The chromatographic analysis (HPLC) of phenolic compounds from different host-plants of bird cherry-oat aphid

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Summary

The host-plants of bird cherry-oat aphid were compared as to the content and composition of phenolic compounds. The gallic, chlorogenic, caffeic, siringic, o- and p-coumaric, tannic, ferulic, sinapinic, salicylic, p-hydroxybenzoic acids and /+-catechin were detected in the all studied host-plants of the bird cherry-oat aphid. Coumarin and vanilic acid only occurred within orchard grass and winter triticale tissues. Predominant compounds within P. padus leaves were /+-catechin, chlorogenic acid, p-hydroxybenzoic acid and tannic acid. Vanilic and salicilic acids were the major compounds of the orchard grass, whereas leaves of winter triticale contained mostly salicilic, chlorogenic and vanilic acids as well as coumarin.

Key words: phenolic compounds, Prunus padus, Dactylis glomerata, triticale, bird cherry-oat aphid, HPLC

INTRODUCTION

Higher plants produce a variety of allelochemicals that play an important role in insect-plant relationships. Among them, plant phenolics show a strong negative effect on aphids’ behavior, physiology and metabolism. It has been found that high concentrations of the phenolics in plant tissues were toxic or deterrent to aphids [1-3]. On the other hand, some of them were also phagostimulants for several aphid species [4]. These substances are considered in particular by the aphid migrants during plant colonization [5].
Bird cherry-oat aphid (*Rhopalosiphum padi* L.) belongs to oligophagous species that seasonally alternate between woody (primary) and herbaceous (secondary) host plants. The aphid’s primary host is bird cherry (*Prunus padus* L.), while its broad spectrum of secondary hosts includes numerous species from *Poaceae* [6, 7].

The aim of this work was to determine the content of the phenolic compounds in leaves of bird cherry and selected secondary hosts (orchard grass, *Dactylis glomerata* L. and winter triticale, Marko cv.) during spring migration of the bird cherry-oat aphid.

**MATERIAL AND METHODS**

**Experimental**

The plant material for chemical analyses was collected in the second or third decade of May in 2004–2006 (depending on aphid’s migration time) in Aleksandria Park, Siedlce. Samples were collected from plants non-infested by aphids, immediately transferred to the laboratory and freeze-dried.

**Extraction and separation of phenolic compounds**

At first, the powdered samples of plant material (1 g each) were extracted with chloroform and then with 80% methanol using a Soxhlet apparatus. The methanolic extract was evaporated under vaccum and the residue was dissolved in 10 cm$^3$ of 80% methanol. Obtained extract was filtered (Supelco IsoDisc PTFE, 25 mm x 0.45 μm) and subjected to HPLC. Separation of the phenolic compounds was performed with help of isocratic Varian system, equipped with Microsorb MV 100-5 C18 (250 x 4.6 mm) column and two detectors (UV-VIS ProStar 325 and fluorescence ProStar 363). The detection wavelengths applied were as follows: UV detector $\lambda=300$ nm, fluorescence detector $\lambda_{ex}=230$ nm and $\lambda_{em}=350$ nm. The mobile phase consisted of methanol:water (25:75, v/v) and 1% of acetic acid (v/v) [8]. All separations were performed at the temperature of 25°C and the flow rate was 1 cm$^3$ · min$^{-1}$. Identification of the phenolic compounds was carried out by comparing their retention times with standards and by adding internal standards into the samples. All the analysis were done in three independent replicates. Concentration of the identified phenolics within plant material was expressed as mean values of three years (2004–2006), in mg·g$^{-1}$ dry weight. Differences in content of the phenols between host-plants of *R. padi* were analysed by the analysis of variance followed by Duncan’s test.
RESULTS AND DISCUSSION

The HPLC separations allowed to identify 14 phenolic compounds in the tissues of studied plants (tab. 1). Twelve of them were detected by means of the UV detector and two (\(+/-\)catechin, \(p\)-hydroxybenzoic acid) with use of fluorescence detector.

The gallic, chlorogenic, caffeic, siringic, \(o\)- and \(p\)-coumaric, tannic, ferulic, sinapinic, salicylic, \(p\)-hydroxybenzoic acids and \(+/-\)catechin were detected in tissues of all studied host-plants of the bird cherry-oat aphid (tab. 1). Coumarin and vanilic acid only occurred in orchard grass and winter triticale tissues – plants belonging to \(Poaceae\) family. Predominant compounds within \(P.\ padus\) leaves were \(+/-\)catechin, chlorogenic acid, \(p\)-hydroxybenzoic acid and tannic acid. These four substances together make up about 75\% of total phenolic compounds that were identified in the bird cherry tissues. Vanillic and salicylic acids were the major compounds of the orchard grass, whereas leaves of winter triticale contained mostly salicylic, chlorogenic and vanilic acids as well as coumarin. When comparing total content of the identified phenolic compounds in tissues of the studied plants during \(R.\ padi\) migration it was noted that secondary hosts contained about 30\% less of the identified phenolics than the primary one (fig. 1).

