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Component composition of organic compounds of the assimilative apparatus of different species of the genus *Aesculus* affected by *Cameraria ohridella* (Lepidoptera, Gracillariidae)

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The most important disease for plants of the *Aesculus* genus is leaf spot caused by the leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae), which limits the use of *Aesculus* in landscaping in urban areas. The leaf miner *C. ohridella* is an insect widely spread in most European countries. The study was carried out in an urban area with six species of the genus *Aesculus* L. (*Ae. hippocastanum* L., *Ae. pavia* L., *Ae. parviflora* Walt., *Ae. flava* Sol., *Ae. glabra* Willd. and hybrid *Aesculus × carnea*). The article reflects the results of research on the influence of the leaf miner *C. ohridella* on the peculiarities of changes in the chemical composition of chestnut leaf extracts. The component composition of the leaves of different chestnut species was determined using chromatographic analysis. The study shows the results of the phytochemical screening of the leaves of different species from genus *Aesculus*, uninfested and infested with *C. ohridella*. A total of 186 chemical components were identified from the leaf extracts of *Aesculus* plants with six main groups of chemical components – carbohydrates, polyphenols, steroids, hydrocarbons, fatty acids and miscellaneous. The carbohydrates group is represented in the leaves of all studied *Aesculus* species in the maximum degree. The obtained data demonstrate significant change in the number of all chemical components under the influence of *C. ohridella*. The studied species showed an increase in carbohydrates content with the influence of leaf miner *C. ohridella*, except for such species as *Ae. flava* and *Ae. parviflora*. The qualitative composition of polyphenolic compounds was species-specific. The analysis of the composition of extracts of uninfested leaves established that four components were contained in extracts of *Ae. hippocastanum* and *Ae. pavia*, but they were different in their composition. In comparison with the composition of polyphenols in leaves without damage and leaves infected with *C. ordidella*, the absence of (2R-trans)-catechine in extracts from infected leaves of *Ae. hippocastanum* and *Ae. glabra* was found. Using principal component analysis, it was established that *Ae. glabra* shows a clear separation between chemical composition of the infested and uninfested samples. We identified two components of a phenolic nature only in extracts of infested leaves of *Ae. flava,* (trimethylsilyl catechollactate tris(trimethylsilyl) ether and epigallocatechin (6TMS)), and quercetin was identified only in infected leaf extracts of *Ae. glabra*. These results can indicate a greater potential resistance of these *Aesculus* species to damage by the studied herbivory.

Keywords: GC-MS; ICP-MS; LC-ESI-MS/MS; *Aesculus*; chemical composition; leaf miner.

Introduction

Today, the decorative value of a plant is subjective and is determined by assessing the esthetic significance of flowers, fruits, leaves and the overall structure of the plant. *Aesculus* species from the subfamily Hippocastanaceae can be easily recognised by their palmate leaves, decorative flowers and specific seeds, which are believed to be the basis of their common name. Because of its beautiful foliage, effective shape of inflorescences with a long blooming period and dense spherical crown, this tree has a unique aesthetic appearance, especially in urban areas, as it is well adapted to anthropogenic conditions. The autumn colour of the species is also highly variable within species and is used as a breeding tool. This is an important component in the selection of woody plants for greenery (Lovynska et al., 2022; Shupranova et al., 2024). For this reason, the frequently decorative species of the *Aesculus* genus are commonly used for landscaping along roads and streets, and are also widely used as ornamental plants in parks and squares. *Aesculus hippocastanum* is one of species that maintain a decorative appearance throughout the growing season. *Aesculus parviflora* has the most persistent bright yellow autumn colour of all species. *Aesculus glabra* can have a beautiful orange and/or red colour if the foliage has not been damaged in summer (Brickell, 1992; Benvie, 2000). *Aesculus* are among the first plants to lose their leaves in autumn, thus revealing the branched structure of the plants. All *Aesculus* species, except *Ae. parviflora*, have a pronounced and coarse texture that contrasts with other plants used for landscape decoration. The majority of *Aesculus*, including the most famous species *Ae. hippocastanum* and *Ae. pavia*, bloom in mid-spring and have spectacular flower panicles.

