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ORIGINAL PAPER

The content of long-chain hydrocarbons in the bark of selected willow cultivars in the context of cervids' food preferences

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ABSTRACT

The content of long-chain hydrocarbons in the bark of several willow cultivars was investigated. The research material consisted of 10-cm-long apical one-year shoots harvested from 3-year-old carps. The content and composition of hydrocarbons were determined by gas chromatography coupled with mass spectrometry. All analyzed cultivars contained 19 hydrocarbons in various amounts. Hydrocarbon content was highest in the bark of *Salix purpurea* clone 1126, mainly due to a high concentration of 9,12-octadecadienoic acid. In turn, eicosanoic acid was found in trace amounts. A correlation analysis showed a positive relationship between browsing intensity and the content of the following compounds in willow bark: hexadecanoic acid, 11-octadecenoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, docosanoic acid, and octadecanoid acid.

KEY WORDS

deer, long-chain hydrocarbons, willow browsing by cervids

Introduction

Almost all species of trees and shrubs in Poland and Europe are browsed by deer. Damage caused by these animals in forests, mainly in young stands, is a significant economic problem and a breeding challenge for foresters and hunters. In order to minimize these losses, various protective measures are taken, mainly by building wire mesh fences and permanent fences, securing the apex buds with various methods and creating buffer zones. Numerous scientific studies are also carried out to improve feeding conditions for game animals as a method used to limit the damage caused by these animals (Drogoszewski and Kokocki, 2003; Budny *et al.*, 2021a, b). For many

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years in Europe, including Poland, chemical protection of trees in plantations and young forests has been also used, *i.e.*, repellents. Research on the effectiveness of repellents in forest protection were successfully conducted in some regions of the country (Szukiel and Borowski, 2000). Moreover, each species of deer feeds differently, which should be taken into account when protecting crops. Roe deer *Capreolus capreolus* L. mainly bites donuts, and in winter also chews the bark with a sip (Pielowski, 1999). The red deer *Cervus elaphus* L. eats everything young and burns the trunks of young trees, mainly pine (Bobek et al., 1992). The moose *Alces alces* L. capsizes or breaks whole young trees and chooses what it likes (Bobek *et al.*, 2021).

Various protective measures are being implemented to minimize the losses caused by cervid foraging in forests and farmland. Attempts have been made to introduce alternative food sources for deer, for example by cultivating willow browse in forest ecosystems. Interestingly, deer forage on only several willow cultivars out of some 450 willow genotypes growing in Poland. Our previous studies and other authors' findings suggest that the attractiveness of willow browse for deer is determined by the chemical composition of tissue (Drogoszewski and Wlazełko, 1980; Drogoszewski, 1999; McCabe and Barry, 2009; Tajchman, 2020; Budny *et al.*, 2021a,b). Our recent studies analyzed the relationships between the content of soluble carbohydrates and phenols in willow branches (young shoots with leaves) and their attractiveness for foraging animals, and significant positive or negative correlations between the concentration of some of these compounds and the intensity of browsing by deer were found (Budny *et al.*, 2021a, b). However, the content of long-chain hydrocarbons and phenols which could act as potential attractors for deer, in particular in winter and spring, has never been examined in willow bark (excluding other tissues).

Long-chain hydrocarbons are the main constituents of waxes. Waxes are a highly diverse group of organic compounds that occur in nature. They are composed mainly of higher fatty acid esters, monohydroxy alcohol esters, free fatty acids and alkanes. Long-chain hydrocarbons play mainly protective roles in animals and plants. However, research has shown that plant waxes contain fatty acid esters and alcohols that play dietary and regulatory roles in animals (Hargrove *et al.*, 2004). Waxes that occur on the surface of plants have mainly protective functions. They protect leaves and stems against excessive transpiration and, consequently, wilting in periods characterized by high temperature and insolation. Surface waxes also protect plants against insects, fungi, bacteria and viruses that cause plant diseases. The dietary and health-promoting roles of long-chain hydrocarbons have been investigated mainly in the context of human and livestock nutrition, but their nutritional value for plants and wild animals has been rarely analyzed. The impact of long-chain hydrocarbons on the taste and aroma of forage plants for animals, in particular free-living animals, has never been studied in the literature.

