Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2002
Volume 5
Issue 2
Series BIOLOGY

URBAŃSKA A., LESZCZYŃSKI B., MATOK H., DIXON A.F.G. 2002. CYANIDE DETOXIFYING ENZYMES OF BIRD CHERRY OAT APHID **Electronic Journal of Polish Agricultural Universities**, Biology, Volume 5, Issue 2.
Available Online http://www.ejpau.media.pl

CYANIDE DETOXIFYING ENZYMES OF BIRD CHERRY OAT APHID

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ABSTRACT

The activity of beta-cyanoalanine synthase and rhodanese within the tissues of the three generations of the bird cherry-oat aphid that are specialized to feed on the cyanogenic spring foliage of bird cherry was assessed. The order of beta-cyanoalanine synthase activity in the three aphid generations was: generation 1> generation 2> generation 3; whereas that of rhodanese was: generation 3> generation 1> generation 2. The possible role of the bird cherry – oat aphid enzymes in metabolizing dietary cyanide is discussed.

Key words: beta-cyanoalanine synthase, rhodanese, cyanide detoxication, bird cherry-oat aphid, *Rhopalosiphum padi,* bird cherry, *Prunus padus*

INTRODUCTION

Herbivorous insects may induce cyanogenesis while feeding on cyanogenic plants and as a consequence are exposed to the toxic effects of the released cyanide [8, 15]. One such insect is the bird cherry-oat aphid, *Rhopalosiphum padi* L., which feeds in spring on the highly cyanogenic leaves and young shoots of bird cherry, *Prunus padus* L, its primary (winter) host-plant. Cyanide is toxic for many herbivorous insects, but there are specialists that can feed on highly cyanogenic plants.

Some herbivorous insects, including aphids, e.g., *Therioaphis trifoli*, are tolerant of cyanide and even prefer cyanogenic plants [7, 11, 18]. These insects use enzymes to metabolize cyanide. One such enzyme, rhodanese (thiosulphate: cyanide sulphur transferase; EC 2.8.1.1) converts cyanide into the less toxic thiocyanate. As a consequence, the rhodanese activity in insects that feed on cyanogenic plants has been widely studied [2, 8, 10,

12, 13, 14, 16]. The conclusion is that although rhodanese may be involved in the tolerance of cyanide its primary role is not the detoxification of cyanide in these insects. Insensitivity of insects to cyanide is attributed to another enzyme, beta-cyanoalanine synthase (beta-CAS; EC 4.4.1.9), although not many data were provided in this aspect [1, 5]. This enzyme is thought to 'transfer' cyanide into the amino acid pool by catalyzing the first in a series of reactions. Another enzyme, beta-cyanoalanine hydratase (EC 4.2.1.65) participates in this process and the sequential action of these two enzymes constitutes an effective mechanism for detoxifying HCN [6, 20].

In this paper we report on the activity of rhodanese and beta-cyanoalanine synthase in the tissues of the first three generations of the bird cherry-oat aphid, which feed on the highly cyanogenic spring foliage of bird cherry.

MATERIALS AND METHODS

Aphids. The overwintering eggs that the bird cherry-oat aphid lays in autumn hatch in spring and give rise to three parthenogentic generations of aphids on bird cherry. The third generation individuals are winged and on completing their development fly off and colonize a variety of grasses, which are the secondary host plants of this host-alternating aphid. The first generation individuals are green in colour, those of the second generation are mainly black and lack wings and those of the third generation are similar in colour but have wings. Aphids were collected weekly, beginning in late April and ending in the second third of May. The spring migrants that colonize cereals and other grasses were caught in suction traps in the second third of May. Immediately after collection, the aphids were homogenized in an appropriate buffer and enzymes were prepared.