Plants of the *Aesculus* genus are susceptible to foliar problems. The most important disease is leaf spot caused by the chestnut leaf miner, which limits the use of *Aesculus* in landscaping in urban areas. The leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae) is an insect from the family Lepidoptera (Gracillariidae). The insect larvae feed on leaf tissue and make long paths inside the chlorenchyma of the host plant, thus 'mining' the leaves. As a result, they form round, dark brown patches. Baraniak, Walczak & Zduniak (2005) described that damage to the plant is caused by the larvae living inside leaves and feeding on the sap and palisade parenchyma. Foliar damage occurs when larva tunnel into the leaves of the tree, causing physical destruction of leaf tissue (Perciva et al., 2011). The patches are called mines and gradually cover more and more leaf area (Paterska et al., 2025). *Cameraria ohridella* attacks the white-flowering chestnut tree (*Aesculus hippocas-*

tanum L.) more often than red horse chestnut (*Aesculus carea*) (Oszmiański et al., 2014). The number of larvae and the leaf damage on the red horse-chestnut are relatively low, i.e., a maximum of 10% of the leaves is damaged throughout the vegetative period (Dzięgielewska & Kaup, 2007).

The level of expansion of the leaf miner *C. ohridella* is truly impressive. Indeed, the first occurrence of this pest was recorded in 1984 in Macedonia, after which the pest spread incredibly rapidly in most European countries, as well as in Western and Central Asia (Didmanidze et al., 2000; Sefrova & Skuhravy, 2001). The main factors of the rapid spread of the leaf miner are usually the low efficiency of their natural enemies, high reproduction rate, and the emergence of several generations during the year (Gilbert et al., 2005). Larvae of the leaf miner *C. ohridella* cannot change their feeding site and must feed on leaves chosen by the egg-laying females (Maria, 1988). This type of feeding requires high-quality food which is rich in carbohydrates and amino acids.

The main host plant of the leaf miner is the horse chestnut (*Ae. hippocastanum*). The larvae of *C. ohridella* damage the leaves by tunnelling into the palisade layer of the parenchyma, leading to severe damage and defoliation by the end of July (Tomiczek & Krechan, 1998). Tree decline as a result of repeated defoliation may occur. In contrast to *Ae. hippocastanum*, the *Aesculus* × *carnea* hybrid was found to be less sensitive. The species that is one of the most susceptible to damage by the leaf miner *C. ohridella* is *Ae. turbinata* (Paterska et al., 2025). According to recent studies, the resistant species of chestnuts include *Ae. parviflora*, *Ae. wilsoni*, *Ae. assamica*, *Ae. californica*, and *Ae. indica* (Bogoutdinova et al., 2024). A number of authors have demonstrated that the chemical content of chestnut tree leaf blades affects the susceptibility of trees to *C. ohridella* (Paterska et al., 2017, 2025). In particular, tree species with leaves that are enriched in carbohydrates show a higher susceptibility to *C. ohridella* (Bogoutdinova et al., 2024). Trees resistant to *C. ohridella* had leaves with higher tannin and catechin content (Materska et al., 2022). Volatile organic compounds also often play a key role in plant-insect interactions and can determine plant resistance or susceptibility (Scala et al., 2013).

The aim of this study was to determine the chemical components of the extract from the leaves of *Aesculus* species when damaged by *C. ohridella*. Since the chemical composition of the *Aesculus* species that are infested with *C. ohridella* (CO) have species-specific peculiarities, this study aimed to conduct the phytochemical screening of the leaves of *Aesculus* species infested with CO in the non-native range in urban areas. The following hypotheses were formulated.

Hypothesis 1. Chemical composition of the leaves of *Aesculus* species in the non-native range is different.

Hypothesis 2. Chemical composition in the leaves of *Aesculus* species changes under CO infestation.

Hypothesis 3. CO lead to changes of the compound content of the polyphenol group in the leaves of *Aesculus* species.

Materials and methods

The study was conducted in Dnipro city, which is in the Ukrainian North Steppe subzone. The research objects were species of the genus *Aesculus* L. (*Ae. hippocastanum* L. (AH), *Ae. pavia* L. (AP), *Ae. parviflora* Walt. (APF), *Ae. flava* Sol. (AF), *Ae. glabra* Willd. (AG) and *Aesculus* × *carnea* Zeyh. (AC). The leaf samples were collected from the collections of the Botanical Garden of Oles Honchar Dnipro National University. Species identification was performed by Dr. I. Ivanko. Herbarium specimens are stored in the herbarium of the Institute of Biology of Oles Honchar Dnipro National University.

