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THE EFFECT OF *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae) FEEDING ON THE CONTENT OF SELECTED BIOLOGICALLY ACTIVE SUBSTANCES IN DIFFERENT SPECIES OF HOST PLANTS

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Abstract. The choice of host plants by the phytophagous insects depends on a number of factors, including secondary metabolites which as "specific plant substances" have a significant influence not only on the physiology and behaviour of plant-eating insects but also on the chemism of plants' defense. The present paper determined the effect of the feeding of *Acrobasis advenella* caterpillars on the content of flavonoids, phenolic acids and tannins in the inflorescences of two species of host plants, namely *Aronia melanocarpa* and *Sorbus aucuparia*. The content of flavonoids and phenolic acids in the extracts of both plants where caterpillars were feeding was lower as compared to the extracts from control plants. It was found out that the content of tannins in the plant material of *S. aucuparia* where caterpillars were feeding increased in comparison to the control material. In 2009, their content was found to be more than 1.5 times higher, reaching over 13% of DW. A reverse reaction was observed in the case of *A. melanocarpa*, where the content of tannins dropped significantly. The value of that parameter in 2009 decreased more than 1.5 times and it was 5.42% of DW, while in 2010 the decrease was 2-fold, reaching 2.61% of DW.

Key words: *Aronia melanocarpa, Sorbus aucuparia*, secondary metabolites, phenolic acids, flavonoids, tannins, plant–insect interaction

INTRODUCTION

Black chokeberry is considered to be a plant which is very resistant to diseases and pests, which – according to Scott and Skirvin [2007] – is caused by a high content of polyphenols. Studies conducted in Poland in the years 1987–1989 on chokeberry planta-

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tions did not show any presence of pests [Kozłowski et al. 1990]. Later studies pointed out that leaf mining moths, moths from the tortrix family (Tortricidae), winter moths (*Operophtera brumata* L.), aphids (Aphididae), pear and cherry *slug* sawflies (*Caliroa limacina* Retz.) and apple fruit moths (*Argyresthia conjugella* Zell.) can sporadically occur on chokeberry [Chlebowska 1999, Chlebowska and Smolarz 1988, Eggert 1986]. The latest information from the first decade of the 21st c. refer to the species *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae), whose caterpillars feed on chokeberry inflorescences [Górska-Drabik 2009]. This moth belongs to the commonly occurring species and so far only plants from genera Crataegus, Sorbus and Prunus have been quoted as hosts to caterpillars [Goater 1986, Palm 1986, Slamka 1997]. The choice of host plants by the phytophagous insects depends on a number of factors, including secondary metabolites which as "specific plant substances" have a significant influence not only on the physiology and behaviour of plant-eating insects but also on the chemism of plants' defense. This group comprises chemical compounds that have a positive effect on insects. Most of them, however, have a negative influence, having a deterrent, toxic or inhibiting effect on the feeding by insects. The factor determining the properties of those compounds is their concentration in the tissues of host plants. The studies conducted so far point out that secondary metabolites, localized mainly in the peripheral tissues, can have a significant role in interactions between plants and insects possessing a chewing type of the mouthparts.

The aim of this study was to compare the content of selected secondary metabolites in inflorescences of two host plants species of *A. advenella* and determine the changes that occur in them due to the feeding of their caterpillars.

MATERIAL AND METHODS

The studied material were inflorescences of *Aronia melanocarpa* [Michx.] Elliot and *Sorbus aucuparia* L. (buds together with petioles), both colonized and not colonized by caterpillars of *A. advenella*. Due to the large difference in the size of inflorescences studied plant species various number of them were analyzed. The inflorescences were collected randomly from the host plants (approximately 200 inflorescences of *A. melanocarpa* and 50 of *S. aucuparia*). Inflorescences were collected at the end of the feeding of caterpillars, namely in the second 10-day-long period of May 2009 and 2010. The feeding caterpillars were taken from the inflorescences. Next, the inflorescences that were both colonized and not colonized by caterpillars were dried at the temperature of up to 35°C.

