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DOES CYANOGENESIS INFLUENCE HOST ALTERNATION OF BIRD CHERRY-OAT APHID?

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ABSTRACT

Changes in content of cyanogenic glycosides and in cyanogenesis potential within the bird cherry leaves during occurrence of the bird cherry-oat aphid, *Rhopalosiphum padi* L. and while its spring host-plants alternation have been studied. The highest content of the cyanogenic glycosides and the highest cyanogenesis potential was found in the youngest leaves of the primary host during occurrence of the first fundatrices. When the aphid population started to build up, a decrease in content of the cyanogenic glycoside and in the cyanogenesis potential was observed. Finally, when the winged migrants began to fly off from the primary host onto cereals, pretty low amount of the plant xenobiotics was recorded within the bird cherry leaves. Possible role of the cyanogenesis in host alternation of the bird cherry-oat aphid is discussed.

Key words: bird cherry-oat aphid, *Rhopalosiphum padi*, *Prunus padus,* host alternation, cyanogenesis, cyanogenic glycosides

INTRODUCTION

Plant xenobiotics e.g. phenolic compounds, hydroxamic acids, alkaloids or furanocoumarins are highly toxic to phytophagous insects, including cereal aphids. They negatively influence the aphid feeding behaviour, prolong growth and development, reduce fecundity and lower intrinsic rate of natural increase and modulate activity of the aphid enzymes [22, 23, 27]. Among them, cyanogenic glycosides are serious problem to numerous herbivores since they are widely distributed and generate toxic HCN upon plant tissues disruption and/or ingestion of animal enzymes [15, 25].

Cyanogenesis is especially dangerous for generalist phytophagous insects because the cyanide similarly to carbon oxide, nitrogen oxide or azides is binding of O_2 to heme unit of the terminal cytochrome oxidase and blocks electron transport through the respiratory chain [7]. Thus the generalists usually do not feed on plants rich in the cyanogenic glycosides and in that case cyanogenesis might play an important role in plant chemical defence. Monophagous insects that are specialised to feed on cyanogenic plants developed specific enzymes: β - cyanoalanine synthetase, sulphur transferase (rhodanese) and/or linamarase that allows them to detoxify the highly toxic cyanide [1, 3, 5, 8, 10, 31].

There are also insect species that need more than one hosts during their life cycle, and occur on distantly related plant species. One of them is bird cherry-oat aphid, *Rhopalosiphum padi* (L.) that alternates between woody bird cherry (*Prunus padus* L.) and herbaceous *Gramineae*. Phenology of this phenomenon has been extensively studied [9, 18, 19, 20, 21]. However, a little information is provided on chemistry of this process [14, 17], and no data on role of the cyanogenesis in *R. padi* host-plant alternation is available.

The present paper reports on changes in content of the cyanogenic glycosides and cyanogenesis potential in tissues of the bird cherry foliage during *R. padi* occurrence on the primary host and while its spring host alternation.

MATERIALS AND METHODS

Aphids. The bird cherry-oat aphid *R. padi* population occurred on the primary host was used in the experiments. The studied aphid morphs were: *fundatrix* (the first morph hatched from the winter eggs), *fundatrigeniae* and winged migrants (*alatae*).

Chemicals and columns. Commercial standards of prunasin and amygdalin and other chemicals used in the experiments were purchased from Sigma Chemical Company. The chromatography columns for purification and separation of the cyanogenic glycosides came from Waters Associates, Milford, MA, (USA) and Saulentechnik, Homburg, (Germany), respectively.

Field experiments. A population of the bird cherry-oat aphid was monitored weakly on fifty randomly chosen young shoots of the bird cherry trees growing in Aleksandria Park, Siedlce. Our observation started from the end of April and was terminated at the beginning of June, and population dynamics of the aphid on the primary host was established. During this observation, two groups of the bird cherry leaves were collected: (1) uninfested (control), and (2) infested by the bird cherry-oat aphid. After removing of the aphids from the infested leaves, all samples were divided into two portions. The first one was freeze-dried and kept at 0°C until cyanogenic glycoside analysis. The second part was immediately used for determination of the cyanogesis potential.

