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Changes in the Sets and Levels of Flavonoids and Phenolcarboxylic Acids in the Leaves of *Spiraea betulifolia* subsp. *aemiliana* (Rosaceae) during Introduction into Novosibirsk Conditions

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Abstract

The set and levels of phenolic compounds in water-ethanol extracts from the leaves of *Spiraea betulifolia* subsp. *aemiliana* (Schneid.) Hara (Rosaceae) were studied under natural conditions and after introduction into the Novosibirsk (Russia) environment. The samples were collected in two natural coenopopulations (Kunashir Island) and in the Introduction Division of CSBG SB RAS (Novosibirsk) in 2016–2019. Flavonoids and phenolcarboxylic acids were found in the extracts. Using high-performance liquid chromatography, we detected 19 phenolic compounds in the water-ethanol extracts from plant leaves of the natural populations and 26 in the introduced population. Thus, differences in high-performance liquid-chromatography profiles of phenolic compounds were found between the natural populations and the introduced population. Five new phenolic acids and one flavonol were discovered in the leaves of the introduced plant population. Additionally, the levels of some phenolic compounds in the leaves of *S. betulifolia* subsp. *aemiliana* changed as a result of the transfer from the wild to the new environment. Hyperoside (3.36–9.99 mg/g) is the major phenolic compound in the leaves of the natural populations, while quercetin (1.23–5.07 mg/g) is the major phenolic compound in the leaves of the introduced population. The differences in the leaf concentrations of quercetin and hyperoside between the natural and introduced populations were statistically significant ($P \geq 0.05$). Concentrations of taxifolin, isoquercitrin, rutin, avicularin, astragalin, kaempferol, and chlorogenic, *p*-coumaric, and cinnamic acids in the leaf extracts were found to be similar between the introduced and natural (Kunashir Island, Lake Kipyashcheye shore) populations. Across plant development phases, inhomogeneous distributions of individual phenolic compounds were revealed in the leaf extracts from the introduced *S. betulifolia* subsp. *aemiliana*.

Keywords: *Rosaceae, Spiraea betulifolia* subsp. *aemiliana*, flavonoid, phenolcarboxylic acid, HPLC, high-performance liquid chromatography

INTRODUCTION

Phenolic compounds are among the most widespread representatives of secondary metabolism in plant tissues. They play a vital role in structural integrity, UV protection, regulation of physiological functions, and transmission of the signals of plant cells [1, 2]. Due to the high biological activity, plant polyphenols are successfully used in industry, as well as in medicine and pharmacology

as the substances possessing antioxidant, neuroregulatory, capillary-strengthening, immunomodulating, anticancer and other properties [2–6]. The synthesis and accumulation of the substances of secondary metabolism with time depend on plant species, type of secondary metabolism and its physiological role, and especially on external factors. There are likely to be no general patterns in the changes of secondary metabolism in plant ontogenesis [7–9]. Because of this, a comparative investigation of the sets and levels of phenolic compounds in plants in nature and under introduction, as well as the dynamics of their accumulation, is to be carried out individually for each plant species.

The representatives of the *Spiraea* L. species are distinguished by the high decorative characteristics, have many forms and varieties, are honey-bearing plants, possess diverse biological activities and other useful properties [10, 11]. The *S. betulifolia* subsp. *aemiliana* (Schneid.) Hara. (syn. *S. aemiliana* Schneid.) species is a component of the polymorphous complex of plants of the *Calospira* C. Coch. section of *Spiraea* genus (Rosaceae Juss.); it grows only on the eastern island part of Russia (Sakhalin island, Kuril islands) and in Japan. The distinguishing features of the sub-species are: height up to 30 cm, compact crown, dense white corymbiform blossom clusters, small rounded leaf blade, and the absence of trichomes of the blossom cluster ramules, as well as some metric indices for which the average values do not overlap with the average values of closely related taxons *S. betulifolia* Pall. and *S. beauverdiana* C. K. Schneid. [12–14]. Investigation of the set and levels of phenolic compounds in the leaves of *S. betulifolia* subsp. *aemiliana* from natural populations revealed the promising character of this plant as a source of flavonoids, especially flavonols – hyperoside, isoquercitrin, rutin, quercetin, kaempferol, astragalin [15]. Flavonoids exhibit antioxidant, anti-inflammatory, neurotropic and other essential pharmacological properties [2]. Closely related species to *S. betulifolia* subsp. *aemiliana* possess economically significant properties. The *S. betulifolia* plant exhibits antimicrobial activity, it is used in Chukotka as a substituent for tea, and has also importance as forage. The juice of leaves demonstrated phytoncide activity [10, 16]. The leaves and flowers of *S. betulifolia* and *S. beauverdiana* contain various biologically active substances and exhibit high antioxidant and antiviral activity [17, 18].