The study material was collected from the plantation of black chokeberry situated at Boduszyn (15 km away from Lublin, Poland), where the treatments of pruning the branches and fertilization were performed every few years. Inflorescences of *S. aucuparia* were collected from the trees of the green areas of Lublin. The trees grew in small groups and they were subject to pruning every few years. No pesticide treatments were used in those places.

Biochemical analysis. The chemical analysis was carried out of the air dry plant material with the aim of determining the content of selected secondary metabolites. The dry weight (DW) was determined using the drying method [Chmielewska 1955], while the weight loss after drying was established using the gravimetric method according to the Polish Pharmakopoeia VI [2002].

In order to find out the presence of biologically active compounds, screening studies were conducted of selected secondary metabolites such as phenolic acids, flavonoids and tannins [Polish Pharmakopoeia VI 2002, Strzelecka et al. 1987, Borkowski 1973, Drost-Karbowska et al. 1994].

The percentage content of flavonoids and the sum of phenolic acids were established using the spectrophotometric method [Polish Pharmakopoeia VI 2002]. The percentage content of flavonoids was converted into quercetin, while the percentage of phenolic acids was converted in caffeic acid. The percentage content of tannins was established by means of the weight titration method according to Polish Pharmakopoeia IV [1970].

Statistic analysis. For all statistical analyses of the results, the software package Statistica 9.1 (StatSoft) was used. ANOVA with Tukey simultaneous test was applied, with the criterion of $p < 0.01$ and $p < 0.05$. All analyses were conducted in three replicates.

RESULTS

As a result of chemical analysis conducted in 2009 and 2010 on the plant material, no statistically significant differences were found in the mean content of the dry weight between control plants of both species and the inflorescences of *S. aucuparia* that were, and were not, colonized by caterpillars. In the case of *A. melanocarpa*, significant differences in the content of dry weight between healthy and damaged inflorescences were shown only in 2010. A comparison was made of the mean content of flavonoids, phenolic acids and tannins in two species of host plants to *A. advenella* which were not damaged by insects.

The mean content of flavonoids in the tissues of control plants of both species of host plants ranged from 0.56% (*A. melanocarpa*) to 0.60% (*S. aucuparia*), whereas the mean content of phenolic acids was 3.93% in the healthy inflorescences of *S. aucuparia* and 4.03% in *A. melanocarpa.* No significant differences were found in the studied plant species as for the content of flavonoids and phenolic acids. The dominating group among the biologically active compounds were tannins. The mean content of tannins in the case of control plants of *S. aucuparia* was 5.88% and it was lower in comparison with *A. melanocarpa* – 6.87%. Statistically significant differences were found only in the content of tannins between the studied plant species (fig. 1).

On the basis of the obtained results it was found out that the percentage content of flavonoids, phenolic acids and tannins differed in successive years of studies. A higher concentration of all compounds, both in those colonized by *A. advenella* caterpillars and the control material, was observed in 2009 (figs 2, 3).

The chemical composition of *S. aucuparia* inflorescences. The obtained results showed that the content of flavonoids in the examined raw materials of *S. aucuparia* which were not colonized by *A. advenella* caterpillars ranged from 0.30% in 2010 to 0.89% in 2009. In inflorescences where caterpillars fed the content of flavonoids was lower – from 0.28% in 2010 to 0.71% in 2009; however, significant differences between the content of this parameter was observed only in 2009 (fig. 2).

