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RESEARCH PAPERS

# Content and Composition of Lipids and Their Fatty Acids in Needles of *Pinus sylvestris* L. and *Picea obovata* Ledeb. upon Cold Hardening in the Cryolithozone of Yakutia

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**Abstract**—The composition of lipids and fatty acids (FAs) and changes in their content in needles of summer- and autumn-vegetating conifer trees growing in the cryolithozone of Yakutia have been studied by thin-layer and gas-liquid chromatography–mass spectrometry. A comparative analysis of the content of total lipids (TL) and phospholipids (PL) has been carried out, and the FA composition of TL in needles of *Pinus sylvestris* L. and *Picea obovata* Ledeb. has been determined for summer and autumn periods. In the course of adaptation to low autumn temperatures of the Yakutian cryolithozone, the TL content in needles of *P. sylvestris* and *P. obovata* significantly (by 30%) increased compared to the summer season. During this period, the phosphatidylcholine content in needles of both species also increased from 13.8 to 31 mg/g dry wt. For both species, the FA lipid composition of needles included a high content of species-specific unsaturated polymethylene-interrupted fatty acids (Δ5-UPIFA). Increase in the content of TL, PL, total FA, and Δ5-UPIFA observed during a temperature drop significantly exceeds that in plants of these species growing in other parts of Siberia. This fact is probably caused by features of low-temperature adaptation of plants in permafrost ecosystems of Yakutia.

*Keywords: Pinus sylvestris*, *Picea obovata*, conifers, needles, lipids, phospholipids, fatty acids, Δ5-unsaturated polymethylene-interrupted fatty acids, low temperatures, cryolithozone

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## INTRODUCTION

The climate of Yakutia, which is situated in the permafrost zone, is characterized by the maximum amplitudes of seasonal temperature variations, which did not occur in any other place of the world. In this region, plants grow under very peculiar and almost unique conditions determined by a combination of permafrost with a high-level solar radiation, low water content in the soil and air (especially in the first half of a vegetation period), and long and hot days with sharp drops of night temperatures occurring during late spring and early autumn frosts compared to the relatively warm but short summer. The wintering of perennial plants in this region is characterized by winter temperatures, which are extremely low and very unique for the Northern Hemisphere (up to −55...−60°C with the average winter temperature below −42.5°C). The survival of tree plant forms under such conditions, as well as their ability to withstand severe low-temperature stress, which represents the main selective factor for adaptation of indigenous species, is a unique phenomenon. This survival under extremely low temperatures is provided by the maintenance of the optimal physiological activity of cell membranes in needle tissues and depends on features of their lipid- and fatty-acid- (FA) composition. The qualitative composition and structure of saturated and unsaturated FAs in membrane lipids provides information about the presence and activity of membrane desaturases catalyzing introduction of a double bond into a hydrocarbon FA chain [1, 2]. In many higher plants, introduction of the first double bond during biosynthesis of unsaturated FAs is realized by a soluble stearoyl-acyl carrier protein desaturase (SAD) [3, 4]. Introduction of the second and third double

*Abbreviations:* FA—fatty acid; FAME—FA methyl esters; DPG—diphosphatidylglycerol; PA—phosphatidic acid; PC—phosphatidylcholine; PE—phosphatidylethanolamine; PI—phosphatidylinositol; PG—phosphatidylglycerol; PL—membrane phospholipids; PUFA—polyunsaturated FA; LDR—linoleyl desaturase ratio; ODR—oleyl desaturase ratio; SDA—stearoyl-acyl desaturase; TL—total lipids; Δ5-UPIFA—Δ5-unsaturated polymethylene-interrupted FAs.