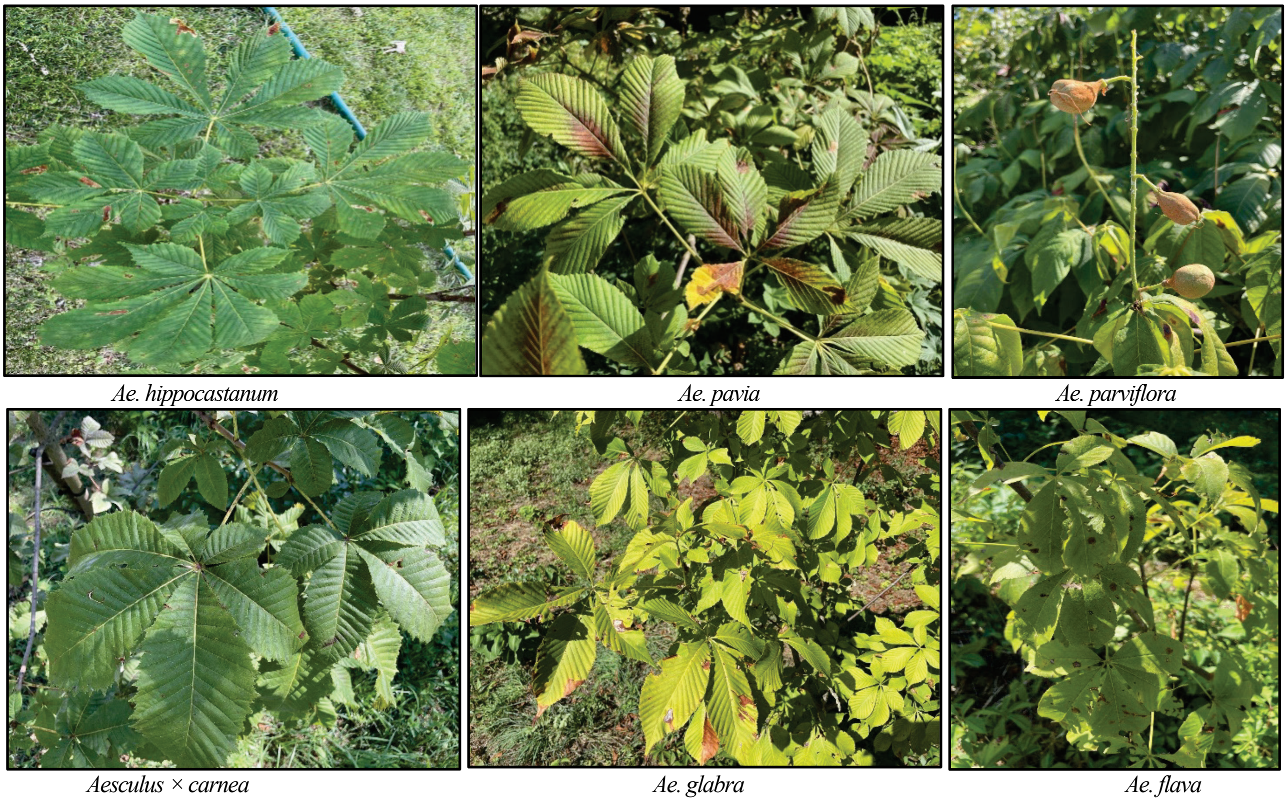

Fig. 1. Plants of genus *Aesculus* affected by herbivory of *Cameraria ohridella*

Leaves of *Aesculus* plants were collected during the vegetation season in July 2023. At this time of the year *Aesculus* trees are already damaged by *Cameraria ohridella* (Seliutina et al., 2020). All trees of the genus of *Aesculus* had symptoms of herbivore damage, except *Ae.* × *carnea*. The plants were not treated with insecticides. For the sampling, study trees with similar morphological and biometric characteristics were selected. Within these trees, leaves of either one of two categories were sampled: i) without damage symptoms (uninfested – U) and ii) infested (I) by CO (Fig. 1), resulting overall in 11 groups. Leaf samples were taken under cloudless conditions at 9–11 a.m, from the lower third of the crown of southern exposure. First, 20 entire leaves of the same category were harvested using scissors, washed on site with tap water and dried at ambient temperature. Then, only the healthy section of the leaf was harvested, avoiding the section which carried the mines in the case of infested leaves The leaves were cut with a scalpel and 20 leaves pooled in one paper bag, representing one sample. All stages of the sampling were carried out with gloves and the instruments used were cleaned with ethanol in between samples. For further analysis, freshly collected leaf samples were used.