The goal of the work was to carry out a comparative investigation of phenolic compounds in the leaves of *S. betulifolia* subsp. *aemiliana,* in nature and under introduction, by means of high performance liquid chromatography (HPLC).

EXPERIMENTAL

The objects for the investigation of phenolic compounds were the leaves of *S. betulifolia* subsp. *aemiliana*. The material was collected in 2016 in two natural populations of Kunashir Island (P1 and P2) and in the introduction field of the Laboratory of Phytochemistry of the Central Siberian Botanical Garden, SB RAS (CSBG SB RAS, Akademgorodok, Novosibirsk) at the phases of budding (I1), blossoming (I2) and fruiting (I3) in 2017, as well as the phase of blossoming in 2018 (I4) and in 2019 (I5) (Table 1). The plants were brought to the introduction field from Kunashir

TABLE 1

Characterization of the studied samples of S. betulifolia subsp. *aemiliana*

Sample	Collection site	Date of sampling	Phase of vegetation
P1	Kuryl Islands, Kunashir Island, Kuryl Reserve, the Kislyi brook, tributary of the Lesnaya river; boggy bank; fir mossy forest	03.07.2016	Blossoming–fruiting
P2	Kuryl Islands, Kunashir Island, Kuryl Reserve, caldera of the Golovnin volcano, side of Lake Kipyashchee; stony detritus, low-bush tundra	27.07.2016	Blossoming–fruiting
I1	Novosibirsk, Academy town, experimental field of CSBG SB RAS	28.06.2017	Budding
I2	The same	17.07.2017	Blossoming
I3	»	30.08.2017	Fruiting
I4	»	19.07.2018 .	Blossoming
I5	»	22.07.2019	The same

island, the caldera of the Golovnin volcano, the side of the Kipyashchee lake (P2). Only healthy individuals unaffected by diseases and pests were chosen for investigation. The regions under investigation differ from each other by climate, namely by the hydrothermal conditions and insolation. The climate of Kunashir Island is typical marine, characteristic of midlatitudes. An insignificant effect of monsoon is observed, along with abundant atmospheric precipitation (up to 1100–1400 mm/year) and high humidity (the average relative humidity of the air is 80 %). The duration of frost-free period is up to 190 days. The sum of active temperatures for Kunashir Island is ~1700 °C on average. A sharp change of weather during the day, mild winter and cool summer are characteristic of the island. Insolation in June is 4.16 kW/m^2 in Yuzhno-Kurilsk according to the data over many years [19]. The climate of Novosibirsk city is continental, the average duration of the frost-free period is up to 120 days, the sum of equivalent active temperatures (above 10 °C) is 1920 °C, the total annual amount of precipitation is 425 mm. The Novosibirsk Region is characterized by higher insolation, which is 5.02 kW/m^2 in June according to the data over many years [20].

The leaves were dried in the air in a shadowed place. After drying, the raw plant material was ground to 2–3 mm, mixed, and a representative sample was taken.

To study phenolic compounds, we used water-ethanol extracts (40 %) from the leaves of *S. betulifolia* subsp. *aemiliana*, obtained through extraction on a water bath. An exactly weighted portion (0.5000 g) of ground air-dry material was extracted twice: at first, with 30 mL for 30 min, then 20 mL for 29 min after filtration, the residue in the flask and on the filter was washed with 5 mL of 40 % ethanol. After that, the united extract was concentrated in porcelain cups to a volume of 10–15 mL (exact volume). Analysis was repeated twice [21].

To remove impurities, 1 mL of water-ethanol extract was diluted with double distilled water to the volume of 5 mL and passed through a Diapak C16 concentrating cartridge (CC BioKhimMak, Russia). The substances were washed from the cartridge with a small amount of 40 % ethanol (3 mL), and then with 96 % ethanol (2 mL). The united eluate was passed through a membrane filter with a pore diameter of 0.45 μm.

Analysis of phenolic compounds present in the eluate was carried out with the help of the analytical HPLC system composed of an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) with the diode matrix detector, auto-sampler and a ChemStation system collecting and processing the chromatographic data. We modified the procedure proposed by T. A. van Beek [22]. The column was Agilent Zorbax SB-C18, 4.6 × 150 mm, 5 μm. Separation was carried out with methanol gradient in the aqueous solution of 0.1% orthophosphoric acid in the mode from 31 to 33 % – for 27 min, then from 33 to 46 % – for 11 min, then from 46 to 56 % – for 12 min and from 56 to 100 % – for 4 min (system I). The rate of eluent flow was 1 mL/min. The column temperature was 26 °C. The volume of the sample introduced into the chromatograph was 10 μL. Detection was performed at wavelengths λ = 254, 270, 290, 340, 360 and 370 nm. Standard samples were prepared using cinnamic acid (Serva, Germany), taxifolin (Austrowaren, Austria), chlorogenic and *p*-coumaric acids, quercetin, kaempferol (Sigma-Aldrich, Germany), isoquercitrin, rutin, avicularin, astragalin and hyperoside (Fluka, Germany). Standard solutions were prepared at the concentration of 10 μg/mL. Known compounds were identified by comparing them with reference samples. Quantitative determination of individual components in plant samples was performed using the external standard method [22]. The concentrations of non-identified components were calculated using the standard areas of hyperoside peaks (for flavonoids) and chlorogenic acid (for phenolic acids) [23, 24].