The cultivation of willow browse as an alternative food source for cervids is one of the many methods of protecting forests against foraging damage. Willows contain phenolic compounds such as salicin, salicortin, tremulacin and other bitter-tasting phenolic glycosides, but this aspect of willow browse cultivation has not been previously explored (Julkunen-Titto and Gebhardt, 1991). These compounds possess anti-inflammatory, antipyretic, antifungal and analgesic properties. Flavonoids and tannins also deliver health-promoting effects (Sulima *et al.*, 2017). These substances improve animal health and well-being (Niemiec and Dubas, 2015), but they impart a bitter taste, thus discouraging animals from choosing plants that contain these compounds. Many studies into willow browsing by cervids focus solely on the quantitative reduction in willow mass without identifying the underlying causes of specific foraging behaviors. In these experiments, only selected *Salix purpurea* L. cultivars were not browsed by deer. Cervids' food preferences could be affected by differences in the content of bitter-tasting and 'discouraging' substances, relative to the concentrations of attractants (vitamins, minerals, sweet proteins, carbohydrates). According to Magac (2017), the maximum level of deer browsing damage should be estimated, but it should not be minimized by building fences or using repellants, but by identifying the key determinants of foraging behaviour. Foresters should not protect tree stands against animals, but should create a nutritionally heterogeneous environment that eliminates the need for protective measures. This goal could be achieved by establishing browse plots containing selected willow cultivars.

The main aim of the study was to establish whether there is a statistically proven relationship between the degree of browsing of the analyzed cultivars and the content of long-chain hydrocarbons.

Materials and Methods

Materials. The content of long-chain hydrocarbons was determined in the shoots of ten willow cultivars. To avoid repetition, the names of cultivars were marked with numbers, and these numbers were used in the subsequent sections of the article (Table 1). Bark and phloem were obtained from freshly harvested one-year-old willow shoots grown in an experimental plot in the Research Station of the Polish Hunting Association in Bieczyny. *Salix fragilis* L. cv. Krakowianka had not been established successfully in the experimental plot, and shoots of this willow cultivar were obtained from the Agricultural Experiment Station in Bałcyny (operated by the University of Warmia and Mazury in Olsztyn) and a thicket in Czempiń. The shoots of willow cultivar No. 5 grown in three nurseries located in two Polish voivodeships (Western Pomerania and Wielkopolska) were compared to determine the influence of location on the concentrations of the analyzed compounds. The evaluated samples were genetically homogeneous because these willows had been propagated from cuttings obtained from a thicket in the Resko Forest District. One-year-old shoots were harvested in late November 2018. Bark and phloem were separated manually, freeze-dried and ground in a hammer mill. Analytical samples of 10 g each were extracted with petroleum ether at a temperature of 40°C for 6 hours. The extract was condensed in a vacuum evaporator at a temperature of 40°C.

Determination of long-chain hydrocarbons and fatty acids in waxes. The content of long-chain hydrocarbons and fatty acids in waxes was determined by gas chromatography coupled with mass spectrometry (GC-MS QP2010 PLUS, Shimadzu, Japan), according to the method described by Czaplicki *et al*. (2016).

Table 1.
List of tested willow cultivars and their designations used later in the work in the tables and figures

Cultivar	Assigned number
Salix purpurea L. 1126	1
Salix fragilis L. Kamon (Bałdy)	2
Salix fragilis Kamon (Czempiń)	3
Salix amygdalina L. 1102	4
Salix amygdalina Krakowianka	5
Salix amygdalina Dunajec	6
Salix laurina Sm. 220/205	7
Salix amygdalina Triandra 1045	8
Salix cordata Michx. 1036	9
Salix pontederana	10

To evaluate the composition of long-chain hydrocarbons and fatty acids, the analyzed samples were dissolved in a mixture of chloroform-methanol-sulfuric acid (100/100/1, v/v/v) and methylated by heating the vials at 70°C for 60 min. Then sulfuric acid was neutralized with the addition of powdered zinc, and the obtained mixture was evaporated under a nitrogen stream. The obtained methyl esters were dissolved in hexane, filtered and analyzed with the use a BPX70 (25m×0.22mm×0.25mm) capillary column (SGE Analytical Science, Victoria, Australia) with helium as a carrier gas at a flow rate of 1.3 ml/min. Column temperature was programmed as follows: increase from 150°C to 180°C at a rate of 10°C/min, increase to 185°C at a rate of 1.5°C/min, increase to 250°C at a rate of 30°C/min, after which column temperature was kept constant for 10 min. The temperature of the interface and the GC-MS ion source was set at 240°C, and electron energy was set at 70 eV. The total ion current (TIC) mode was applied in 45-500 m/z range.

A sucrose experiment was performed to determine the insulating properties of waxes on fresh shoots. The extent to which soluble sugars (such as sucrose) penetrate shoot tissues was examined in shoot samples that had been soaked in a 5% aqueous solution of sucrose at a temperature of 10°C for 24 hours. Shoots were rinsed with tap water after the experiment.