Rhodanese assay. Activity of rhodanese was determined according to Sörbo [19]. The aphids were homogenized in 200 mM ice-cold Na-phosphate buffer, pH 7.5 and centrifuged at 600*g* for 15 minutes. The supernatant was collected and used to determine enzyme activity. The incubation mixture contained: 1.0 ml of the enzyme extract, 1.0 ml of buffered 125 mM potassium cyanide and 1.0 ml of 125 mM sodium thiosulphate. After 30 minutes of incubation at 30°C, the reaction was stopped by addition of 0.5 ml of 37% formaldehyde followed by 2.5 ml of ferric nitrate reagent. Next, the reaction mixture was centrifuged at 1050*g* for 15 minutes, and then absorbance at 460 nm was measured against a control containing formaldehyde. Sodium thiocyanate was used as a standard and activity of the rhodanese was expressed in nmol of thiocyanate/min^{-1}/mg^{-1} protein.

beta-cyanoalanine synthase assay. The beta-CAS activity was determined using a slightly modified method of Blumenthal *et al.* [3]. The aphids were homogenized in ice-cold 50 mM Tris-HCl buffer, pH 8.5. The crude homogenate was centrifuged at 600*g* for 15 minutes and the supernatant was used as the enzyme source. The reaction mixture contained: 1.0 ml of the enzyme extract, 0.5 ml of 50 mM NaCN and 0.5 ml of 10 mM L-cysteine, both buffered in 100 mM Tris-HCl buffer, pH 8.5. This mixture was incubated in a closed vessel for 30 minutes at 30°C. The reaction was stopped by the addition of 0.5 ml of 20 mM N, N-dimethyl-*p*-phenylenediamine sulphate and 0.5 ml of 30 mM ferric chloride. The mixture was centrifuged at 1000*g* for 15 minutes and the absorbance at 650 nm was measured using a Hewlett Packard 8452 A spectrophotometer. Sodium sulfide was used as a standard and activity of the beta-cyanoalanine synthase was expressed in nmol H_2S/min^{-1}/mg^{-1} protein. Three independent replicates of each assay were performed, and the enzyme analyses were carried out at a temperature 0-4°C. Protein content in the enzyme extracts was determined using Bradford method [4] and bovine serum albumin as a standard.

Statistics. Differences in activity of the enzymes in the three aphid generations were analyzed by an analysis of variance followed by Duncan's test. In addition, Pearson's correlation coefficients between enzyme activity and cyanogenesis potential were also calculated.

RESULTS AND DISCUSSION

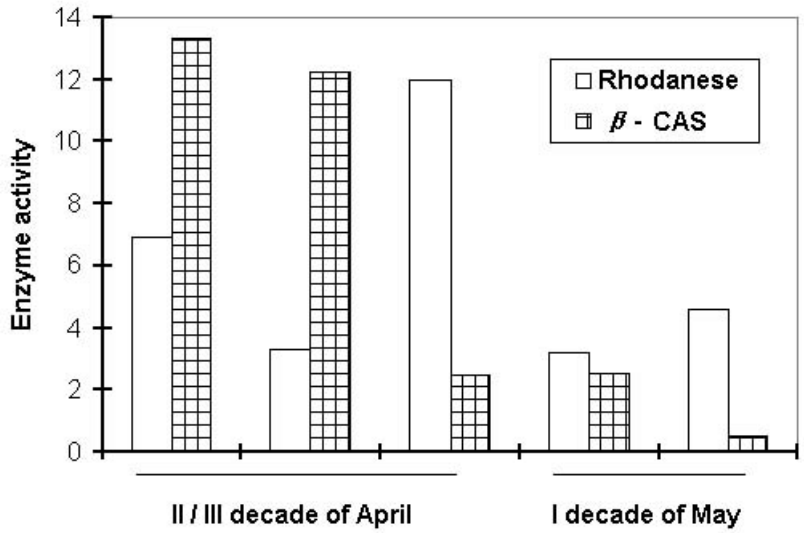
The homogenates of the three generations of the bird cherry-oat aphid contained both rhodanese and beta-cyanoalanine synthase. The order of the rhodanese activity in the three generations was: generation 3 > generation 1 > generation 2. Significantly higher activity of beta-CAS was recorded in homogenates of first generation individuals compared with other generations (Table 1). Generally, the activity of beta-cyanoalanine synthase in the tissues of first generation individuals was about 2-times greater than that of rhodanese. In contrast, the activity of rhodanese was much higher in tissues of individuals of the third generation, which is less exposed to cyanide. Over the spring period while feeding on the primary host the aphid shows fluctuations in the activity of rhodanese and a constant decrease in the activity of beta-cyanoalanine synthase (Fig. 1).

