

# Changes in the Differentiation Program of Phloem Derivatives of Birch Cambium after Trunk Girdling <sup>†</sup>

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**Abstract:** The processes of cambial activity and secondary xylem and phloem differentiation are completely dependent on the influx of photoassimilates. Trunk girdling is a frequently used method for studying cambial growth under conditions of different assimilate supply. We girdled 20-year-old birch trees (*Betula pendula* Roth) and took samples 1 cm (AG1) and 35 cm above the girdle (AG35). Tissues of ungirdled trees served as a control. A sharp increase in carbohydrates level (AG1) inhibited xylogenesis and stimulated phloemogenesis. A moderate increase (AG35) also stimulated phloemogenesis; however, xylogenesis continued. The activity of the *APL* gene encoding a phloem-specific transcription factor correlates with the active phloemogenesis, as it was 2.18 (AG1) and 3 (AG35) times higher than in the control. The *SUC* gene encoding the transmembrane sucrose transporter was up-regulated in the AG1 and AG35 zones by 2.24 and 2.51 times, respectively, compared with the control, which indicates an active sucrose loads into the cells and correlates with the preferential differentiation of parenchyma. The activity of the *PIN1* gene encoding the auxin transporter was highest in zone AG35 (2.1 times higher than in the control). In zone AG1, the *PIN1* activity was 1.7 times lower than that of AG35, which corresponds to the impaired differentiation of the phloem sieve tubes. The data obtained can be useful for a better understanding of physiological processes and predicting changes in the forest's productivity under conditions of a changing climate.

**Keywords:** phloemogenesis; xylogenesis; *APL*; *SUC*; *PIN1*; source–sink relationship



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## 1. Introduction

Molecular genetic mechanisms of plant cell differentiation have been studied for a long time. Nevertheless, there are still very few studies on the formation of conductive tissues in tree trunks. To maintain the processes of xylem and phloem formation, a constant supply of sugars formed during photosynthesis is necessary. Currently, soluble sugars are considered not only as metabolites, but also as signaling molecules involved in the regulation of cells and tissues morphogenesis. Previously, it has been shown in woody plants that an increased sugar content in tissues can affect the expression of a large number of genes [1–4]. The aim of our work was to study the peculiarities of xylo- and phloemogenesis of silver birch (*Betula pendula* Roth) under conditions of an excess of photoassimilates. We performed girdling of birch trunks and compared the observed anatomical changes with the expression of some target genes involved in phloem formation. Trunk girdling is a frequently used methodological technique for studying cambial growth under conditions of different tissue assimilate supply. As a result of girdling in the section of the trunk located above the girdle, sugars accumulate, while in the zone located directly above the girdle, the accumulation of photoassimilates occurs most intensively [5].

## 2. Materials and Methods

We selected 32 birch trees, growing in the same soil and climatic conditions on the experimental site of the Forest Research Institute of the Karelian Research Centre of the Russian Academy of Sciences, 2 km south of Petrozavodsk. Trees were selected that were close in age (19–22 years), height ( $10 \pm 0.5$  m), and trunk diameter at a height of 1.3 m ( $10 \pm 0.2$  cm). All trees had a well-developed crown and had no visible signs of damage.

On 19 June 2017 (period of active cambial growth), girdling was performed on the trunks of 20 birch trees. At a height of 1.3 m, a 5 cm wide girdle of bark tissue was removed with a sharp knife up to the zone of the developing xylem. Twelve trees were marked as control trees; shallow punctures of the bark tissues were made on their trunks in order to mark the beginning of the experiment.

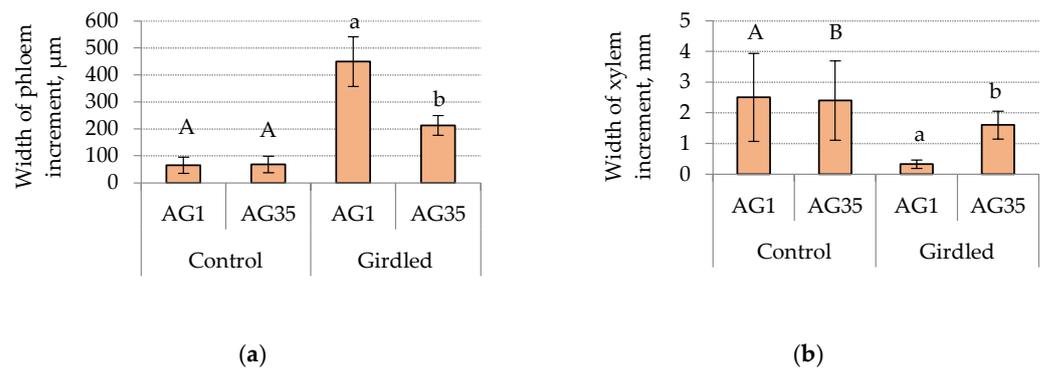
The sampling of trunk tissues was carried out at 2 levels: 1 and 35 cm above the upper border of the girdle (zones AG1 and AG35, respectively). On control trees, samples were taken at a height corresponding to the levels of AG1 and AG35 in girdled trees. Samples of trunk tissues for microscopic analysis were taken 10, 20, and 30 days after girdling, and after the end of the growing season (October). At each time, samples were taken from 3 control and 5 girdled trees. Samples of phloem and cambium tissues for determining the level of gene expression were taken from the same trees 10 days after girdling. Due to the small volume of tissues, the samples for each group of trees were combined into a single sample.