For chemical analysis, the samples were milled and leaf powder (ca. 1 g) was extracted with a methanol-chloroform mixture (1:1, 8 mL). Extraction was carried out for 48 h at room temperature with occasional shaking. The suspension was filtered through a 0.45 µm syringe PTFE membrane filter and 2 mL of each extract was evaporated to dryness in vacuo. Then, each sample was derivatized using 0.2 mL of silylation reagent N,O-bis-(trimethylsilyl) trifluoracetamide (BSTFA) with 5% TMS-Cl by simply heating without solvent for 45 min at 65 °C. The obtained clear solution was directly used for the analysis. The identification and content of specialized metabolites in individual extracts were determined by gas chromato-mass spectrometry (GC-MS) on a Shimadzu GCMS-QP2020 instrument (Kyoto, Japan). Capillary column Restek Rxi-5ms (USA, Crossbond® 5% diphenyl / 95% dimethylpolysiloxane), length 30 m, diameter 0.25 mm, thickness 0.25 µm was used. Carrier gas: helium (99.999%), flow rate 1.5 mL/min (89.5 kPa). The temperature gradients during the analysis were within the range of 50 to 300 °C (first 25 min with gradient 10 °C/min, then isothermally at 300 °C for 25 min), the injector and interface temperature was 280 °C, while the ionic source was 250 °C. Solvent cut time was 5 min, splitless injection mode with the sample injected into a volume of 1 µL. The GC-MS analysis was held for 50 min in total. Identification of the substances was carried out using internal NIST-14 (National Institute of Standards and Technology, USA) library considering mass-spectra patterns. The scanning range (TIC mode) was 20–900 m/z with electron impact ionization energy 70 eV, while the identification of substances credibility was within 70–98%. The compounds were characterized as trimethylsilyl (TMS) derivatives. The following groups of chemical components were identified as a result of chromatographic analysis: carbohydrates (CH), polyphenols (PPh), steroids (S), hydrocarbons (HC), fatty acids (FA), miscellaneous (MC).

Principal component analysis (PCA) was performed using Statistica 10 (StatSoft Inc., USA) on mean values of eleven samples and 1847 variables. Separation of data points: the spread of the points along PC1 indicates the most significant variation in the chemical composition across different species; the spread along PC2 represents the second most important variation in the data; species with similar chemical compositions are clustered together, while those with distinct compositions are farther apart. The position of each plant along PC1 and PC2 suggests which chemical compositions are more similar and which are distinct.

Results

As a result of GC-MS analysis, 186 chemical components were identified from the leaf extract of *Aesculus* plants (Table 1). The leaf extract showed the presence of six main groups of chemical components – carbohydrates, polyphenols, steroids, hydrocarbons, fatty acids and miscellaneous.

Table 1
Number of identified components in the main groups of organic compounds of the species of genus *Aesculus*

Components	AG		AP		AF		APF		AH		AC
	U	I	U	I	U	I	U	I	U	I	U
CH	83	69	117	121	111	123	112	100	94	96	109
PPh	4	5	9	8	2	9	8	5	5	5	9
S	4	3	2	4	3	3	3	3	4	4	5
HC	16	22	16	13	16	19	12	15	17	22	13
FA	14	15	15	16	10	10	11	15	11	12	14
MC	20	27	21	24	24	21	28	37	32	26	21
Total	141	141	180	186	166	185	174	175	163	165	171

Notes: AG – *Ae. glabra*, AP – *Ae. pavia*, AF – *Ae. flava*; APF – *Ae. parviflora*; AH – *Ae. hippocastanum*; AC – hybrid *Aesculus × carnea*; U – uninfested; I – infested; CH – carbohydrates, PPh – polyphenols, S – steroids, HC – hydrocarbons, FA – fatty acids, MC – miscellaneous.

