Secondary phloem traits in mature stems of *Betula ermanii* in stressful environments of volcanic activity

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**Abstract.** This research summarizes the results of study of structural peculiarities of *Betula ermanii* growing around Baransky Volcano close to the thermal Golubye Ozera. We have performed statistical analysis of parameters of the secondary phloem of multiyear shoots and stems and found some features tied to extreme environmental conditions. We have found that structural reaction of the secondary phloem of multiyear shoots and stems of *B. ermanii* to the extreme conditions of post-volcanic activity of Baransky Volcano manifests itself as changes in the geometry of the conductive elements and dilatation of the radial parenchyma in the non-conductive phloem. We believe these changes to be adaptive in nature due to the need for increased conductivity in volcanic landscapes.

1. **Introduction**

The Kuril Islands have unique ecosystems shaped by various environmental factors. One of the key environmental factors changing the islands’ natural systems is magma volcanism [1‒3]. Volcanism is responsible for the dynamics of relief-forming processes; it ensures specific geochemical processes, influences ambient air transparency and composition, and it is also one of the key factors of soil formation which impacts vegetation. History of vegetation in these conditions is closely related to life strategies of the plants aimed at survival of certain species in specific environmental conditions. Such species have developed various adaptive mechanisms ensuring that their life processes adjust to stressful living conditions [4].

Plants with a wide reaction norm and high adaptivity to all kinds of habitats include *Betula ermanii* Cham. (fam. *Betulaceae* S. F. Grai). *B. ermanii* is a monoecious, foliage, anemophilous tree up to 30 m in height or large brush (at the vegetation limit up to three or five meters high). It forms an independent belt of stone birch forests in the mountains and foothills, brushwood at seashores and in the highlands; it can also be found in landscapes changed by current volcanic activity [5]. Area of species includes south of Siberia and a lot of area in the Russian Far East. The species grows in large quantities on Sakhalin Island and the Kuril Islands (Iturup, Kuhashir, Shikotan, Rasshua, Ushishir, Keti, Simushir, Urup) [6]; it is also found in China, the Korean Peninsula, and in Japan [5].

The purpose of this research is to find adaptive peculiarities in the secondary phloem of multiyear shoots (10 years, 23–24 years) and stem (56 years) of *B. ermanii* growing in conditions of post-volcanic activity of Baransky Volcano.

The information on the organization of phloem and sieve tubes as its conductive elements in trees is necessary to model long-distance transport of carbon in woody plants in various environmental
conditions. That data is extremely useful for assessment of changes in carbon production of forests, namely, stone birch ones and those including stone birch, in conditions of changing climate. Right now, those issues are far from certainty. The peculiarities of phloem transport and mechanisms of its modeling in trees were described in a number of studies based on Munch’s theory of phloem transport [7–9].

2. Materials and methods
Samples were collected during expeditions from 2015 to 2018. *B. ermanii* samples were collected in conditions typical for the species in fir–stone birch shrub–forbs forest on Krasnaya Mountain (Susunai Ridge, Sakhalin Island) on October 18, 2015 (figure 1). Samples for reference were collected in stone birch–larch–bamboo forest nearby hot springs around Golube Ozera (Baransky Volcano, Iturup Island) on July 31, 2018 (figure 1). Golube Ozera hot springs are two deep funnels filled with opalescent bluish water, fringed with bright dispersed sulfur rims. The water is of the sulfate-chloride type, ultra-acidic (pH = 1.2), up to 107.5°C hot. The emission gases are nitrogen (51 %); carbon dioxide (38 %), oxygen (9 %), methane and other hydrocarbons (0.05 %) [10].

![Figure 1. Betula ermanii in various environmental conditions: on the left – fir–stone birch shrub–forbs forest on Krasnaya Mountain (Susunai Ridge, Sakhalin Island); on the right – stone birch–larch–bamboo forest nearby hot springs around Golube Ozera (Baransky Volcano, Iturup Island).](image)

Stem samples for anatomical analysis were selected and fixed following the standard techniques suggested in R.P. Barykina et al. [11] and modified in our laboratory [12, 13]. Transverse, radial and tangential 10–25 µm thick microsections of the stems were made with a sliding microtome Microm HM 430c (Thermo scientific) with a fast freezing unit Microm KS34 (Thermo Scientific). The microsections were stained regessively using Safranin and Nile blue. The bark samples were also macerated to reveal the structure of conducting elements of the phloem. Section images were processed with ZEN 2 lite software under light microscope Axio Scope.A1, CarlZeiss for measuring and photography. We analyzed 15 characters of secondary phloem in transverse and longitudinal sections for each model tree. The dataset for each character of each model tree comprised at least 32 measurements. The sample mean and its confidence interval (for 95 % probability) were estimated for each character. Bark tissue descriptions were made relying on the analytical approaches common in xylotomy, and according to current guidelines on bark anatomy of woody plants by the International Association of Wood Anatomists – IAWA [14].

