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COMPARATIVE CHARACTERISTICS OF BUD MERISTEM LIPIDS FROM *Picea obovata* AND *Pinus sylvestris*

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It was shown that the lipid content of Pinus sylvestris *L. was greater than that of* Picea obovata *L. The difference was 43–45% of total lipids in winter. Polar fractions dominated the lipid composition of both conifers. The phospholipid content was greater than the glycolipid content in winter. A significant amount of glycolipids that was equal to that of phospholipids was present simultaneously in meristem of wintering pine buds. Swelling buds of both species showed in spring an elevated content of glycolipids.*

Key words: *Picea obovata* L., *Pinus sylvestris* L., buds, meristem tissue, lipids, fractional composition.

The main portion of the forestry resources of Krasnoyarsk Krai, which consists of about 61.3 million hectares, is conifer (boreal) forests. The pine family (Pinaceae) stands out in this territory with respect to the number of species and expanse of distribution. The output of conifer biomass production is determined to a large extent by the rate of biosynthesis of plant cell components. Lipids are one of the principal classes of biomolecules and play a main role in these processes. The direction of lipid exchange can to a certain degree be inferred from the change of lipid content and composition in plant organs and tissues in different seasons. Herein we present results from a study of structural and chemical changes of meristem cells of frost-resistant conifer species during the yearly cycle. In particular, we studied the seasonal dynamics of the lipid composition and content of bud meristem tissues (needle and runner embryonic tissues) of Siberian spruce (*Picea obovata* L.) and common pine (*Pinus sylvestris* L.).

Seasonal temperature variations drive the rhythm of exchange processes occurring under Central Siberian climatic conditions in the histogenetic centers of conifers. A study of the dynamics of the total lipid (TL) content in meristem from the time that vegetative runners stopped growing (August) until the emergence of young needles (May) showed (Fig. 1) that the amount of TL in the studied species varied considerably during the year. The difference between species in some periods was as much as 100%.

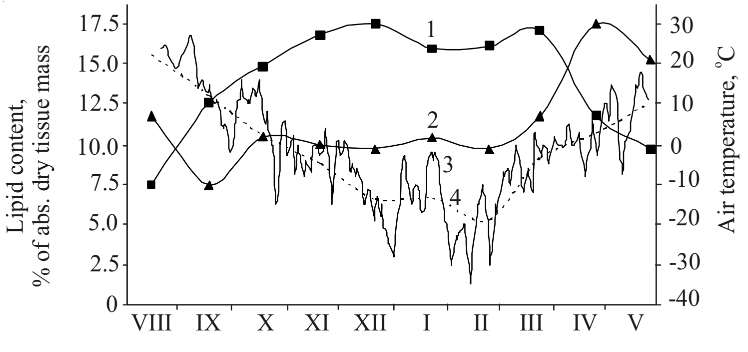

Fig. 1. Seasonal dynamics of total lipid content in bud meristem: pine (1), spruce (2), change of average daily air temperaure (3), change of average monthly air temperature (4).

GOU VPO Siberian State Technological University, 660049, Krasnoyarsk, prosp. Mira, 82, Russia, e-mail: AlaudinovaEV@yandex.ru; e-mail: pvm@sibstu.kts.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 666–669, November–December, 2009. Original article submitted June 18, 2009.

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TABLE 1. Fractional Composition of Bud Meristem Lipids, % of Abs. Dry Tissue Mass

