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Seasonal Development of *Paeonia obovata* and *Paeonia oreogeton* and Their Contents of Biologically Active and Reserve Substances in the Forest-Steppe Zone of Western Siberia

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Abstract: *Paeonia obovata* and *Paeonia oreogeton* belong to the monotypic family Paeoniaceae. Both are popular as ornamental plants. *P. obovata* and *P. oreogeton* have been introduced into Novosibirsk Oblast (Western Siberia) from Primorye (Far East). The aim of the study was to assess their adaptability as well as the effect of seasonal developmental stages on the accumulation of secondary metabolites and reserve substances in the leaves and rhizomes under the conditions of Akademgorodok (Novosibirsk, Russia). According to long-term data (15 years), *P. obovata* and *P. oreogeton* complete the entire growth cycle here, including flowering and fruiting. Both species exhibited abundant flowering, but in the first 3 years, *P. oreogeton* did not bloom; yet under the microclimatic conditions specifically selected for this species (a more shaded area), it started to bloom and fruit yearly. A biochemical analysis (by spectrometric method) of *P. obovata* and *P. oreogeton* grown in Akademgorodok showed that the leaves accumulate higher concentrations of flavonols (*P. obovata*: 1.77%), tannins (*P. oreogeton*: 16.42%), ascorbic acid (*P. oreogeton*: 155.2 mg/100 g), and sugars (*P. obovata*: 20.85%) as compared to the roots. Peony rhizomes contain higher concentrations of protopectins (*P. oreogeton*: 13.03%), saponins (*P. obovata*: 21.06%), and starch (*P. obovata*: 30.20%) than the leaves do. These data can help to increase the levels of these natural compounds in these species. Further investigation into the dynamics of accumulation of biologically active substances in the organs of peonies will help to identify introduced plant species having high biochemical potential for the pharmaceutical industry.

Keywords: *Paeonia*; weather conditions; ascorbic acid; flavonol; tannin; catechin; pectin; protopectin; saponin; sugar; starch

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1. Introduction

The monotypic family Paeoniaceae F. Rudolphi includes only one genus *Paeonia* L., which is a relic of ancient mesophilic arcto-tertiary flora, which includes 33 species growing in Europe, in the Mediterranean, and in East and Southeast Asia [1,2]. Peonies are highly popular in floriculture worldwide; they are both beautifully flowering and decorative foliaceous plants owing to powerful shoots up to 1 m in height with dense foliage. Wild and cultivated peony species are also attractive as medicinal and food plants because they contain biologically active substances: e.g., carbohydrates, terpenoids, steroids, phenol-carboxylic acids, phenol glycosides, flavonoids, tannins, and vitamins [3–6]. Data on pharmacological properties of representatives of the genus *Paeonia* have revealed their antioxidant, anti-inflammatory, antitumor, antibacterial, antiviral, cardiovascular, and neuroprotective effects [7–10]. Peonies are used in conventional medicine in many countries. For example, in Chinese conventional medicine, dried root bark of *P. suffruticosa* Andrews is popular as a febricide; it is used for blood cooling and promoting blood circulation to eliminate

stasis [11]. Roots of *P. lactiflora* Pall. are officially included in the Japanese Pharmacopoeia (JP17), Korean Pharmacopoeia X (KPX), European Pharmacopoeia (EP10.2), and British Pharmacopoeia (BP 2020). *P. anomala* L. is used in Russian conventional medicine [12]. Its alcohol tincture (*Tinctura paeoniae*) is used as a typical medicinal formulation and has a sedative effect; it is indicated for neurasthenia, insomnia, and dysautonomia. For preparation of a peony tincture and peony extract, pharmaceutical manufacturers use grass (shoots), rhizomes, and roots [13,14].

Paeonia anomala grows in steppe zones of the Caucasus and in Southern and Central Europe, China, and Siberia (Figure 1). This is a deciduous herbaceous plant with triternate leaves that are deeply incised and pinnately dissected into lanceolate and linear lobes. Flowers have different colors: purple, dark red, and reddish-pink. The ovary and fruits are covered with felted hairs, rarely almost naked. The fruits are ovoid or oval in shape. Mature fruits are straight or patulous [15,16]. Harvesting of *P. anomala* raw materials in nature causes great damage to coenopopulations because harvesters excessively dig up the largest rhizomes of mature generative plants. Many medicinal species of this genus have not been studied properly except for a few species (*P. anomala*, *P. emodi* Royle, *P. lactiflora*, *P. ostii* T. Hong & J.X. Zhang, and *P. suffruticosa*) widely used in folk medicine [4,10,17,18]. Phytochemical and pharmacological research on other *Paeonia* species may open up new medicinal resources.

Figure 1. Peonies: (**A**) *P. oreogeton*; (**B**) *P. obovata*; (**C**) *P. anomala*. Photo by Olga V. Kalendar.

According to the latest taxonomic studies [19,20], *P. oreogeton* S. Moore is a form of *P. obovata* Maxim.: a species native to the Far East, China, Korea, and Japan (Figure 1). This is a herbaceous plant having biternate or triternate leaves with smooth-edged wide lobes. Flowers of *P. obovata* have different colors: red, pink, purple, and pinkish-violet. *P. oreogeton* has yellow or cream-colored flowers and biternate leaves with smooth-edged wide lobes; it grows in the Far East of Russia and occurs in China, on the Korean Peninsula, and in Japan [21]. The most important advantages of these peonies are their durability in culture and high winter hardiness even in USDA (United States Department of Agriculture) zones 2 and 3. Accordingly, they are economically profitable crops that do not require constant or frequent renewal of flower beds or purchases of plants to restore plantings affected by wintering. In *P. obovata*, the roots are used most often for crude medicinal formulations in traditional Chinese medicine. It is used as a tincture for the treatment of dyspepsia and menopausal disorders. In addition, the roots of this plant are employed as a substitute for medicinal *P. lactiflora* in China for the treatment of chest pain, abdominal pain, eye redness, dysmenorrhea, amenorrhea, blood vomiting, carbuncles, and bruising and serve as antiplatelet, sedative, astringent, and antispasmodic therapeutics [13,22]. The profile of monoterpene compounds, of their glycosides (derivatives of paeoniflorin), and of tannins in the roots of *P. obovata* has been sufficiently studied. Seven proanthocyanidins have been found in the roots of this species [23,24]. Flowers contain anthocyanins: pelargonidin 3-glucoside, cyanidin 3,5- diglucoside, peonidin 3-glucoside, and peonidin 3,5-Di-*O*-β-D-glucopyranoside [25]. Seventy-seven percent of the total content of volatile compounds in *P. obovata* flowers is represented by two monoterpene alcohols. Two sesquiterpenes