Determination of cyanogenic glycosides content. Content of the bird cherry cyanogenic glycosides was determined after HPLC separation according to slightly modified procedure given by Stochmal and Oleszek [34].

1 g of the freeze-dried plant material was extracted with 10 ml of 70% methanol by blending in a Ultra-Turrax T25 blender (IKA, Labortechnik, Germany), followed by 20 min sonification at room temperature. The extract was purified on C18 Sep-Pak cartriges (Waters Associates, Milford, MA, USA), using a solid-phase extraction-purification procedure. This was done by concentration of the extract *in vacuo* (40°C) until almost all the methanol was removed. Next the concentrated extract was passed through the preconditioned Sep-Pak cartridge, which was then washed with 5 ml of 20% MeOH. The elutes were combined, freeze-dried, dissolved in 0.5 ml of the HPLC mobile phase and used for the cyanogenic glycosides determination. 20 µl of the sample was analysed using HPLC unit (Knauer, Berlin, Germany) equipped with a computer system to monitor chromatographic parameters and to process the data and a differential refractometer detector. The sample was injected on a Eurospher RP18 column (Saulentechnik, Homburg, Germany; 25 cm x 4.6 mm i.d. 5 µm particle size). The used mobile phase was H_2O : MeOH : H_3PO_4 (85 : 15 : 0.05) and the system was operated isocratically at a flow rate of 1 ml/min. Quantitation of the cyanogenic glycosides in the plant material was based on external standard method. A standard curve was prepared by plotting a peak area against the concentration of the studied compounds. Content of the cyanogenic glycosides in the bird cherry tissues was expressed as mg/g of freeze-dried material.

Cyanogenesis potential assay. Potential of the cyanogenesis within the bird cherry leaves was measured using slightly modified procedure of Pereira *et al.* [30].
0.5 g of freshly harvested plant material was ground in mortar and pestle with 15 ml of 0.7 M citrate buffer pH 8.7. A portion of 1 ml of the homogenate was added to 4 ml of 0.7 M citrate buffer pH 5.3 in a modified Wartburg flask and sealed with a septum. Next, 0.01 ml of exogenous β-glucosidase from almonds (Sigma-Aldrich Fine Chemicals) was injected into the mixture and incubated at 37°C. Released HCN was absorbed in 5 ml of 5% Na_2CO_3 solution located in a centre well. After 4 hours, 0.5 ml of 2N H_2SO_4 was added from the side arm of the flask and the mixture was incubated for an additional 15 min. When the incubation was ended, the sample was taken from the center well with a syringe and cyanide content was determined spectrophotometrically [6, 13]. To 1.0 ml of sample in a test tube 0.2 ml of 1% aqueous chloramine T solution was added and after one minute, 6 ml of pyridine-pyrazolone reagent, and then the test tube was stoppered and shaken. After next 20 min, the absorbance of the mixture was measured at 630 nm, against control containing buffer instead of the sample. Content of the released cyanide in the reaction mixture was calculated from calibration curve prepared from NaCN standard solution. Cyanogenesis potential within the bird cherry foliage was expresses as μg released HCN/g of fresh material. Chemical analyses were done in three independent replicates.

