Anatomical and Morphological Features of Scots Pine Heartwood Formation in Two Forest Types in the Middle Taiga Subzone

Tatiana V. Tarelkina *, Natalia A. Galibina, Sergei A. Moshnikov, Kseniya M. Nikerova, Elena V. Moshkina and Nadezhda V. Genikova

Forest Research Institute of the Karelian Research Centre of the Russian Academy of Sciences, 11 Pushkinskayast., 185910 Petrozavodsk, Russia; galibina@krc.karelia.ru (N.A.G.); moshniks@krc.karelia.ru (S.A.M.); knikerova@yandex.ru (K.M.N.); lena_moshkina@mail.ru (E.V.M.); genikova@krc.karelia.ru (N.V.G.)

* Correspondence: karelina.t.v@gmail.com

Abstract: Currently, there is no consensus on how growing conditions affect the heartwood formation in Scots pine. Comparing the results obtained by different authors is difficult due to methodological differences and poor descriptions of the objects used. We selected two sample plots in (1) a blueberry pine forest on nutrient-rich and moist soil and (2) a lichen pine forest on nutrient-poor and dry soil and performed their detailed characterization. The sample plots were located 22 km apart in the middle taiga subzone (Karelia Republic, northwest Russia). In each sample plot, we selected five dominant trees (model trees), from which we took cores at different trunk heights (0.3, 1.5, 4.5, 7.5 and 10.5 m). The cores were treated with 2,6-dichlorophenolindophenol to identify the heartwood zone. Additionally, samples were taken to study the structural features of the transition zone between sapwood and heartwood. In both forest types, the number of heartwood rings depended on the cambium age, and the patterns of parenchyma cell death did not differ in the transition zone. These facts point to a predominantly internal regulation of the heartwood formation in Scots pine. The heartwood radius and its proportion on the cross-sections were significantly higher in the blueberry pine forest than in the lichen pine forest, despite the relative values of the annual ring width. Further research is needed to develop successful Scots pine heartwood width models under a wide range of conditions.

Keywords: heartwood age rule; heartwood radius; parenchyma cell death; lichen pine forest; blueberry pine forest

1. Introduction

Scots pine is an economically important species in many European and Asian countries [1]. Pine heartwood is especially prized and differs in its physical and chemical properties from the peripheral sapwood [2–7]. In contrast to sapwood, heartwood has important qualities for the wood processing industry (colour, low moisture content, biodegradation resistance). The darker heartwood colour is due to extractives, which also determine the natural biodegradation resistance [8–11]. The heartwood formation is accompanied by degradation and incrustation of pores in the xylem vascular elements with extractive substances, which significantly reduces the wood permeability and leads to water transport cessation, and also prevents the organism penetration that causes wood decay [12–15]. When heartwood is formed, nutrients are recirculated into sapwood [8,9,16–20]. Due to this fact, in trees growing on poor soils, the nutrient content in sapwood can be comparable to that in trees growing on rich soils [21,22]. Despite the heartwood importance for ecology and economics, the process of heartwood formation in pine remains poorly understood.

Scots pine is a eurybiont that grows in a wide range of conditions, from the tundra border in the north to the forest-steppe in the south [23,24]. It can form completely different...
forests — from highly productive oxalis pine forests to sparse dry lichen and waterlogged sphagnum forests [25,26]. Under different conditions, growth processes vary significantly, affecting the trees’ morphological characteristics and wood properties [27–32]. It is known that, in other woody species, heartwood formation is controlled by diverse environmental conditions and soil fertility [33–36]. Growth-enhancement factors, i.e., irrigation and fertilization, induced heartwood formation in Eucalyptus globulus earlier and at an increased rate [37]. There is currently no consensus on how the growing conditions affect heartwood formation in Scots pine. Some authors argue that heartwood formation is mainly determined by age. Their developed models did not reveal significant correlations between the heartwood amount (the number of annual rings in heartwood) and habitat, stand or tree parameters [4,38,39]. Fertilization experiments and thinning in the pine stands have shown no increase in the area and the number of annual rings in the trunk compared to control conditions [40,41]. At the same time, several studies have shown that the heartwood proportion in the trunk in more productive forest types is higher than in less productive stands of Scots pine trees of the same age [42,43]. Possibly, conflicting opinions regarding the growing conditions influencing the heartwood formation process are due to methodology differences. The above studies used various parameters to evaluate the heartwood formation (number of annual rings, heartwood radius, cross-sectional area). In addition, we could not find published articles that considered the features of heartwood formation in different types of natural forests formed by Scots pine.