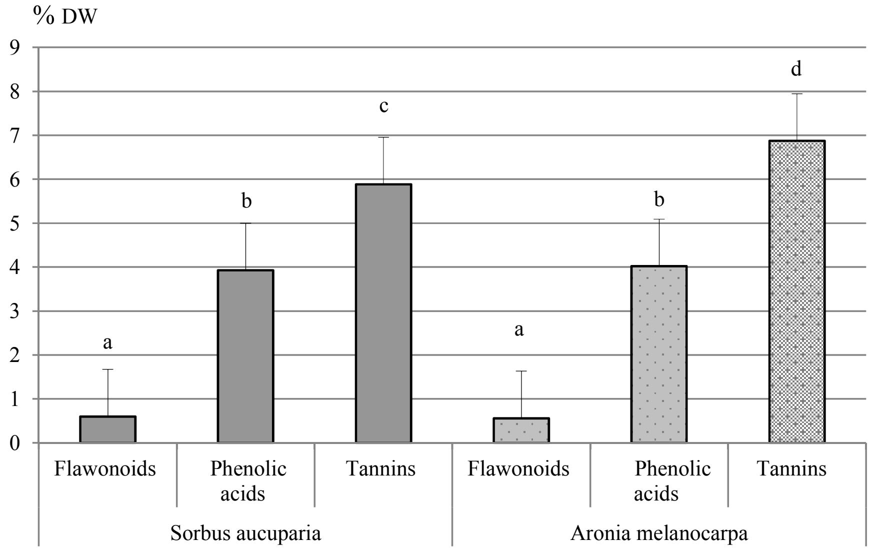

Fig. 1. The percentage of mean content of flavonoids, phenolic acids and tannins in the non – infested inflorescences of *Sorbus aucuparia* L. and *Aronia melanocarpa* [Michx.] Elliot. Means sharing the same letter do not differ significantly at p < 0.05 (Tukey test)

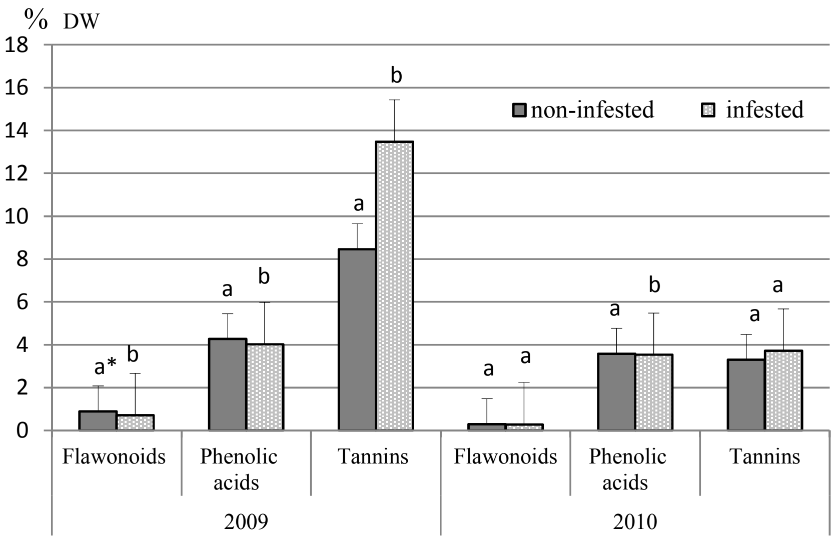

Fig. 2. The percentage of mean content of flavonoids, phenolic acids and tannins in non-infested and infested by caterpillars of *Acrobasis advenella* of *Sorbus aucuparia* in 2009–2010. Means sharing the same letter do not differ significantly at p < 0.05, *p < 0.01 (Tukey test)

