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Influence of bird cherry-oat aphid (*Rhopalosiphum padi* (Linnaeus, 1758)) /Hemiptera, Aphidoidea/ feeding on the activity of β-glucosidase within tissues of its primary host

HUBERT SYTYKIEWICZ

University of Podlasie, Department of Biochemistry and Molecular Biology,
Prusa 12, 08-110 Siedlce, Poland
huberts@ap.siedlce.pl

Introduction

Rhopalosiphum padi (Linnaeus, 1758) represents one of the dominating species of the aphid fauna settling cereals agrocenoses in the United States (ZWEINER *et al.*, 2005), in Western Europe (ROSCHEWITZ, 2005) and in Poland (CIEPIELA *et al.*, 2006). The life cycle of this hemipteron is connected with the migration of winged specimens between philogenetically distant hosts. Bird cherry (*Prunus padus* L.) is its primary host in Europe, whereas a wide spectrum of secondary hosts embraces numerous species of the Poaceae family, along with some representatives of the Cyperaceae, Iridaceae, Juncaceae and Typhaceae (STOETZEL & MILLER, 2001; ASLAN & UYGUN, 2005).

The glucohydrolase β-D-glucopiranosides (β-glucosidase, E.C. 3.2.1.21) is a biocatalyser hydrolyzing β-glucosides containing in their molecule β-glicosidic bonds (GERARDI *et al.*, 2001; HAYASHI *et al.*, 2004; SPEROTTO *et al.*, 2008). It also performs important biochemical and physiological functions in plants' organs, in the conditions of uninhibited growth and development and during a modulating reaction of abiotic and biotic stresors (BLANCHARD *et al.*, 2001; SAKAI *et al.*, 2008; SPEROTTO *et al.*, 2008). The aim of this research was to determine the impact of bird cherry-oat aphid on the level of catalytic activity of β-glucosidase in the leaves of *P. padus*.

Material and methods

Field experiment

The observation of the population growth of the studied aphid species was carried out on the shrubs of *P. padus* in Municipal Park Alexandria in the town of Siedlce, in the natural settlement of these shrubs by *R. padi*. Three morphs of *R. padi* were embraced by entomological tests: fundatrices, wingless virginoparae (fundatrigeniae) and migrantes. The observation was carried out in three subsequent growth seasons (2001-2003). It was initiated directly after the appearance of fundatrices on *P. padus* shoots. This took place in 2001 and 2003 in the III decade of April (22.04 and 29.04, respectively), and in 2002 on 11.04. Entomological observations were carried out in weekly intervals, each time recording the type of morph and its number. The applied technique involved direct counting of the *R. padi* specimens on 50 side shoots of *P. padus* selected at random (each was about 40 cm long) and subsequently isolated with bolting-cloth. The observations were carried out until the decline of the *R. padi* population on isolated shoots of the primary host (02.06.2001, 23.05.2002, and 06.06.2003). The results constituted the basis to assess the course of the number dynamics of the studied aphid species on isolated shoots of the primary host.

The experiment concerning the impact of the feeding of *R. padi* on the activity of the identified enzyme (β-glucosidase) in the tissues of the primary host was carried out on shrubs of *P. padus* in the Municipal Park Alexandria in the town of Siedlce. After the hatching of fundatrices from overwintering eggs, 100 side shoots of *P. padus* (each about 40 cm long) with fundartices on them were selected at random along with the same number of checked shoots (without aphids). These shoots were isolated with bolting-cloth, followed by the collection of plant material (side shoots) for biochemical analyses. The research material was collected each time in the afternoon in five selected terms (during *R. padi* presence on the primary host) in 2001-2003 (Tab. 1).