are also present in its flowers [26]. A relatively recent study by Bae et al. [27] showed that a methanolic extract of *P. obovata* has an antiulcerogenic activity. The other species, *P. oreogeton*, has been studied insufficiently.

In this regard, the aim of this study was to analyze seasonal development of *P. oreogeton* and *P. obovata* and their levels of biologically active and reserve substances under ex situ conditions and to determine the optimal time for harvesting of raw material in the form of leaves and rhizomes.

2. Materials and Methods

2.1. Plant Material

The study was conducted in the Central Siberian Botanical Garden, the Siberian Branch of the Russian Academy of Sciences (CSBG SB RAS, Novosibirsk, Russia); N 54.819308, E 83.102064. We transferred *P. oreogeton* and *P. obovata* (growing in the monsoon climate of the Russian Far East) to the CSBG SB RAS (Western Siberia) and analyzed *P. anomala* brought from natural Siberian habitats. *P. oreogeton* was brought from Primorye, Khasansky district (De Livron Island) N 42.694390, E 131.365044, and *P. obovata* was brought from Primorye, Khasansky district (environs of Slavyanka village); N 42.911206, E 131.338515. The plants were identified by an expert from the CSBG SB RAS (Novosibirsk, Russia). Voucher specimens were deposited in the Plant Material Storage Room in the Laboratory of Introduction of Ornamental Plants (CSBG SB RAS).

The plants were grown on experimental plots of the Laboratory of Introduction of Ornamental Plants (CSBG SB RAS); this territory has an irrigation system. Soils in these plots are gray forest soils with a bulk of density in the 0-20 cm layer: 0.8-1.18 g/cm^3. The content of humus in the 0-20 cm layer is 2-4%, and at a depth of 50-60 cm, no more than 0.8%. The total natural reserves of nutrients are low, and therefore organic and mineral fertilizers were applied. Winter sheltering of the plants was not carried out. Mycological analysis and identification of peony lesions caused by *Cronartium flaccidum* (Alb. et Schw.) Wint. were performed by Dr. I.G. Vorobieva. The identification of pathogens and measures to combat them are implemented by the Plant protection group in the CSBG SB RAS.

For the analysis of biologically active substances of *P. oreogeton* and *P. obovata*, the leaves and rhizomes were collected at the beginning of the growing season (May 2017) and rhizomes at its end (17 August 2017) from Collections of Living Plants Indoors and Outdoors (unique scientific unit No. 440534 of CSBG SB RAS) in a garden plot located at a forest-steppe site with gray forest soil and mean yearly temperature/precipitation of 1.8 °C/448 mm. For biochemical analysis, samples of the raw material from above-ground and underground organs were taken from mature generative plants: ontogenetic state g2. The raw material was dried and ground to obtain a representative sample for the analysis.

2.2. Seasonal Development Analysis

Rhythms of growth and development of peonies were studied by the phenological observation technique developed earlier [28,29]. For convenience, start dates of the main plant phenophases are presented in phenospectra. Qualitative traits of ontogenetic states were described according to a scale of ontogeny periodization [30,31]. *P. anomala* (a representative of local flora) served as a control for comparative analyses of biological characteristics.

2.3. Extract Preparation

Approximately 0.5 g (accurately weighed) of the raw material ground to a particle size of ~3 mm was placed into a 100 mL flask and exhaustively extracted with 70% ethanol. Extraction completeness was verified by means of a reaction with a 5% sodium hydroxide solution (until discoloration). After that, the volume of the filtered extract was measured.

Moisture was quantified via drying of a sample to constant weight at 105 °C in a thermostat. Biochemical parameters were calculated for absolutely dry weight of the raw material. All chemical analyses were performed on two biological replicates and three technical replicates [32].

2.4. Determination of Flavonol Contents

This procedure was performed by a spectrophotometric method based on the complexation reaction between flavonols and aluminum chloride [33]. An extract (0.1 mL) was placed into two 5 mL test tubes, 0.2 mL of a 2% ethanol solution of aluminum chloride was added into one test tube, 1–2 drops of 30% acetic acid were added into the other one, and the solution was brought to the nominal volume with 96% ethanol. The solutions were mixed, and after 40 min, optical density of the solution containing aluminum chloride was measured on an SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at a wavelength of 415 nm in a cuvette with a 1-cm light path, using a solution of acetic acid as a control. The amount of flavonols in each sample was determined by means of a calibration curve built based on rutine (Chemapol, Mumbai, MH, India).

2.5. Quantification of Catechins

The content of catechins was determined spectrophotometrically by the method based on the ability of catechins to produce a crimson color in a solution of vanillin in concentrated hydrochloric acid [34,35]. A 0.8 mL aliquot of an extract was placed into two test tubes. Next, 4 mL of a 1% solution of vanillin in concentrated hydrochloric acid was poured into one of them, and the volumes were adjusted to 5 mL in both tubes with concentrated hydrochloric acid. A tube without vanillin served as a control. In the presence of catechins, the sample became pink, raspberry, or orange-red. After 5 min, the intensity of colors was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at 504 nm in a cuvette with a light path of 1 cm. The standard curve was constructed with (±)-catechin (Sigma, St. Louis, MO, USA).