The level of individual components (C_x) was calculated using equation (in mg/g of the mass of absolutely dry raw material):

$$C_x = C_{st} S_1 V_1 V_2 / (S_2 M V_3)$$

where C_{st} is the concentration of the corresponding standard solution of flavonol, mg/mL; S_1 is the area of flavonol peak in the sample under analysis, in the optical density units (o. d. u.); V_1 is the volume of eluate after elution of flavonols from the concentrating cartridge, mL; V_2 is the total volume of the extract, mL; S_2 is the area of the peak of standard flavonol, o. d. u.; M is the mass of sample portion, g; V_3 is the volume of extract taken for analysis, mL.

To reveal statistically significant differences, we used dispersion analysis (ANOVA) followed by Tukey test (HSD). We discuss the differences which are significant at the significance level of $P \geq 0.05$. Statistical treatment of the results was carried out with Statistica 10 software package. The values are given as arithmetic mean ± standard error.

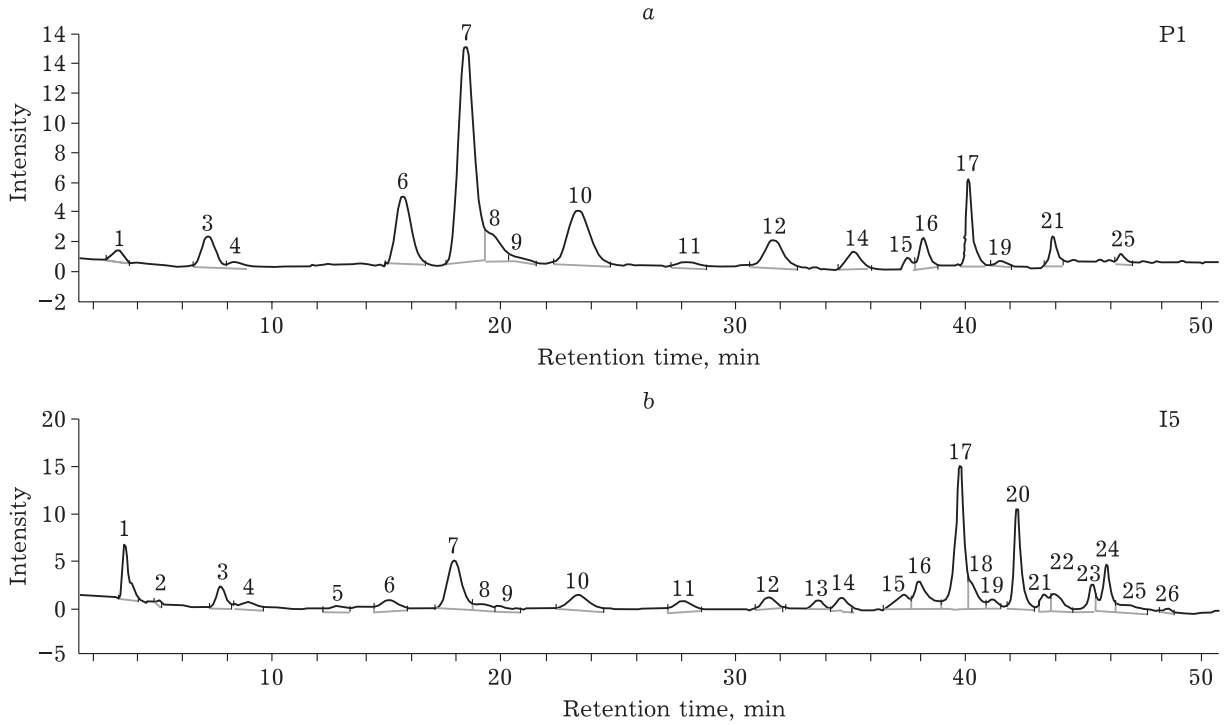

Fig. 1. Chromatograms of 40 % water-ethanol extracts from the leaves of *S. betulifolia* subsp. *aemiliana*. Measurements were carried out at the detector wavelength λ = 360 nm; peak numbers correspond to the compounds listed in Table 2.