The fixation of the samples and the production of cross sections of the tissues were carried out according to the generally accepted methods [6]. Anatomical measurements were performed using ImageJ. The level of gene expression was assessed by real-time PCR as described previously [3]. The data were statistically processed using the Statistica software.

## 3. Results and Discussion

At the start of the experiment, in all trees, the early phloem (part of the conducting phloem increment, which is formed at the beginning of the growing season) was fully formed, and active xylem formation was in progress. Comparison of the samples taken at different times (10, 20, and 30 days after the start of the experiment) allows us to conclude that in girdled trees in the AG1 zone, immediately after girdling, the active formation of late phloem began. At the same time, the deposition of new xylem elements by cambium in the AG1 zone did not occur for 20 days after girdling. In the AG35 zone of girdled trees, both xylogenesis and phloemogenesis were continued for 30 days after the start of the experiment. In control trees, the processes of xylem and late phloem formation proceeded at the time typical for the species [7].

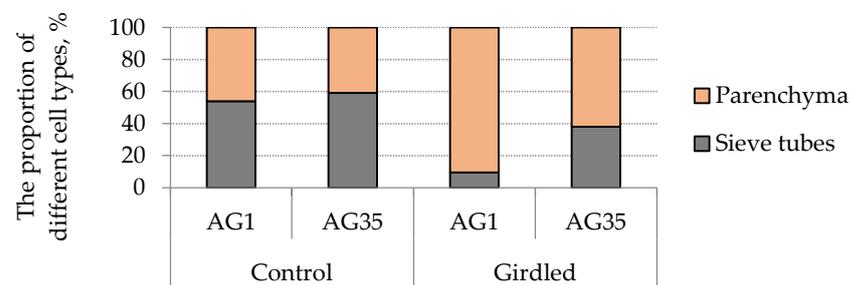
Ten days after girdling in both zones, a barrier zone was formed in the xylem of girdled trees, which was the response to injury. After girdling, the expansion of xylem derivatives already deposited by cambium was disrupted, resulting in the formation of a layer of radially flattened cells with thick secondary walls [8]. In the xylem of the control trees, we also observed a narrow barrier zone (3–5 cell layers) formed in response to the puncture of the bark tissues performed at the beginning of the experiment. Thus, the barrier zone in the xylem of control and girdled trees can serve as a marker of the beginning of the experiment. In the samples taken at the end of the growing season, we measured the width of the increments of xylem and phloem formed after girdling. In the xylem, this parameter was measured from the border of the barrier zone to the cambium; in the phloem, from the cambium to the border of the late phloem. The data obtained are in good agreement with the anatomical picture observed in the samples taken at different times after girdling (Figure 1).



**Figure 1.** Width of phloem (a) and xylem (b) increments, formed after the start of the experiment in zones AG1 (1 cm above the girdle boundary) and AG35 (35 cm above the girdle boundary) of control and girdled trees of *B. pendula*. Different letters indicate a significant difference at  $p < 0.05$ .