2.6. Quantification of Tannins

This assay of tannins (hydrolyzable tannins) was performed by the method proposed by L.M. Fedoseeva [36]. An extract (10 mL) was placed into a 100 mL volumetric flask, and 10 mL of a 2% aqueous solution of ammonium molybdate was introduced. The content was brought to the nominal volume with purified water and incubated for 15 min. The intensity of the resulting color was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at 420 nm in a cuvette having a 1-cm light path. A government standard sample of tannin (Sigma, St. Louis, MO, USA) served as a standard.

2.7. Quantification of Saponins

The concentration of saponins was determined by the gravimetric method. Approximately 2 g of the air-dry material was extracted with chloroform in a Soxhlet apparatus until complete discoloration to remove lipids and resins, which hamper saponin analysis. The samples were dried and extracted in a water bath at 70 °C for 30 min successively with 50%, 60%, and 96% ethanol. The combined extract was evaporated to 5 mL, and sevenfold volume of acetone was added. After 18 h, the formed precipitate was filtered off, dried at 70 °C, and weighed, and the percentage of saponins was calculated [37]. Qualitative assays (reactions) for detecting saponins were as follows: 1) foaming equal in volume and stability is achieved after shaking of the extract with an acidic or alkaline solution; 2) acetone added to the extract induces the formation of a white flocculent precipitate, which indicates the presence of triterpene saponins in samples.

2.8. Quantification of Ascorbic Acid

This procedure was performed by titration based on its reducing properties [32]. A raw-material sample (2–5 g), thoroughly ground up in a mortar to a homogeneous mass, was extracted with 20 mL of a 1% hydrochloric acid solution. After that, the extract was poured into a 100 mL volumetric flask and brought to the nominal volume with a 1% oxalic acid solution, which improves the stability of ascorbic acid in the extract. The extract was incubated for 5 min, then filtered and titrated until staining clear pink with the Tillmans reagent. For preparation of a 0.001N dye solution, 60 mg of 2,6-dichlorophenolindophenol

was dissolved in 200 mL of warm distilled water; 4–5 drops of 0.01N sodium hydroxide were added; after 10 min of vigorous shaking, the solution was passed through a dense filter into a dry flask. A mixture of 1% hydrochloric acid and 1% oxalic acid at a ratio of 1:5 was used as a control.

2.9. Quantification of Pectins and Protopectins

Pectin substances (protopectins and pectins) were quantitated by the carbazole-free method based on specific yellowish-orange staining of uronic acids in the presence of thymol in a sulfuric acid solution [32,38]. A 0.5–1.0 g ground-up sample of the air-dry material was extracted thrice with hot 80–82% ethanol (40, 30, and 25 mL) in a boiling water bath under reflux for 20–30 min (to extract free carbohydrates, which hamper pectin analysis) and passed through a paper filter into a flask. The filtered sample was dried at 50 °C until the smell of alcohol disappeared.

2.9.1. Extraction of Water-Soluble Pectin (Extract I)

Fifty milliliters of distilled water heated to 45 °C was added to the dried residue of the raw material; extraction in the water bath was performed at 45 °C for 1 h. The liquid was filtered into a 100 mL volumetric flask; after cooling, the volume was brought to the mark with water.

2.9.2. Extraction of Protopectin (Extract II)

Fifty milliliters of 0.3N hydrochloric acid was poured into an extraction flask containing the residual raw material; the solution was heated for 30 min in a boiling water bath under reflux. Then, the solution was filtered into a 200 mL volumetric flask, and the extract was washed with 50 mL of hot water. The filter and the precipitate were placed into the same extraction flask filled with 50 mL of a 1% ammonium citrate solution, which was then kept in the boiling water bath for 30 min. After that, the solution was filtered into a flask containing the filtrate of the hydrochloric acid extract and washed with hot water. After cooling, the volume was brought to the mark with water.

2.9.3. The Reaction with Thymol

Concentrated sulfuric acid cooled to 4 °C was added dropwise with cooling to 0.5 mL of cooled extracts I and II and thoroughly shaken; the test tubes were heated for 6 min in the boiling water bath and cooled; 0.1 mL of a 0.2% alcohol solution of thymol was added, and the solution was thoroughly mixed. After the reaction with thymol, optical density of the colored solutions was measured using the Agilent 8453 spectrophotometer at 480 nm in a cuvette with a 1-cm light path. The control was 96% ethanol. Pectins and protopectins were quantified with the help of a calibration curve plotted using galacturonic acid standards (Merck, Rahway, NJ, USA).

2.10. Quantification of Carotenoids

The concentration of carotenoids was determined in an acetone–ethanol extract [32,39]. A 0.1 g air-dried sample was ground in a mortar to a homogeneous mass during successive addition of 0.1 g of calcium carbonate (to neutralize organic acids because carotenoids are unstable in an acidic environment), 1 mL of dimethylformamide (for pigment stability), and 2 g of anhydrous sodium sulfate. Carotenoids were extracted first with acetone (20 mL: one time, and 5 mL: two times), and then the extraction was continued with 96% ethanol (5 mL: three times) to extract lycopene. Next, exhaustive extraction was continued with acetone until discoloration. The volume of the combined extract was measured. The carotenoid content was measured at 662 nm (for chlorophyll a), 644 nm (for chlorophyll b), and 440.5 nm (for carotenoids) using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) in a cuvette with a 1-cm light path. The control was 96% ethanol. The concentration of carotenoids (mg/dm^3) was computed using the formula:

$$C_{car} = 4.695 \times D440.5 - 0.268 \times (5.134 \times D662 - 20.436 \times D644),$$

where D is optical density of the extract, and C_{car} is the concentration of carotenoids, mg/dm^3.