Evaluation of willow browsing. A 5-point scale developed by Bukiewicz (1960, 1963) was used to assess the damage caused by game in the occlusal plots, which was only slightly modified. It is as follows: 1 – no damage, no traces of biting shoots, buds and leaves; 2 – small damage, not on all shrubs, should be completely regenerated, loss calculation factor 0.05 (5%); 3 – medium damage, the upper part of the main shoot is bitten, possibly of the side shoots, visible losses in foliage, signs of bark tearing on short sections, no top bud; the effect may be the inhibition of shoot growth and the deterioration of the shrubs' condition; loss calculation factor 0.15; 4 – large lesions, nibbled main and side shoots, buds, partial or complete defoliation, nibbled suckers from carp; shoots stripped of bark more than half; the effect can be a significant reduction in the growth of shrubs or, in unfavorable conditions, the separation of carp, loss calculation coefficient 0.50; 5 – no shoot, shoots bitten and dead as a result of damage, coefficient for calculating losses 1.00.

The intensity of browsing damage in the experimental plots was determined based on the following assumptions: the experimental plots contained different numbers of the analyzed willow cultivars; therefore, browsing damage to each cultivar was assessed along five segments of 20 plants each, both in fenced plots and after fencing had been removed.

Statistical analysis. The results were processed statistically in Dell Statistica 13.0 data analysis software system (Dell Inc.). The significance of differences between mean hydrocarbon concentrations in the examined cultivars was determined by Tukey's (HSD) test at a significance level of 0.01.

Results

A total of 19 hydrocarbons were identified in the chromatographic analysis of the mixture extracted with petroleum ether from the bark and phloem of 10 willow cultivars (Table 2, Fig. 1). The content of 9,12-octadecadienoic acid was high in all cultivars, in particular in *Salix purpurea*. The examined cultivars differ significantly in the concentration of 9,12-octadecadienoic acid which ranged from 74 μg/g DM in *S. cordata* Michx. to 2389 μg/g DM in *S. purpurea*. All of the analyzed cultivars were abundant in two unsaturated fatty acids: 9,12,15-octadecatrienoic acid and hexadecanoic acid (Table 2). In turn, other hydrocarbons were identified in trace amounts or at the limit of detection. Trace amounts that were detected, but not quantified for statistical and methodological

Table 2.

The content of long-chain hydrocarbons (μg/g DM) in the bark and the phloem of one-year-old shoots of selected willow cultivars grown in experimental plots. Shoots were harvested from four-year-old carps. Mean values and SD are shown. Tr – trace amounts, EM – methyl ester