Table 1. Activity of cyanide metabolizing enzymes in the tissues of individuals of the first three generations of *R. padi* (means ± SE, n=3)

Generation	Rhodanese (nM SCN/min^{-1}/mg^{-1} protein)	beta-CAS (nM H_2S/min^{-1}/mg^{-1} protein)
First	6.90 ± 0.11 [a]	12.77 ± 0.05 [a]
Second	5.42 ± 0.35 [a]	1.52 ± 0.12 [b]
Third	7.96 ± 0.41 [a]	1.11 ± 0.08 [b]

Values in columns not followed by the same letter are significantly different at 0.01 (Duncan's test)

Fig. 1. Activity of rhodanese (nmol SCN × min^{-1} × mg protein^{-1}) and beta-CAS (nmol H_2S × min^{-1} × mg protein^{-1}) within the bird cherry-oat aphid tissues.



The results indicate that the generations of *R. padi* that fed on the foliage of bird cherry in spring show both rhodanese and beta-CAS activity, and thus are able to convert HCN into less toxic thiocyanate and/or to include it into the amino acid pool. Rhodanese activity has been widely reported from a variety of herbivorous insects, including the aphids *Aphis loti* and *Acyrthosiphon loti,* which are known to feed on cyanogenic plants [2]. beta-CAS activity has also been found for numerous arthropods including: polydesmoid millipedes, *Oxidius gracilis* and *Harpaphe haydeniana*, larvae of the noctuid moths, *Heliothis zea* and *Spodoptera exiqua*, and adults of the lygaeid bug *Oncopeltus fasciatus* feeding on cyanogenic plants [12].

The results presented here indicate that rhodanese activity in *R. padi* is not directly associated with the cyanogenesis potential of bird cherry foliage (unpublished data). First generation individuals of the bird cherry-oat aphid that fed on the highly cyanogenic youngest leaves of bird cherry did not show an increased rhodanese activity. Thus, similarly to [16], we conclude that rhodanese is not *R. padi* sole defense against dietary cyanide. The primary role of the rhodanese in cyanide detoxication, assumed in earlier studies, has not be supported by subsequent studies on a wide range of herbivorous insects including *Coleoptera, Lepidoptera* and also *Homoptera* that feed on cyanogenic *Lotus corniculatus* and *Vicia sativa* [2]. According to Jones [14] other sulphurtransferases such as 3-mercaptopyruvate sulphurtransferase (EC 2.8.1.2) and thiosulphate: thiol transferase (EC 2.8.1.3.) are involved in HCN-detoxication.

Our results indicate that dietary cyanide may have induced the high activity of beta-CAS in the bird cherry-oat aphid tissues. First generation individuals have the highest beta-CAS activity and fed on the youngest leaves that have the highest cyanogenesis potential (unpublished data). This suggests that beta-cyanoalanine synthase is an important enzyme in the detoxication of dietary cyanide in *R. padi*. Larvae of *Heliconius melpomene* fed on cyanogenic plants show beta-CAS activity, but not those fed on non cyanogenic plants [9]. In addition beta-CAS activity was much higher in *Synanthedon exitiosa* insensitive to amygdalin in their diet than in *S. pictipes*, which is sensitive to this cyanogenic glycoside [17]. According to Brattsten [6] it is possible that beta-CAS is an important enzyme protecting insects from prolonged exposure to low concentrations of cyanide.

In conclusion, the bird cherry-oat aphid is adapted to detoxify the cyanide produced by the leaves of its primary host-plant. However, beta-cyanoalanine synthase plays a major role in the metabolism of cyanide in the bird cherry-oat aphid.

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