Lipid fraction	Sample collection time, month									
	VIII	IX	X	XI	XII	I	II	III	IV	V
					Picea obovata L.					
NL	3.9±0.1	2.2±0.1	3.8±0.1	3.3±0.1	3.3±0.1	3.6±0.1	3.5±0.1	4.6±0.2	8.1±0.2	4.0±0.1
GL	3.5±0.1	1.6±0.1	0.8±0.1	1.0±0.1	0.9±0.1	0.8±0.1	0.7±0.1	2.0±0.1	4.5±0.1	5.5±0.2
PL	4.3±0.1	4.6±0.2	6.1±0.1	6.0±0.2	5.9±0.2	5.7±0.1	5.9±0.2	5.1±0.1	3.5±0.1	4.9±0.2
					Pinus sylvestris L.					
NL	3.5±0.1	4.6±0.2	4.8±0.2	5.2±0.2	5.3±0.2	5.3±0.1	5.7±0.2	6.2±0.3	6.0±0.2	3.2±0.1
GL	6.5±0.3	5.4±0.2	4.8±0.2	4.4±0.2	4.1±0.1	4.1±0.1	3.9±0.1	4.6±0.2	5.3±0.2	5.9±0.2
PL	3.8±0.2	6.1±0.2	7.5±0.3	8.4±0.4	8.9±0.4	8.2±0.3	8.1±0.2	7.3±0.3	4.4±0.2	5.7±0.2

The TL content in spruce, about 12% of the absolute (abs.) dry tissue mass, was high in August in formed buds after growth processes in the wood stopped. The reduction of the TL level to the minimum in September might have been due to their consumption for the synthesis of water-soluble cytoplasmic substances, the content of which for spruce increased dramatically in autumn [1, 2]. The reduction in this same period of lipid levels in buds of apple, poplar, and birch has already been studied [3–5]. We obtained analogous results previously [6] during a study of bud meristem of *Larix sibirica* L. The amount of TL in spruce bud meristem increased in October by about 20% of their September content despite the positive monthly average air temperatures (data from Krasnoyarsk Center of Hydrometerology and Environmental Monitoring). Their content remained relatively stable during the autumn-winter period (about 10% of abs. dry tissue mass). The TL level in meristem began to increase again in spring and reached the maximum (about 16% of abs. dry tissue mass) toward the end of April. This was associated with significant structural and chemical rearrangements in meristem cells in preparation for vegetation in addition to the need to store additional reserves of energetic substances for the future growth of needles. The reduction in the amount of TL in meristem during swelling and emergence of buds was explained by their consumption by intensifying growth processes. The nature of the TL dynamics and content in pine bud meristem differed substantially (Fig. 1).

The TL content was minimal at the end of August. The accumulation of TL in pine began when growth processes stopped and bud formation was finished even with positive monthly average temperatures at the start of September, reaching a maximum in December. The content in winter was relatively stable. The average winter TL content in pine was significantly (by about 43–45% of TL) greater than in spruce. It was assumed that the higher TL accumulation was due to morphological features of pine buds [7] and the involvement of certain lipids in the development of low-temperature resistance of meristem bud tissue associated with this. As a rule, researchers link a high lipid level in plants to a high resistance to hypothermia, i.e., they consider lipid accumulation to be one of the indicators of adaptation to low winter temperatures. However, pine is less frost-resistant than larch or spruce [8]. This suggests that these conifer species have different types of biochemical transformation of bud meristem tissue that are associated with lipids and produce similarly low-temperature resistance in the meristem. The TL content in pine started to decline at the end of March and continued up to the emergence of young needles. The lack of a spring TL maximum (Fig. 1) might have been due to the storage during autumn and winter of a reserve sufficient to support active spring growth processes.

It was necessary to study the fractional lipid composition because of the variety of functions carried out by different lipid groups in the living plant cell and the observed interspecies and seasonal differences in the TL content in spruce and pine. A review of the literature showed that existing information on the fractional composition of lipids from various organs and tissues of conifers has been obtained mainly from the study of samples with different compositions and anatomical structures (needles, whole buds, bark, roots, trunk wood, runners, cambial zone, etc.) [9–14]. In our instance, the study of bud meristem allowed all specifics of the lipid composition and its changes during the yearly cycle to be related only to living bud tissues. This means that all changes of cell membrane lipids can be interpreted with high reliability. Table 1 gives the fractional TL composition and its seasonal variations. Neutral lipids (NL), which most researchers consider to act as reserve compounds in cells, are the most available energy source supporting exchange processes in plant ontogenesis. However, the physiological role of NL cannot be only an energy reserve because these compounds can act as a source of structural elements such as glycerine and fatty acids, which are the initial products during biosynthesis of various compounds, including those forming the cryoprotective structure of plant cell membranes.

