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BRIEF MESSAGES

Seasonal Dynamics of Polysaccharides in Bark of *Ulmus laciniata* (Trautv.) Mayr in the Nutritional Aspect of *Cervus elaphus xanthopygus* (Milne–Edwards) in the Southern Part of the Russian Far East (Primorskii Krai)

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Abstract—*Ulmus laciniata* is a strategic food resource for *Cervus elaphus xanthopygus*, especially in autumn and spring. The results of a study of the seasonal content of water-soluble polysaccharides, their monosaccharide composition, extracted from the bark and bast of *U. laciniata,* are presented. Two maxima in the content of water-soluble polysaccharides were revealed: during the shoot growth period (June) and during a slightly lower one during the preparation for winter dormancy (October). The minimum content is observed during the beginning of the growing season (April–May). The content of pectin substances, unlike water-soluble polysaccharides, changed slightly during the year. The groups of substances studied are classified as the most bioavailable polysaccharides with extensive physiological activity. In addition, the data obtained may explain the feeding behavior of *C. elaphus xanthopygus*, which lives in Primorskii krai.

Keywords: Ulmus laciniata, water-soluble polysaccharides, pectins, seasonal dynamics, Cervus elaphus xanthopygus, Russian Far East

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INTRODUCTION

The majority of the planet's organic matter consists of carbohydrates, of which polysaccharides form the bulk of the dry mass of plants. Polysaccharides are high-molecular carbohydrates consisting of identical or different monosaccharide residues linked by glycosidic bonds. According to their functional properties, the following groups of polysaccharides are distinguished: water-soluble, which are highly hydrated and protect cells and tissues from drying out; reserve, which serve as an energy resource; and structural, which give the cells and tissues of the mechanical strength of the body (Gorshkova et al., 2013). Most polysaccharides are soluble in water and practically insoluble in ethyl alcohol; this property is the basis of the isolation method of polysaccharides from plant materials by aqueous extraction followed by precipitation with 96% ethyl alcohol.

The complex of chemical compounds contained in plants exhibits a wide range of physiological activity, exerting a multifaceted effect on the bodies of humans and animals. The biological activity of plant polysaccharides depends on the composition, structure, and molecular weight. Water-soluble polysaccharides (WSPS) and pectin substances (PSs) are considered to be the most bioavailable polysaccharides, possessing hepatoprotective, antihypoxic, and sorption activities. The mentioned compounds also exhibit immunomodulatory, hypolipidemic, and anti-inflammatory properties and have antitumor and prebiotic effects, while a very low level of toxicity, or the absence of such, is noted (Sychev, 2008; Zueva et al., 2010).

Particular attention among this extensive group of compounds is given to pectin polysaccharides, one of the most complex and structurally dynamic groups of biopolymers. Pectin substances are a structural component of the intracellular matrix of higher plants and are also found in some aquatic plants (*Zostera marina* L.). By determining the strength and extensibility of the cell walls, pectin substances determine the plant's resistance to abiotic stressors, provide water-salt balance, are characterized by a high gelling capacity, and play an important role as a reserve energy substrate (Paulsen and Barsett, 2005; Ovodov, 2009; Zueva et al., 2010). The accumulation of polysaccharides by plants as reserve nutrients not only ensures their own existence, but is also the key to survival of a number of animals during the cold season.

Red deer across their range feed on the bark of more than 20 tree species, often causing significant

(a) (b)

Fig. 1. Feeding of *Cervus elaphus xanthopygus* on the bark of *Ulmus laciniata*. (a) Damage to the trunk of a large elm after removing the bark. Trial area, UUD. April 21, 2022. Photo by M. Maslov. (b) A young male red deer eats the cambium of a large lobed elm. Ussuriiskii Nature Reserve. March 21, 2021. Bushnell camera trap.

damage to forests. Numerous publications note instances of bark consumption from *Ulmus laciniata* (Trautv.) Mayr and *Ulmus japonica* (Rehder) Sarg. by two species of deer (Cervidae), the Manchurian wapiti *Cervus elaphus xanthopygus* (Milne–Edwards) and the sika deer *Cervus nippon* Temminck in the southern part of the Russian Far East (Kaplanov, 1948; Mikhaylovsky, 1975; Bromley and Kucherenko, 1983; Danilkin, 1999; Makovkin, 1999; Gaponov, 1991, 2006). It has been shown that feeding on *U. laciniata* bark dominates the feeding behavior of *C. elaphus xanthopygus* in Primorskii krai during the nonvegetation period. For example, L.G. Kaplanov (1948) noted this phenomenon in the Sikhote-Alin State Nature Biosphere Reserve, and V.V. Gaponov (1991) observed it in the Chuguevskii Municipal District.