Compound name	Cultivar (No)									
	1	2	3	4	5	6	7	8	9	10
Hexadecanoic acid, EM	113.19±0.35 B	35.83±0.39 H	64.52±0.78 D	81.87±1.18 C	51.21±0.81 E	45.98±0.99	48.50±0.46 EF	37.89±0.59 H	45.89±0.15 G	66.37±0.74 D
Octadecanoic acid, EM	37.76±0.28 B	3.89±0.50 G	6.41±0.49 CD	6.87±0.56 C	4.79±0.29 FG	5.47±0.33 EF	4.12±0.18 G	3.56±0.12 G	3.66±0.09 G	6.41±0.25 CD
11-Octadecenoic acid, EM	61.20±0.43 A	5.06±0.10 F	12.47±0.50 C	14.78±0.52 B	6.85±0.19 DE	7.39±0.42 D	5.25±0.14 F	5.24±0.34 F	8.05±0.08 D	8.21±0.33 D
9,12-Octadecadienoic acid, EM	2389.22±311 A	82.56±0.52 A	170.37±2.57 A	243.19±4.71 A	102.01±0.91 A	116.31±0.78 A	120.07±6.41 A	91.39±1.57 A	74.07±0.73 A	152.10±2.68 A
9,12,15-Octadecatrienoic acid, EM	149.22±2.12 B	30.46±0.97 J	48.69±0.47 G	99.69±1.33 C	43.46±0.57 H	54.48±0.33 F	65.62±0.99 E	39.34±0.27 I	39.92±0.32 HI	90.79±1.39 D
Pentacosane	86.20±0.94 B	29.62±0.56 F	82.66±1.54 C	13.21±0.25 H	41.81±1.07 D	28.56±0.75 F	21.3±1.31 G	19.57±0.73 G	8.67±0.27 I	27.83±0.50 F
Eicosanoic acid, EM	3.19±0.08 B	Tr F	2.62±0.09 BC	2.41±0.40 C	2.84±0.13 BC	1.69±0.18 E	2.36±0.04 CD	1.73±0.13 E	1.79±0.14 D	1.71±0.15 E
cis-11-Eicosenoic acid, EM	7.83±0.14 A	Tr E	Tr E	Tr E	Tr E	1.83±0.11 C	Tr E	Tr E	0.61±0.07 D	Tr E
Octacosane	84.73±0.55B	44.13±0.88 G	53.35±0.71 CD	8.99±0.66 I	47.96±1.00 EF	50.72±0.67 DE	8.88±0.07 I	33.32±0.99 H	30.05±0.83 H	45.98±1.47 FG
Docosanoic acid, EM	6.28±0.21 A	3.71±0.10 A	6.49±0.74 A	6.37±0.82 A	5.89±0.22 A	3.59±0.27 A	Tr F	3.08±0.16 A	3.13±0.08 A	4.06±0.14 A
Tetratriacontane	18.08±0.66 A	3.05±0.10 H	4.29±0.16 FG	4.71±0.13 EF	2.93±0.12 H	9.31±0.36 C	Tr I	3.56±0.07 GH	5.38±0.05 E	3.58±0.10 GH
Tricosanoic acid, EM	5.07±0.14 C	Tr F	3.77±0.28 CD	6.69±1.01 B	3.89±0.21 CD	Tr F	3.33±0.52 DE	2.04±0.09 E	2.78±0.56 DE	3.62±0.35 CD
Tetracosanoic acid, EM	8.45±0.19 CD	9.09±0.51 C	13.32±0.52 A	5.73±0.52 FG	10.61±0.62 B	3.59±0.26 H	4.52±0.13 GH	7.43±0.13 DE	4.66±0.18 GH	6.30±0.26 EF
Pentacosanoic acid, EM	7.63±1.19 C	2.17±0.10 EF	9.81±0.24 B	3.74±0.11 D	2.88±0.08 DE	Tr H	3.32±0.11 DE	1.66±0.09 FG	0.90±0.07 G	3.10±0.15 DE
Hexacosanoic acid, EM	14.51±0.25 E	24.62±0.35 B	44.63±1.11 A	13.62±0.52 E	24.64±0.57 B	4.59±0.17 G	18.42±0.68 D	21.33±1.40 C	4.35±0.17 G	8.10±0.19 F
Heptacosanoic acid, EM	26.18±0.63 B	7.21±0.19 DE	27.46±1.20 B	7.12±0.25 DE	8.19±0.24 D	7.65±0.47 D	5.46±0.13 F	6.62±0.20 DE	3.10±0.11 G	5.56±0.21 EF
Octacosanoic acid, EM	45.10±0.14 F	85.59±1.24 C	100.07±1.14 A	26.71±01.55 GH	92.13±1.76 B	22.81±2.88 HI	28.42±0.64 G	73.69±1.31 D	19.30±0.53 I	23.86±0.88 GH
1,1-dimethoxy-dodecane	58.43±0.55 A	Tr G	6.49±0.47 EF	1.82±0.09 G	4.14±0.11 FG	19.79±0.80 C	0.58±0.04 G	9.61±0.93 E	14.18±0.18 D	4.10±0.32 FG
Triacontanoic acid, EM	47.27±0.77 D	60.75±0.50 B	38.07±0.78 F	15.28±0.82 H	56.14±0.61 C	32.22±0.77 G	7.01±0.22 I	57.04±1.07 C	34.87±0.82 G	38.02±0.59 F

reasons (measurement errors at very small values) were marked with the symbol *Tr*. These compounds include cis-11-eicosenoic acid, eicosanoic acid, tricosanoic acid, pentacosanoic acid, docosanoic acid and tetratriacontane (higher alkane $CH_3(CH_2)_{33}CH_3$).

The sucrose content of one-year-old shoots soaked in water (control) and a 5% aqueous solution of sucrose for 24 hours is compared in GC chromatograms in Figure 2. The purpose of the experiment presented in this figure was to determine the insulating properties of waxes (including the analyzed hydrocarbons) on the surface of willow shoots. The statistical analysis revealed no significant differences between samples.