RESULTS AND DISCUSSION

Investigation of the set of phenolic compounds showed that 26 compounds are present in water-ethanol extracts from the leaves of *S. betulifolia* subsp. *aemiliana* (Fig. 1, Table 2). Among them, the list of identified compounds includes cinnamic acid and oxycinnamic acids (chlorogenic and *p*-coumaric), flavonols (quercetin, kaempferol, hyperoside, isoquercitrin, rutin, avicularin, astragalin) and dihydroflavonol (taxifolin). Other components are not identified, but their UV spectra were recorded while chromatographing in the online mode. According to the spectra characteristics, non-identified compounds belong to flavonols (the wavelength of absorption maximum is λ_{max} = 250–270, 350–390 nm), phenolcarboxylic acids – oxybenzoic acids (λ_{max} = 235–270, 290–305 nm) or oxycinnamic acids (λ_{max} = 230–240, 290–320 nm) and flavons (λ_{max} = 250–270, 210–350 nm) [1, 25]. The set of phenolic compounds from the leaves of Spiraea is mainly represented by flavonols and phenolcarboxylic acids. One flavon was also detected in the leaves of the plant under investigation.

The number of compounds detected in water-ethanol extracts of Spiraea leaves is 17 in each of the two natural populations (Table 2). There are some differences in phenol profiles of the extracts from the leaves of *S. betulifolia* subsp. *aemiliana* from natural populations. The plants from P1 population contain taxifolin and flavonol 15, which are absent from the leaves of plants from the other population. The leaves of plants from P2 population contain additional compounds (phenolic acid 13 and flavonol 26), which were not revealed in population P1. The total number of compounds that were detected in the leaves of Spiraea from natural populations is (see Table 2). The major compounds determined in *S. betulifolia* subsp. *aemiliana* from natural populations are hyperoside, isoquercitrin, astragalin, quercetin, *p*-coumaric acid, flavonols 6 and 16, flavone 10 (see Fig. 1, Table 3). In addition to the listed compounds, the leaves of plants from population P1 also contain cinnamic acid, and the plants from population P2 contain kaempferol as major compounds.

The set and levels of phenolic compounds in the leaves of *S. betulifolia* subsp. *aemiliana* samples from the introduced population taken from P2 (the side of Lake Kipashchee) were studied in the phase of blossoming in 2017 (I2), 2018 (I4) and 2019 (I5) (see Tables 1 and 2). The phenol profile of the leaves of plants from the the introduced population of *S. betulifolia* subsp. *aemiliana* changed significantly in comparison with the natural populations (see Fig. 1, Table 2). The leaves of the samples of introduced population were

TABLE 2

Characterization of phenolic compounds discovered in 40 % water-ethanol extracts from the leaves of *S. betulifolia* subsp. *aemiliana* plants

Peak No.	Compound	Retention time (t_R), min	Spectral characteristics (λ_{max}), nm	Sample
1	Chlorogenic acid	3.2	244, 300 sh., 330	P1, P2, I1–I5
2	Phenolic acid*	4.7	235, 315	I2–I5
3	*p*-Coumaric acid	7.9	226, 293, 320	P1, P2, I1–I5
4	Taxifolin	8.5	290	P1, I1–I5
5	Phenolic acid*	13.8	235, 320	I2–I5
6	Flavonol	15.2	250, 265, 355	P1, P2, I1–I5
7	Hyperoside	18.0	225, 268 sh., 355	P1, P2, I1–I5
8	Isoquercitrin	19.3	259, 266 sh., 358	P1, P2, I2–I5
9	Rutin	20.0	256, 358	P1, P2, I2–I5
10	Flavon*	23.8	250, 340	P1, P2, I1–I5
11	Avicularin	28.4	260, 270 sh., 360	P1, P2, I1–I5
12	Astragalin	32.5	265, 300 sh., 350	P1, P2, I1–I5
13	Phenolic acid*	34.2	240, 300 sh., 330	P2, I1–I5
14	Cinnamic acid	35.9	216, 270	P1, P2, I2–I5
15	Flavonol*	37.8	260, 300 sh., 360	P1, I2–I5
16	Flavonol*	38.1	265, 300 sh., 355	P1, P2, I1–I5
17	Quercetin	40.6	255, 372	P1, P2, I1–I5
18	Phenolic acid*	41.5	225, 300 sh., 315	I2–I5
19	Flavonol*	42.0	255, 300 sh., 355	P1, P2, I1–I5
20	Phenolic acid*	43.1	255, 265 sh., 315	I1–I5
21	Phenolic acid*	44.0	235, 300 sh., 315	P1, P2, I1–I5
22	Component 22	44.4	–**	I1–I5
23	Flavonol*	45.7	270, 300 sh., 350	I1–I5
24	Phenolic acid*	46.1	270, 300 sh., 315	I1–I5
25	Kaempferol	46.9	266, 370	P1, P2, I1–I5
26	Flavonol*	48.3	250, 300 sh., 360	P2, I2–I5

Note. Sh. means a shoulder.

* Group of substances was determined from spectral characteristics [1, 25].