Over the years, the authors have repeatedly encountered characteristic damage to *U. laciniata* caused by red deer within the Ussuriiskii State Nature Reserve (territory of the Land of the Leopard National Park), which was confirmed by data from camera traps installed in the wintering areas of these animals (Fig. 1). *U. laciniata* trees with stripped bark were also noted in the Udege Legend National Park in the Bolshaya Ussurka River valley (Krasnoarmeiskii District) (45°45′52″ N, 135°28′35″ E), in the upper reaches of the Perevoznaya River (Nadezhdinskii District) (43°32′88″ N; 132°05′99″ E), as well as in the Shkotovskii and Mikhailovskii districts. Since 2021, continuous observations have been carried out in the Ussuriiskii urban district (UUD) on the territory of the forest area of the Primorskii State Agrarian and Technological University in the vicinity of the village of Kamenushka (43°37′23″ N, 132°13′50″ E) (Belyaev and Maslov, 2022). An important aspect of studying the nature of the eating behavior of *C. elaphus xanthopygus* in Primorskii krai is determination of the dynamics of accumulation of metabolites in the bark *U. laciniata*, which have nutritional value for animals.

The bark of woody plants consists of an outer element, the crust, and an inner element, the bast. The bark is a covering tissue that performs protective and gas exchange functions; the bast or phloem, being, in particular, a conducting and storage tissue, implements transport and storage functions, ensuring the downward transport of organic substances from the photosynthetic or storage tissues of the plant. Phloem parenchyma cells provide metabolic reactions and accumulate various compounds: polysaccharides, fats, proteins, tannins, resins, and crystals (Zitte et al., 2007). Phytochemical study of *U. laciniata* was conducted in the 1970s and 1980s, mainly by Japanese researchers. The following groups of substances were found in the wood: sesquiterpenoids, phenolic acids, coumarins, lipids, and flavonoids were found in the leaves (*Rastitel'nye resursy*..., 2008). We have not found any data on the study of polysaccharides of *U. laciniata*.

The aim of this work is to analyze the dynamics of the content water-soluble polysaccharides and pectin substances in the bark and bast of *U. laciniata* during the year.

MATERIALS AND METHODS

The subject of this study is the raw material of the bark and bast of *U. laciniata*, harvested in the vicinity of the village of Kamenushka, Ussuriiskii district, Primorskii krai (territory of the Land of the Leopard National Park (43°37′49″ N; 132°13′49″ E). *U. lacini-*

ata is a Far Eastern species, a summer-green tree taller than 10 m, a progressive relict, that grows in mixed forests on mountain slopes and foothills, but is rare in valley forests. The trees reach 25 m in height and 55 cm in trunk diameter. It lives up to 200–230 years (Bezdelev and Bezdeleva, 2006; Usenko, 2009). Plant samples were identified by Doctor of Biological Sciences T.A. Bezdeleva and are stored in the herbarium of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences (VLA 93599, VLA 93824).

In order to minimize damage to the bark of objects, the seasonal dynamics of the polysaccharide content was studied on combined samples from five different trees of approximately the same age, growing in the same type of biotopes, similar in the composition of tree and shrub vegetation. Samples of raw materials were collected by the authors of the study in the second ten-day period of each month from May 2021 to April 2022 using a metal sampler with an adjustable cutting depth from 0.5 to 2.5 mm at a height of 100–150 cm from the base of the trunk. The entry of trees into phenological phases was assessed based on a set of characteristic features (Zaitsev, 1981). Drying of raw materials was carried out using the air-shadow method, followed by drying to 12% moisture content using convective electric drying at 35–40°C (Kitfort KT-1912, China). Air-dried raw materials were ground in a VLM-6 laboratory mill (Vilithek, Russia) to a particle size of 1–2 mm.

To separate alcohol-soluble substances, the raw material was extracted twice with 70% ethyl alcohol in a ratio of 1 : 10 at room temperature for 120 min (maceration). After preliminary extraction of polyphenolic compounds, a water-soluble polysaccharide complex (WSPS) was obtained from the air-dried meal (air-shadow drying). A 5-g sample of air-dried meal was extracted with 100 mL of purified water at 80°C for 90 min with constant stirring. Exhaustive extraction of polysaccharides was performed twice at a ratio of 1 : 20. The plant material was separated by centrifugation. The combined extracts were evaporated to 1/3 of the original volume. Polysaccharides were precipitated with a threefold volume of 96% ethyl alcohol relative to extraction at room temperature. The precipitate was filtered, washed with ethanol then acetone, and then dried and weighed. Low molecular weight impurities precipitated together with WSPS are removed by purifying the latter by washing the WSPS precipitate with ethanol, acetone and filtration. The analysis was carried out in threefold analytical replicates; the results are presented as the average value of three replicates.

