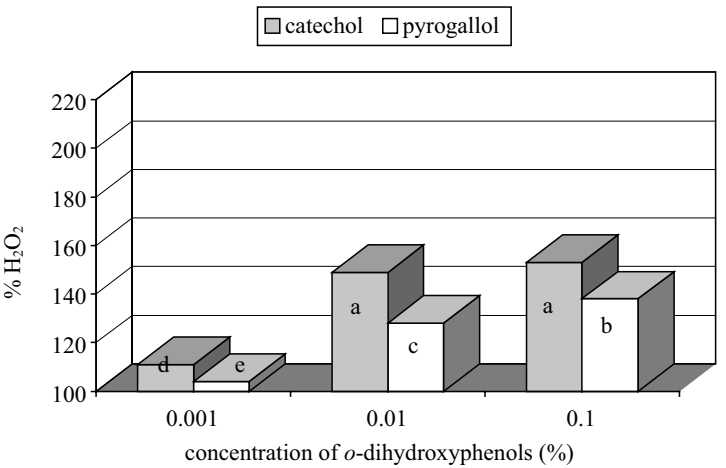

Figure 4. Effect of benzoic acid derivatives on content of the hydrogen peroxide content within tissues of the grain aphid (control without tested phenols = 100%). Values not followed by the same letter are significantly different at level $p \leq 0.01$ (Duncan's test).

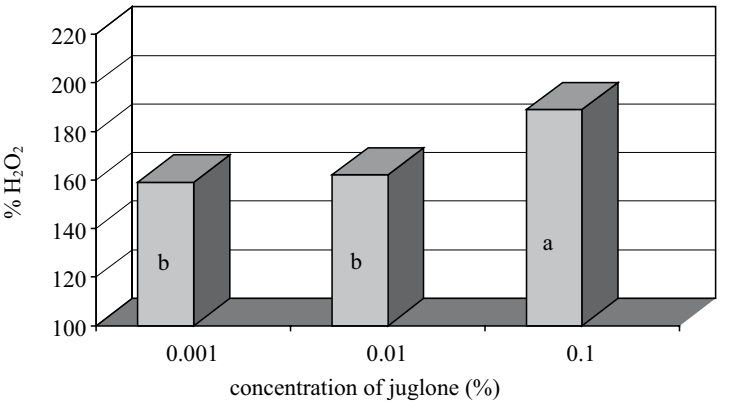

Figure 5. Effect of quinone (juglone) on content of the hydrogen peroxide content within tissues of the grain aphid (control without tested phenols = 100%). Values not followed by the same letter are significantly different at level $p \leq 0.01$ (Duncan's test).

Phenolic compounds represent a major line of the chemical plant defence against herbivores [12] and may produce reactive oxygen species by undergoing spontaneous oxidation to semiquinones [13]. The conducted experiments showed that all of the tested *o*-dixydroxyphenols and quinone contributed to the oxidative burst within the grain aphid tissues. The studies related to *Lepidoptera* demonstrated that the ingestion of oxidizable allelochemicals, such as tannins and other phenols, can induce oxidative stress within herbivores tissues [7, 8, 13]. It may lead to damage of midgut epithelial proteins and lipids [14], lethal deformations [15] and reduced availability of essential amino acids [16]. Among the studied allelochemicals the caffeic acid exerted the strongest effect on accumulation of the hydrogen peroxide within the grain aphid tissues. Monitoring the aphid stylet pathways within plant tissues using electrical penetration graphs (EPG) method showed that caffeic acid reduced duration of probing and decreased salivation and ingestion from sieve elements [1]. Previous studies indicated that *trans*-cinnamic acid derivatives, caffeic acid and chlorogenic acid, had the greatest potential effects on lipid peroxidation, protein oxidation and ascorbate depletion within midgut tissues of *Helicoverpa zea* (*Lepidoptera*, *Noctuidae*) [6]. The juglone at the highest concentration affected hydrogen peroxide content similarly to caffeic acid. In our previous experiments this quinone showed the strongest effect on the depletion of thiol groups within the cereal aphid proteins [17]. Thiobaldeaux et al. [18] proved that juglone caused partial loss of epithelial midguts and decreasae in content of glutathione within larvae of *Actias luna* and

Callosoma promethea. In addition, toxic effects of juglone include DNA strand scission [19], oxidative damage from excessive production of reactive oxygen species, depletion of GSH [20] and inhibition of glutathione transferase [21].

In conclusion, the results presented here demonstrated that the plant *o*-dihydroxyphenols and quinones enhanced reactive oxygen species level within the grain aphid tissues. It suggests that the induction of oxidative stress may be an important component of phenolics toxicity to sucking-piercing insects.

REFERENCES

[1] Leszczynski B., Tjallingii W.F., Dixon A.F.G., Swiderski R., Entomol. Exp. Appl., 1995, 76, 157-162.
[2] Tosh C.R., Powell G., Holmes N.D., Hardie J., J. Insect Physiol., 2003, 49, 1173-1182.
[3] Eleftherianos I., Vamvatsikos P., Ward D., Gravanis F., J. Appl. Entomol, 2006, 130(1), 15-19.
[4] Kalyanaraman B., Meth. Enzymol., 1990, 186, 333-343.
[5] Downer R.G.H., Comprehensive insect physiology, biochemistry and pharmacology, (Kerkut G.A., Gilbert L.I., Eds.), Pergamon Press, Oxford, UK 1986, pp. 77-113.
[6] Summers C.B., Felton G.W., Insect Biochem. Mol. Biol., 1994, 24, 943-953.
[7] Barbehenn R.V., Bumgarner S.L., Roosen E.F., Martin M.M., J. Insect Physiol., 2001, 47, 349-357.
[8] Krishnan N., Kodrik D., Turanli F., Sehnal F., *ibid*., 2007, 53, 67-74.
[9] Green M.J., Hill H. A., Meth. Enzymol., 1984, 105**,** 3-22.
[10] Bradford M.M., Analyt. Biochem., 1976, 72, 258-254.
[11] Sohal R.S., Allen R.G., Adv. Free Radical Biol. Medicine, 1986, 2, 117-160.
[12] Harborne J.B., Nat. Prod. Rep., 2001, 18, 361-379.
[13] Barbehenn R.V., Cheek S., Gasperut A., Lister E., Maben R., J. Chem. Ecol., 2005, 31, 969-988.
[14] Bi J.L., Felton G., *ibid*., 1995, 21, 1511-1530.
[15] Barbehenn R.V., Martin M.M., *ibid*., 1994, 20, 1985-2001.
[16] Felton G.W., Donato K.K., Del Vecchio R.J., Duffey S.S., *ibid*., 1989, 15, 2667-2694.
[17] Łukasik I., Leszczyński B., Aphids and other Homopterous Insects, 2005, 11, 117-125.
[18] Thiobaldeaux R.L., Lindroth R.L., Tracy J.W., Comp. Biochem. Physiol., 1998, 120 C, 481-487.
[19] Nutter L.M., Ngo E.O., Fisher G.R., Gutierrez P.L., J. Biol. Chem., 1992, 267, 2474-2479.

[20] Bellomo G., Mirabelli F., DiMonte D., Richelmi P., Thar H., Orrenius C., Orrenius S., Biochem. Pharmacol., 1987, 36, 1313-1320.
[21] Van Ommen B., Ploemen J.H.T.M., Bogaards J.J.P., Monks T.J., Lan S.S., van Bladeren P.J., Biochem. J., 1991, 276, 661-666.