This work is part of a project investigating the heartwood formation process in Scots pine. The project is being carried out in the Karelia Republic (northwest Russia), where pine forests cover 65% of the forest fund area [44]. Earlier heartwood formation patterns were shown depending on the tree age and cambial age (C.A.) within the same tree in Scots pine in a middle taiga lingonberry pine forest [45]. This article presents the results of the heartwood study in Scots pine trees at the age of 70–80 years, growing in closely located areas in blueberry (Myrtillus) pine forest and lichen (Cladonia) pine forest. Blueberry pine forests are a highly productive type of forest that forms on soils with a sufficiently high fertility level and moisture. Lichen pine forests are low-productive forests formed on poor and dry soils. These forests were chosen because blueberry pine forests are the second most widespread forest type in the study area. The lichen type, on the contrary, is quite rare in the Karelia Republic and practically escapes the researchers’ attention (Figure 1). At the same time, lichen pine forests are widely represented in Northern Fennoscandia and Western Siberia; in the latter, lichen forests account for 30% of pine forests [46,47].

![Figure 1. (a) Study area location (red dot). The main areal of Pinus sylvestris L. is highlighted in green. (b) Pine forests distribution area by the main types in the middle taiga zone of the Karelia Republic are presented [25,26].](image-url)
The transformation process of sapwood to heartwood occurs in the transition zone, the innermost sapwood layers located at the heartwood boundary [48]. Various cell changes can be observed at the microscopic level in the transition zone of Scots pine trees. In the tracheids, bordered pits are aspirated, and extractives and lignin are deposited on the pits membrane, which leads to transport function loss and water disappearance from the tracheids [49–52]. In the field, the border between sapwood and heartwood is most often determined by moisture difference, clearly visible on cores and cuts of Scots pine trunks [53–55]. More significant changes occur in living parenchyma cells. Starch disappears from the parenchyma cells [56–59], which is confirmed by biochemical analyses [60–63]. The cell walls of the radial parenchyma are lignified [51,58,64]. The final stage in the sapwood transformation into heartwood is nuclei destruction and parenchyma cell death [56,58,64].

This article presents data on the number of heartwood rings’ distribution along the trunk (HW), heartwood radial width (R(HW)) and heartwood proportion on the cross-section (HW (%)) for pine trees in two forest types. We have also studied the transition zone anatomical structure for the first time using various cytological markers to assess how the transformation of sapwood to heartwood is related to the tree’s growing conditions. A detailed sample plot description, the use of the exact sampling and processing techniques and the same set of measured parameters will allow comparison of the results obtained for different forest types, both within the project framework and in the future.

2. Materials and Methods

2.1. Sample Plot Characteristics

2.1.1. Selection and Description

The study was conducted in the middle taiga subzone (Karelia Republic, northwest Russia). Two sample plots (SPs) were selected at 22 km distance from each other in a lichen pine forest (SP 8) and a blueberry pine forest (SP 2) at 70–80 years of trees age.

SPs were established in May 2021. A forest stand inventory was carried out using a commonly accepted methodology, OST 56-69–83 [65]. The age of the sampling cores was determined with the Pressler age borer at the root collar in 15–20 trees. A continuous count of trees was carried out in 2 cm steps of diameter on the SPs. The heights were measured for 20–25 pine trees. The following stand characteristics were determined: tree species composition, density, the sum of cross-sectional areas (basal area per hectare), average diameter and height, growing stock (Table 1).

Table 1. A summary of the sample plots and model trees used in the study.