Table 1. Time of plant material collection to chemical analyses (2001-2003)

Number of shoots collection*	2001	2002	2003
I.	22.April	11.April	29.April
II.	29.April	18.April	06.May
III.	05.May	25.April	20.May
IV.	19.May	09.May	27.May
V.	02.June	23.May	06.June

* numbers (I-V) of shoot collection for chemical analyses corresponded to different cycles of *R. padi* population development and were used throughout the remaining part of the research as developmental stages of *R. padi*: I – first occurrence, II – increase, III – maximum, IV – decrease, V – disappearance;

β-glucosidase assay

The extraction of β-glucosidase out of the leaves of *P. padus* was made by means of homogenization of 500 mg of preparation of acetone of these organs in 20cm^3 0.1 M phosphoric buffer with pH 5.8. The obtained suspension was centrifuged with 13000g for 30 min, and in the obtained supernatant the enzyme activity was identified using the Katagiri method modified by CHARARAS & CHIPOULET (1982). The reaction mixture contained: 0.2 cm^3 of enzymatic preparation, 0.2 cm^3 of substrate solution (50 mM p-nitrophenyl-β-D-glucopiranoside in 0.1 M phosphate buffer with pH 5.8) and 0.1cm^3 of 0.1 M phosphate buffer. The mixture was incubated for 1 hour in 30°C, and after the assessed time the enzymatic reaction was interrupted by adding 3 cm^3 of 2% of Na_2CO_3 solution. Next, the absorbantion value was measured with λ =400 nm, while the control sample (synthetic substrate was replaced with the same volume 0.1 M of phosphate buffer with pH 5.8). The concentration of released p-nitrophenol during the reaction of hydrolyse of the synthetic substrate was read out from the standard curve made for the following concentration of this chemical compound: 0.1; 0.5; 10; 20; 30 and 50 μM. The specific activity of the analysed enzyme was expressed in μM p-nitrcphenol min.$^{-1}$ mg^{-1} of protein. The protein content in enzyme extracts was assessed according to LOWRYS *et al.* method (1951).

Statistical analysis

Chemical analyses were made in three replications The significance of differences between mean numbers in the level of activity of the studied enzyme in leaves of *P. padus* during the studied growth seasons was estimated with a Duncan's multiple test, with significance levels of $p \leq 0.01$ and $p \leq 0.05$. The significance of differences in the degree of catalytic activity of the identified biocatalyser between the settled and checked leaves was assessed using the t-Student's test, with significance levels of $p \leq 0.01$ and $p \leq 0.05$.

Results

The observations of changes in the number of *R. padi* on isolated side shoots of *P. padus* showed its significant diversity during the studied seasons. In all the years of the research (2001-2003) the course of number dynamics of the studied aphid species had the form of a curve for one pick (Fig. 1). The highest mean numbers of aphids (an individual per one side shoot) was recorded in the growth season of 2002, while the lowest in 2001. Meanwhile, it was pointed out that the period of *R. padi* feeding on the primary host's shoots was the longest in 2002 (amounting to 44 days), whereas the shortest – in the last of the studied seasons (38 days). Moreover, it was found out that the period of

bird cherry-oat aphid population development from the day of its appearance until reaching their maximum number was the longest in the last analysed year (21 days), and in 2001-2002 it amounted to 13 and 14 days respectively (Fig. 1).