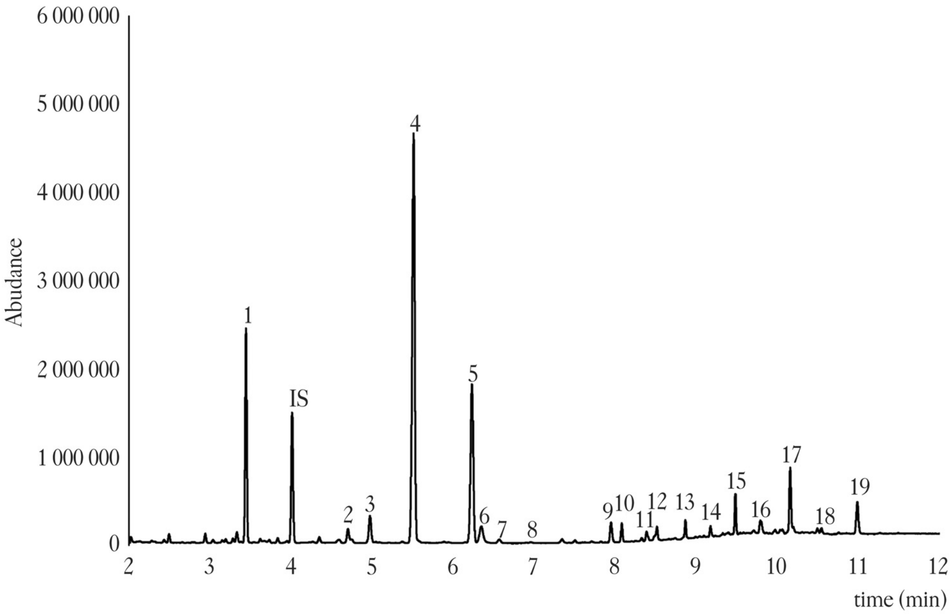

Fig. 1.

A chromatogram of hydrocarbons in *S. amygdalina* cv. Krakowianka (No. 5). Identification of compounds: 1) Hexadecanoic acid, methyl ester; IS (internal standard) – Heptadecanoic acid, methyl ester; 2) Octadecanoic acid, methyl ester; 3) 11-octadecenoic acid, methyl ester; 4) 9,12-octadecadienoic acid, methyl ester; 5) 9,12,15-octadecatrienoic acid, methyl ester; 6) Pentacosane; 7) Eicosanoic acid, methyl ester; 8) cis-11--eicosenoic acid, methyl ester; 9) Octacosane; 10) Docosanoic acid, methyl ester; 11) Tetratriacontane; 12) Tricosanoic acid, methyl ester; 13) Tetracosanoic acid, methyl ester; 14) Pentacosanoic acid, methyl ester; 15) Hexacosanoic acid, methyl ester; 16) Heptacosanoic acid, methyl ester; 17) Octacosanoic acid, methyl ester; 18) 1,1-dimethoxy-dodecane; 19) Triacontanoic acid, methyl ester

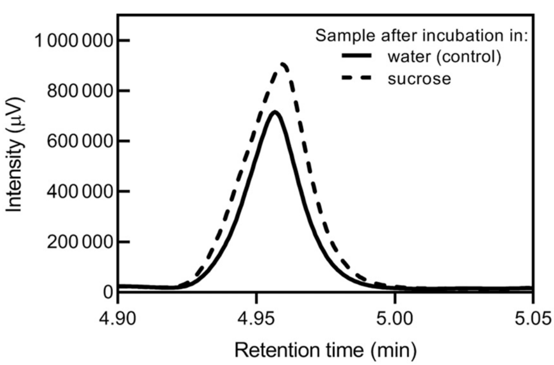

Fig. 2.

Peak areas of TMS-derivatives of sucrose in GC chromatograms of extracts from one--year-old willow shoots soaked in water (control – solid line) and a 5% aqueous solution of sucrose (dotted line) for 24 hours.

Total hydrocarbon concentrations were highest in cultivar No. 1 which was one the least damaged willow cultivars (Table 3). The above suggests that the total content of long-chain hydrocarbons in bark and phloem was not directly correlated with the intensity of browsing damage caused by cervids. However, a significant positive correlation was observed between the content of selected hydrocarbons and the extent of browsing damage (Table 4).

The correlation analysis revealed a positive relationship between the extent of browsing damage and the content of the following long-chain hydrocarbons in willow bark: hexadecanoic

Table 3.

Content of hydrocarbons in the bark and phloem of selected willow cultivars and their attractiveness for cervids based on the extent of browsing damage in the experimental plots

Cultivar number	Hydrocarbon content [μg/g d.w.]	Proportion of browsed shoots in in Bieczyny [%]*	Mean proportion of browsed shoots in three plots [%]*
1	**4189.95±238**	2.70±0.07	5.03±0.28
2	427.7±4±23.1	4.09±0.02	4.59±0.22
3	695.46±31.2	**8.76±0.05**	11.13±0.93
4	562.80±25.8	**27.96±1.21**	18.04±1.09
5	512.38±22.1	4.94±0.22	15.23±0.78
6	282.32±22.9	2.75±0.13	5.48 (2 plots)
7	347.23±16.7	4.55±0.20	5.45 (2 plots)
8	411.94±25.3	2.57±0.11	10.90±0.78
9	305.36±13.9	7.52±0.32	8.99±0.47
10	499.70±25.8	3.53±0.15	5.92±0.29

* according to Budny et al. (2021b). Standard deviation was included in the data

Table 4.