In the number of components, the carbohydrates group is represented in the leaves of all studied chestnut species to the maximum degree. The amount of miscellaneous is about four times less. The third and fourth positions are occupied by components of the hydrocarbon group and fatty acids. In terms of the number of available components, polyphenols and steroid groups are the least represented. It was found that in the carbohydrates group, the largest share is represented by such components as sucrose, D-pinitol, thymol-beta-d-glucopyranoside, methyl galactoside. The polyphenolic group is represented mainly by 2R-cis-catechine. In the steroid group, the following compounds were found in the largest amount: (24S)-3-beta-hydroxy-stigmast-5-ene, stigmasterol; in the fatty acids group – nonanoic acid, 2,4-pentanedioic acid, methyl 2-hydroxypentacos-2-enoate, stearic acid, palmitic acid, 2-hydroxyhexacosanoic acid; hydrocarbons – phytol, squalene, n-tetrapentacontane. In the content of miscellaneous compounds, the most abundant were tocopherol, chlorogenic acid, 1,2,3-butanetriol, quininic, ascorbic acid.

The ratio between the groups of studied chemical components of leaf extracts significantly depended on the plant species (Fig. 2). The obtained data demonstrate a change in the number of components in the groups under the influence of *Cameraria ohridella*.

According to the results of chromatographic analysis, the CH content ranged from 43.97% to 73.25 % depending on the species. Almost all of the studied species showed an increase in CH content when leaf leaves were damaged by leaf miner *C. ohridella*. Exceptions were found for the species *Ae. flava* and *Ae. parviflora*, where the CH content was higher in intact plants compared to other species studied. The lowest amount of CH component was found for *Ae. glabra*. The content of MC components was also high enough in the leaves and varied from 9.44% to 21.33%. In contrast to the above mentioned chemical group, the relative content of MC in leaves infected with *C. ohridella* was lower compared to healthy plants. A difference in the effect was observed only for the species *Ae. glabra*, for which the amount of MC was higher for unaffected plants. In comparison with the described component groups, the content of PPh, S, HC and FA was significantly lower in all studied species and ranged from 4.61–12.99%, 0.67–3.34%, 3.27–16.0% and 3.33–12.39%, respectively. As can be seen from the data presented, the lowest amounts of steroid components were found in plants both infested and uninfested with leaf miner.

Discussion

Due to the rapid spread of *C. ohridella* across Europe, this pest has recently been studied by many researchers (Grabenweger, 2004; Kuldová et al., 2007). However, studies of the life cycle, functioning, and criteria for the spread of this insect are mainly based on the determination of the factor influence of environmental conditions. Indeed, the weather conditions, in particular, air temperature, solar radiation and air humidity, are extremely important factors affecting the intensity of chestnut leaf miner *C. ohridella* infestation (Cedro & Nowak, 2022; Bogoutdinova et al., 2023). That is why in our work, the primary task was to collect research material from plants that grew under the same environmental conditions for all the species studied. We have not found any similar scientific works on the comparison of the chemical composition of extracts from the leaves of different species of the genus *Aesculus*.

The key factors that determine the synthesis of specialised metabolites in plants are abiotic environmental factors (climate, weather regime, soil composition and microflora) and biotic interactions between plants and insects (Seliutina et al., 2020). The presence of specialised metabolites of phenolic nature is often considered as a chemical defence against herbivory (Uzelac et al., 2023). The studied species are species that grow in non-native habitats, so their chemical composition was formed under the complex influence of urban environmental factors, among which *C. ohridella* infection was chosen as the key one and the change in chemical composition components under its action was studied. Seven components belonging to the group of polyphenolic compounds were identified in the methanol-chloroform extracts of leaves of the studied six species of the genus *Aesculus* (Table 2, Fig. 3).