The concentration of carotenoids (mg/100 g) was found by means of the formula:

$$X = C_{car} \times V \times V_2 \times 100/(M \times V_1 \times 1000),$$

where C_{car} is the concentration of carotenoids, mg/dm^3; V is the volume of the initial extract, mL; V_1 is the volume of the initial extract used for dilution, mL; V_2 is the volume of the diluted extract, mL; and M is absolute dry weight of the raw material, g.

2.11. Quantification of Sugar and Starch

For sugar quantification, the method proposed by A.S. Shvetsova and E.Kh. Lukyanenko was employed. It involves the reduction of potassium ferricyanide with reducing sugars to ferrocyanide in an alkaline medium. The latter causes stable blue staining with iron sulfate in the presence of gelatin [32]. An extract (0.1 mL) and an alkaline solution of potassium ferricyanide (2 mL; 1.65 g of ferricyanide and 10 g of sodium carbonate dissolved in 1 L of water) and distilled water (2 mL) were poured into 50 mL measuring tubes up to the 10 mL mark. The tubes were shaken and heated in a boiling water bath for 15 min. After that, the tubes were cooled, and 4 mL of a ferrous sulfate solution was poured into each measuring tube. To prepare the assay solution, 1 g of ferrous sulfate was dissolved in 10 mL of concentrated sulfuric acid in a 1 L flask, and water was added to bring the volume to the mark. A working solution of ferrous sulfate was prepared on the day of use via mixing of a 10% gelatin solution with the ferrous sulfate solution at a ratio of 1:20. The solution was then stirred, and the volume in the test tube was adjusted to the mark of 50 mL with distilled water. After vigorous stirring, optical density of the solution was measured using the SF-56 spectrophotometer (Lomo, St. Petersburg, Russia) at 690 nm (glucose absorption maximum) in a cuvette with a 1-cm light path. The sugar concentration was calculated by means of a calibration curve plotted for glucose (Reachem, Russia). A blank sample was used as a control.

For starch quantification, the extract was hydrolyzed with a 1% hydrochloric acid solution for 6 h [40]. After neutralization of the extract, the content of sugars was determined by the above method, and the previously determined content of sugars was subtracted. Given that 1 g of glucose corresponds to 0.89996 g of starch, the resulting difference (in %) was multiplied by 0.89996 to determine the starch concentration in an analyzed sample.

3. Results

3.1. Seasonal Development of Paeonia in the Continental Climate

P. oreogeton and *P. obovata* grow naturally in Primorye with its monsoon climate. In our experiments, *P. oreogeton*, *P. obovata*, and *P. anomala*, which are representative of local flora, were grown ex situ under the conditions of the continental climate (Western Siberia). According to long-term data (15 years), *P. obovata* and *P. oreogeton* complete the entire growth cycle here, including flowering and fruiting. Both species exhibited abundant flowering, but in the first 3 years, *P. oreogeton* did not bloom; yet under the microclimatic conditions specifically selected for this species (a more shaded area), it started to bloom and fruit yearly.

Table S1 presents a comparison of the main climatic characteristics of the two climate types. Figures 2–4 illustrate phenophases of *P. oreogeton*, *P. obovata*, and *P. anomala* (control) under the conditions of the forest-steppe zone of Western Siberia during three growing seasons. During the observation period from 2007 to 2018, the 2012 growing season in Akademgorodok (Novosibirsk) was abnormally dry (Table S2), and the 2013 season was abnormally humid (Table S3).

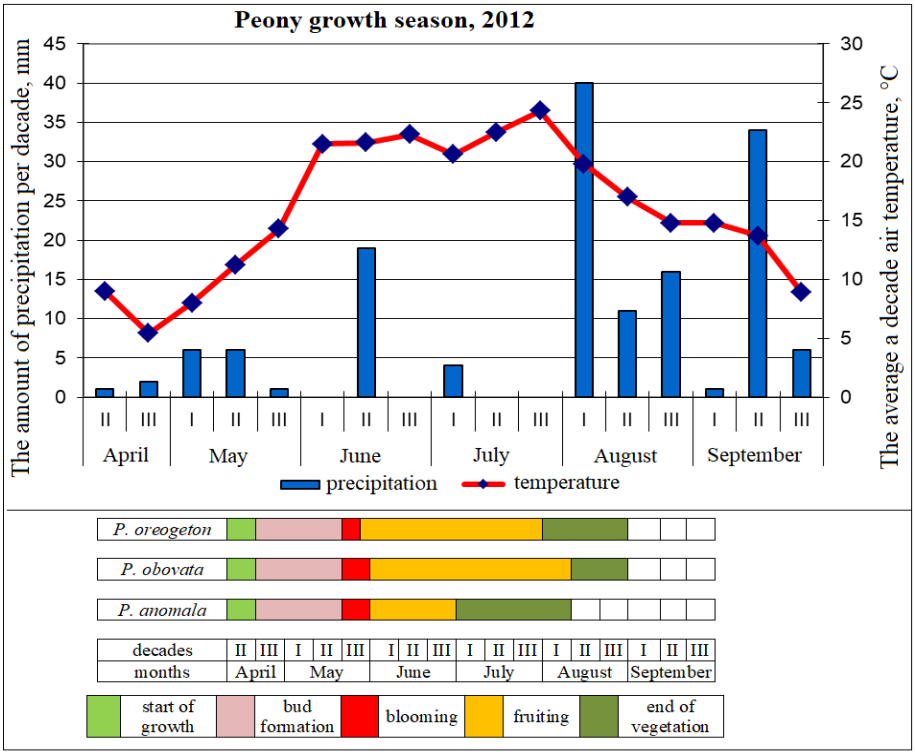

Figure 2. Hydrothermal conditions and peony phenophases in Western Siberia in 2012.