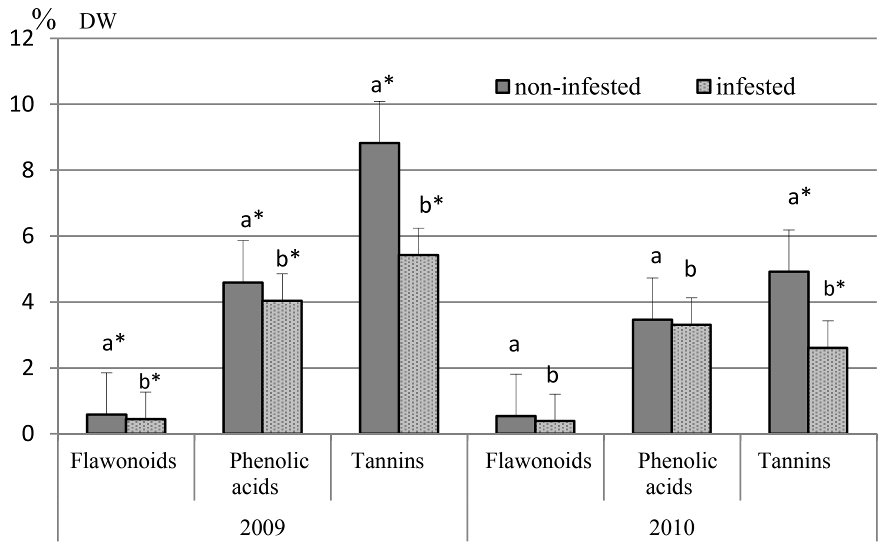

Fig. 3. The percentage of mean content of flavonoids, phenolic acids and tannins in non-infested and infested by caterpillars of *Acrobasis advenella* of *Aronia melanocarpa* in 2009–2010. Means sharing the same letter do not differ significantly at $p < 0.05$, $^*p < 0.01$ (Tukey test)

The content of phenolic acids in the plants of *S. aucuparia* which were free from the feeding caterpillars ranged from 3.58% in 2010 to 4.27% in 2009. It was found out that the content of those compounds in inflorescences colonized by caterpillars decreased, showing significant differences, to the level 3.53% in 2010 and 4.02% in 2009.

The content of tannins in the studied control raw materials of *S. aucuparia* ranged from 3.3% in 2010 to 8.46% in 2009. The content of tannins in the plant material where caterpillars fed increased. The content of tannins was statistically significantly different between the injured and healthy inflorescences in 2009. We observed an over 1.5-fold increase of the content of tannins, reaching more than 13% of DW (fig. 2).

The chemical composition of *A. melanocarpa* inflorescences. The proportion of flavonoids in plant raw materials of *A. melanocarpa* which were not colonized by caterpillars ranged from 0.54% in 2010 to 0.58% in 2009. It was observed that the content of flavonoids in inflorescences colonized by the pest was lower and it significantly differed as compared to the plants that were not damaged: respectively, 0.39% in 2010 and 0.45% in 2009. The studies showed significant differences between the control and colonized inflorescences in both years of studies (fig. 3).

The percentage content of phenolic acids in the plants material of *A. melanocarpa* which was not colonized by the pest ranged from 3.46% (2010) to 4.59% in 2009. The content of those compounds significantly differed in the extracts from inflorescences that were colonized by caterpillars. The percentage of those compounds decreased to the level of 3.31% in 2010 and 4.04% in 2009.

The percentage content of tannins in healthy inflorescences from *A. melanocarpa* ranged from 4.92% in 2010 to 8.82% in 2009. Statistically significant differences were found in the content of tannins between healthy and colonized material. Contrary to *S. aucuparia*, where the content of tannins in the plant material damaged by caterpillars was higher, in the case of *A. melanocarpa* the content of tannins dropped significantly. The value of this parameter in 2009 dropped more than 1.5 times and was 5.42% of DW, while in 2010 the decrease was almost 2-fold, reaching the value of 2.61% of DW (fig. 3).

DISCUSSION

Biologically active substances present in plants are the object of extensive studies considering their usefulness for people. They are aimed at finding out the qualitative and quantitative composition of bioactive compounds showing specific properties in human organism. The fruit of black chokeberry were very well studied in respect of the chemical content. A number of authors draw attention to the abundance of polyphenols occurring in this plant, which – in turn – have the effect of counteracting a lot of diseases having the basis in oxidative stress [Kokotkiewicz et al. 2010, Niedworok and Brzozowski 2001, Wawer 2010, Wolski et al. 2007, Zielińska-Przyjemska et al. 2007].

Despite the fact that many authors describe examples of both plants' resistance to and their attractiveness for pests [Bennewicz 1989, Łuczak 1998], the available literature lacks any information on the chemical composition of rowan as a factor affecting the choice of the host plant and the plants' reaction to the feeding of insects.