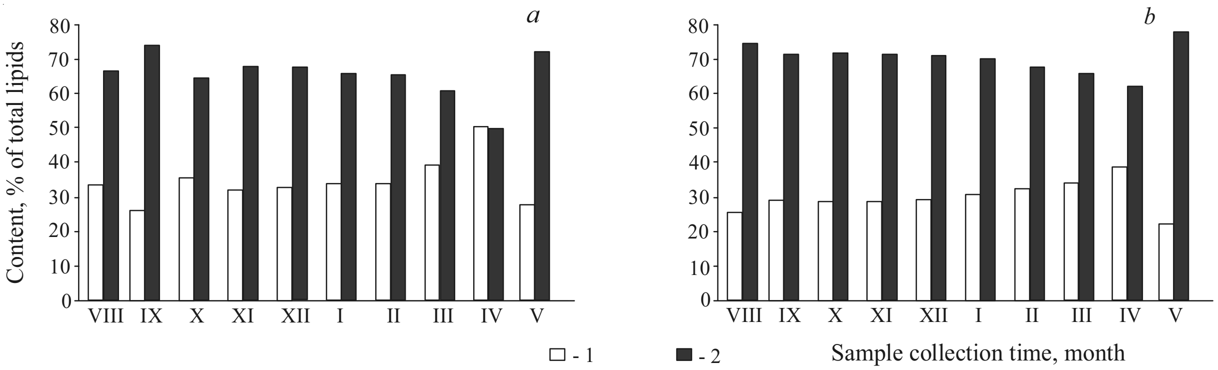

Fig. 2. Seasonal dynamics of neutral (1) and polar (2) lipids in bud meristem of Siberian spruce (*a*) and common pine (*b*).

The greatest variations of NL levels were observed in spruce bud meristem (Table 1). Their content in September decreased to the minimum (about 2.2%), which might have been due to consumption in various structural and chemical transformations that adapted the cell meristem to low winter temperatures. The amount of NL increased again in October to approximately their level in formed buds (August) and remained relatively stable until March. More NL were synthesized in preparation for vegetation. Their content reached a maximum (about 8%) in April. However, the content of this lipid fraction was reduced by half in swelling buds before the emergence of needles. The NL dynamics in pine bud meristem (Table 1) differed from that in spruce primarily by the lack of a distinct autumn minimum and spring maximum. A slight increase in their fraction in March-April was found only by calculating the NL content in total lipids (Fig. 2).

NL started to accumulate gradually in meristem tissue when the growth stopped in August with positive daily average temperatures. Their content remained relatively stable during senescence. The NL level in pine in winter, like TL, was higher by about 30–40% than in spruce. The content continued to increase in February-March. In April, the NL level did not change significantly and was about 6% of abs. dry tissue mass. This was 10–12% greater than the average winter levels. In May, when pine and spruce buds emerged, the NL content was halved because they were utilized in vigorous growth processes. The reduction of the NL level in spruce and pine bud meristem before emergence of needles agreed with data obtained earlier for the cambial zone and meristem bud tissue of *Larix sibirica* L. [6, 9]. The general features of the change of lipid content was probably explained by the analogous nature of compound exchange occurring in meristem of swelling buds and the cambial zone of conifers in spring.

The results showed that a significant part of the conifer bud meristem lipids was polar lipids (PL) (Fig. 2). The quantitative ratio of NL and PL in spruce and pine was similar in meristem of wintering buds. Changes of PL in plants under the conditions of Central Siberia are due primarily to a qualitative restructuring of cell membranes during formation of the cryoresistant state of living tissues in autumn-winter and a loss of low-temperature resistance in the vegetative period. Therefore, the seasonal transformations of the content and composition of PL had to be studied in order to find the physicochemical cryoresistance mechanisms of meristem of frost-resistant conifers.