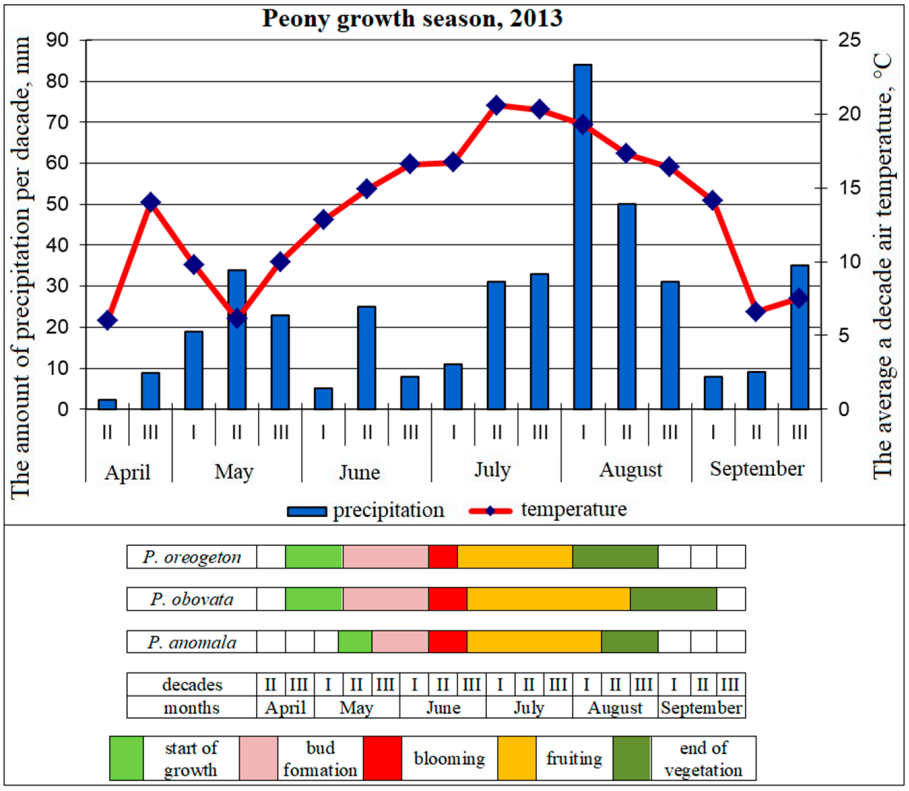

Figure 3. Hydrothermal conditions and peony phenophases in Western Siberia in 2013.

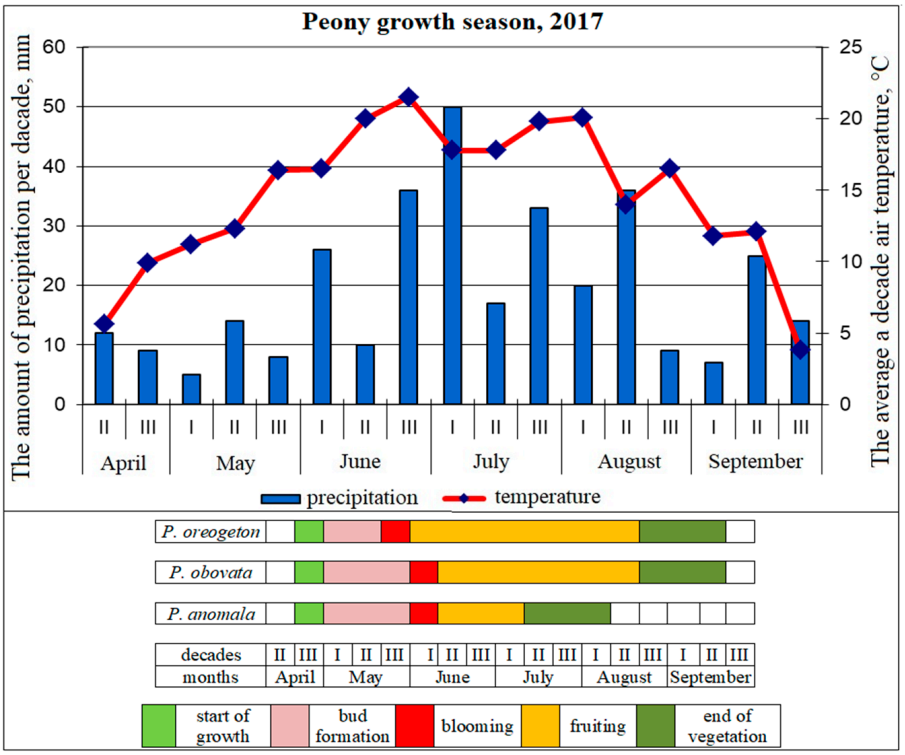

Figure 4. Hydrothermal conditions and peony phenophases in Western Siberia in 2017.

The examination of seasonal development and amplitude of adaptations showed that the above-ground shoots of peonies were damaged by high temperatures during moisture deficiency under abnormally dry weather conditions in 2012 (Figure 5A). Increased ambient humidity and low temperatures caused fungal diseases under abnormally humid weather conditions in 2013 (Figure 5B).

Figure 5. Peony shoots: (**A**) *P. anomala* shoots damaged by low humidity in Western Siberia in 2012; (**B**) *P. obovata* shoots damaged by rust in Western Siberia in 2013.