Scott and Skirvin [2007] say that black chokeberry is a plant free from pests due to the high content of certain polyphenols. As stated by Harborne [1997] and Baldwin [2001], insects can biochemically adapt themselves to digesting new plant foods, develop new feeding habits or new taste preferences with the aim of avoiding the plants' resistance system. *A. advenella* is the species commonly occurring on plants from genera Sorbus and Crataegus. The presence of this species on *A. melanocarpa* was observed in south-eastern Poland in 2004 [Górska-Drabik 2009]. Studies conducted by Górska-Drabik (unpublished data) show that this species commonly occurs on black chokeberry plantations in the area of all Poland, and the population of this pest is higher as compared to the populations occurring on the "primary host", which is *S.aucuparia.* A comparison of the mean content of flavonoids, phenolic acids and tannins in control extract of *S. aucuparia* and *A. melanocarpa* presented in fig. 1 shows that the content of flavonoids and phenolic acids does not differ in a significant manner. On the other hand, significant differences were found between the studied plants in the content of tannins.

Among substances deterring insects from feeding on plants include the compounds of secondary metabolism [Harborne 1997]. The majority of secondary metabolites have a negative effect on the growth and development of herbivorous animals. The quantity and quality of these compounds can directly or indirectly affect the growth and feeding, the body's dimensions of particular developmental stages and the fertility of plant-eating insects [Ramiro et al. 2006, Onyilagha et al. 2004]. For some herbivorous, the coevolution between plants and the herbivorous leads to specialization that the herbivorous may adapt to secondary metabolites [Ehrlich and Raven 1964, Awmack and Leather 2002].

Plant tannins, occurring commonly and in relatively high concentrations, belong to the compounds which deter the herbivorous. They can cause serious problems in digesting the plant food, hence, they fulfill an important function in affecting the biology of the herbivorous [Zucker 1983]. Certain insects show susceptibility to the presence of tannins in food which constitute the main barrier from feeding by having a toxic effect on the insect gut. They have the potential to form complexes with proteins and/or inactivate digestive enzymes and they often act as a pest deterrents or inhibitors [Feeny 1976, Elliger et al. 1980, Hagerman and Robbins 1987, Appel 1993, Simmonds 2003]. The available literature points to the negative effect of tannins on insects. Studies conducted by Fornal et al. [1991] pointed out that tannins occurring in the bean tegument are an important anti-feeding factor affecting seed infection by bean weevil (*Acanthoscelides obtectus*). Janiuk [2012] showed that the content of tannins in particular varieties of northern highbush blueberry was the decisive factor for the population of dark fruit-tree tortrix (*Pandemis heparana*) feeding on the highbush blueberry leaves. Cv. "Northland", containing the highest concentration of tannins, was not willingly colonized by caterpillars. Similar results were obtained by Feeny [1970], who showed that caterpillars of the winter moth occurring on oak leaves stop feeding at the moment when those compounds considerably increase their content in the plant.

The present studies show that a high content of tannins in *A. melanocarpa* inflorescences (fig. 1) does not make a barrier for this plant's colonization by *A. advenella* caterpillars. Our findings and research of other authors support the view that tannins do not act as a primary of defense against herbivores [Costa et al. 2011, Queiroz et al. 2012]. Moreover, these compounds can play a very important role in the plant's defence mechanisms. It was observed that following the feeding of caterpillars in *A. melanocarpa* inflorescences, there was a drop in the content of all the studied compounds. This drop was observed in both studied years. In the case of *S. aucuparia,* significant differences in the content of all compounds between control plants and those that were colonized by the pest were observed only in 2009, while in 2010 significant differences were found only in the content of phenolic acids. As pointed out by Leszczyński [2001], the plants' reaction to the attack of the herbivorous is a very complex reaction and it depends on the host plant. The plants' resistance to the phytophagous insects can be related to resistance mechanisms which effectively protect plants from the attack. It can also result from the host's ability to defense reactions, which are a response to the insect's attack. Studies conducted by Kiełkiewicz et al. [2011] on the feeding of *Phytoptus tetratrichis* Nalepa on the leaves of *Tilia cordata* Mill. and *Tilia tomentosa* Moench point out that tannins are the compounds which strongly react to the feeding by the Eriophyoidea. The Authors showed that on the leaves of *T. cordata* where a small number of galls formed by the Eriophyoidea was observed a drop in the content of tannins occurred, while with a big number of galls, a 2.5-fold increase in the content of those compounds was found. On the other hand, an increase in the content of tannins always took place on the leaves of *T. tomentosa,* regardless of how big the population of the pest was. With a big population on the leaves, that was an even 8-fold increase. Gantner [2007] observed that a slight increase in the content of tannins took place in the hazelnut of cv. 'Lamberta Biały', which is most resistant to aphids, under the effect of feeding by *Myzocallis coryli* Goetze, which – according to the Author – can testify to a stronger systemic reaction of this cultivar as compared to others.