Spearman rank correlations between the intensity of browsing damage (in the experimental plot in Bieczyny) and the content of long-chain hydrocarbons in the bark of the analyzed willow cultivars

Cultivar number	Compound name	Spearman rank correlation
1	Hexadecanoic acid, EM	0.45*
2	Octadecanoic acid, EM	0.26*
3	11-Octadecenoic acid, EM	0.43*
4	9,12-Octadecadienoic acid, EM	0.43*
5	9,12,15-Octadecatrienoic acid, EM	0.42*
6	Pentacosane	–0.10
7	Eicosanoic acid, EM	0.13
8	cis-11-Eicosenoic acid, EM	0.11
9	Octacosane	–0.18
10	Docosanoic acid, EM	0.31*
11	Tetratriacontane	0.10
12	Tricosanoic acid, EM	0.04
13	Tetracosanoic acid, EM	0.07
14	Pentacosanoic acid, EM	0.18
15	Hexacosanoic acid, EM	0.10
16	Heptacosanoic acid, EM	0.02
17	Octacosanoic acid, EM	0.03
18	1,1-dimethoxy-dodecane	–0.44*
19	Triacontanoic acid, EM	–0.35*

* R significant at the level of 0.05

acid, methyl ester (R=0.45), 11-octadecenoic acid, methyl ester (R=0.43), 9,12-octadecadienoic acid, methyl ester (R=0.43), 9,12,15-octadecatrienoic acid, methyl ester (R=0.42), docosanoic acid, methyl ester (R=0.31), and octadecanoic acid, methyl ester (R=0.26) (Table 3). A weak negative correlation was noted between the intensity of browsing damage and the concentrations of 1,1--dimethoxy-dodecane (R=–0.44) and triacontanoic acid, methyl ester (R=–0.35).

The comparison of long-chain hydrocarbon content in the shoots of *S. amygdalina* L. Krakowianka collected from the three localities showed significant differences in the case of 9,12-octadecadienoic acid, EM, and octacosanoic acid, EM. Smaller, but statistically significant differences were also noted in the concentrations of other hydrocarbons (such as hexadecanoic acid, EM). In several comparisons, differences in the content of the studied compounds were determined only at the significance level (such as pentacosanoic acid, EM).

Discussion

Numerous research studies have demonstrated that willow browse can be an alternative food source for cervids, in particular in periods of drought and when food resources are scarce in animal habitats (Drogoszewski, 1991, 1995, 1999; McCabe and Barry, 2009; Kokocki and Kowalski, 2012).

Cervids rely on the senses of taste, smell and sight when selecting specific willow cultivars (if such a choice exists). The extent to which long-chain hydrocarbons play a role in the foraging behavior of deer remains unknown. It should be noted that long-chain hydrocarbons constitute the surface layer of potential browse plants and influence the animals' taste perception upon contact.

Table 5.

Composition (content) of long-chain hydrocarbons (µg/g DM) in the bark and phloem of willow cv. Krakowianka grown in experimental plots in the Resko Forest District, in the Research Station of the Polish Hunting Association in Czempiń, and in Bieczyny. Mean values and SD are shown

Compound name	Resko Forest District	Czempiń	Bieczyny
Hexadecanoic acid, EM	26.84±1.11	81.87±4.60	51.21±0.81
Octadecanoic acid, EM	3.22±0.23	6.87±0.21	4.79±0.29
11-Octadecenoic acid, EM	5.11±0.20	6.08±0.34	6.85±0.19
9,12-Octadecadienoic acid, EM	49.67±1.87	243.19±13.03	102.01±0.91
9,12,15-Octadecatrienoic acid, EM	**37.64±2.08**	**89.70±5.11**	**43.46±0.57**
Pentacosane	23.15±1.26	13.21±0.72	41.81±1.07
Eicosanoic acid, EM	0.81±0.07	2.41±0.13	2.84±0.13
cis-11-Eicosenoic acid, EM	traces	traces	traces
Octacosane	26.72±1.47	8.99±0.41	47.96±1.00
Docosanoic acid, EM	4.98±0.20	6.40±0.29	5.89±0.22
Tetratriacontane	1.23±0.07	4.71±0.27	2.93±0.12
Tricosanoic acid, EM	5.13±0.31	6.68±0.30	3.89±0.21
Tetracosanoic acid, EM	7.03±0.42	5.73±0.29	10.61±0.62
Pentacosanoic acid, EM	2.84±0.26	3.74±0.16	2.88±0.08
Hexacosanoic acid, EM	16.37±0.79	13.62±0.65	24.64±0.57
Heptacosanoic acid, EM	traces	7.12±0.42	8.19±0.24
Octacosanoic acid, EM	**32.00±2.02**	**26.71±1.83**	**92.13±1.76**
1,1-dimethoxy-dodecane	3.61±0.19	1.82±0.10	4.14±0.11
Triacontanoic acid, EM	22.06±1.45	15.28±0.84	46.14±0.61
Total	268.41±15.89	544.13±28.33	502.37±29.56