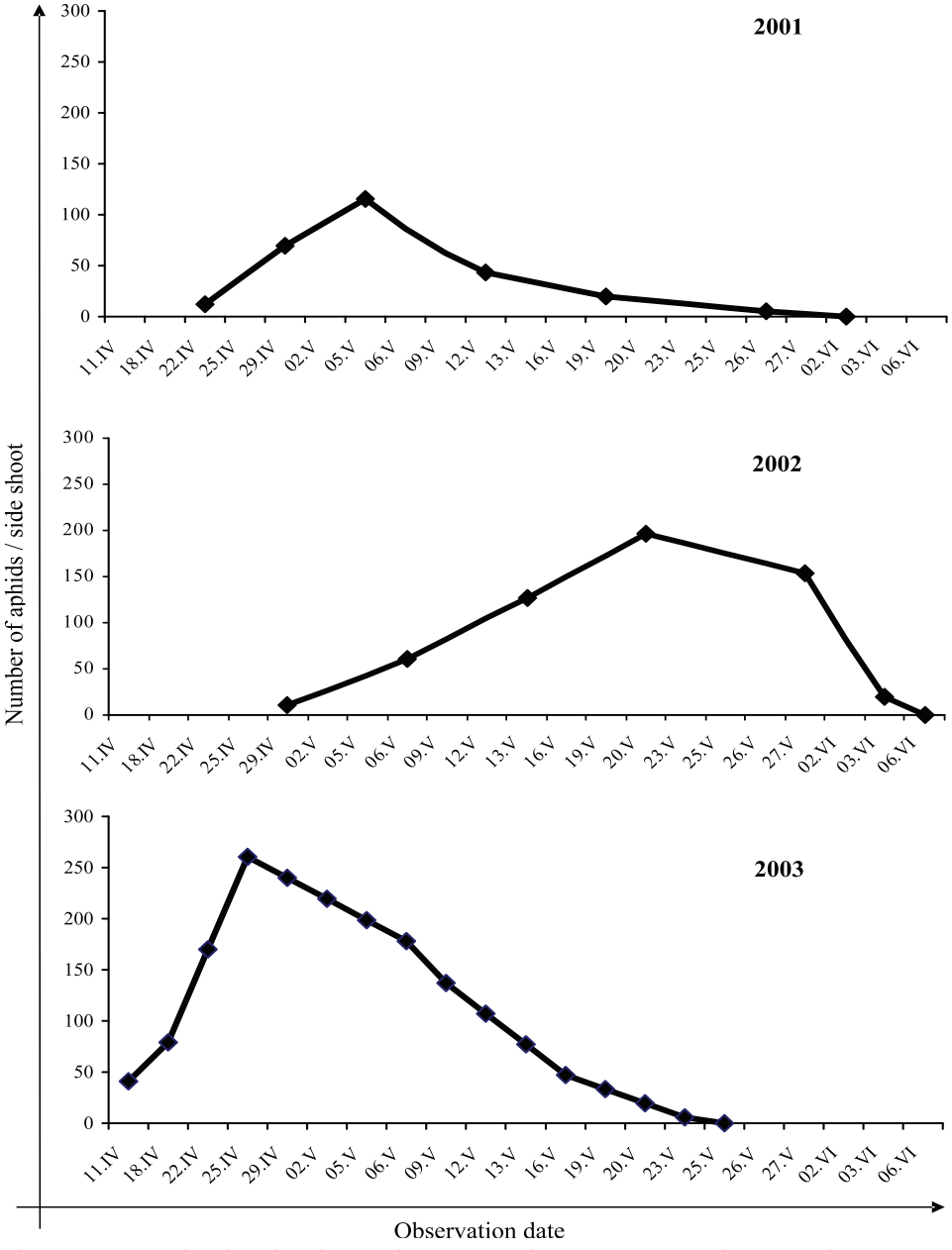


Figure 1. Changes in *Rhopalosiphum padi* numbers on isolated shoots of primary host in 2001-2003

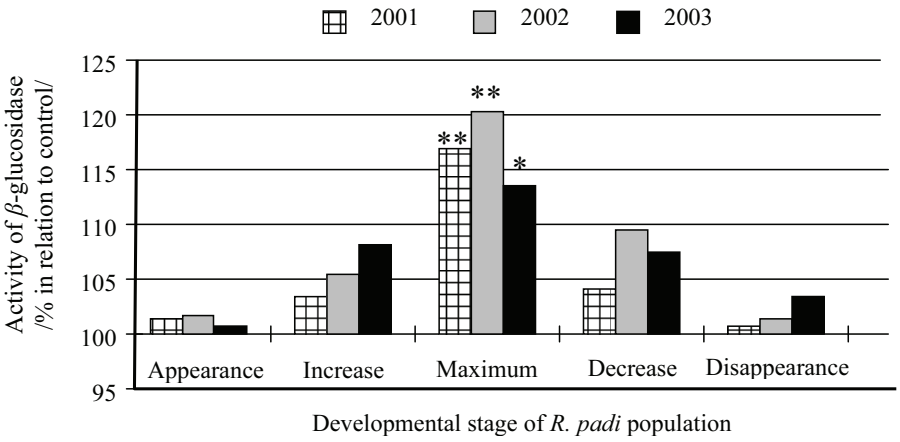
In 2001 the appearance of the studied aphid species on the *P. padus* shoots was noted on April 22. After that day a gradual increase in the size of the analysed population was recorded, which reached the maximum density on May 5. The following days a reverse tendency was recorded, i.e. a decrease in aphid number, and on June 2 the lack of *R. padi* specimens on the primary host was recorded.