3. Results and discussion
Secondary phloem is a tissue derivative of cambium. It transports products of photosynthesis. Functionally it can be divided into conductive and non-conductive phloem. It includes segments of
sieve tubes with companion cells, the axial and radial parenchyma [15]. In the conducting phloem of *B. ermanii* the axial parenchyma is striped in structure; the sieve tubes are located in groups between the radial parenchyma. Sieve tubes contain complex sieve plates of step and net structure. Rays in the phloem are homo and heterocellular. Rays are single-row, double-row, triple-row, rarely even four rows. The non-conductive phloem, in addition to the elements listed before, contains multiple sclereid groups formed by sclerification of parenchyma cells [16].

During ontogenesis, secondary phloem in the bark of *B. ermanii* was found to increase in all studied habitats. Near thermal lakes the increase in the secondary phloem of *B. ermanii* is even more significant. It exceeds the norm by 34 % and can be as great as 1553.12±35.64 µm (while the norm is 1172.12±36.71 µm). At 24 years of age, conductive phloem width in samples from thermal lakes exceeds the norm (102.15±3.51 µm) by 15 %. In the stem, secondary phloem width in the stem part near thermal lakes is 62 % less than normal (3772.58±143.28 µm), due to reduction of non-conductive phloem, because in the stem part the width of conductive phloem near thermal lakes is similar to background conditions. Increased width of non-conductive phloem in multiyear shoots in thermal lakes conditions may be linked to active dilatation of cells of the axial and radial parenchyma. The share of secondary phloem in the total width of the bark near thermal lakes in a ten-year-old shoot is 58 %; in a 24-year-old shoot it’s 71 %; in the stem part (56 years) it’s 55 %. At the same time, the share of non-conductive phloem in the bark width in background conditions is 47 % in a ten-year-old shoot; 46 % in a 24-year-old shoot; 71 % in the stem part. Therefore, the share of non-conductive phloem in the bark in conditions of thermal lakes increases expectedly, exceeding the norm by 11 % in a ten-year-old shoot, by 25 % in a 24-year-old shoot, and in the stem part its share in the bark is 16 % less than normal.

In multiyear stems close to thermal lakes, the radial diameter of sieve tubes increases by 45 % at ten years of age, up to 26.40±1.93 µm; at 24 years of age the increase is 37 %. In a year-old shoot of *B. ermanii*, we also observe the same increase in the radial size of sieve tubes [17, 18]. In the stem part the radial diameter of sieve tubes remains normal. However, we see a reduction in their tangential diameter by 26 % versus the norm (45.42±1.94 µm). Length of sieve tube segments near thermal lakes changes in different ways: at ten years of age, we see a 19 % decrease in this parameter versus the norm (511.73±35.94 µm); then, at 24 years of age, the length of the sieve tube segments exceeds the norm (553.95±37.09 µm) by 22 %, and in the stem part this parameter is within the norm (745.09±45.92 µm).

In the secondary phloem near thermal lakes, we see a decrease in the total number of phloem rays per 1 mm of cross-section versus the norm: by 10 % at ten years of age; by 23 % at 24 years of age. We also see a decreased number of single-row rays per 1 mm near thermal lakes: by 12 % in a ten-year-old shoot; by 27 % in a 24-year-old shoot. In the stem part, this parameter is normal (4.39±0.40 pcs).

Specific volume of elements of secondary phloem per 1 mm of cross-section in the conductive phloem at specific ages near thermal lakes remains within norm, while in non-conductive phloem specific volume of radial parenchyma per 1 mm near thermal lakes exceeds the norm by 15 % in multiyear shoots and by 25 % in the stem part. Increase in the specific volume of radial parenchyma is due to active dilatation of that tissue in non-conductive phloem. As a result, specific volume of sieve tubes in the non-conductive phloem in multiyear shoots and in the stem part reduces by 9 %.

4. Conclusion
Statistical analysis of structural parameters of the secondary phloem of *B. ermanii* growing around the thermal lakes of Baransky Volcano (Iturup Island) shows, that is a clear reaction of structural elements of that tissue. It manifests itself as increased parenchymatization of secondary phloem in the non-conductive zone. This may be related to the need for an additional reservoir for water since it is insufficient due to volcanic salinization. Another structural change in phloem of *B. ermanii* is increased size of sieve tubes. We believe these changes to be adaptive in nature due to the need for increased conductivity in volcanic landscapes. Our results match those found in some research of the recent years studying the role of phloem in how plants react to drought [19]. The research shows that the conductive channels of the phloem must be surrounded by living cells if they are to maintain
transportation capability of the phloem at low water potential in tissues when water is scarce. Those groups of living cells, the phloem parenchyma, can control water content and serve as a water reservoir quite independently of wood during drought. We agree with the researchers [19] thinking that the reaction of the phloem to drought can be a key to predicting plants’ survival time when water is scarce or the plants’ restorative capability after drought.

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