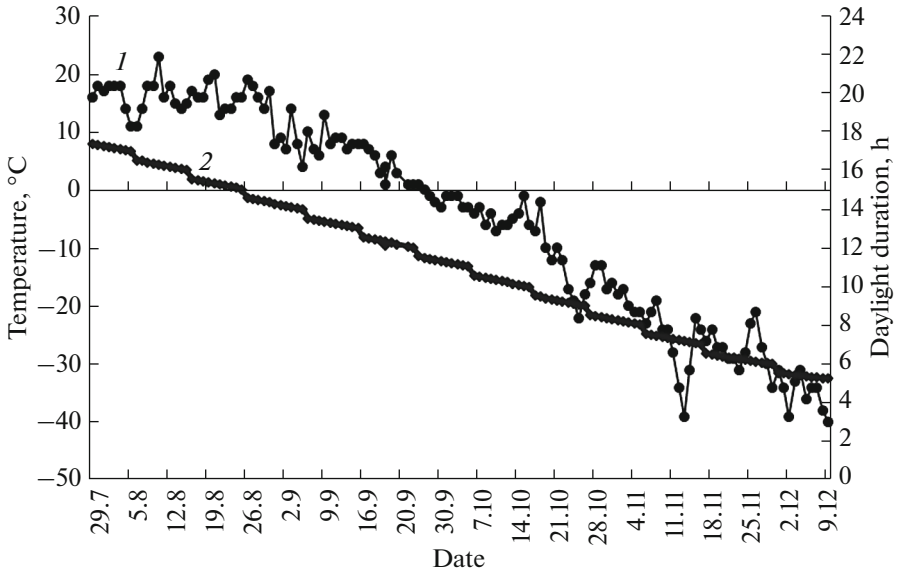


**Fig. 1.** Seasonal changes in air temperature (*1*) and daylight duration (*2*) in Central Yakutia (62°15′ N, 129°37′ E) in 2014. Air temperature is represented by mean daily values.

bonds into 18C-unsaturated FAs in chloroplast membranes is performed by acyl-lipid ω-6 (*Fad5* and *Fad6*) and ω-3 (*Fad7* and *Fad8*) membrane desaturases [4–6].

In spite of a good understanding of biochemical processes providing adaptive changes in the content and composition of a membrane lipid complex in some cultivated plant species (Arabidopsis, maize, tobacco, etc.) in response to low temperatures, such processes in tree plant species still remain poorly studied. Temperature-dependent changes in the FA composition of plant lipids represent a common phenomenon and permanently occur in different plant habitats, including unusual permafrost and temperature conditions of Yakutia. Therefore, investigation of qualitative and quantitative changes in the composition of total lipids (TL), membrane phospholipids (PL), and FAs of TL, which occur in the summer–autumn period in needles of the basic forest-forming tree species of the region (Scotch pine, *Pinus sylvestris* L., and Siberian spruce, *Picea obovata* Ledeb.), is of special interest.

The purpose of this study was a comparative analysis of the content and composition of TLs, PLs, and also the FA composition of TLs in needles of *P. sylvestris* and *P. obovata* under cryolithozone conditions of Yakutia.

## MATERIALS AND METHODS

**Plant material and growing conditions.** The objects of the study were second-year needles of *Pinus sylvestris* L. and *Picea obovata* Ledeb. trees growing in Central Yakutia. The needles were collected from the well-illuminated middle part of the crown. The collection of field materials was carried out from tree samples of each species grown in permanent sample areas of the forest park zone of the Botanical Garden of the Institute of Biological Problems of the Cryolithozone (Siberian Branch, Russian Academy of Sciences) located on the second terrace above a floodplain of the Lena river, seven kilometers to the west of Yakutsk (62°15′ N, 129°37′ E). The age of *P. sylvestris* and *P. obovata* trees included in the study was 60–65 years, and the height was approximately 12–13 m.

Air temperature at the sample areas was registered using DS 1922 LiBitton thermographs (DallasSemiconductor, United States). The measurements were performed every hour with the accuracy of ±0.5°C (Fig. 1). The average air temperature during the vegetation period (May–September) slightly exceeded 14°C, and the total precipitation was 163 mm. During winter, the minimal air temperature did not fall below −48°C. The snow depth in December–January was 44–48 cm. Weather conditions during the experiment were typical for Central Yakutia. First frosts were observed in the middle of the third decade of September, while the stable transition of night temperatures below 0°C was registered in the beginning of October.

**Extraction and analysis of total lipids.** For lipid extraction, a sample of a plant material (0.5 g) was fixed in liquid nitrogen. After the addition of 0.001% ionol, the sample was ground to obtain a homogeneous mass. Then 10 mL of a chloroform : methanol mixture (1 : 2, v/v) was added, and the solution was thoroughly mixed and left for 30 min to provide a complete diffusion of lipids into a solvent. The solution was quantitatively transferred into a separating funnel through a filter with triple washing of the mortar and the filter with the same mixture of solvents. To remove nonlipid components, some water was added.

For the TL analysis, the bottom chloroform fraction was separated. Chloroform (research grade, stabilized with 0.005% amylene) was removed from the lipid extract under a vacuum using a RVO-64 rotary evaporator (Czech Republic). To check the lipid extraction efficiency (%), the known amount of nonadecanoic acid ($C_{19:0}$) was added at the homogenization stage. FA methyl esters (FAME) were obtained from the extract according to the Christie method [7]. Additional FAME purification was carried out by TLC on a glass plates with silica gel (KSK, Russia) placed in a chamber with benzene as a mobile phase. To visualize the FAME zone ($R_f$ = 0.71–0.73), plates were sprayed with 10% $H_2SO_4$ solution in MeOH and heated in a drying chamber at 100°C. The FAME zone was removed from a plate with a spatula, eluated from the silica gel by *n*-hexane, and analyzed by gas-liquid chromatography using a 5973/6890N MSD/DS chromatography-mass spectrometer (Agilent Technologies, United States) equipped with a quadrupole mass spectrometer as a detector. Ionization was performed by the electron impact, and the ionization energy was 70 eV. The analysis was performed in the total ion current registration mode.