The concentration of identified free phenols (mg·g\(^{-1}\) dry weight, mean ± S.D.) within bird cherry, orchard grass and winter triticale tissues

<table>
<thead>
<tr>
<th>phenolic compound</th>
<th>bird cherry</th>
<th>orchard grass</th>
<th>triticale</th>
</tr>
</thead>
<tbody>
<tr>
<td>gallic acid</td>
<td>0.48±0.07 b</td>
<td>0.18±0.06 c</td>
<td>0.63±0.09 a</td>
</tr>
<tr>
<td>chlorogenic acid</td>
<td>6.64±0.82 a</td>
<td>0.79±0.10 c</td>
<td>1.97±0.29 b</td>
</tr>
<tr>
<td>vanilic acid</td>
<td>-</td>
<td>5.82±0.70 a</td>
<td>1.48±0.19 b</td>
</tr>
<tr>
<td>caffeic acid</td>
<td>0.31±0.04 b</td>
<td>0.49±0.07 a</td>
<td>0.52±0.03 a</td>
</tr>
<tr>
<td>siringic acid</td>
<td>0.98±0.11 a</td>
<td>0.71±0.06 b</td>
<td>1.02±0.07 a</td>
</tr>
<tr>
<td>(p)-coumaric acid</td>
<td>0.16±0.02 a</td>
<td>0.08±0.02 b</td>
<td>0.08±0.01 b</td>
</tr>
<tr>
<td>tannic acid</td>
<td>1.53±0.14 a</td>
<td>0.41±0.05 b</td>
<td>0.37±0.04 b</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>0.17±0.03 a</td>
<td>0.15±0.02 a</td>
<td>0.08±0.02 b</td>
</tr>
<tr>
<td>sinapinic acid</td>
<td>0.08±0.03 c</td>
<td>0.23±0.06 b</td>
<td>0.96±0.09 a</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>0.41±0.07 b</td>
<td>3.41±0.45 a</td>
<td>2.77±0.32 a</td>
</tr>
<tr>
<td>coumarin</td>
<td>-</td>
<td>0.20±0.04 b</td>
<td>1.37±0.15 a</td>
</tr>
<tr>
<td>(o)-coumaric acid</td>
<td>0.06±0.01 b</td>
<td>0.27±0.04 a</td>
<td>0.06±0.01 b</td>
</tr>
<tr>
<td>(+/-)catechin</td>
<td>4.28±0.39 a</td>
<td>0.33±0.05 c</td>
<td>0.91±0.11 b</td>
</tr>
<tr>
<td>(p)-hydroxybenzoic acid</td>
<td>3.64±0.46 a</td>
<td>0.68±0.09 b</td>
<td>-</td>
</tr>
</tbody>
</table>

Values followed by the same letter (in rows) are not significantly different at \(p\leq0.05\) (Duncan’s test).
Figure 1.  Total amount of the identified phenolic compounds in leaves of the studied host-plants during *R. padi* migration

The HPLC analysis revealed clear differences in the phenolics composition among studied host-plants of the bird cherry-oat aphid, especially between primary and secondary hosts. High level of chlorogenic, *p*-hydroxybenzoic and tannic acids as well as /+-catechin in the bird cherry leaves might be a reason for *R. padi* spring migration onto the orchard grass and winter triticale. It has been shown that chlorogenic acid and catechin exerted a strong negative effect on the bird cherry-oat aphid and other cereal aphid species [9, 10]. Leszczyński et al. [11] found that chlorogenic acid at a concentration of 62.5 mg · dm$^{-3}$ reduced the growth and development of *R. padi*. However, some of the phenolic compounds (e.g. coumarin) were reported to act as attractants to insect and allowed them to recognize plants among certain genus [12]. Thus, coumarin that occurred in orchard grass and triticale played an important role in the secondary host plant selection by the bird cherry-oat aphid.

CONCLUSION

The obtained results suggest that qualitative and quantitative differences in the phenolics composition between bird cherry, orchard grass and winter triticale may affect the life cycle of the bird cherry-oat aphid.

REFERENCES


ANALIZA CHROMATOGRAFICZNA (HPLC) ZWIĄZKÓW FENOLOWYCH WYBRANYCH ŻYWICIELI MSZYCY CZEREMCHOWO-ZBOŻOWEJ

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S t r e s z c z e n i e

W prezentowanej pracy analizowano skład jakościowy i ilościowy związków fenolowych roślin żywicielskich mszy cy czeremchowo-zbożowej, w okresie jej wiosennych migracji. We wszystkich badanych roślinach żywicielskich stwierdzono obecność kwasów: galusowego chlorogenowego, kawowego, o- i p-kumarowego, taninowego, ferulowego, synapinowego, salicylowego, p-hydroksybenzoesowego oraz /+/-katechiny. Kumaryna i kwas vanilinowy występowały tylko w tkankach kupkówki pospolitej i pszenżyta ozimego. W liściach czeremch zwyczajnej w największych ilościach występował kwas chlorogenowy, p-hydroksybenzoesowy i taninowy oraz /-katechyna. Kumaryna i kwas vanilinowy występowały tylko w tkankach kupkówki pospolitej i pszenżyta ozimego. W liściach czeremch zwyczajnej w największych ilościach występował kwas chlorogenowy, p-hydroksybenzoesowy i taninowy oraz /-katechyna. Rośliny kupkówki pospolitej charakteryzowały się wysokimi stężeniami kwasu vanilinowego i salicylowego, natomiast w tkankach pszenżyta odnotowano stosunkowo wysoką zawartość kwasu salicylowego, chlorogenowego i wanilinowego oraz kumaryny.

Słowa kluczowe: związki fenolowe, Prunus padus, Dactylis glomerata, pszenżyto, mszyca czeremchowo-zbożowa, HPLC