** Absent.

determined to contain 26 substances of phenolic nature. Some components were determined as additional: phenolic acids 2, 5, 18, 20 and 24, flavonol 23 and component 22. The entire range of compounds was detected in the water-ethanol extracts from the plants of the introduced population. The changes were detected not only in the set but also in the level of phenolic compounds in the leaves of introduced samples. The major components in the leaves of the introduced population are hyperoside, astragalin, quercetin, flavone 10, flavonol 16, phenolic acids 20 and 24 (see Fig. 1, Table 3).

The levels of phenolic compounds in the leaves of *S. betulifolia* subsp. *aemiliana* was calculated for the identified compounds and the major non-identified substances with the concentrations in extracts higher than 1 mg/g (see Table 3). The leaves of plants from natural population P1 demonstrated the highest level of *p*-coumaric (1.37 mg/g) and cinnamic (0.92 mg/g) acids, hyperoside (9.99 mg/g), isoquercitrin (1.11 mg/g), astragalin (1.90 mg/g) and flavonol 6 (3.05 mg/g). For the plants from population 2, the highest level of the major component flavonol 16 was established (2.96 mg/g). The difference in the levels of these components is statistically significant with $P \geq 0.05$. The concentration of the major component flavonol 6 in the leaves of *S. betulifolia* subsp. *aemiliana* from natural populations (3.05 mg/g – in P1 and 1.63 mg/g – in P2) is statistically reliably higher than in the leaves of the plants from the introduced population (0.50–0.85 mg/g). The level of chlorogenic, *p*-coumaric and cinnamic acids, taxifolin, isoquercitrin, rutin,

TABLE 3

Level of phenolic compounds, identified and major non-identified ($C \geq 1$ mg/g) in the extracts from the leaves of *S. betulifolia* subsp. *aemiliana* plants (mg/g of the mass of absolutely dry raw material)

Peak No.	Compound	Sample				
		Natural population		Introduced population		
		P1	P2	I2	I4	I5
1	Chlorogenic acid	0.42±0.03 b	0.16±0.03 b	0.32±0.03 b	0.79±0.06 a	1.01±0.09 a
3	*p*-Coumaric acid	1.37±0.07 a	0.59±0.04 b, c	0.34±0.04 c	0.54±0.05 b, c	0.84±0.09 b
4	Taxifolin	0.59±0.05 a	–	0.22±0.03 b	0.24±0.07 b	0.56±0.05 a
6	Flavonol 6	3.05±0.10 a	1.63±0.10 b	0.50±0.02 b	0.67±0.04 b	0.85±0.06 b
7	Hyperoside	9.99±0.13 a	3.36±0.07 b	2.20±0.10 c	1.39±0.06 d	1.70±0.10 c, d
8	Isoquercitrin	1.11±0.06 a	0.63±0.10 b	0.49±0.06 b, c	0.21±0.09 c	0.28±0.03 b, c
9	Rutin	0.27±0.07 a	0.18±0.07 a	0.40±0.05 a	0.14±0.03 a	0.19±0.03 a
10	Flavone 10	1.13±0.05 a	1.19±0.06 a	0.77±0.04 b	1.31±0.06 a	1.33±0.05 a
11	Avicularin	0.53±0.08 a, b	0.32±0.07 b	0.20±0.05 b	0.34±0.05 a, b	0.68±0.05 a
12	Astragalin	1.90±0.09 a	0.78±0.08 c	0.78±0.05 c	1.24±0.06 b	0.65±0.06 c
14	Cinnamic acid	0.92±0.07 a	0.09±0.06 c	0.28±0.03 b, c	0.27±0.04 b, c	0.43±0.07 b
16	Flavonol 16	0.88±0.07 c	2.96±0.07 a	1.43±0.08 b	1.00±0.04 b	1.56±0.11 b
17	Quercetin	1.43±0.08 d	2.02±0.13 c	2.97±0.07 b	2.86±0.14 b	5.07±0.07 a
20	Phenolic acid 20	–	–	2.22±0.09 b	1.90±0.10 b	2.66±0.09 a
24	Phenolic acid 24	–	–	0.88±0.04 b	0.92±0.07 b	1.49±0.04 a
25	Kaempferol	0.29±0.03 b	0.77±0.05 a	0.32±0.04 b	0.19±0.06 b	0.75±0.10 a

Note. Here and in Table 4: 1. Dash means that the substance was not detected. 2. Values are given as the average over three measurements ± standard error. 3. a–d – results of comparison according to Tukey test (significance level $P \leq 0.05$).

avicularin, kaempferol and other non-identified flavonols and phenolic acids is equal in the extracts from the leaves of plants from the introduced population and from the natural population P2, from which the cultivated plants were taken.