The FAME mixture was separated using a HP-INNOWAX capillary column (30 m × 250 μm × 0.50 μm) with polyethylene glycol as a stationary phase. Helium was used as a carrier gas, and the flow rate was 1 mL/min. The temperatures of the evaporator, ion source, and detector were 250, 230, and 150°C, respectively; the temperature of the AUX line was 280°C. The scanning range was 41–450 a.m.u. The volume of injection was 1 μL, and the flow divider was 5 : 1. A FAME mixture was separated under isothermal conditions at 200°C. FAs were identified using a NIST 08 mass spectral library and the Christie archive of FAME mass spectra [8]. The relative FA content was determined by the internal normalization method via weight percent (wt, %) of their total content in a sample with allowance for the FA response coefficient.

The level of lipid unsaturation was characterized using an unsaturation coefficient (*k*) and a double bond index (DBI) [9]:

$$k = \sum P_{\text{unsaturated}} \Big/ \sum P_{\text{saturated}}, \quad (1)$$

$$\text{DBI} = \sum P_j n_j / 100, \quad (2)$$

where $P$ and $P_j$ represent the FA content, %, and $n_j$ is the number of double bonds in each FA.

Activity of acyl-lipid ω9-, ω6-, and ω3-membrane desaturases responsible for the introduction of double bonds into hydrocarbon chains of oleic (C18:1(n-9)), linoleic (C18:2(n-6)), and α-linolenic (C18:3(n-3)) FAs was calculated as the stearoyl- (SDR), oleyl- (ODR), and linoleyl- (LDR) desaturase ratios [10] using the following formulas:

$$\text{SRD} = (\%\text{C18:1}) / (\%\text{C18:0} + \%\text{C18:1}), \quad (3)$$

$$\text{ODR} = (\%\text{C18:2} + \%\text{C18:3}) / (\%\text{C18:1} + \%\text{C18:2} + \%\text{C18:3}), \quad (4)$$

$$\text{LDR} = (\%\text{C18:3}) / (\%\text{C18:2} + \%\text{C18:3}). \quad (5)$$

Quantification of FLs and their division into separate lipids were carried out by a two-dimensional TLC using two solvent systems. For the first direction, the solvent system was chloroform : methanol : benzene : 28% ammonia (water solution) at a ratio of 65 : 30 : 10 : 6. For the second direction, a mixture of chloroform : methanol : acetic acid : acetone : benzene : water (70 : 30 : 4 : 5 : 10 : 1) was used. Detection and identification of FLs in plant material was performed using specific reagents, such as molybdenum blue for phosphorous-containing components [11], Dragendorff's reagent prepared according to Wagner et al. [12] for choline-containing lipids, and 0.2% ninhydrin solution in acetone for amine-containing lipids [13]. The PL content was quantified according to the Vaskovsky's method [11].

**Statistical data treatment**. The data shown in the tables represent means and standard deviations calculated for 3–6 biological replications. Experimental data were statistically analyzed using the Microsoft Office Excel 2010 software package. A statistical significance of differences between the compared mean values was evaluated by a *t*-test ($P < 0.05$).

## RESULTS AND DISCUSSION

Assessment results of summer and autumn–winter changes of the absolute TL content in needles of *P. sylvestris* and *P. obovata* under conditions of Central Yakutia are shown in Table 1. From summer to winter periods, the TL content in needles of *P. sylvestris* was one and a half times higher on average than that in *P. obovata*; the maximum (1.6×) difference between the species was observed for August samples (after the termination of the bud mass increase stage). The higher content of lipids in *P. sylvestris* was found to be species-specific. During vegetation, the TL content in needles of *P. sylvestris* changed from 211.7 ± 9.5 (July) to 314.5 ± 18.6 (December) mg/g dry wt reaching a 1.5-time increase. The relative TL accumulation in needles of *P. obovata* during the same period increased 1.4 times (from 145.8 ± 8.4 to 204.9 ± 12.3 mg/g dry wt). In September, when conifer trees came into the first cold-hardening phase characterized by lowered and low positive air temperature

**Table 1.** Seasonal changes in the total lipid (TL) content in needles of *Pinus sylvestris* and *Picea obovata* (mg/g dry wt)

| Sampling date | Developmental stage; hardening phase | TL content | |
|---|---|---|---|
| | | *Pinus sylvestris* | *Picea obovata* |
| 29.07 | Bud breaking; beginning of extra-bud growth | 211.7 ± 9.5 | 145.8 ± 8.4 |
| 05.08 | Bud mass increase; beginning of the organic dormancy phase | 225.6 ± 8.7 | 139.2 ± 8.9 |
| 20.09 | I cold hardening phase | 273.1 ± 10.7 | 189.1 ± 11.2 |
| 09.12 | Induced dormancy | 314.5 ± 18.6 | 204.9 ± 12 |

$n = 3$, the differences are significant for all variants ($P < 0.05$).