<table>
<thead>
<tr>
<th>SP</th>
<th>Age (Years)</th>
<th>FSC ¹</th>
<th>DBH ²/H ³</th>
<th>D ⁴</th>
<th>V ⁵</th>
<th>Basal Area per Hectare (m²)</th>
<th>UTSC ⁶</th>
<th>NE ⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>80</td>
<td>95% <em>Pinus sylvestris</em> 1% <em>Picea</em> 4% <em>Betula</em></td>
<td>22.9/23.3</td>
<td>0.96</td>
<td>377/25</td>
<td>35.6</td>
<td>70% Spruce 30% Birch/0.33</td>
<td>62°17’48.3” N 34°00’31.7” E</td>
</tr>
<tr>
<td>8</td>
<td>70–80</td>
<td>100% <em>Pinus sylvestris</em></td>
<td>15.0/11.6</td>
<td>1.80</td>
<td>109/0</td>
<td>18.4</td>
<td>100% Pine/43</td>
<td>62°28’41.6” N 33°48’21.6” E</td>
</tr>
</tbody>
</table>

¹ FSC—forest species composition; ² DBH—average diameter at the breast height; ³ H—average tree height; ⁴ D—tree density; ⁵ V—the trunk volume of trees per area unit (growing stock/standing deadwood); ⁶ UTSC—undergrowth tree species composition; ⁷ NE—geographical coordinates.

Within the SPs, the field layer cover species composition was revealed. The general projective coverage of the living ground cover, moss-lichen and herb-dwarf shrub layers and each plant species were assessed (Table 2). Latin names for vascular plants and mosses are given according to www.theplantlist.org, for lichens, according to [66].
Table 2. Geobotanical characteristics of sample plots.

<table>
<thead>
<tr>
<th>SP Geobotanical Characteristics</th>
<th>SP Photo</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 2, Blueberry pine forest, 80 years</td>
<td><img src="image" alt="SP2_photo" /></td>
</tr>
<tr>
<td>General projective coverage—80%, moss-lichen layer—70%, herb-dwarf shrub layer—60%. In the living ground cover, 11 species were recorded, including Vaccinium myrtillus, V. vitis-idaea, Calluna vulgaris, Lycopodium annotinum L., Melampyrum pratense L., Deschampsia flexuosa. In the moss-lichen layer there are Pleurozium schreberi, Hylocomium splendens, Dicranum polysetum.</td>
<td></td>
</tr>
</tbody>
</table>

| SP 8, Lichen pine forest, 70–80 years | ![SP8_photo](image) |
| General projective coverage—90%, moss-lichen layer—90%, herb-dwarf shrub layer—20%. In the living ground cover, there are Calluna vulgaris, Vaccinium vitis-idaea, Arctostaphylos uva-ursi, Diphasiastrum complanatum, Empetrum nigrum and Vaccinium myrtillus. In the moss-lichen layer there are Pleurozium schreberi and Cladonia rangiferina, Cl. arbuscula. |

2.1.2. Soil Sampling and Chemical Soil Analysis

Soil sections were laid in pine forests (blueberry, lichen) in inter-crown spaces (at least 1.5 m from tree trunks). According to the soil classification, the soils are classified as Albic Podzols Arenic and Entic Podzol [67]. These soils are widespread in Karelia [68]; they have a genetic profile typical of podzols.

A thick forest litter forms on the surface (horizon O), subdivided into sub-horizons O(L) 0–2 (3) cm and O(F + H) 2 (3)–4 (6) cm. Horizon O(L) is fresh plant litter consisting of needles, cones, live mosses and lichens. The O(F + H) sub-horizon is dark brown, represented by organic substances of different decomposition degrees. Due to its low thickness in the lichen pine forest, the forest litter is not differentiated into sub-horizons. The podzolic horizon (E) is morphologically distinct but small thickness—2–3 cm. According to the granulometric composition, the illuvial-ferruginous, yellowish-brown BF horizon lies...
at 20–50 cm depth—loose and cohesive sand. The BF horizon is characterized by a density of 1.36–1.49 g/cm³. Horizon BC is formed at a 50–100 cm depth and is represented by yellowish-brown, structureless, loose, sandy sediments. The density of the soil mineral horizons increases with the profile depth from 1.26 to 1.56 g/cm³ (Figure 2).

![Figure 2.](image)

**Figure 2.** (a) Albic Podzol Arenic soil of SP 2, Blueberry pine forest, 80 years. (b) Entic Podzol soil of SP 8, Lichen pine forest, 70–80 years. The rulers are 90 cm.