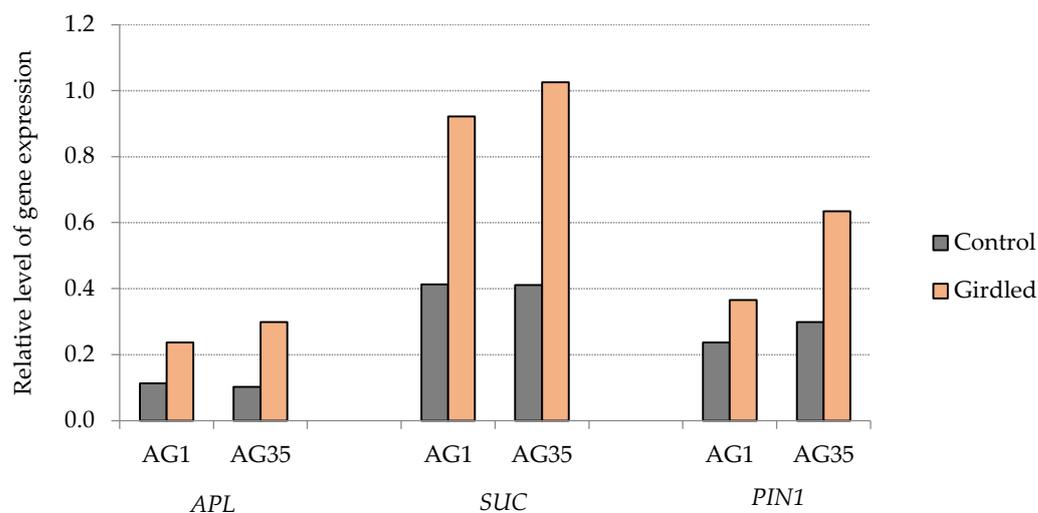
Previously, experiments with girdling of various coniferous and deciduous trees have shown an increase in the trunk diameter above the girdle [5,9–11]. At the same time, it was shown for *Pinus strobus* L. that girdling causes a more significant intensification of phloem growth in comparison with xylem [12]. In our experiment, birch experienced a significant decrease in the radial growth from the xylem side as compared to the control, which was not compensated by the increase in phloem growth. The fact that an increase in the sucrose level in birch tissues stimulated phloemogenesis and inhibited xylogenesis is consistent with the higher sensitivity of xylem derivatives of cambium to the level of sugars [13].

Since the regulatory mechanisms of phloemogenesis in woody plants remain poorly understood [14], in our further work we focused on the process of phloem formation. We determined the proportion of cells of different types in the composition of late phloem. In girdled trees, in the absence of sugar transport to the underlying sinks (zone AG1), late phloem was almost completely represented by parenchyma cells, and small sieve tubes were rare. In the AG35 zone in the same trees, parenchyma cells also dominated in the late phloem; however, the proportion of sieve tubes was higher than in the AG1 zone, which indicates the predominance of processes associated with storage while maintaining the transport function of tissues. In control trees, the late phloem structure was typical of the species (Figure 2). A disturbance in the sieve tubes' differentiation and an increase in the proportion of parenchyma in the phloem were observed in experiments with girdling of peach and lemon trees [15].



**Figure 2.** The proportion of different cell types in the composition of the late phloem in zones AG1 (1 cm above the girdle boundary) and AG35 (35 cm above the girdle boundary) of control and girdled trees of *B. pendula*.

The observed anatomical picture corresponded to changes in gene expression levels 10 days after girdling (Figure 3).



**Figure 3.** Relative level of gene expression in phloem and cambium in zones AG1 (1 cm above the girdle boundary) and AG35 (35 cm above the girdle boundary) of control and girdled trees of *B. pendula* 10 days after girdling.

In the phloem of girdled trees, the activity of the *APL* gene encoding the transcription factor involved in the differentiation of phloem elements was higher in the AG1 and AG35 zones as compared to the control trees, which corresponds to the wider phloem increments. The expression level of the *SUC* gene encoding the transmembrane sucrose transporter was higher in the AG1 and AG35 zones as compared to the control, which indicates an active loading of sucrose into cells and correlates with the volume of living parenchyma cells. Girdling also caused the interruption of the downward polar transport of auxin. The level of expression of the *PIN1* gene encoding the auxin transporter was highest in the AG35 zone, where it was higher compared to the control. In the AG1 zone, the activity of *PIN1* was lower compared to AG35, which corresponds to the impaired differentiation of the phloem sieve tubes, which requires auxin. Thus, the active loading of sugars into cells and a decrease in the polar auxin transport, apparently, served as a signal for a change in the program of cambial derivatives' development in the direction of differentiation of the parenchyma cells. It can be concluded that girdling is a valuable tool for studying the processes of xylogenesis and phloemogenesis in forest trees [16].

#### 4. Conclusions

Interruption of the downward transport of assimilates and the accumulation of soluble sugars over the girdle stimulated phloemogenesis and suppressed xylogenesis. The proportions of different types of cells in the conducting phloem have changed, which indicates a change in the differentiation program of cambial derivatives under the conditions of an excess of sugars. This assumption is also supported by changes in gene expression. The results obtained are important for understanding the regulation of phloemogenesis in trunks and source–sink relationships in the body of a woody plant as a whole.

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