The content of glycolipids (GL) during the studied period varied in spruce from 0.7 to 5.5%; in pine, from 3.9 to 6.5% of the abs. dry tissue mass (Table 1). The nature of the GL dynamics was similar in these species. In August, the GL content was elevated in young formed buds. The GL content in spruce was 20% less than that of phospholipids (PL); in pine, it exceeded the PL content by almost twice. However, the GL content in meristem began already in September to decrease rapidly. It was obvious that this was mainly due to an extensive structural rearrangement of the meristem cells. Furthermore, the GL could be a unique source of carbohydrates accumulated in buds during formation of the low-temperature resistance of the tissues in autumn-winter [2]. As a result, the GL level in spruce in winter was less than 1% of the abs. dry tissue mass; in pine, about 4%. Pine bud meristem in a state of low-temperature resistance in winter contained almost four times more GL than spruce and larch bud meristem [6]. This means that GL were during adaptation to cold temperatures very important to the structural organization of pine bud meristem cell membranes. In March, the GL content in the studied species began to increase. The numerical values of the two polar lipid forms in swelling buds were already similar. This indicated that the ultrastructure of green cell plastids had stabilized on going from senescence to preparation of the photosynthetic apparatus for photosynthesis.

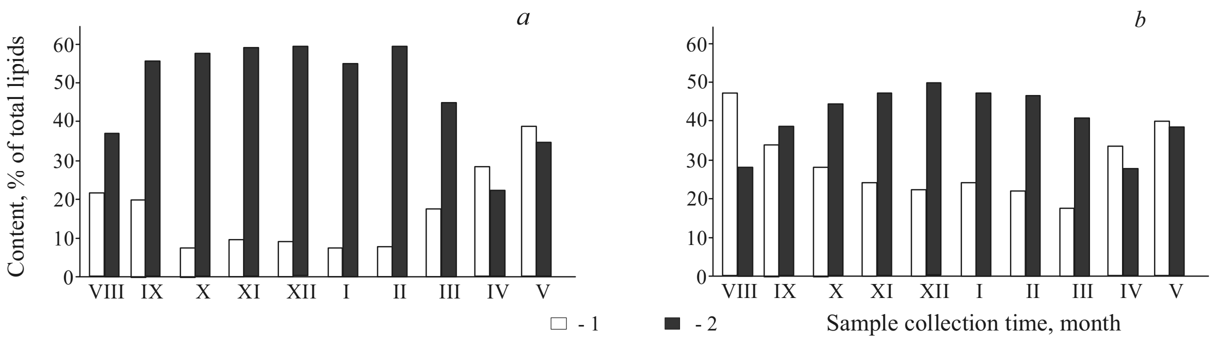

Fig. 3. Seasonal dynamics of glyco- (1) and phospholipids (2) in bud meristem of Siberian spruce (*a*) and common pine (*b*).

PL dominated the TL of spruce and pine during autumn-winter (Fig. 3).

The PL began to accumulate at the end of August. The PL content in spruce was maximum in October; in pine, later in November. The maximum PL content coincided in time with the minimum GL content, i.e., PL membrane structures dominated in a state of low-temperature resistance in spruce and pine bud meristem cells. A period of stability was observed in winter months. In March, the PL content in spruce and pine decreased by a factor of about 1.5. The low content of this lipid form in conifer bud meristem in spring coincided in time with the structural reearrangement of cell membranes upon loss of low-temperature resistance and preparation for vegetation. The PL content increased again at the start of May. This was explained by the rapid growth of young needles within the buds and the formation of new cell structures related to this, which contained PL. The observed considerable increase of PL content in this period occurred as the overall amount of TL (Fig. 1) and NL (Table 1) decreased.

Thus, the investigation produced the following information on the content and fractional composition of lipids from forest-forming conifers of Krasnoyarsk Krai. Individual features of the seasonal dynamics of TL content in bud meristem of *P. obovata* and *P. sylvestris* growing in a single geographical region were found. From September through May, *P. sylvestris* had a higher TL content than *P. obovata.* In winter, the difference reached 43–45% of TL. Polar fractions dominated in both species over the whole observation period. The yearly dynamics of GL and PL contents were opposite in nature. In autumn-winter, the dominant component of polar lipids was PL; during formation of low-temperature resistance of meristem in autumn, their content increased to 50–60% of TL. In spring, the GL content increased in meristem of swelling buds (GL/PL ratio came the closest to unity). The most substantial changes of TL content and their fractional composition in bud meristem of *P. obovata* and *P. sylvestris* agreed clearly with the seasonal development cycle and the role that various lipid groups play in the metabolism of living tissues. The change of individual lipid group content could become a reliable marker characterizing the condition of living bud tissues because they differed greatly in their physical and chemical properties.

