Changes in the content of low-molecular antioxidants and carbohydrates in *pinus silvestris, rosa acicularis, ledum palustre* in the context of the transition to winter dormancy in Yakutia

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Abstract. The accumulation of low-molecular antioxidants and carbohydrates in three wintering plants of Yakutia in the summer-autumn period (July, August, September) was studied. Determining the content of the sum of low-molecular antioxidants (LMAO), the sum of flavonoids, ascorbic acid, and the sum of carbohydrates was conducted spectrofotometrically in the needles of *pinus silvestris* L. and annual shoots of *ledum palustre* L., *rosa acicularis* Lindl. The research shows that in general a decrease of temperature (a transition from summer months to autumn months) increases the content of LMAO in the three examined plants. The study also shows that an increase of the LMAO amount in the content descends to a greater extent due to the accumulation of ascorbic acid. In the dynamics of accumulation of flavonoids during the studied period, the maximum was noticed in August and followed by a decline in September. In the dynamics of the content of carbohydrates (reducing sugars), as well as the LMAO, was revealed a gradual increase in the period from July to September. The wide fluctuations in the content of the sum of LMAO, ascorbic acid, and flavonoids with decreasing temperature were found in the *rosa acicularis* Lindl, as well as in the sugars in the needles of *pinus silvestris* L. Thus, the revealed dynamics of accumulation of LMAO in wintering plants (fraction of flavonoids, ascorbic acid) in the period of preparation for the transition to winter dormancy did not show the presence of an explicit dependence on the accumulation of the main cryoprotectants of plants, i.e. carbohydrates.

1. Introduction

Many experimental studies have shown the protective role of antioxidants in the oxidation chain of lipids as an explanation of some aspects of the nature of plants frost resistance. Thus, with increasing frost resistance by tempering plants, the content of phenolic compounds increases [1]. Also, resistance to low temperatures is characterized by a high accumulation of ascorbic acid and intensive oxidation-reduction processes. In this regard, it should be noted that in the needles of various tree species vitamin C content more in the winter months, when it is lowered [2]. According to A. D. Egorov [3], in the needles of the Yakut pine (*pinus silvestris* L.) is found that the maximum accumulation of ascorbic acid occurs in winter than in other seasons of the year.

The purpose of this work was to determine the dynamics of accumulation of low-molecular antioxidants and carbohydrates in the wintering plants in the summer-autumn period.
2. Materials and Method

The Republic of Sakha (Yakutia) is a territorial entity of the Russian Federation. The entire territory is located in the continuous distribution of permafrost zone. The climate of Central Yakutia can be characterized by sharp continentality, which causes great contrast of the seasons of the year. The average annual air temperature is -10.3°C, the average monthly temperature is -43.2°C to -45.2°C in winter (December, January).

The most important period for the plant’s life with an active temperature of 10°C and above lasts 95-98 days, from May 28th to September 4th. The summer period is short, hot and dry, includes sharp changes in daily temperatures. The average monthly temperature in July, August, and September is +18.8°C, +14.9°C, +5.7°C [4].

The object of the study was the 3 species of plants, *pinus silvestris* L., *ledum palustre* L., *rosa acicularis* Lindl. The pine pine (*pinus silvestris* L.), growing in the bearberry-pine forest 7 km to the west of Yakutsk (62°N, 130°E), is a coniferous evergreen tree of the *Pinaceae* family, up to 50 meters high. The prickly wild rose (*rosa acicularis* Lindl.) is a prickly bush of the *Rosaceae* family up to 2 m high. The Swamp Ledum (*ledum palustre* L.) is an evergreen shrub of the heather family *Ericaceae*, reaching 50-90 cm in height. For the study at the end of the July, August, September months were collected: the needles of the second year of Scots Pine and the annual shoots of the Swamp Ledum, Prickly wild rose. Determination of the amount of LMAO, ascorbic acid and flavonoids was carried out by the spectrophotometric method in ethanol extracts [5].

The determination of the amount of carbohydrates (reducing sugar) was carried out according to Bertrand [6]. Statistical data processing was carried out using the BIOSTAT and Microsoft Excel software. The data in the tables are presented in the form M±m, where M is the mean, m is the error of the mean. The reliability of the differences was assessed by the Student t-test, p<0.05.

3. Results

In general, the results of the studies indicate that a decrease in temperature (the transition from summer months to autumn ones) increases the content of low-molecular antioxidants in the three investigated plants. Results of the research:

1. The content of LMAO in the stalk of the *ledum palustre* increased by 29.6% (p<0.05) from July to September, *pinus silvestris* by 16.8% (p<0.05), and in the stem of *rosa acicularis* by 19.6% (p<0.05) (Table 1).

2. The increased content of LMAO in a greater degree descends due to the accumulation of ascorbic acid: in the stalk of *ledum palustre*, the content of ascorbic acid increased almost in 2 times (p<0.05), 38.5% (p<0.05) in *pinus silvestris*, and by 54% (p<0.05) in the stalk of *rosa acicularis*.