(2°C) and daylight reduction (Fig. 1), and also at the beginning of the dormancy period, the TL content in photosynthesizing organs of pine and spruce trees increased 1.3 times compared to the summer season.

In general, TL content in needles of Scotch pine and Siberian spruce growing in Central Yakutia demonstrated a regular tendency to increase from summer to autumn (Table 1), since lipid accumulation indicates plant adaptation to low-temperature stress. Our results correspond to the earlier-obtained data that the TL content in *Larix gmelinii* Rupr., *L. sibirica* Ledeb., and *Picea obovata* grown in Krasnoyarsk krai and Eastern Siberia increased in the autumn period by ~20% compared to the summer [14, 15]. At the same time, in the case of a cryolithozone, a more significant increase (30%) in the TL content in spruce needles was observed.

Analysis of a qualitative PL composition in needles of the studied species showed PC to be the basic PL group in plants during the studied period (Figs. 2 and 3). The PC content in *P. sylvestris* exceeded that in *P. obovata* that probably represents a species-specific feature. In autumn, when growth processes are completed and metabolism intensity decreases, the PC content in needles of *P. sylvestris* and *P. obovata* increased in 2.2 and 1.4 times, respectively, as compared with the summer values. Such increase in the PC content during the formation of cryoresistance of plant cells is probably one of the mechanisms allowing a plant to escape the transition of the lipid bilayer of separate membrane regions into an inverted hexagonal phase. A large polar head of strongly hydrated PC provides a lower temperature of its phase transition during hydratation caused by extracellular ice formation. As the temperature and illumination decreased, the PI content in needles of *P. obovata* increased by up to 2.9 mg/g dry wt compared to the summer period. In needles of *P. sylvestris*, the PI content during the summer–autumn period varied from 9.6 to 11.2 mg/g dry wt. The content of PG in *P. sylvestris* was high in summer, which was probably provided by the maximum chlorophyll concentration in needles during this period [16]. No significant difference in the PE content in *P. sylvestris* was observed between the summer and autumn periods (10.6–11.5 mg/g dry wt). At the same time, the PE content in needles of *P. obovata* in autumn was 1.2 times lower than in summer. *P. sylvestris* in autumn was characterized by a heightened PA content, while the same parameter in *P. obovata* did not change at the lowered temperature and made approximately 5.3 mg/g of dry wt (8–9% of the total PL content). The DPG content in *P. sylvestris* exceeded that in *P. obovata* reaching

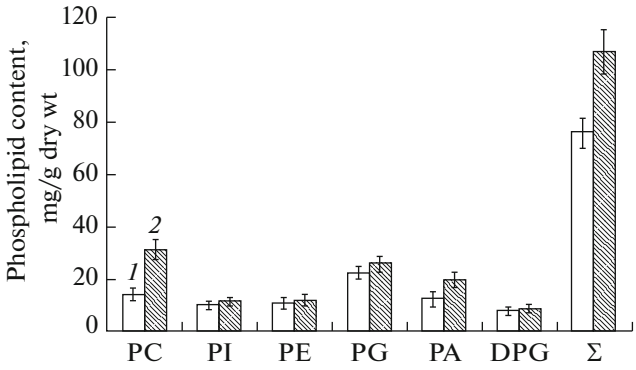


**Fig. 2.** Changes in the content of individual phospholipids in needles of *Pinus sylvestris* during summer (*1*) and autumn (*2*) periods. PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; DPG, diphosphatidylglycerol; Σ, total phospholipids.

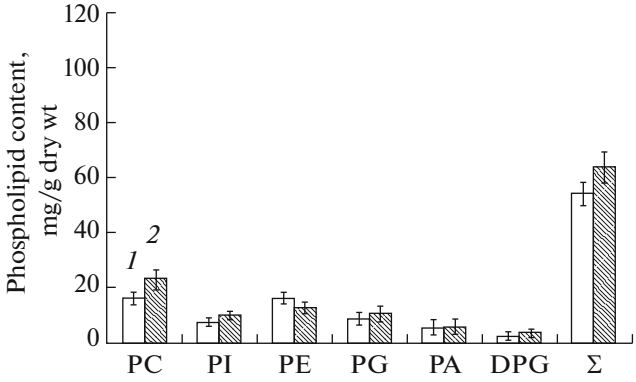


**Fig. 3.** Changes in the content of individual phospholipids in needles of *Picea obovata* during summer (*1*) and autumn (*2*) periods. PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PA, phosphatidic acid; DPG, diphosphatidylglycerol; Σ, total phospholipids.