3.2. Contents of Biologically Active and Reserve Substances in the Leaves and Roots of Paeonia

The year 2017 was chosen as a model one to study the content of biologically active and reserve substances because this year did not show strong deviations of mean monthly

hydrothermal characteristics. We measured the contents of phenolic compounds (flavonols, catechins, and tannins) and of ascorbic acid, pectin substances, saponins, and carotenoids in the leaves and rhizomes of two peony species—*P. obovata* and *P. oreogeton*—at the beginning of the growing season (Table 1). The content of flavonols that was found in the peony leaves at the beginning of the growing season was not very high. In the leaves of *P. obovata*, the content of flavonols reached 1.77%, which slightly exceeded that in the leaves of *P. oreogeton*. Flavonols were not found in rhizomes of the analyzed peonies. Carotenoids and flavonols were detectable only in peony leaves. Their level in the leaves of *P. obovata* (89.2 mg/100 g) was three-fold higher than that in *P. oreogeton* (29.1 mg/100 g). The level of tannins was sixfold higher in the leaves of *P. oreogeton* (16.42%) than in its rhizomes (2.56%). Concentrations of tannins were comparable between leaves and roots of *P. obovata*. The content of catechins was higher in the leaves of *P. oreogeton* (68.3 mg/100 g) and *P. obovata* (84.4 mg/100 g) as compared to the rhizomes. The content of ascorbic acid was fivefold higher in the peony leaves than in the peony rhizomes. The highest content of saponins was found in the leaves of *P. obovata* (21.06%), while their content in the rhizomes was lower. Levels of pectins in the peony leaves and rhizomes were not very high. Their content in *P. oreogeton* was higher in the leaves (1.89%), whereas in *P. obovata,* it was higher in the rhizomes (2.02%). The concentration of protopectins significantly exceeded that of pectins. In the underground organs of peonies, the level of protopectins was higher than that in the leaves. Protopectins mostly accumulated in the rhizomes of *P. oreogeton* (13.03%).

Table 1. The contents of biologically active and reserve substances in peonies at the beginning of the growing season in Western Siberia.

Substances	*P. oreogeton*		*P. obovata*	
	Rhizomes	**Leaves**	**Rhizomes**	**Leaves**
Moisture (% dry weight)	66.86	80.39	78.17	74.63
Flavonols (% dry weight)	no	1.17 ± 0.04^{b}	no	1.77 ± 0.05^{a}
Catechins (g (100 g dry weight)$^{-1}$)	28.1 ± 0.48^{d}	68.3 ± 1.17^{b}	44.0 ± 0.76^{c}	84.4 ± 1.45^{a}
Tannins (% dry weight)	2.56 ± 0.02^{d}	16.42 ± 0.14^{a}	11.59 ± 0.10^{c}	12.81 ± 0.11^{b}
Ascorbic acid (g (100 g dry weight)$^{-1}$)	30.5 ± 1.22^{b}	155.2 ± 6.21^{a}	31.5 ± 1.26^{b}	151.8 ± 6.07^{a}
Pectins (% dry weight)	1.15 ± 0.01^{b}	1.89 ± 0.06^{a}	2.02 ± 0.10^{a}	0.86 ± 0.01^{c}
Protopectins (% dry weight)	13.03 ± 0.26^{a}	5.99 ± 0.01^{c}	7.02 ± 0.09^{b}	3.96 ± 0.02^{d}
Saponins (% dry weight)	13.37 ± 0.51^{b}	11.25 ± 0.43^{c}	21.06 ± 0.80^{a}	12.94 ± 0.49^{b}
Carotenoids (g (100 g dry weight)$^{-1}$)	no	29.1 ± 0.29^{b}	no	89.2 ± 0.87^{a}
Sugar (% dry weight)	5.97 ± 0.21^{c}	17.44 ± 0.61^{b}	17.22 ± 0.60^{b}	20.85 ± 0.73^{a}
Starch (% dry weight)	25.31 ± 0.94^{a}	-	10.51 ± 0.39^{b}	-

Note: mean values and standard deviation (n = 3) are presented; no, not found; "-", not tested; means with different superscript letters (a, b, c, or d) in the same row are significantly different ($p \leq 0.05$) according to Tukey's honestly significant difference (HSD) test.

We determined contents of reserve substances (starch and sugars) in the leaves and rhizomes of Far Eastern peonies at the beginning of the growing season (Table 1). The content of sugars in *P. oreogeton* (17.44%) and *P. obovata* (20.85%) was higher in the leaves. The rhizomes of the Far Eastern peony *P. oreogeton* accumulated up to 25.31% of starch.

In addition, we studied the content of biologically active compounds and reserve substances (starch and sugars) in the rhizomes of Far Eastern peonies at the end of the growing season in Western Siberia (Figures 6 and 7). At the beginning of the growing season, protopectins accumulated mainly in the rhizomes of *P. oreogeton* (13.03%). The contents of protopectins in the rhizomes of *P. obovata* were similar when we compared the beginning (7.02%) and the end (7.62%) of the growing season (Figure 6). Contents of saponins (17.9%), catechins (44.3 mg/100 g), and sugars (7.36%) in the rhizomes of *P. oreogeton* were higher at the end of the growing season, whereas in *P. obovata* (21.06%,

44.0 mg/100 g, and 17.22%, respectively), this parameter was higher at the beginning of the growing season. At the end of the growing season, the content of saponins in *P. obovata* rhizomes dropped fivefold (to 4.49%). In *P. oreogeton* and *P. obovata*, ascorbic acid (45.6 and 50.9 mg/100 g, respectively) and starch (27.13% and 30.2%, respectively) accumulated by the end of the growing season (Figures 6 and 7). At the beginning of the growing season, the content of pectins was low and reached 1.15% in *P. oreogeton* and 2.02% in *P. obovata*; by the end of the growing season, this parameter decreased.