The absence of significant differences in the sucrose content of willow shoots soaked in water and in a 5% aqueous solution of sucrose indicates that the outer waxy layer constitutes a highly effective barrier.

The role played by long-chain hydrocarbons in animals, including cervids, remains unknown. Olfaction is one of the most highly developed senses in animals, in particular wild animals. In cervids, the olfactory organ (olfactory epithelium, olfactory receptors, olfactory bulb) contains 297 million olfactory receptors, as compared to only 5 million in humans (Tajchman, 2020). Sensory perception is largely determined by substances dissolved in cell juice, including sugars, proteins and fats. Willow shoots are abundant in aromatic compounds, including polyphenols, that also play a very important role in the foraging behavior of cervids (Budny *et al.* 2021a).

It is difficult to determine, based on the results of this study, whether waxes present in selected willow cultivars (such as *Salix purpurea* and *Salix fragilis* cv. Kamon) can mask the taste perception of polyphenols. Long-chain hydrocarbons do not have a distinct aroma; therefore, the presence of these compounds is more likely to be masked by aromatic compounds, mainly polyphenols (Budny *et al.*, 2021b). McCabe and Barry (2009) reported very interesting results of an experiment investigating the nutritive value and digestibility of tree willow (*Salix matsudana* × *S. alba*) and osier willow (*Salix viminalis* L.) for goats, sheep and deer. Willow intake was associated with lignin and tannin concentrations in shoots. The authors concluded that willow could be used most efficiently when fed to goats, followed by deer, whereas sheep were least efficient. They also observed that selected willow cultivars could be used as supplementary feed for deer, especially during summer droughts. Changes in the content of long-chain hydrocarbons in willow shoots during the growing season and in winter have not been investigated to date. However, salicylate concentrations were 25% higher in the bark of 2-year-old willows harvested in autumn than in the bark of 1-year-old willows harvested in spring (Kenstovicienė *et al.*, 2009)

The intensity of willow shoot and bark browsing by cervids can be affected by the availability of other food sources. Rajsky (2008) studied the effect of various supplementary feeds on spruce bark browsing by red deer under experimental conditions. Bark browsing decreased with an increase in the availability of supplementary feeds. Browsing intensity increased when red deer were disturbed by hunters or when their daily grazing cycle was impaired.

The feeding behavior of cervids cannot be changed, but the diversity of deer diets can be increased by establishing browse plots in the vicinity of forage crops that are frequently grazed by deer. In addition to willows, other tree species such as beech, linden, maple and pine can also be planted in browse plots.

Conclusions

The analyzed willow cultivars differ in the content of long-chain hydrocarbons in bark and phloem.

Hydrocarbon concentrations in the bark and phloem of the same willow genotype differ between plants grown in various locations.

The concentrations of several hydrocarbons affected the intensity of willow browsing by cervids. However, the total hydrocarbon content of shoots had no effect on the extent of browsing in the examined willow cultivars. Based on previous research carried out by our team, we believe that the content of bitter phenols, especially salicin, salicortin and soluble sugars, has a greater impact on the bite attractiveness of willow shoots than the content of long-chain hydrocarbons. We would like to point out, however, that the taste and smell of these compounds was examined on the example of humans, not deer, which have a much wider spectrum of food recognition.

Authors' contributions

M.B. – conceptualization, formal project preparation, establishment and maintenance of experimental plots, data collection, data curation and analysis, manuscript preparation and editing; K.Z. – conceptualization, methodology, preparation of materials for laboratory analysis, manuscript preparation and editing; J.S. – result review and manuscript editing; S.C. – gas chromatographic analysis; A.O. – statistical analysis.

Conflicts of interest

The authors declare the absence of potential conflicts of interest.