**Table 2.** Changes in the fatty acid (FA) composition of total lipids in needles of *Pinus sylvestris* from Central Yakutia occurring in the summer–autumn period

| Fatty acids | Summer period (29.07) | | Autumn period (20.09) | |
|---|---|---|---|---|
| | mg/g dry wt | % of the total FA | mg/g dry wt | % of the total FA |
| C14:0 | 5.3 ± 0.9 | 2.5 ± 0.5 | – | – |
| C15:0 | 0.5 ± 0.1 | 0.2 ± 0.0 | 0.7 ± 0.1 | 0.3 ± 0.0 |
| C16:0 | 41.9 ± 4.9 | 19.8 ± 1.7 | 54.7 ± 3.6 | 20.0 ± 0.2 |
| C16:1(n-9) | 0.6 ± 0.1 | 0.3 ± 0.1 | 1.0 ± 0.5 | 0.4 ± 0.1 |
| C16:1(n-7) | 0.5 ± 0.0 | 0.3 ± 0.0 | – | – |
| C16:1(n-5) | 2.7 ± 0.4 | 1.3 ± 0.1 | 1.1 ± 0.1 | 0.4 ± 0.0 |
| C17:0-a | 2.5 ± 0.2 | 1.2 ± 0.1 | 5.2 ± 0.3 | 1.9 ± 0.0 |
| C17:0 | 1.1 ± 0.1 | 0.5 ± 0.0 | 1.2 ± 0.1 | 0.4 ± 0.0 |
| C16:3(n-3) | 1.0 ± 0.2 | 0.5 ± 0.1 | 2.5 ± 0.8 | 0.9 ± 0.2 |
| C18:0 | 6.0 ± 0.7 | 2.8 ± 0.2 | 5.8 ± 0.1 | 2.1 ± 0.1 |
| C18:1(n-9) | 14.8 ± 1.1 | 7.0 ± 0.6 | 17.0 ± 0.7 | 6.2 ± 0.1 |
| C18:1(n-7) | 1.8 ± 0.2 | 0.8 ± 0.1 | 2.9 ± 0.2 | 1.1 ± 0.1 |
| C18:2(Δ5,9) | 0.9 ± 0.2 | 0.4 ± 0.1 | 1.1 ± 0.0 | 0.4 ± 0.0 |
| C18:2(n-6) | 47.6 ± 1.9 | 22.5 ± 0.7 | 61.6 ± 3.1 | 22.6 ± 0.1 |
| C18:3(Δ5,9,12) | 9.7 ± 1.9 | 4.6 ± 1.0 | 24.3 ± 1.2 | 8.9 ± 0.9 |
| C18:3(n-3) | 55.1 ± 4.2 | 26.0 ± 1.1 | 47.5 ± 2.2 | 17.4 ± 0.8 |
| C18:4(Δ5,9,12,15) | 2.9 ± 0.4 | 1.3 ± 0.2 | 6.8 ± 0.4 | 2.5 ± 0.3 |
| C20:0 | 1.4 ± 0.2 | 0.7 ± 0.1 | 4.5 ± 0.3 | 1.6 ± 0.2 |
| C20:1(n-11) | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.9 ± 0.1 | 0.3 ± 0.0 |
| C20:2(n-9) | 0.9 ± 0.1 | 0.4 ± 0.0 | 4.1 ± 0.2 | 1.5 ± 0.2 |
| C20:3(Δ5,11,14) | 9.8 ± 0.6 | 4.6 ± 0.3 | 16.3 ± 0.8 | 6.0 ± 0.8 |
| C20:3(Δ7,11,14) | 0.9 ± 0.0 | 0.4 ± 0.0 | 3.1 ± 0.0 | 1.1 ± 0.4 |
| C20:3(Δ11,14,17) | 0.4 ± 0.1 | 0.2 ± 0.0 | 1.6 ± 0.0 | 0.6 ± 0.1 |
| C20:4(Δ5,11,14,17) | 1.4 ± 0.1 | 0.6 ± 0.0 | 2.4 ± 0.0 | 0.9 ± 0.1 |
| C22:0 | 1.7 ± 0.1 | 0.8 ± 0.0 | 6.8 ± 0.7 | 2.5 ± 0.3 |
| $\Sigma_{\Delta 5\text{-UPIFA}}$ | 24.6 ± 1.2 | 11.6 ± 0.7 | 50.9 ± 2.1 | 18.6 ± 0.9 |
| Σ | 211.7 ± 9.5 | 100.0 | 273.1 ± 10.7 | 100.0 |
| $\Sigma_{\text{saturated}}$ | 60.4 ± 5.5 | 28.5 ± 1.7 | 78.9 ± 5.1 | 28.9 ± 1.1 |
| $\Sigma_{\text{unsaturated}}$ | 151.3 ± 5.8 | 71.5 ± 1.7 | 194.3 ± 10.0 | 71.1 ± 0.9 |
| *k* | | 2.51 ± 0.21 | | 2.46 ± 0.02 |
| DBI | | 1.73 | | 1.75 |
| SDR | | 0.71 ± 0.04 | | 0.74 ± 0.01 |
| ODR | | 1.47 ± 0.01 | | 0.87 ± 0.02 |
| LDR | | 0.54 ± 0.02 | | 0.44 ± 0.02 |