Hyperoside is the major component in the extracts of the leaves of natural population. The highest hyperoside level was detected in natural population P1 (9.99 mg/g); it was three times lower in population P2 (3.36 mg/g). A decrease in hyperoside level may be traced in the leaves of *S. betulifolia* subsp. *aemiliana* from the introduced population. The highest hyperoside level (2.20 mg/g) was detected during the second year of Spiraea cultivation at the CSBG SB RAS (I2). Later on, hyperoside concentration in the leaves of Spiraea decreased by a factor of 1.6 in the third year of introduction (I4) and by a factor of 1.3 in the fourth year (I5) in comparison with the first year of plant cultivation. The difference in hyperoside level in the leaves of natural and introduced populations is statistically significant with $P \geq 0.05$. Quercetin is the major component of the leaves of plants from the introduced population. Its concentration in the leaves in comparison with natural populations (1.43 mg/g – P1 and 2.02 mg/g – P2) increases substantially from 2.86 mg/g (2018) to 5.07 mg/g (2019). The difference in quercetin level in water-ethanol extracts from the leaves of natural and introduced populations is statistically significant with $P \geq 0.05$. A gradual increase in the level of other identified components in water-ethanol extracts from the leaves may also be traced (for example, chlorogenic, *p*-coumaric, cinnamic acids, kaempferol, taxifolin, avicularin, flavonol 6, flavone 10 and phenolic acid 24). The maximal level of these compounds was detected in the leaves of the introduced population in 2019. Quite the contrary, the level of rutin, isoquercitrin and hyperoside decreases. Their maximal concentration is detected in the leaves of plants in 2017. This may be the route along which the level of different compounds is regulated to provide gradual adaptation of phenol metabolism in *S. betulifolia* subsp. *aemiliana* to the conditions existing in the Academy town of Novosibirsk. Some decrease in the concentrations of hyperoside, isoquercitrin, rutin, kaempferol, flavonol 16 and phenolic acid 20 in the leaves of cultured Spiraea was detected in 2018 in comparison with the samples collected in 2017 and 2019.

The revealed differences in the set and level of phenolic compounds in the leaves of Spiraea from natural and introduced samples brought from natural populations are likely to be due to different climatic conditions of the regions. The synthesis of phenolic compounds is most strongly affected by UV radiation and hydrothermal stress. An increase in flavonoid level in the plants under the action of increased insolation has been detected [26, 27]. In the leaves of *Lonicera caerule*a subsp. *altaica* (Pall.) Gladkova, an increase (by a factor of more than 2) in the level of the derivatives of hydroxycinnamic acids and flavonols was observed in the dry year 2012 in comparison with the wet and cool year 2013 [28]. An increase in the total level of flavonoids in the leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja is detected at lower temperatures. This feature is connected with the high level of active oxygen forms. Flavonoids act as inhibitors of the active forms of oxygen [29]. Changes in the set of phenolic compounds in plants caused by transfer to introduction conditions do not occur in all cases. The level of substances in plants often changes while the chromatographic profile remains the same. These features are observed for some representatives of the Rosaceae family during introduction under the conditions existing in the Academy town of Novosibirsk. For instance, comparative analysis of HPLC patterns showed that in general the phenol profile and total level in the leaves of *Sibiraea altaiensis* (Laxm.) Schneid. plant, which is close to Spiraea plants, from the natural populations of Mountainous Altai is close to the corresponding parameters of the introduced population in Academy town of Novosibirsk [30]. However, it was revealed for the introduced plants that the sum of quercetin glycosides decreases by a factor of 3, isorhamnetin glycosides – by a factor of 2, while the sum of the glycosides of kaempferol increases in comparison with plants from natural population. Therefore, the quantitative level of separate components changes while the set of the substances remains the same. Similar features are observed in the investigation of the level of separate flavonoids in another representative of Rosaceae family – *Pentaphylloides fruticosa* (L.) O. Schwarz [27]. Under the conditions of the Republic of Altai, the organs of the above-ground parts of *P. fruticosa* exhibit an increased level of hyperoside, isoquercitric, rutin, the glycoside of ellagic acid, quercetin in comparison with the individuals introduced in Novosibirsk. An ecologically optimal state is likely to occur for these plants under the conditions of introduction, so a decrease in the level of phenolic compounds occurs. In the case of worsening of growth conditions in culture (in comparison with the natural conditions), their concentrations in the extracts of plants increase [28, 31]. During the introduction of *Lonicera caerulea* subsp. *pallasii* from the southern taiga subzone and Mountainous Altai into the conditions of the Ob river right-bank forest-steppe (Novosibirsk), the level of dicofeylquinic acid, flavonols and flavones increases many fold in comparison with the natural populations. The authors suppose that low water supply is the major stress factor causing a sharp increase in the level of accumulation of flavonoids and hydroxycinnamic acids in the forest-steppe zone for *L. caerulea* subsp. *pallasii* [28]. The marine climate of Kunashir Island is distinguished by increased humidity, which promotes the accumulation of the glycosides of quercetin (hyperoside, rutin, isoquercitrin) in the leaves of Spiraea. The continental climate of Novosibirsk is characterized by higher insolation and a lower amount of atmospheric precipitation. Most probably, under the conditions of Academy town, with increased insolation and drier climate, Spiraea plants implement the potential possibility of the synthesis of additional compounds (phenolcarboxylic acids and flavonoids) and a decrease in the concentrations of hyperoside, rutin and isoquercitrin along with an increase in the level of chlorogenic, *p*-coumaric and cinnamic acids, kaempferol, taxifolin, avicularin, flavonol 6, flavone 10 and phenolic acid 24. A decrease in the concentrations of hyperoside, isoquercitrin, rutin, kaempferol, flavonol 16, quercetin and phenolic acid 20, which was observed in 2018, in the leaves of Spiraea from the introduced population (in comparison with the samples collected in 2017 and 2019) is likely to be connected with climatic conditions, too.