The meal obtained after WSPS extraction was used for the extraction of pectin substances (PSs). PS meal was extracted with a mixture of 0.5% solutions of oxalic acid and ammonium oxalate (1 : 1) in a ratio of 1 : 20 at 85°C for 120 min. Exhaustive extraction of PSs was performed twice, followed by centrifugation and precipitation with 96% ethanol. The resulting sediment was processed using the above method (Bubenchikova and Kondratova, 2008).

The determination of the monosaccharide composition of WSPS was carried out in combined samples according to the phases of the vegetative state of the plant, by acid hydrolysis of the fraction with 2 N sulfuric acid in a test tube with a reflux condenser in a boiling water bath for six hours. Monosaccharides in hydrolysates were determined by paper chromatography in comparison with standard samples of monosaccharides in the solvent system: n-butanol–pyridine–water (6 : 4 : 3) and ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4) (Drozdova, 2004). After drying, the chromatograms were treated uniformly with a solution of 0.86 g sulfanilamide and 0.83 g *o*-phthalic acid in 50 mL ethanol and dried at 103 ± 2°C for 5 min. Monosaccharides appeared as reddish brown and purple-brown spots.

RESULTS

Seasonal monitoring of the dynamics of WSPS and PS accumulation revealed changes in the content of metabolites in the bark of *U. laciniata*. In the accumulation of WSPS (Fig. 2) during the year, two maxima are observed in summer and autumn and one minimum, in April–May. We explain the spring minimum by the beginning of growth and development of leaves. It is known that the synthesized polysaccharides remain in the leaf until they reach a size of at least 1/2 of the total leaf area (Heldt, 2011). As the leaf develops, the outflow of WSPS increases, and up to half of the synthesized carbohydrates are transported to the organs that require the products of photosynthesis through the phloem, which confirms the observed maximum of WSPS in the bark in June at 21.15%. Later in the season, a decrease in the WSPS content to 16.45% is observed in September, which is probably explained by a slowdown in growth processes. In October, an increase in WSPS to 18.3% is observed, which is probably associated with the preparation of the plant organism for the winter dormancy phase and the acquisition of frost resistance. During the winter dormancy period, the amount of WSPS is recorded in the range from 16.9% in November to 16.2% in February. In the spring, with the onset of active life, swelling of the buds, intensification of respiration, and sap flow, a decrease in WSPS to an annual minimum of 11.85% in April is observed.

It should be noted that the content of easily digestible polysaccharides in the bark and bast of *U. laciniata* is quite high throughout the season, so the bark and bast can potentially be a significant source of easily digestible polysaccharides in the food balance of red deer, especially in winter.

The seasonal variability of the pectin content (Fig. 3) in the bark of *U. laciniata* also has an oscillatory char-

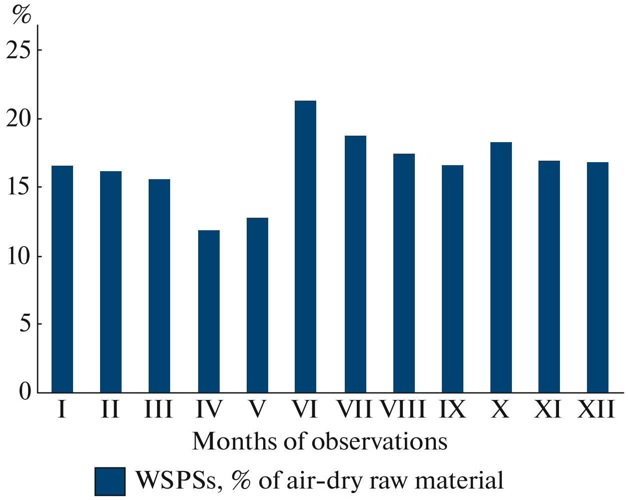

Fig. 2. Accumulation of water-soluble polysaccharides in the bast and bark of *U. laciniata* during the year.