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STRESZCZENIE

Zawartość węglowodorów długołańcuchowych w korze wybranych kultywarów wierzb w kontekście preferencji żywieniowych jeleniowatych

W pracy analizowano zawartość węglowodorów długołańcuchowych w korze i łyku wybranych kultywarów wierzb na poletkach doświadczalnych założonych przez Stację Badawczą PZŁ w Czempiniu. Wyniki doświadczeń laboratoryjnych porównano z obserwacjami zgryzień przez jeleniowate w celu znalezienia zależności między składem chemicznym a atrakcyjnością żerową poszczególnych kultywarów. Jak wiadomo, węglowodory długołańcuchowe są głównym składnikiem związków nazywanych woskami, które w przyrodzie występują np. na powierzchni pędów i liści roślin. Ich główną funkcją w przypadku roślin jest ochrona, np. przed suszą, nadmierną transpiracją czy silnym nasłonecznieniem. Ponadto zabezpieczają rośliny przed owadami, wirusami, grzybami i bakteriami, które powodują ich liczne schorzenia.

Do analizy wykorzystano kultywary występujące na poletku ogryzowym w miejscowości Bieczyny. Na potrzeby niniejszej pracy, w celu ułatwienia interpretacji wyników poszczególnych kultywarów, zastosowano ich numerację (tab. 1). Korę z łykiem pobierano ręcznie i poddawano dalszej obróbce mechanicznej i termicznej w celu wykonania ostatecznej analizy biochemicznej. W jej wyniku zidentyfikowano 19 węglowodorów długołańcuchowych występujących we wszystkich kultywarach w różnych ilościach: od bardzo wysokiego stężenia do minimalnego, na granicy odczytu (ryc. 1). Zależności pomiędzy występowaniem węglowodorów w poszczególnych kultywarach przedstawiono w tabeli 2. Dodatkowo, w celu porównania, w jakim stopniu miejsce uprawy wierzb wpływa na zawartość analizowanych związków, wykorzystano kultywar *Salix amigdalina* 'Krakowianka' (nr 5) uprawiany na różnych poletkach położonych w dwóch województwach: zachodniopomorskim i wielkopolskim. Analizowane próbki były jednorodne genetycznie, ponieważ nasadzenia wykonano z materiału (sztobry) otrzymanego z matecznika Nadleśnictwa Resko. Analiza wykazała, że miejsce występowania roślin (gleba, wilgotność, nasłonecznienie) może mieć wpływ na ilość występujących węglowodorów długołańcuchowych (tab. 5). Istotne jest, że w przypadku niektórych analizowanych związków różnice są bardzo duże i istotne statystycznie (np. zawartość kwasu 9,12-oktadekadienowego, EM; kwasu oktakozanowego, EM). W przypadku innych różnice są istotne, ale mniejsze (np. zawartość kwasu heksadekanowego, EM).

W kilku porównaniach różnice w ilości analizowanych związków są jedynie na poziomie istotności (np. kwasu pentakozanowego, EM).

W celu sprawdzenia izolujących właściwości wosków (w tym analizowanych węglowodorów) na świeżych pędach wykonano próbę z sacharozą wykazującą, jak cukrowce rozpuszczalne (na przykładzie sacharozy) wnikają do wnętrza pędów po ich 24-godzinnym moczeniu w temp. 10°C. Zastosowano 5-procentowy wodny roztwór cukru, a po doświadczeniu pędy opłukano wodą wodociągową. Wyniki doświadczenia przedstawiono na rycinie 2. Przeprowadzona analiza statystyczna nie wykazała istotnych różnic miedzy analizowanymi próbami, co dowodzi, że bariera stworzona przez warstwę wosków jest bardzo skuteczna.

W celu odpowiedzi na pytanie, czy ilość węglowodorów długołańcuchowych jest skorelowana z ilością uszkodzeń spowodowanych przez jeleniowate, zestawiono wyniki biochemiczne z danymi dotyczącymi uszkodzeń na tym poletku (tab. 3). Jako wyniki porównawcze przedstawiono dodatkowo zgryzienia występujące na innych poletkach uprawianych przez Stację Badawczą PZŁ w Czempiniu, gdzie również analizowano atrakcyjność żerową tych samych kultywarów. Największe stężenie węglowodorów łącznie występowało w kultywarze nr 1, który wykazywał jeden z najniższych poziomów uszkodzeń (tab. 3). Sugeruje to brak bezpośredniego związku między zawartością wszystkich węglowodorów długołańcuchowych w korze z łykiem a stopniem zgryzania pędów przez jeleniowate. Natomiast w przypadku niektórych węglowodorów stwierdzono istotny dodatni związek między ich zawartością a stopniem zgryzania (tab. 4).