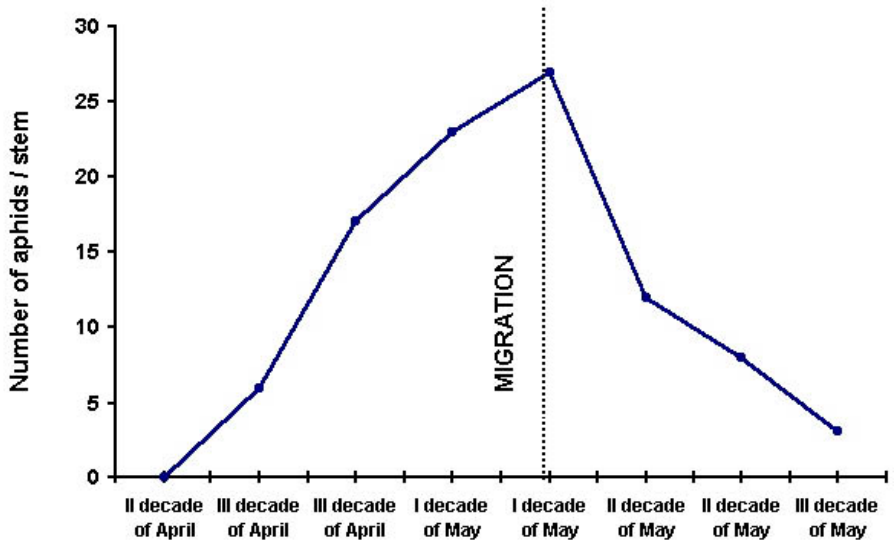
Statistics. Differences in content of the cyanogenic glycosides and in the cyanogenesis potential within the bird cherry leaves were analysed by an analysis of variance followed by Duncan's test. The Pearson's correlation coefficient between content of the cyanogenic glycosides within the uninfested bird cherry leaves and the cyanogenesis potential in these tissues was calculated.

RESULTS

Dynamics of the bird cherry-oat aphid population on the primary host.

The first fundatrices of *R. padi*, hatched from the winter eggs were observed on the bird cherry in the end of April. These morphs fed on the opening buds and then on the undersides of young leaves and soon after the first fundatrigeniae were found. While increase in size of the colonies, the aphids spread along leaf stalks, young shoot stems and inflorescence. The bird cherry-oat aphid population reached maximum on winter host in the middle of May (Fig. 1). At the same time, winged migrants alternating into summer hosts were gradually formed and most of them left the primary host immediately after being created. So, there were always no more than a few winged aphids among still quite abundant wingless fundatrigeniae. With appearance of the alates on the bird cherry, the level of the *R. padi* population rapidly declined as a result of the spring migration (Fig. 1).

Fig. 1. Dynamics of *R. padi* population on primary host-plant



Changes in content of the cyanogenic glycosides within the bird cherry leaves.

The highest content of the cyanogenic glycosides (0.62 mg/g freeze-dried mater) was found within the uninfested youngest leaves and was at the time of appearance of the first fundatrices of *R. padi* on the primary

host (Table 1). While the aphid population started to build up, a rapid decrease of the cyanogenic glycosides within the uninfested bird cherry leaves was found. It was continued until the third decade of May, when almost all of the aphids left the primary host-plant. The aphid infestation, at first brought slight increase in content of the cyanogenic glycosides, this was followed by a 30% decrease in their content, during the second decade of May, when the aphid population was mostly abundant (Table 1).

Table 1. Comparison of the cyanogenic glycosides content (mg/g freeze dried weight) within the bird cherry leaves

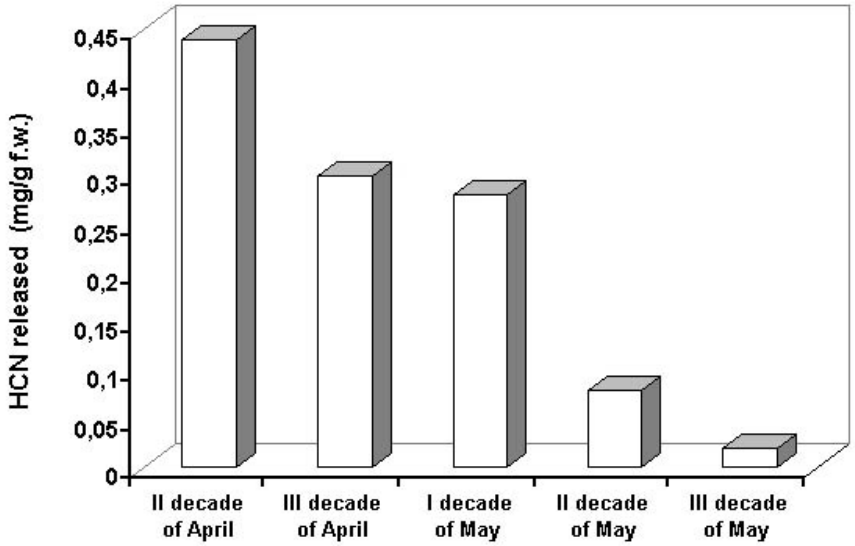
	Uninfested foliage	Infested foliage
II decade of April	0.62[a]	0.61[a]
III decade of April	0.52 [a]	0.54 [a]
I decade of May	0.38 [b]	0.36 [b]
II decade of May	0.18 [c]	0.12 [d]
III decade of May	0.12 [d]	0.11 [d]