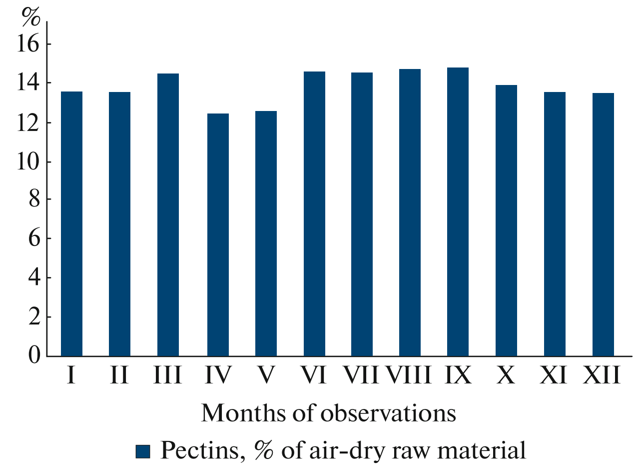

Fig. 3. Accumulation of pectin substances in the bast and bark of *U. laciniata* during the year.

acter, although smoother. One pronounced minimum is observed at the beginning of the growing season (April–May), when the PS content decreases to 12.4%; during the period of growth and development of shoots (June–July), the PS content increases, reaching maximum values by the end of the latent growth period at 14.85% (September).

In the dormancy preparation phase, the amount of pectin decreases (13.9%), possibly due to an increase in the lipid fraction in the cell membranes associated with the process of formation of frost resistance (Trunova, 2007; Petrov et al., 2011), taking values of 13.5–13.6% in the dormancy phase (November–February). The hydrophilic properties of pectins during plant growth probably play an important role in water exchange. Water bound by pectin substances does not freeze and evaporates with difficulty; therefore, pectins give young plants and tissues resistance to freezing and drought (Fengel, 1988). In March, during the period of emergence from dormancy, the PS content in the cortex increases to 14.5%, possibly as a result of the outflow of WSPS to awakening organs.

DISCUSSION

It is known that the effect of low temperatures causes a number of effects at the cellular level: an increase in membrane viscosity, a slowdown in metabolism, and an increase in the concentration of cellular solutions, which is ensured by a complex of adaptive reactions in which non-structural carbohydrates (monosaccharides and WSPS) play a significant role (John et al., 2016; Nikolaeva et al., 2017; Bhandari, 2018; Dong and Beckles, 2019; Chen and Yang, 2020). It can be assumed that changes in the content of WSPS and PS are regulated by physiological patterns (metabolism) of the annual cycle of *U. laciniata*. The coefficient of variation of the seasonal dynamics for WSPS CV, % = 16.27, for PSs CV, % = 5.92, which corresponds to the average (WSPS) and very low (PS) degrees of variability (Mamaev, 1972). Considering the role of WSPS as plastic and energetic elements in plant physiology, it can be assumed that among the total pool of polysaccharides, soluble polysaccharides have a "fast response" function, which reflects a higher variation coefficient.

The study of the monosaccharide fraction of WSPS (Table 1, Fig. 4) revealed the presence of glucose (Glc), fructose (Fru), galactose (Gal), rhamnose (Rha), and xylose (Xyl). The data of quantitative analysis of the WSPS complex hydrolysates, determined densitometrically, are presented in Table 1.

The analysis showed that the monosaccharide composition of WSPS bark and bast of *U. laciniata* as the annual physiological cycle progresses changes both quantitatively and qualitatively. During the period of shoot growth (VI), two monosaccharides are fixed in the phloem: Fru and Glc in almost equal proportions: 52.95 and 47.05%, respectively. In samples from the latent growth period (VII–IX), the pool of monosaccharides is replenished with rhamnose (Rha), the amount of which approaches a third (31.34%) of the total amount of identified monosaccharides, while the content of Fru (44.27%) significantly exceeds Glc (24.38%).

With the onset of cold weather and the transition to the period of preparation for dormancy (X), a decrease in the amount of Glc and Fru to 20.38 and 25.17% is noted. Gal (4.9%) and Xyl (12.9%) appear in the composition, and the level of Rha increases to 36.66% of the fraction of identified monosaccharides. During the transition to a period of deep dormancy, in the WSPS complex of the bark and bast of *U. laciniata* there is a slight increase in Xyl to 17.27%, Rha to 36.98%, and Gal to 5.27%, while the proportion of