The obtained results of the studies indicate that metabolic changes, which are directed at triggering defense reactions, take place under the effect of feeding by *A. advenella.* Such a reaction was observed in the case of *S. aucuparia,* where an increase of the main anti-feeding compound, which were tannins, was found. On the other hand, in the case of *A. melanocarpa* the content of tannins decreased, which can prove the lack of this plant's resistance to the feeding by *A. advenella* caterpillars.

The studies can confirm the existing hypothesis that secondary metabolites (in this case, tannins) which affect insects have been and are synthesized by plants for the defense against the herbivorous. Insects which formed the proper ways of their detoxication can begin to feed only on this plant.

CONCLUSIONS

1. The content of flavonoids and phenolic acids in the extracts of *A. melanocarpa* and *S.aucuparia* where caterpillars were feeding was lower as compared to the extracts from control plants.
2. It was found out that the content of tannins in the plant material of *S. aucuparia* where caterpillars were feeding increased in comparison to the control material. In 2009, their content was found to be more than 1.5 times higher.
3. A reverse reaction was observed in the case of *A. melanocarpa*, where the content of tannins dropped significantly. The value of that parameter in 2009 decreased more than 1.5 times, while in 2010 the decrease was 2-fold.
4. High content of tannins in *A. melanocarpa* inflorescences does not make a barrier for this plant's colonization by *A. advenella* caterpillars.

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WPŁYW ŻEROWANIA GĄSIENIC *Acrobasis advenella* (Zinck.) (Lepidoptera, Pyralidae) NA ZAWARTOŚĆ WYBRANYCH SUBSTANCJI BIOLOGICZNIE CZYNNYCH W KWIATOSTANACH RÓŻNYCH GATUNKÓW ROŚLIN ŻYWICIELSKICH

Streszczenie. Wybór roślin żywicielskich przez fitofaga zależny jest od wielu czynników, wśród których metabolity wtórne jako „swoiste substancje roślin" wykazują istotny wpływ nie tylko na fizjologię i zachowanie się roślinożernych owadów, ale także na chemizm obrony roślin. Celem pracy było porównanie zawartości wybranych metabolitów wtórnych w kwiatostanach dwóch gatunków roślin żywicielskich *Acrobasis advenella* (*Sorbus aucuparia* i *Aronia melanocarpa*) oraz określenie zmian zachodzących w nich pod wpływem żerowania gąsienic. W ekstraktach zarówno aronii, jak i jarzębiny, w których żerowały gąsienice, zawartość flawonoidów i kwasów fenolowych była mniejsza w porównaniu z ekstraktami roślin kontrolnych. Stwierdzono, że w materiale roślinnym *S. aucuparia*, w którym żerowały gąsienice, zawartość garbników wzrosła w porównaniu z materiałem kontrolnym. W roku 2009 ich zawartość była 1,5-krotnie większa i osiągnęła ponad 13% suchej masy. Przeciwną reakcję obserwowano w przypadku *A. melanocarpa*, gdzie zawartość garbników znacznie spadła, w 2009 r. ponad 1,5-krotnie i wyniosła 5,42% suchej masy, a w roku 2010 – 2-krotnie, osiągając 2,61%.

Słowa kluczowe: *Aronia melanocarpa, Sorbus aucuparia*, metabolity wtórne, flawonoidy, kwasy fenolowe, garbniki, interakcje roślina–owad

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