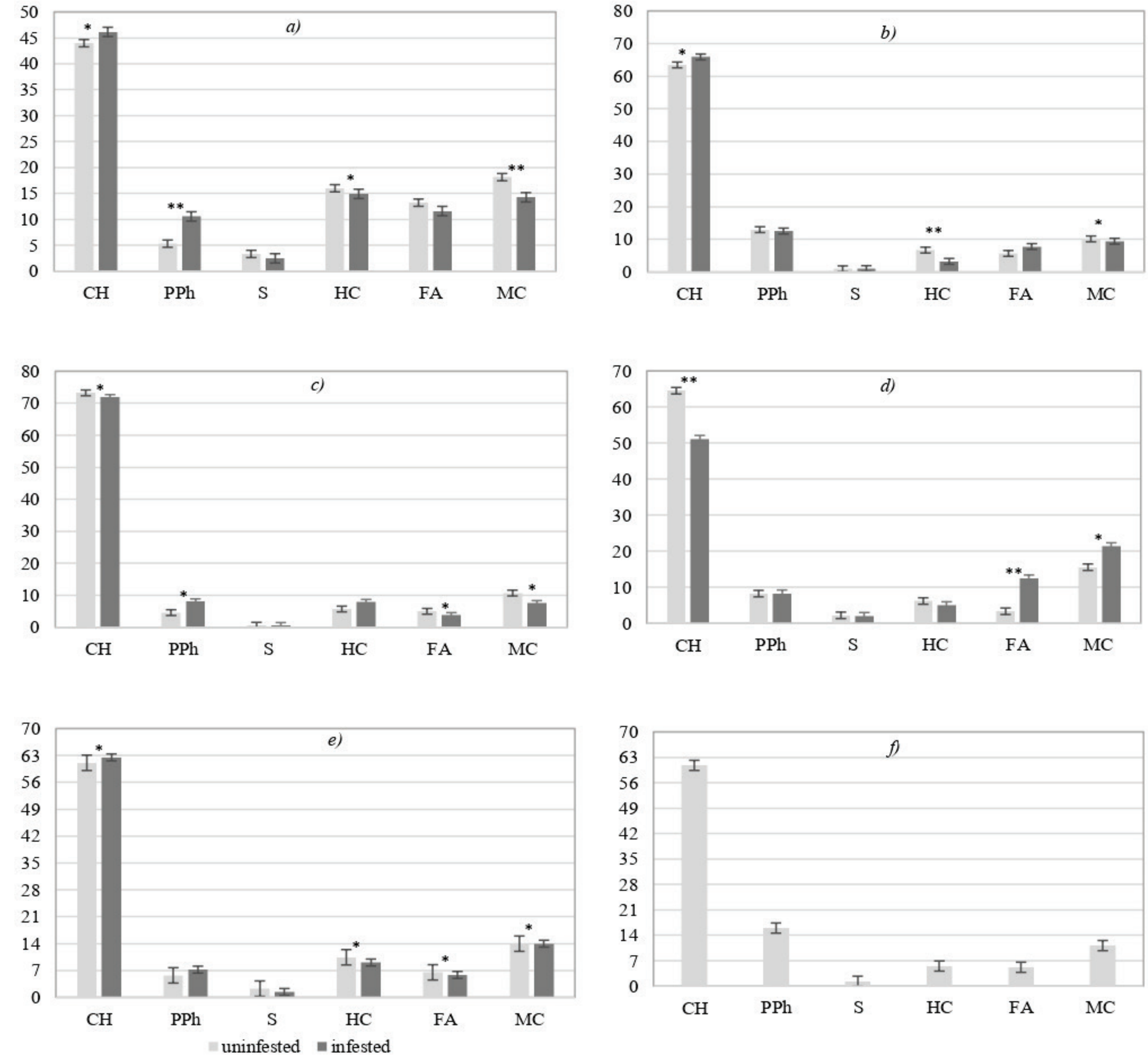

Fig. 2. Chemical composition (%) of leaves depending on chestnut species of genus *Aesculus* and mine leaf miner damage: *a – Ae. glabra*; *b – Ae. pavia*; *c – Ae. flava*; *d – Ae. parviflora*; *e – Ae. hippocastanum*; *f – Aesculus × carnea*; CH – carbohydrates, PPh – polyphenols, S – steroids, HC – hydrocarbons, FA – fatty acids, MC – miscellaneous; presented values shows means ± SE; P-value: * < 0.05, ** < 0.01