One can see in the climatogram (Fig. 2) that June and July in 2017 were distinguished by abundant rains in comparison with the parameters averaged over many years. July in 2019 was rainy, while the previous month, June, was cool and dry. July in 2018 did not differ from the data of many years on air temperature and the amount of precipitation. Increased humidity in July 2017 and July 2019 was likely to promote the higher accumulation of some compounds in the leaves of *S. betulifolia* subsp. *aemiliana* during these vegetation seasons. The dependence of the level of phenolic compounds in the plants on the amount of atmospheric precipitation during specific veg-

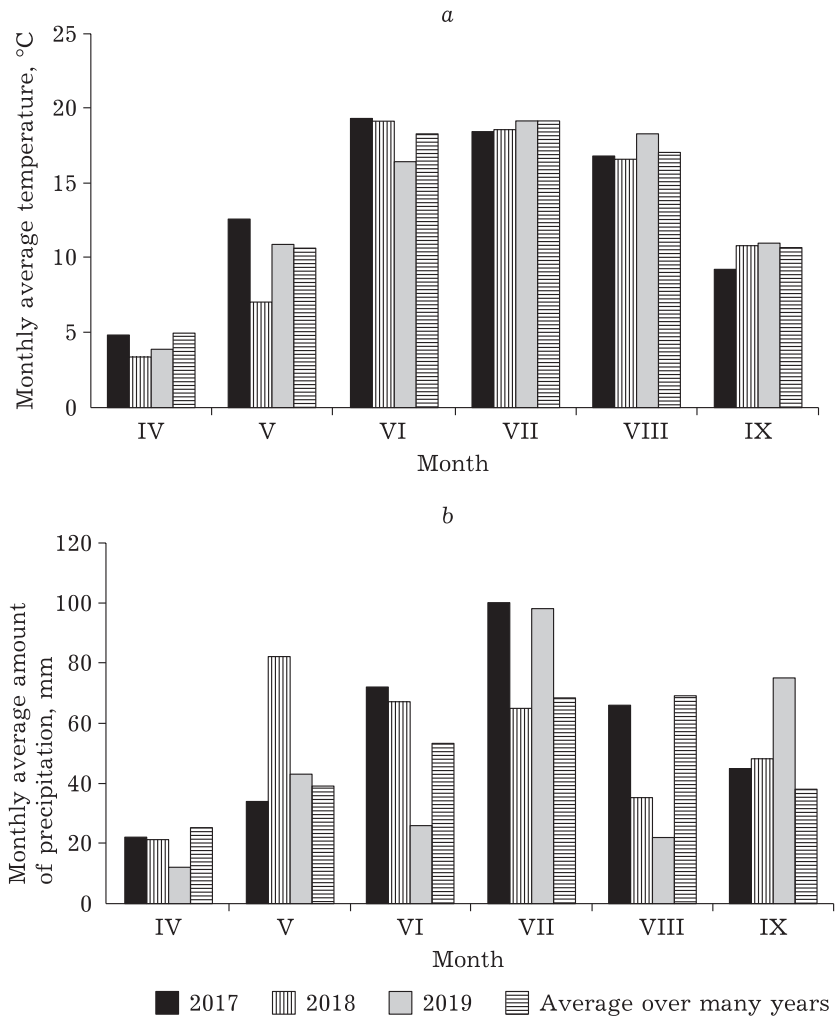

Fig. 2. Monthly average temperatures and average monthly temperatures over years (*a*) and monthly average amounts of precipitation (*b*) in Novosibirsk [32].

etation seasons was revealed previously. In some cases, increased level of phenolic compounds in plants was detected in dry years [28], while, quite contrary, during other years a decrease in the level of phenolic compounds under dry conditions was observed [33]. For instance, a substantial and sharp increase in the amount of precipitation caused an increased synthesis of rosmarinic acid in the leaves of *Salvia officinalis* L. [34]. The response of plants to stress factors (including the amount of atmospheric precipitation) is individual for each species, and it is impossible to reveal any general patterns.