"–", not detected; $\Sigma_{\text{saturated}}$, total saturated FA; $\Sigma_{\text{unsaturated}}$, total unsaturated FA; *k*, unsaturation coefficient; DBI, double bond index. The data represent mean values and their standard deviations calculated for 3–6 biological repetitions.

7–8 mg/g dry wt in the summer–autumn period; in the case of *P. obovata,* this parameter significantly increased during the autumn cold hardening and exceeded its summer value 1.7 times. In general, the autumn increase of the total PL was especially noticeable in needles of *P. sylvestris*: the summer value of this parameter was 75.6, while it reached 106.7 mg/g dry wt in autumn. In the case of *P. obovata*, this change was less noticeable.

Since reliable data on the dynamics of changes in the FA composition during cold hardening require determination of the absolute FA content per dry weight rather than only FA percentages [17, 18], we determined both these indices. In total, lipids from *P. sylvestris* and *P. obovata* included 25 and 26 FAs, respectively (Tables 2 and 3). Palmitic acid (C16:0) prevailed among saturated FAs; its relative content during summer and autumn periods varied from 16.32 to 20.02%. Among unsaturated FAs, linoleic (C18:2) and linolenic (C18:3) acids prevailed, as well as FAs of the Δ-5 type, which are observed in conifer plants and some other ancient taxa. The content of oleic acid (C18:1) in *P. obovata* doubled in autumn compared to the summer period. Oleic acid is a substrate for biosynthesis of linoleic, α-linolenic, and Δ5-unsaturated polymethylene-interrupted FAs (Δ5-UPIFA) in cell

**Table 3.** Changes in the fatty acid (FA) composition of total lipids in needles of *Pícea obovata* from Central Yakutia occurring in the summer–autumn period