Table 1. Monosaccharide content, % of WSPS complex

Phase	Month	Monosaccharide, %				
		Xyl	Rha	Gal	Fru	Glc
Dormancy	I–III	5.53	12.58	1.80	8.75	5.47
Start of vegetation	IV–V	–	4.35	–	9.82	4.05
Shoot growth	VI	–	–	–	15.06	13.38
Latent growth period	VII–IX	–	6.62	–	9.35	5.15
Preparation for dormancy	X	4.85	13.78	1.84	9.46	7.66
Deep dormancy	XI–XII	6.1	13.06	1.86	8.18	6.12

Glc and Fru continues to decrease to 17.33 and 23.16%, respectively. The ratio of monosaccharides remains at almost the same level during the period of forced dormancy (16.2, 36.86, 5.27, 16.03, 25.64%, respectively). Thus, as the plant enters the period of winter dormancy (X), the spectrum of monosaccharides expands due to xylose (Xyl) and galactose (Gal), while the amount of Xyl increases somewhat towards the phases of deep (XI–XII) and forced dormancy (I–III), the amount of Gal remains practically constant.

It can be assumed that the identified monosaccharides are partly products of hydrolysis of oligosaccharides, presumably raffinose and sucrose. For the development of frost resistance, a prerequisite is the accumulation in plant tissues of a sufficient amount of soluble carbohydrates, both mono- and oligosaccharides (Ding et al., 2019, Belyavskaya et al., 2020). It is known that Gal is a product of raffinose hydrolysis, while disaccharides and oligosaccharides of the raffinose family belong to the group of carbohydrates involved in cold acclimation reactions of plants. At temperatures close to 0°C, energy- and material-intensive substances, in particular sugars, are produced in large quantities in the plant cell, which in turn is an important component of the adaptation of herbivores to the cold season (Petrov et al., 2011; Wang et al., 2018; Leuendorf et al., 2020; Belyavskaya et al., 2020).

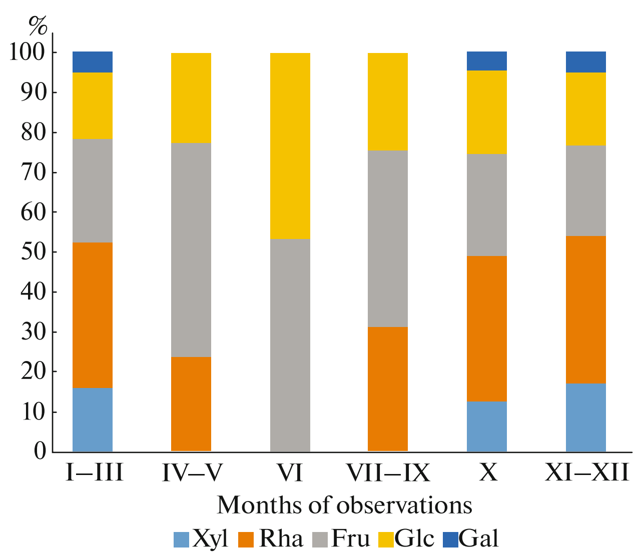

Fig. 4. Quantitative ratio of individual saccharides in the WSPS complex of *U. laciniata*, % of the pool of the monosaccharide complex of the bark and bast.

With the beginning of the growing season (IV–V), the composition of the hydrolyzed fraction of WSPS changes significantly: Xyl and Gal were not detected; 53.16% of the total amount of monosaccharides is Fru; 22.34% is Glc; and Rha was detected in an amount of 23.87%.

CONCLUSIONS

According to research on the role of elm bark in the nutrition of *C. elaphus xanthopygus*, it is a strategically important food resource, especially in the autumn and spring periods. Study of the content and composition of water-soluble polysaccharides of the bark and bast of *U. laciniata*, growing in the southern part of the Russian Far East, showed that two maxima of WSPS accumulation are observed: during the period of shoot growth (June) and a slightly smaller one during the period of preparation for winter dormancy (October). The minimum WSPS content is observed during the beginning of the growing season (April–May). In the WSPS fraction, five monosaccharides are recorded throughout the year: for periods characterized by positive temperatures, a limited monosaccharide composition is noted (Fru, Glc, Rha), and Xyl and Gal are detected in periods with negative temperatures. The quantitative content of pectins also changes during the year: a minimum is recorded during the beginning of the growing season, and an unexpressed maximum, during the latent growth period. It should be noted that the minimum and maximum values of the pectin content are close, which reflects a variation coefficient corresponding to a very low degree of variability. The conducted studies of the dynamics of accumulation and content of WSPS and PSs in the bark of lobed elm may explain the feeding behavior of red deer living in the forests of Primorskii krai.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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