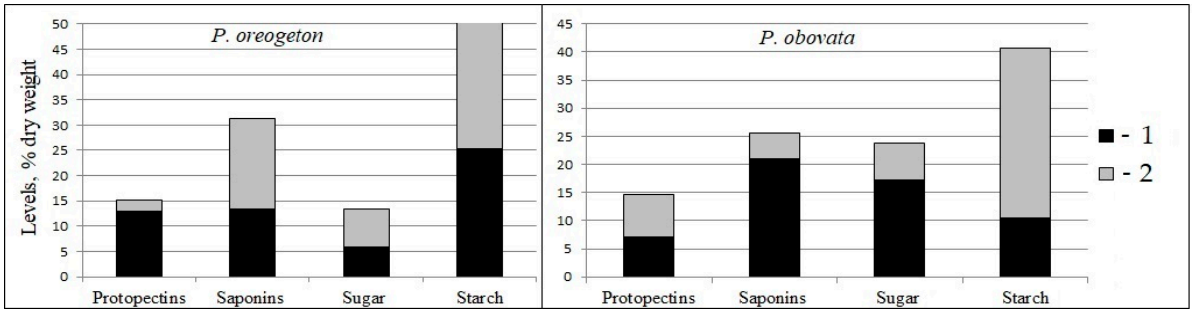

Figure 6. Levels of protopectins, saponins, sugars, and starch in peony rhizomes at the beginning (1) and end (2) of the growing season in Western Siberia.

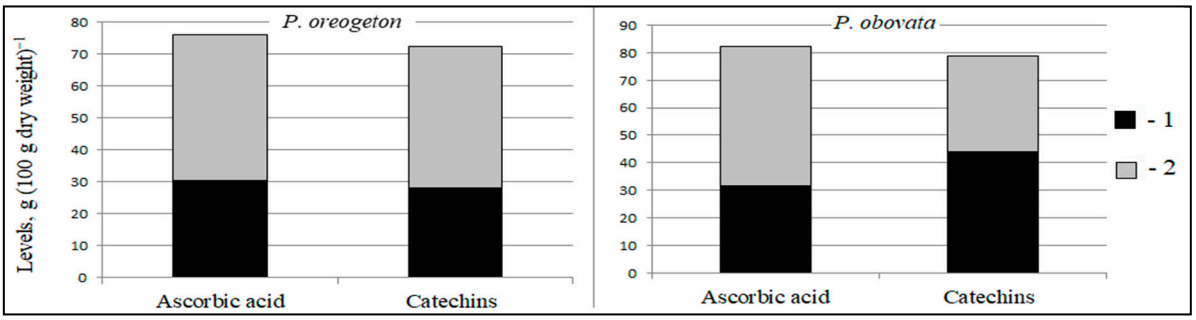

Figure 7. Levels of ascorbic acid and catechins in peony rhizomes at the beginning (1) and end (2) of the growing season in Western Siberia.

4. Discussion

The synthesis and accumulation of biologically active and reserve substances in medicinal plants are dynamic processes that change during plant ontogenesis and depend on numerous environmental factors [41–45]. Results of research by other authors [46,47] show that the concentration of biologically active substances (which in natural habitats perform an adaptive function) diminishes in some medicinal plants growing in areas strongly affected by agriculture. The accumulation of biologically active and reserve substances is influenced by hydrothermal conditions of growing seasons.

An analysis of phenophases by means of long-term phenological observation of representatives of the genus *Paeonia* grown in the forest-steppe zone of Western Siberia revealed that *P. obovata*, *P. oreogeton*, and *P. anomala* start to grow between the second decade of April and the first decade of May [48].

Under the abnormally dry weather conditions in 2012, since the first days of the second decade of April, all three species exhibited abundant growth due to early onset of the spring and a warm first decade of April: the mean 10-day temperature was 4.9 °C, and an excess of the mean monthly temperature was 4.1 °C (Table S2). Budding and flowering stages were registered at similar time points, but abundant flowering of *P. obovata* and *P. anomala* lasted longer as compared to *P. oreogeton*, which is more mesophilic (Figure 2).

It should be noted that in Primorye [49], the duration of flowering of *P. oreogeton* varies from 15 to 23 days, whereas ex situ flowering of this plant is slightly longer (by 5–6 days). At low humidity, in our study, the fruiting stage in all three species was found

to be shifted by one decade, whereas in *P. anomala*, it terminated 8 days earlier. In 2012 and subsequent years, the time point of the end of the growing season for *P. anomala* was easily distinguished because in natural habitats, this plant prepares for the wintering period earlier as compared to *P. obovata* and *P. oreogeton*, whose growing seasons are longer on the Primorye territory.

A gradual temperature increase in early April in abnormally humid 2013 stimulated the mean annual growth of the Far Eastern species, but the decreased temperatures in the first and second decades of May shifted the budding and flowering stages by 10–13 days (Table S3, Figure 3). The duration of flowering lengthened by 2–6 days. Fruit ripening was longer, and fungal diseases afflicted the leaves of all species in the period from the second decade of July to the third decade of August owing to a large amount of precipitation and a cool period at the end of the summer (Figure 5b). This fact and weather conditions did not allow to harvest valuable plant raw materials.

We noticed that the growing season of 2017 (model weather conditions) is characterized by smooth temperatures and uniform precipitation levels, which were favorable for preservation of assimilating leaves on the shoots of Far Eastern peonies. Just as in coastal spring conditions, cool and humid weather induced earlier longer flowering of *P. oreogeton*. The local species *P. anomala* completed its growth at the time point optimal for preparation for wintering and differentiation of underground renewal buds. In 2017, hydrothermal conditions in the second decade of May and at the end of the second decade of August were favorable for harvesting of valuable plant materials (Figure 4).

Under drier weather conditions in 2012, the flowering stage of *P. oreogeton*, which exhibits greater mesophilicity, began early (3rd decade of May) and lasted 2–3 days less (6 days) than in abnormally humid 2013 (2nd decade of June, 8 days). Cool wet weather was similar to that during the coastal spring in 2017 (model weather conditions) and stimulated earlier (3rd decade of May) prolonged flowering (10 days) of *P. oreogeton*.