3. The maximum accumulation of the flavonoids dynamics content was in the period from July to September. The maximum accumulation was observed in August: in the stem of the *ledum palustre*, the content of flavonoids increased 2 times from July to August (p <0.05); it decreased 2.2 times (p<0.05) further in September; in needles of *pinus silvestris*, the contest of flavonoids increased in August by 1.3 times (p <0.05) and decreased in September by 1.7 times (p <0.05); in the stem of *rosa acicularis* in August, there was a tendency to increase by about 20% and a decrease in September by 20% (Table 1).

4. In the dynamics of carbohydrate content, as well as the LMAO, a gradual increase is observed from July to September: 9.5% in the stem of the *ledum palustre*, by 28% (p<0.05) in *pinus silvestris*, and by 22% (p<0.05) in the stem of *rosa acicularis* (Table 2).
Table 1. The content of low-molecular antioxidants in wintering plants in the summer-autumn period.

| Definition parameters          | Months     | Ledum palustre L. | Pinus silvestris L. | Rosa acicularis Lindl. |
|-------------------------------|------------|-------------------|---------------------|------------------------|
| Amount of LMAO, µg/g          | July       | 590±26,4          | 308±16,4            | 769±32,5               |
|                               | August     | 698±12,3*         | 349±15,3*           | 880±11,2*              |
|                               | September  | 765±6,4**         | 360±8,2**           | 920±12,5**             |
| Ascorbic acid, µg/g           | July       | 92,2±1,2          | 130,5±8,9           | 110,8±8,7              |
|                               | August     | 180,5±6,2*        | 180,4±4,4*          | 180,4±4,4*             |
|                               | September  | 180,6±9,4**       | 180,8±8,9**         | 170,8±8,7**            |
| Flavonoids, mg/%              | July       | 1,78±0,4          | 1,89±0,3            | 1,24±0,2               |
|                               | August     | 3,63±0,6*         | 2,39±0,2*           | 1,54±0,1               |
|                               | September  | 1,62±0,3**        | 1,43±0,1**          | 1,23±0,1               |

The statistical significance of the differences in comparison with the July data * p <0.05, with the August data ** p <0.05.

Table 2. The content of carbohydrates (mg / g) in plants in the summer-autumn period.

| Plants                | July         | August       | September    |
|-----------------------|--------------|--------------|--------------|
| Ledum palustre L.     | 21,5±1,4     | 23,3±3,2     | 23,2±2,5     |
| Pinus silvestris L.   | 45,3±2,3     | 56,6±2,2*    | 58,6±1,5*    |
| Rosa acicularis Lindl.| 18,7±2,5     | 20,1±1,8     | 22,2±1,3*    |

The statistical significance of differences in comparison with July data * p <0.05.

4. Discussion

A.D. Egorov explains the periodicity of content change of ascorbic acid in pine needles by depending on the season of the year, by shifting the balance between the processes of synthesis and disintegration, oxidation and reduction. As the temperature of the medium decreases in autumn, the rate of irreversible oxidation of ascorbic acid decreases and the processes of its recovery and formation conversely increase, which entails a rapid increase in the total content of vitamin C in needles [3]. In our opinion, this can also be associated with an increase in the LPO intensity due to cryopreservation of cell membranes and the need to create a reserve of LMAO.

The study on the features of the accumulation of flavonoids in some species of plants in Yakutia shows that, in general, this process is natural and closely related to the developmental phases and growing conditions of plants [7]. The general tendency of increasing the accumulation of flavonoids during the transition of plants to reproductive processes and under favorable temperature and humidity conditions of growth has been established. Probably, this regularity is associated with a general increase in the intensity of metabolism of the plant organism. According to the obtained general dynamics of the accumulation of flavonoids in three studied plants, we can assume that from August to September (after the end of the reproductive processes), the content of flavonoids decreases due to a deduction in the intensity of photosynthetic processes, respiration, mineral nutrition, and the processes of movement of the starting materials and the activity of enzymes for the flavonoids synthesis.

In order to determine the interaction between the accumulation of low-molecular antioxidants and the accumulation of basic cryoprotectants, we also studied the dynamics of the total carbohydrate content using the same plants in the same time period.

5. Conclusion

The research finds that in the three wintering plants that we examined the total content of LMAO and ascorbic acid, and the amount of carbohydrates increases with the transition from July to September.

At the same time, the largest fluctuations in the content of LMAO, ascorbic acid, and flavonoids are observed in ledum palustre, when carbohydrates differences were found in *Pinus silvestris*. In general, the revealed dynamics of LMAO accumulation (fraction of flavonoids, ascorbic acid) in
wintering plants in the transition preparation period to winter rest did not show the presence of an explicit dependence on the accumulation of the main cryoprotectants of plants, i.e. carbohydrates. In our opinion, the reason for this is an increase in the importance of LMAO with a decrease in the ambient temperature in protection from the intensification of LPO, i.e. the subordination to the “principle of complementarity” of the individual functioning groups of cryoprotectants.

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