| Fatty acids | Summer period (29.07) | | Autumn period (20.09) | |
|---|---|---|---|---|
| | mg/g dry wt | mg/g dry wt | mg/g dry wt | % of the total FA |
| C12:0 | 1.5 ± 0.2 | 1.0 ± 0.1 | 2.7 ± 0.6 | 1.4 ± 0.4 |
| C14:0 | 4.4 ± 0.9 | 3.0 ± 1.1 | 4.8 ± 0.3 | 2.5 ± 0.1 |
| C15:0 | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.4 ± 0.1 | 0.2 ± 0.0 |
| C16:0 | 23.8 ± 2.9 | 16.3 ± 1.5 | 33.0 ± 3.7 | 17.4 ± 0.5 |
| C16:1(n-9) | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.7 ± 0.1 | 0.4 ± 0.0 |
| C16:1(n-7) | 0.3 ± 0.0 | 0.2 ± 0.0 | – | – |
| C16:1(n-5) | 1.2 ± 0.1 | 0.8 ± 0.1 | 1.5 ± 0.3 | 0.8 ± 0.1 |
| C17:0-a | 4.9 ± 0.1 | 3.4 ± 0.3 | 6.2 ± 0.4 | 3.3 ± 0.0 |
| C17:0 | 1.4 ± 0.2 | 1.0 ± 0.3 | 1.4 ± 0.1 | 0.7 ± 0.1 |
| C16:3(n-3) | 0.7 ± 0.1 | 0.5 ± 0.1 | 0.9 ± 0.1 | 0.5 ± 0.0 |
| C18:0 | 3.1 ± 0.0 | 2.1 ± 0.2 | 7.4 ± 1.1 | 3.9 ± 0.2 |
| C18:1(n-9) | 12.1 ± 2.7 | 8.3 ± 2.0 | 19.1 ± 1.7 | 10.1 ± 1.9 |
| C18:1(n-7) | 1.3 ± 0.1 | 0.9 ± 0.0 | 1.4 ± 0.2 | 0.7 ± 0.1 |
| C18:2(Δ5,9) | 2.7 ± 0.6 | 1.9 ± 0.8 | 2.4 ± 0.6 | 1.3 ± 0.4 |
| C18:2(n-6) | 23.3 ± 2.7 | 16.0 ± 1.4 | 31.4 ± 2.9 | 16.6 ± 1.3 |
| C18:3(Δ5,9,12) | 7.1 ± 1.4 | 4.9 ± 1.0 | 10.2 ± 1.9 | 5.4 ± 1.1 |
| C18:3(n-3) | 40.3 ± 1.3 | 27.6 ± 3.5 | 44.6 ± 4.1 | 23.6 ± 2.1 |
| C18:4(Δ5,9,12,15) | 5.0 ± 0.3 | 3.4 ± 0.6 | 5.0 ± 0.8 | 2.6 ± 0.5 |
| C20:0 | 1.0 ± 0.6 | 0.7 ± 0.5 | 2.0 ± 0.3 | 1.1 ± 0.6 |
| C20:1(n-11) | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.4 ± 0.0 | 0.2 ± 0.0 |
| C20:2(n-9) | 0.9 ± 0.0 | 0.6 ± 0.1 | 1.1 ± 0.1 | 0.6 ± 0.1 |
| C20:3(Δ5,11,14) | 5.7 ± 0.4 | 3.9 ± 0.3 | 7.1 ± 0.4 | 3.7 ± 0.7 |
| C20:3(Δ7,11,14) | 0.8 ± 0.2 | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.4 ± 0.0 |
| C20:3(Δ11,14,17) | 0.3 ± 0.0 | 0.2 ± 0.0 | 0.4 ± 0.0 | 0.2 ± 0.0 |
| C20:4(Δ5,11,14,17) | 1.7 ± 0.2 | 1.1 ± 0.3 | 1.7 ± 0.1 | 0.9 ± 0.1 |
| C22:0 | 1.3 ± 0.8 | 0.9 ± 0.3 | 2.5 ± 0.8 | 1.3 ± 0.2 |
| $\Sigma_{\Delta 5\text{-UPIFA}}$ | 22.2 ± 1.8 | 15.3 ± 1.2 | 26.3 ± 1.9 | 13.9 ± 1.4 |
| $\Sigma$ | 145.8 ± 8.4 | 100.0 | 189.1 ± 11.2 | 100.0 |
| $\Sigma_{\text{saturated}}$ | 41.8 ± 3.4 | 28.6 ± 1.6 | 60.5 ± 5.5 | 32.0 ± 1.8 |
| $\Sigma_{\text{unsaturated}}$ | 104.0 ± 5.0 | 71.4 ± 1.9 | 128.6 ± 10.3 | 68.0 ± 2.1 |
| *k* | | 2.49 | | 2.13 |
| DBI | | 1.79 | | 1.65 |
| SDR | | 0.79 | | 0.72 |
| ODR | | 0.84 | | 0.80 |
| LDR | | 0.63 | | 0.59 |

"–", not detected; $\Sigma_{\text{saturated}}$, total saturated FA; $\Sigma_{\text{unsaturated}}$, total unsaturated FA; *k*, unsaturation coefficient; DBI, double bond index. The data represent mean values and their standard deviations calculated for 3–6 biological repetitions.

membranes of needles of conifer plants [19]. Increase in the absolute content of oleic acid (C18:1) observed in the autumn period was accompanied by a simultaneous increase in the content of arachidonic (C20:0) and behenic (C22:0) acids from 0.9 to 4.5%. Both C20:0 and C22:0 FAs are present in photosynthesizing tissues of almost all gymnosperms in contrast to angiosperms. The seasonal dynamics of the absolute FA content in TL showed a noticeable tendency to the growth of this parameter from the summer to autumn period. For example, the total FA content in needles of *P. obovata* measured in September exceeded the same value observed in July by 43 mg/g; in the case of *P. sylvestris*, it was 1.3 times higher than in the summer period. The maximum content of unsaturated FAs was also observed in the autumn period, when their biosynthesis is influenced by both low temperatures and low illumination. These two environmental factors inhibit photosynthesis in chloroplast membranes of needles and, probably, result in the increase in the absolute content of α-linolenic acid (C18:3 n-3) in chloroplasts during plant adaptation to such conditions [20]. According to some data obtained for conifer plants (including *P. sylvestris*) of Central Siberia, lipids

of winter-hardy plants, including tree species, are characterized by an increased content of polyunsaturated FAs, such as linoleic and linolenic acids [21]. The absolute content of the total essential saturated FAs in *P. sylvestris* significantly varied across the seasons (60.4–78.9 mg/g dry wt), while these changes were less expressed in *P. obovata*. In contrast to summer values, no C14:0 (myristic acid) and C16:1(n–7) (palmitoleic acid isomer) were observed in plant tissues in autumn.