The coastal species *P. obovata* successfully adapted to the continental climate of Western Siberia, and its flowering stages turned out to be similar to those of the local species *P. anomala*. Under abnormally dry weather conditions in 2012, flowering began in the 3rd decade of May and lasted for 10 days. Abnormally humid weather conditions in 2013 caused flowering to start at the mean annual time point of flowering (2nd decade of June), which lasted for 13 days. In 2017 (model weather conditions), the flowering stage began on June 1 and lasted for 10 days. In Primorye, the period from the beginning of growth to flowering is 35–48 days for *P. obovata* [49], and during its introduction into Western Siberia (CSBG SB RAS), this period reached 28–40 days due to a more intense heat gain at the beginning of the growing season; this phenomenon is typical of the south of Siberia.

When species are grown as sources of biologically active substances, it is not recommended to harvest raw material of above-ground vegetative mass of peonies during excessively humid periods (as in 2013 for example) because leaves are affected by rust, spotting, and fungal spores, which can get into medicines and dietary supplements during industrial processing. It is better to harvest the above-ground part of these plants during the growing seasons that do not show substantial deviations in terms of hydrothermal parameters (as in 2017 for example).

The above analysis of concentrations of reserve substances and biologically active compounds indicates that there are differences in levels of biologically active and reserve substances between the two introduced species of peonies (*P. oreogeton* and *P. obovata*) grown under the conditions of the CSBG SB RAS. This can be attributed to a difference in adaptation mechanisms among the specimens collected in nature. Individual plants respond differently to the change of environmental conditions from the monsoon Far Eastern climate to the continental Siberian climate. Multifunctional adaptation of vegetative organs of peonies proceeds at morphological, anatomical, physiological, and biochemical levels; this adaptation improves active life of cultivars under specific growth conditions and facilitates selection of promising cultivars and species from various regions of Russia and abroad [4,6,50–56]. For example, the total content of flavonoids in the leaves

and stems of *P. lactiflora* grown in the Botanical Garden of the Mongolian Academy of Sciences (Ulaanbaatar) decreases linearly during its growth, and the highest content is observed at the flowering stage [51]. Nonetheless, the differences in the accumulation of substances may be an invariable species trait fixed at the genetic level. To test the latter hypothesis, it is necessary to analyze accumulation of substances in plants collected under natural conditions.

Levels of some substances in the Far Eastern species *P. oreogeton* and *P. obovata* grown in Akademgorodok (Novosibirsk) are less than those in the Siberian species *P. anomala* (our data) and *P. hybrida* and the Siberian-Far Eastern species *P. lactiflora* [56]. The content of some substances in the leaves and rhizomes of the above species has been reported previously [56]. For example, the content of flavonols is higher in the leaves of *P. hybrida* (1.96%) and *P. anomala* (1.95%), the content of catechins is higher in the rhizomes of *P. lactiflora* (1287.8 mg/100 g), the content of ascorbic acid is higher in the leaves of *P. anomala* (1205.69 mg/100 g), and the content of protopectins is greater in the rhizomes of *P. anomala* (15.9%), whereas the content of reserve substances, namely, sugars, is higher in the rhizomes of *P. lactiflora* (22.06%), and the content of starch is greater in the rhizomes of *P. anomala* (31.2%). On the other hand, the concentration of tannins is higher in the leaves of *P. oreogeton* (16.42%), the concentration of saponins is greater in the rhizomes of *P. oreogeton* (21.06%), and the concentration of pectins is higher in rhizomes of *P. obovata* (2.02%). Higher contents of tannins, saponins, and pectins are most likely due to adaptation of the Far Eastern peony species to the Siberian conditions. It should be noted that the Siberian and Far Eastern species grown in Akademgorodok exhibited higher levels of some substances. Thus, species that are not used as medicinal plants in Russia can replace the official medicinal species *P. anomala*. Levels of some substances, such as catechins, sugars, tannins, saponins, and pectins, is lower in *P. anomala* than in some Siberian and Far Eastern species.

5. Conclusions

The study leads to the conclusion that introduced species *P. obovata* and *P. oreogeton* are promising for further research under the conditions of Akademgorodok (Novosibirsk). In this environment, *P. obovata* and *P. oreogeton* start to grow between the second decade of April and the first decade of May, go through the entire growth cycle, including flowering and fruiting, but *P. oreogeton* requires appropriate microclimatic conditions (a more shaded area). To prevent fungal spores from getting into medicines and dietary supplements during industrial processing, peony aerial shoots as a medicinal raw material should not be harvested during periods of excessive humidity.

A biochemical analysis of *P. obovata* and *P. oreogeton* grown in Akademgorodok showed that the leaves accumulate higher concentrations of flavonols (*P. obovata*: 1.77%, and *P. oreogeton*: 1.17%), tannins (*P. oreogeton*: 16.42%, *P. obovata*: 12.81%), ascorbic acid (*P. oreogeton*: 155.2 mg/100 g, *P. obovata*: 151.8 mg/100 g), and sugars (*P. oreogeton*: 17.44%, *P. obovata*: 20.85%) than the roots do. Peony rhizomes contain higher concentrations of protopectins (*P. oreogeton*: 13.03%, *P. obovata*: 7%), saponins (*P. oreogeton*: 17.90%, *P. obovata*: 21.06%), and starch (*P. oreogeton*: 27.13%, *P. obovata*: 30.20%) than the leaves do. We believe that species of the genus *Paeonia* grown in the CSBG SB RAS are a promising plant material. Further investigation into the dynamics of accumulation of biologically active substances in the organs of peonies will help to identify introduced plant species having high biochemical potential for the pharmaceutical industry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9010102/s1, Table S1: The main climatic characteristics of the growing seasons in Novosibirsk and Vladivostok; Table S2: Main climatic characteristics of the 2012 growing season (Novosibirsk); Table S3: Main climatic characteristics of the 2013 growing season (Novosibirsk).

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