Only the presence of one component of (2R-cis)-catechine was found in all samples, both infected by herbivory and in leaves without signs of any damage. The qualitative composition of polyphenolic compounds was species-specific. The analysis of the composition of extracts of uninfested leaves established that four components were contained in extracts of *Ae. hippocastanum* and *Ae. pavia*, but they were different in their composition. In comparison with the composition of polyphenols in leaves without damage and leaves infected with *C. ordidella*, the absence of (2R-trans)-catechine in extracts from infected leaves of *Ae. hippocastanum* and *Ae. glabra* was interesting. The racemic catechine was identified in both healthy and infected leaves of two of the studied species in *Ae. hippocastanum* and *Ae. pavia*, while in *Ae. parviflora* and *Ae. flava*, this component was detected only in extracts of infected leaves. The presence of two components 3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid and epigallocatechin in infected leaves of *Ae. flava* and quercetin in infected leaves of *Ae. glabra*, which were absent in undamaged leaves, may indicate a greater potential resistance of these species to damage by the studied herbivory. The structures of the major polyphenolic compounds are shown in Figure 3. The presence of phenolic compounds in *Ae. hippocastanum* had been reported in the literature (Dudek-Makuch & Matlavska, 2011). Seventeen phenolic compounds were identified in methanolic extracts from white and red chestnut leaves damaged by the leaf-eating insect *C. ohridella*, analysed by the LC-ESI-MS/MS system. Thus, three hydroxycinnamic acids, eight flavan-3-ols: (-)-epicatechin, five procyanidins of type A and two dimers of procyanidins of type B; six flavonols – four quercetin derivatives and two kaempferol derivatives – were detected. The quercetin derivatives were represented by quercetin-3-O-rhamnoglucoside (rutinoside), 3-O-galactoside, 3-O-arabinoside and 3-O-rhamnoside. A number of quercetin flavonoid glycosides, such as rutin, isoquercitrin, quercetin 3-O-arabinoside and the corresponding camperfol glycosides, were also found in leaf tissues (Bombardelli et al., 1996).

The PCA scatter plot visually represents the variation in the chemical composition of different species genus *Aesculus*, with the first principal component (PC1) on the x-axis and the second principal component (PC2) on the y-axis (Fig. 4).

Table 2
Presence/absence of polyphenolic components in different *Aesculus* species under the influence of *C. ohridella*

Polyphenol compounds*	AH		APF		AF		AG		AP		AC
	U	I	U	I	U	I	U	I	U	I	U
(2R-*cis*)-Catechine (5TMS derivative)	+	+	+	+	+	+	+	+	+	+	+
(2R-*trans*)-Catechine (5TMS derivative)	+	–	+	+	+	+	+	–	+	+	+
2-{3,4-Bis[(trimethylsilyl)oxy]phenyl}-3,5-bis[(trimethylsilyl)oxy]-3,4-dihydro-2H-chromen-7-yl)oxy](trimethyl)silane	+	+	–	+	–	+	–	–	+	+	–
2,3,7,8-Tetrakis[(trimethylsilyl)oxy]chromeno[5,4,3-cde]chromene-5,10-dione	+	–	+	+	–	+	–	–	+	+	+
Quercetin (5TMS derivative)	–	–	–	–	–	–	–	+	–	–	–
Trimethylsilyl catechollactate tris(trimethylsilyl) ether	–	–	–	–	–	+	–	–	–	–	–
Epigallocatechin (6TMS derivative)	–	–	–	–	–	+	–	–	–	–	–

Notes: the compounds were characterized as trimethylsilyl (TMS) derivatives.