EXPERIMENTAL

Results of investigations carried out from August 2004 to May 2008 were summarized. We studied meristem tissue of vegetative buds from spruce *P. obovata* and pine *P. sylvestris*. These species were selected because frost-resistant evergreen conifers, in particular pine and spruce, have buds with different morphologies [6]. Runners of the last year were collected every month in Minin forestry district (Krasnoyarsk suburbs) from trees of class II–III age. Meristem tissues were isolated from vegetative buds. The resulting samples were preserved using $CHCl_3$:*i*-PrOH (1:2 v/v) [15, 16] with added ionol (1%). Embryonal tissues were homogenized and lipids were extracted in a cold room at 0–2°C using cooled glassware and reagents. The TL fraction was purified of non-lipid impurities by gel filtration through a column of Sephadex G-25. The purified lipid extract was evaporated in a rotary evaporator in vacuo at 36–38°C and separated into fractions using preparative column chromatography [17]. The adsorbent was Bio-Sil A 100–200 mesh silica gel. The yield of lipid fractions was monitored by TLC. The mass was determined gravimetrically.

Results are presented as arithmetic means of three biological and three analytical repetitions. Runners from 10 trees were used as the biological repetition in each experiment. The significance of the differences was estimated by comparing averages using the Student criterion at the $P = 0.05$ significance level [18].

REFERENCES

1. S. Yu. Simkina, (Thesis), Krasnoyarsk, 2008.
2. E. V. Alaudinova and P. V. Mironov, *New Achievements in the Chemistry and Chemical Engineering of Plant Raw Material* [in Russian], N. G. Bazarnova and V. I. Markin (eds.), Izd. Alt. Univ., Barnaul, 2009, Book 2, 253.
3. A. A. Okanenko, *Fiziol. Biokhim. Kul′t. Rast.*, **6**, 90 (1974).
4. C. O. Tsaregorodtseva, *Biol. Nauki (Moscow)*, **12**, 89 (1975).
5. L. V. Vetchinnikova, *Birch, Problems of Variability* [in Russian], Nauka, Moscow, 2004.
6. E. V. Alaudinova, P. V. Mironov, and S. M. Repyakh, *Khim. Prir. Soedin.*, 259 (2002).
7. P. V. Mironov, E. V. Alaudinova, and S. M. Repyakh, *Low-temperature Resistance of Living Conifer Tissues* [in Russian], Sib. Gos. Tekh. Univ., Krasnoyarsk, 2001.
8. N. A. Khlebnikova, G. I. Girs, and R. A. Kolovskii, in: *Tr. Inst. Lesa Drev. Akad. Nauk SSSR Sib. Otd.*, **60**, Krasnoyarsk, 1963, p. 5.
9. L. P. Rubchevskaya and E. D. Levin, *Khim. Drev.*, **4**, 106 (1981).
10. N. S. Polezhaeva, *Khim. Drev.*, **1**, 94 (1987).
11. Yu. E. Novitskaya, *Physiological and Biochemical Studies of Northern Pines* [in Russian], Inst. Lesa, Karel. Fil. Akad. Nauk SSSR, Petrozavodsk, 1978.
12. E. V. Ignatova, (Thesis), Krasnoyarsk, 1992.
13. V. S. Rodionov, M. K. Il′inova, and T. A. Shulyakovskaya, *Lipidnyi Obmen Drev. Rast. Usloviyakh Sev.*, 69 (1983).
14. L. P. Rubchevskaya, E. V. Ignatova, and S. M. Repyakh, *Khim. Prir. Soedin.*, 549 (1998).
15. J. Folch, M. Lees, and G. H. Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
16. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
17. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York, 1973.
18. A. Bernstein, *Handbook of Statistical Methods* [in Russian], Statistika, Moscow, 1968.