The observations carried out in 2002 allowed one to state that the first fundatrix specimens settled the side shoots of the primary host on April 11. Following that week, i.e. from April 18 onwards, a sudden increase in the number of aphids was recorded; *R. padi* population reached the maximum in the third decade of April (April 25). Following that date a gradual decrease in the number of *R. padi* population was registered leading to its complete disappearance in III decade of May (May 25).

Entomological observations carried out in the last of the analysed seasons (year 2003) pointed out that the appearance of *R. padi* on the primary host took place in the last days of April (April 29). Maximum aphid density (number of individuals/ per one side shoot) was recorded on May 20, and the disappearance of the studied population was recorded in the first decade of June (June 6).

During the direct counting of *R. padi* specimens on the primary host's shoots in 2001-2003, macroscopic changes in leaf blades of *P. padus* were observed. They were manifested by the yellowing of places in which aphid colonies were present and deformations of leaf blade shape – rolling of ridges and twisting as well as folds of their surface.

The results of biochemical analyses proved that bird cherry-oat aphid feeding on the primary host leaves in all the studied seasons stimulated the activity of β-glucosidase (Fig. 2). The highest level of activity of the marked enzyme was registered in the period of *R. padi* maximum presence, while the lowest in the beginning and at the end of the experiment. The statistical analysis of the obtained data confirmed the significance of activity increase of the studied enzyme (between checked leaves and those settled by aphids) only for the phase of *R. padi* maximum density on isolated shoots of the primary host.



*significant differences by p≤ 0.05; ** significant differences by P ≤ 0.01 (t-Student test)

Figure 2. Changes in activity of β-glucosidase in bird cherry leaves stimulated by *Rhopalosiphum padi* feeding (2001-2003)

Discussion

Entomological observations which were carried out in 2001-2003 proved that the dynamics of *R. padi* population developing on isolated shoots of the primary host (*P. padus*) were characterised by phonologically conditioned differences in the degree of its density. Among the most important abiotic factors which determine aphid population number on host plants one has to include weather conditions, such as precipitation, sunlight, temperature and relative air humidity (Morgan, 2000; Stary & Lukasova, 2002; Jaśkiewicz, 2004; Legrand *et al.*, 2004). Intense precipitation and low temperature lowered the degree of density of these hemipterons on host plants. Among the biotic factors which negatively influence the level of phytophages it is worth mentioning the following: a parasitoid attack, a development of entomopathogenic fungi and an impact made by aphid predators (Feng & Chen, 2002; Sigsgaard, 2002; Chen & Feng, 2004; Legrand *et al.*, 2004). The application of partly controlled conditions in the presented experiment contributed undoubtedly to the formation of specific microclimate inside the isolators (such as higher temperature and relative air humidity as well as partial protection against wind) which favoured *R. padi* population dynamic development. The use of isolators for a test allowed to eliminate the impact of aphid parasitoids and predators, and competition between the species (for nutrient and feeding place) on the size of this arthropod's species population.

Aphid feeding induces the occurrence of multilayered changes of a physiological-biochemical nature taking place in tissues of host plants. The results of biochemical assignments let one state that the feeding of *R. padi* induced the activity of the analysed glucosidase in the leaves of the primary host. It was found out, meanwhile, that the highest increase of the activity of the studied biocatalyser took place when *R. padi* number was the most dense, and the lowest – during the phase of the aphid's appearance and disappearance on isolated shoots. The research by Van de Ven *et al.* (2000) confirms the above results. They proved that the feeding of *Bemisia argentifolii* (Hemiptera: Aleyrodidae) on the leaves of common pumpkin (*Cucurbita pepo* L.) cv. 'Chefini' to a large degree stimulated the activity of β-glucosidase in relation to the check. Moreover, the results of analyses carried out by these researchers proved that the leaves of common pumpkin, which were attacked by *B. argentifolii*, showed an increased transcription activity of *SLW3* gene which codes N-glycon β-glucosidase.