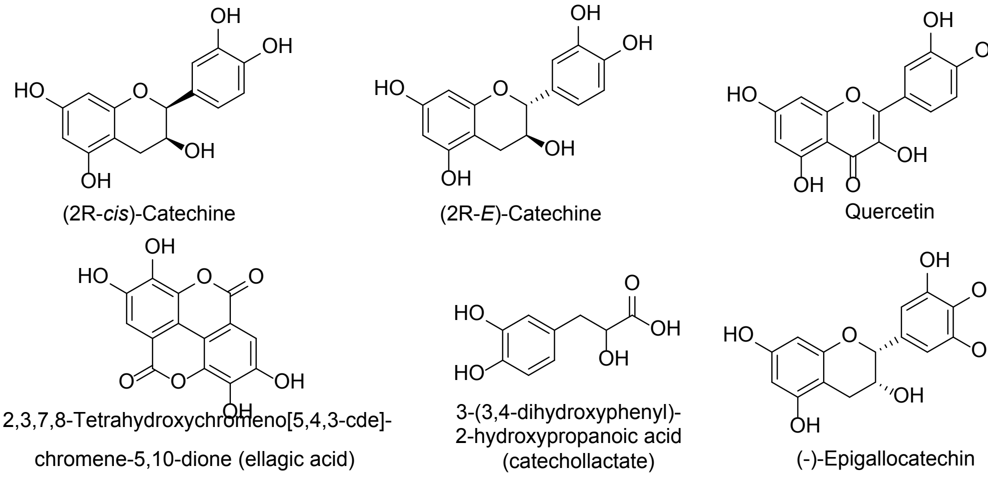

Fig. 3. Structures of major polyphenolic compounds

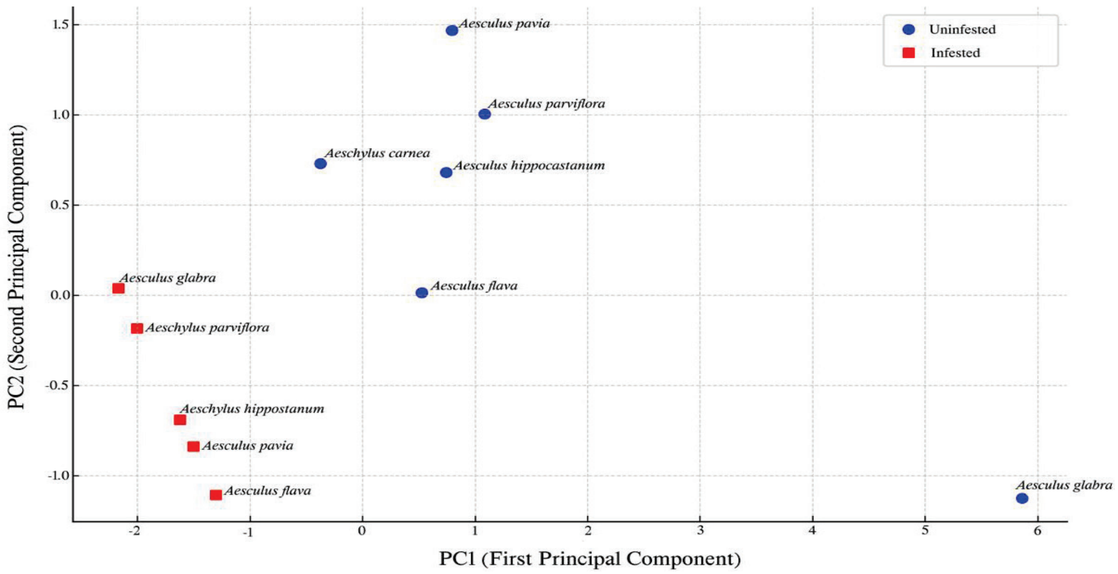

Fig. 4. Loading plot for principal component analysis variation of the chemical composition of the six species of genus *Aesculus*: blue circles represent uninfested leaves, red squares represent infested leaves

The infested and uninfested plants cluster separately, it indicates that infestation significantly affects the plant's chemical profile. Both the infested and uninfested samples of the same plant are close together, the chemical changes due to infestation are less significant. Since PC1 has a much larger spread than PC2, it captures most of the variation in the data. This suggests that the primary factor differentiating plant samples is their overall chemical composition rather than smaller, secondary variations. It was established that *Ae. glabra* shows a clear separation between chemical composition of the infested and uninfested samples. This suggests that infestation significantly alters the chemical composition of these plants. Other species of the genus *Aesculus* have their infested and uninfested samples closer together, meaning the infestation had a less dramatic effect on their chemical composition.

Conclusions

The main group of the chemical compounds of the methanol-chloroform extracts of the leaves *Ae. glabra*, *Ae. pavia*, *Ae. flava*, *Ae. parviflora*, *Ae. hippocastanum* and *Aesculus* × *carnea* are carbohydrates, polyphenols, steroid, hydrocarbon and fatty acids.

The maximum number of chemical components was identified in the extract of *Ae. flava* leaves infested with *C. ohridella* – 186, and in the extract of *Ae. pavia* leaves infected with *C. ohridella*, – 185. The component composition of extracts of uninfested and infested leaves of *Ae. glabra* had the same number of identified chemical components – 141. *Ae. glabra* demonstrated a clear separation between the chemical composition of extracts from infested and uninfested leaves, indicating a significant change in the chemical composition of this species under infestation by *C. ohridella*. Two components of a phenolic nature, trimethylsilyl catechollactate tris(trimethylsilyl) ether and epigallocatechin (6TMS), were identified only in infected leaves of *Ae. flava*, and quercetin was identified only in infected leaves of *Ae. glabra*, which may indicate a greater potential resistance of these *Aesculus* species to damage by the studied herbivory.

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