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

Cyanogenesis potential within the bird cherry leaves.

Obtained results showed that there was a highly significant, positive correlation (r = 0.97) between content of the cyanogenic glycosides within the uninfested bird cherry leaves and level of the cyanogenesis in their tissues. The youngest leaves of the bird cherry had the greatest potential to generate HCN. Such leaves, after mechanical maceration, released about 0.44 µg of HCN/g of fresh weight (Fig. 2). During development of the *R. padi* population a constant decrease in the cyanogenesis potential of the bird cherry foliage was observed Finally, in the end of May homogenates of the bird cherry leaves released only traces of the toxic cyanide (Fig. 2).

Fig. 2. Changes in HCN released (µg/g fresh weight) by macerated tissues of uninfested leaves of the bird cherry.



DISCUSSION

Cyanogenesis has been proved to be a serious problem for many generalist insects [15, 29, 32, 36]. However, specialists often occur preferentially on the cyanogenic plants [4, 33]. There are also aphid species, for example *Therioaphis trifolii* that prefer cyanogenic plants and other (*Aphis craecivora, Nearctaphis bakeri*) occurring mostly on acyanogenic hosts [12, 29]. During stylet punctures the aphids destroy plant cells, release endogenous β-glucosidase and secrete within saliva their own enzymes into the plant [28]. As a result of this wounded effect changes in metabolism of plant xenobiotics, including cyanogenic glycosides occur within the aphid-infested plant tissues.

The obtained results suggest that appearance of various morphs of the bird cherry-oat aphid was strictly related to variation in cyanogenesis potential within the bird cherry foliage. Such behaviour was described previously for other herbivorous insects feeding on *Lotus corniculatus* and *Trifolium repens* [16]. When there is no host alternatives, the first fundatrices of *R. padi* specialise on highly cyanogenic leaves. Morphs feed on less cyanogenic *P. padus* leaves, and winged migrants when almost all cyanogenic compounds disappear from the bird cherry leaves and when their nutritional value is dramatically declined [23, 24, 26].

Moreover, the winged migrants of *R. padi* colonise young highly cyanogenic secondary hosts such as: wheat, rye, triticale, sorghum, barley and oats containing numerous cyanogenic glycosides e.g. dhurrin, linamarin, lotaustralin and/or epilotaustralin [16]. Its well known, that prunasin is a major cyanogenic glycoside within the bird cherry leaves [2]. This suggests that cyanogenesis is not serving as a major plant defence mechanism towards the bird cherry-oat aphid. On the other hand, it has been demonstrated that procyanidin isolated from sorghum was twice more deterrent to another cereal aphid, *Schizaphis graminum* than dhurrin [11]. In addition, the bird cherry-oat aphid is pretty well adapted to the cyanogenic compounds, since it uses β-cyanoalanine synthetase and rhodanese to detoxify the toxic cyanide. This is especially true for the first fundatrices and migrants of *R. padi* that showed the highest activity of these enzymes [35]. Summing up, the results presented here suggest that the spring host alternation of the bird cherry-oat aphid is related to the cyanogenic status of its host-plants.

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