The level of phenolic compounds and all other secondary metabolites depends on the phase of plant vegetation. Differences in the dynamics of the accumulation of separate phenolic compounds are observed even in closely related species [35]. Investigation of the seasonal features of the accumulation of phenolic compounds is extremely important for choosing the terms for collecting the raw material for further application in the pharmaceutical or food industry. [34]. Analysis of the seasonal dynamics of the accumulation of phenolic compounds in the leaves of *Spiraea media* var. *sericea* (Turcz.) Regel showed that the level of flavonologlycosides of hyperoside and rutin was gradually increasing and reached a maximum at the phase of fruiting, while some flavonologlycosides dominated at the phase of blossoming [36].

Accumulation of the identified flavonoids and phenolcarboxylic acids in the leaves of *S. betulifolia* subsp. *aemiliana* turned out to be nonuniform over the phases of vegetation (Table 4). The set and level of phenolic compounds in the leaves of this plant from the introduced population were studied in 2017 over the phases of plant development: budding (I1), blossoming (I2) and fruiting

TABLE 4

Level of phenolic compounds, identified and major non-identified ($C \geq 1$ mg/g) in the extracts from the leaves of *S. betulifolia* subsp. *aemiliana* depending on vegetation phase (mg/g of the mass of absolutely dry raw material)

Peak No.	Compound	Sample		
		I1	I2	I3
1	Chlorogenic acid	0.75±0.05 a	0.32±0.03 b	0.43±0.07 b
3	*p*-Coumaric acid	0.26±0.06 a	0.34±0.04 a	0.48±0.03 a
4	Taxifolin	0.15±0.04 a	0.22±0.03 a	0.34±0.06 a
6	Flavonol 6	0.27±0.04 c	0.50±0.02 b	0.85±0.05 a
7	Hyperoside	0.39±0.05 c	2.20±0.10 a	1.47±0.09 b
8	Isoquercitrin	–	0.49±0.06 a	0.22±0.07 a
9	Rutin	–	0.40±0.05 a	0.24±0.06 a
10	Flavone 10	0.30±0.05 c	0.77±0.04 b	1.32±0.03 a
11	Avicularin	0.47±0.03 a	0.20±0.05 b	0.34±0.05 a, b
12	Astragalin	0.49±0.04 c	0.78±0.05 b	1.28±0.04 a
14	Cinnamic acid	–	0.28±0.03 a	0.28±0.07 a
16	Flavonol 16	0.40±0.05 c	1.43±0.08 a	0.95±0.10 b
17	Quercetin	1.23±0.07 c	2.97±0.07 a	1.96±0.09 b
20	Phenolic acid 20	2.13±0.08 a	2.22±0.09 a	1.45±0.06 b
24	Phenolic acid 24	0.69±0.04 b	0.88±0.04 a	0.59±0.04 b
25	Kaempferol	0.23±0.03 a	0.32±0.04 a	0.18±0.05 a

Note. For designations, see Table 3.

(I3) (see Table 1). Not all compounds were detected in the leaves of Spiraea at the phase of budding: rutin, isoquercitrin, cinnamic acid, phenolic acids 2 and 5 were absent (see Table 2). During other phases, the entire range of substances is present in the extract of leaves. The highest level of chlorogenic acid (0.75 mg/g) and avicularin (0.47 mg/g) in the leaves may be observed at the phase of budding. Then the level of these compounds decreases at the phase of blossoming, while at the phase of fruiting it somewhat increases. The amount of cinnamic acid does not change over the phases of plant development. The concentrations of *p*-coumaric acid, taxifolin, astragalin, flavonol 5, flavon 10 increase gradually from the budding stage to fruiting. The maximal level of other flavonols – hyperoside (2.20 mg/g), isoquercitrin (0.49 mg/g), rutin (0.40 mg/g), quercetin (2.97 mg/g), kaempferol (0.32 mg/g), flavonol 16 (1.43 mg/g), phenolic acid 20 (2.22 mg/g) and phenolic acid 24 (0.88 mg/g) – is observed at the phase of blossoming, and it decreases during the phases of budding and fruiting.

CONCLUSION

By means of HPLC, 26 phenolic compounds with high biological activity were detected in water-ethanol extracts of the leaves of *S. betulifolia* subsp. *aemiliana*. Differences in the profiles of phenolic compounds were revealed between the plants from the introduced and natural populations. Changes in the level of separate phenolic compounds in the leaves of Spiraea were detected after the plants were transferred from natural conditions into the conditions of introduction. A major component in the leaves of plants from natural populations is hyperoside, while in the leaves of the plants of the introduced population quercetin is the major component. Nonuniformity of the distribution of separate phenolic compounds over the phases of plant development was revealed for the extracts from the leaves of the introduced *S. betulifolia* subsp. *aemiliana* plants. The described changes in the set and level of phenolic compounds in the leaves may have a substantial effect on the biological activity of these samples when introduced into the conditions of Academy town in Novosibirsk, in comparison with natural individuals.

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