According to Ahn *et al.* (2004) glycolization of secondary plant metabolites leads to an increase of the degree of solubility of these allelo-compounds in the water environment. A significant part of the total pula of these chemical components is to be found in a cell in the form of glycon (glycoalcaloids, phenolic and cyanogenous glycosids, glucosinolans or sapponins). The increase of the level of activity of plant glucosidase leads to an increased decomposition of these glycolconjugates. According to Sakai *et al.* (2008) endogenic glucosidase form an element of chemical protection of plants which is stimulated as a result of phytophag's feeding or pathogens' attack. This mechanism is connected with the freeing of toxic, free aglycons (eg. hydroxamic acids from glycosids of these compounds) in tissues of the settled plant organs. Moreover, β-glucosidase is involved in the process of lignification of the cell wall (it takes part in hydrolysis of monolignol glycosids, including coniferyl-, sinapic- or cumaric-alcohol conjugates with the saccharic rest). An increase in the activity of this biocatalyser may also lead to an intensification of the building process and the enhancing of ultrastructure of cell walls in tissues of host plants which are damaged because of phytophags' feeding (Ballhorn *et al.*, 2006; Escamilla-Trevino *et al.*, 2006; Mazura *et al.*, 2006).

According to Escamilla-Trevino *et al.* (2006) the β-glucosidases are involved in a number of fundamental physiological processes which take place in plant cells. Mazura *et al.* (2006) stated that β-glucosidase Zm-p60.1 isolated from sweet corn tissues characteristically has the ability to dissociate the β-glycosidase binding in molecule of glycosids of cytokinine. This reaction leads to the freeing of biologically active cytokinines which regulate a wide range of processes taking place during the ontogenetic development of tissues and organs of plants. A wide spectrum of biological functions of β-glycosidase embraces also a premature activation of the ageing process, provocation of dege-

nerating changes and initiation of a programmed cell death in plant tissues (Sperotto *et al.*, 2008).

To sum up it has to be highlighted that an increase in the activity of the analysed enzyme in the leaves of *P. padus* caused by the feeding of *R. padi* may lead to multilayered modifications of the course of diverse biochemical processes taking place in the cells of the settled organs. A complex character of these changes may embrace induced, local protective reactions in the host, such as: 1) an increased concentration of allelo-compounds which are toxic for the feeding aphids, and thus limit their number, 2) the reconstruction of damage of primary and secondary cell wall (caused by *R. padi* feeding) and the strengthening of its ultrastructure.

Conclusions

1. Birdcherry – oat aphid feeding on shoots of primary host stimulated the level of catalytic activity of β-glucosidase.
2. The greatest increase in the level of activity of the identified enzyme took place during the maximum presence of the studied phytophages when wingless virginoparae (fundatrigeniae) dominated in the population structure.

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Wpływ żerowania mszycy czeremchowo-zbożowej (*Rhopalosiphum padi* (Linnaeus, 1758)) /Hemiptera, Aphidoidea/ na aktywność β-glukozydazy w tkankach żywiciela pierwotnego

Streszczenie

Żerowanie mszycy czeremchowo-zbożowej na zaizolowanych pędach żywiciela pierwotnego (*Prunus padus* L.) powodowało indukcję aktywności β-glukozydazy w liściach czeremchy zwyczajnej. Najwyższy przyrost aktywności analizowanego biokatalizatora odnotowano podczas maksymalnego zagęszczenia tej mszycy, rozwijającej się na pędach bocznych gospodarza pierwotnego. Najniższy wzrost aktywności badanego enzymu obserwowano natomiast podczas pojawu oraz w okresie zaniku występowania owada na czeremsze zwyczajnej. Analiza statystyczna uzyskanych wyników dowiodła, że różnice w poziomie aktywności oznaczanej glukozydazy między liśćmi zasiedlonymi przez mszyce a organami kontrolnymi, były istotne jedynie w okresie najwyższej liczebności badanego pluskwiaka podczas rozpatrywanych sezonów.