One of the important characteristics of lipids from conifer needles is the presence of "relic" Δ5-UPIFA. During a low-temperature (autumn) period, the absolute content of Δ5-UPIFA in needles of *P. sylvestris* increased 2.06 times reaching 50.9 mg/g dry wt, while this index reached 26.3 mg/g dry wt in *P. obovata*. Calculation of SDR, ODR, and LDR desaturase ratios characterizing the activity of acyl-lipid ω9-, ω6-, and ω3-desaturases showed that the ODR value varied from 0.87 to 1.47 in both tree species, whereas SDR and LDR values varied from 0.44 to 0.74, which indicated a higher activity of oleate desaturase probably caused by a higher expression level of a *fad*2 gene encoding chloropolast ω6-desaturase in evergreen conifers [22].

Lipid composition of needles was characterized by a high diversity of unsaturated FAs with the carbohydrate chain length C ≥ 20, which was provided by elongation of molecules of oleic and linoleic acids with the formation of C20:1 (eicosenoic acid), C20:2 (eicosadienoic acid), and other FAs. The FA range in *P. sylvestris* and *P. obovata* included the following Δ5 acids: taxoleic—C18:3(Δ5,9); pinolenic—C18:3(Δ5,9,12); coniferonic—C18:4(Δ5,9,12,15); sciadonic—C20:3(Δ5,11,14); and juniperonic—C20:4(Δ5,11,14,17). The dynamics of the Δ5-UPIFA content is similar to the general changes in the total unsaturated FA content. According to the existing data [15, 22], *P. sylvestris* and *P. obovata* growing in Eastern Siberia are characterized by a significantly lower content of Δ5-UPIFA than plants of the same species growing in the cryolithozone of Yakutia. This difference reached 27.5 and 12 mg/g dry wt in *P. sylvestris* and *P. obovata,* respectively. A high content of these acids in chloroplast membrane lipids of *P. sylvestris* and *P. obovata* needles is usually considered to be associated with cold adaptation of plants [23–25]. In autumn, lowered illumination, together with low temperatures, represents a stress factor for needles of evergreen plants of Central Yakutia and results in increase in the content of almost all unsaturated FAs in the TL composition. Judging by the published data, such increase in the autumn period is determined by both the beginning of deep physiological dormancy (first cold hardening stage) and reorganization of chloroplast membranes in needles caused by illumination and temperature decrease and occurred due to the increase in the surface area of grana, number of thylakoids per granum, and the total number of thylakoids per chloroplast [22]. The physiological role of some unsaturated FAs in both chloroplast and microsomal membranes in conifer trees still remains unclear in many respects. Cytochrome $b_5$, which represents one of the key enzymes in the PUFA biosynthesis in animal and plant cells, plays an important role in these processes [26]. As we mentioned earlier, Δ5-UPIFA probably play a crucial role in the cold adaptation of plants, which is evidenced by the replacement of α-linolenic acid with pinolenic acid in the lipid composition of conifer trees' seeds. It is proposed that pinolenic acid may be equivalent to γ-linolenic acid as an intermediate link preserved in the course of evolution for the biosynthesis of arachidonic and eicosapentaenoic acids with the further changes from Δ6,9,12-C18 (pteridophytes) to Δ5,9,12-C18 (conifers). Significant amounts of arachidonic and eicosapentaenoic acids are observed in microorganisms, mosses, lichens, fungi, and animals [27], but they are hardly revealed in lipids of conifer seeds and needles [26, 28]. The evolutionary order of metabolic pathways of these FAs is clearly traced from lower to higher plants [29]. The PUFA biosynthesis in lower plants follows a ω6 pathway with the formation of linoleic–γ-linolenic–arachidonic–eicosapentaenoic acids, whereas the PUFA biosynthesis in higher plants follows a ω3-pathway with the formation of linoleic and α-linolenic acids and possible exclusion of the arachidonic acid stage preceding the synthesis of eicosapentaenoic acid [30].

Thus, the revealed features typical for needles of evergreen plants of Yakutia include (a) high absolute content of TLs, PLs, and FAs, especially their unsaturated forms, which influence the stabilization of cell membranes, and (b) a high diversity and a high content of unique Δ5-UPIFA. These features may play a significant role in a formation of resistance of such tree species to low-temperature (up to –60°C) stress under permafrost conditions.

## COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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