Samples were collected from soil genetic horizons and analysed following the techniques commonly used in soil science. The physical and chemical soil properties were analysed separately for each sample. The soil samples were milled to pass through a 2 mm sieve. Fresh soil samples were used for the determination of pH and mineral nitrogen. pH H2O and pH in 1 N KCl was performed potentiometrically (pH-meter Hanna 2210, HANNA Instruments Deutschland GmbH, Vöhringen, Germany) in a 1:25 soil-H2O. The contents of mineral nitrogen compounds (ammonium nitrogen (in H2O) and nitrate nitrogen (in 1% potassium aluminium sulphate solution) were measured potentiometrically (Anion-4100, Infraspak-Analit, Novosibirsk, Russia). The soil samples were then dried at room temperature and in an oven at 105 °C until constant weight. Field soil moisture was calculated from the relationship between evaporated water and dry soil weights after soil sample drying. Total C and N were determined using the ultimate CHNS analyser (Perkin Elmer’s 2400 Series II CHNS/O, Perkin Elmer, Norwalk, Connecticut, USA). Soil humus was calculated using C total content. Labile P content was determined in the soil extracts (0.2 N HCl) with ammonium molybdate spectrophotometrically (OKB Spektr SF-2000, St. Petersburg, Russia). Hydrolyzable N content was determined in the soil extracts (0.5 N H2SO4) with Nessler’s reagent spectrophotometrically (OKB Spektr SF-2000, St. Petersburg, Russia). The analysis of labile K content was performed by a flame atomic spectrophotometer (Shimadzu AA 7000, Shimadzu, Kyoto, Japan). We calculated C total, N total, N mineral, N hydrolysable, humus, P2O5 and K2O reserves that were available to plants for all selected horizons of soil substrates. We considered the litter for organic horizons and measured the thickness of mineral horizons. The reserves were calculated for the 0–50 cm layer because it is there that the main forms of the nutrients available to plants are accumulated. Furthermore, this soil layer contained a large mass of woody plant roots. Nutrient reserves determined for individual soils were recalculated for the areas occupied by the soils in the landscape [69] (Table 3).
Table 3. C, N, P, K reserves and water supply in the 50 cm soil layer of the studied pine forests.

<table>
<thead>
<tr>
<th>Soil Horizon</th>
<th>Horizon Thickness, cm</th>
<th>C, tons/ha</th>
<th>N, kg/ha</th>
<th>Mineral N, kg/ha</th>
<th>Hydrolysable N, kg/ha</th>
<th>Humus, tons/ha</th>
<th>P$_2$O$_5$, kg/ha</th>
<th>K$_2$O, kg/ha</th>
<th>Water Supply (m$^3$/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 2, blueberry pine forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest litter</td>
<td>4</td>
<td>11.7</td>
<td>344</td>
<td>1.09</td>
<td>0.03</td>
<td>20.1</td>
<td>8.5</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>Mineral layer</td>
<td>50</td>
<td>11.4</td>
<td>945</td>
<td>3.94</td>
<td>0.69</td>
<td>19.7</td>
<td>2357</td>
<td>115</td>
<td>359</td>
</tr>
<tr>
<td>In total</td>
<td></td>
<td>23.1</td>
<td>1289</td>
<td>5.03</td>
<td>0.73</td>
<td>39.8</td>
<td>2366</td>
<td>144</td>
<td>386</td>
</tr>
<tr>
<td>SP 8, lichen pine forest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest litter</td>
<td>2</td>
<td>3.5</td>
<td>83</td>
<td>0.19</td>
<td>0.01</td>
<td>6.0</td>
<td>1.8</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Mineral layer</td>
<td>50</td>
<td>15.6</td>
<td>1700</td>
<td>3.18</td>
<td>0.62</td>
<td>27.0</td>
<td>2400</td>
<td>201</td>
<td>166</td>
</tr>
<tr>
<td>In total</td>
<td></td>
<td>19.1</td>
<td>1783</td>
<td>3.37</td>
<td>0.62</td>
<td>32.9</td>
<td>2402</td>
<td>206</td>
<td>174</td>
</tr>
</tbody>
</table>

The soils of all SPs are characterized by a deficiency of the essential elements of mineral nutrition. The forest litter in the lichen pine forest is less thick and contains less organic matter, N, P, K, than the blueberry pine forest.

2.2. Model Trees Characteristics

Five model trees were selected from each SP. The dominant trees without oppression signs were chosen as model trees. For model trees, the following parameters were determined: age, height, diameter at breast height (DBH), length of the living crown (L(Cr)) (Table 4).

Table 4. Information of randomly selected stems (for model trees).

<table>
<thead>
<tr>
<th>Site</th>
<th>№</th>
<th>Age (Years)</th>
<th>DBH $^1$ (cm)</th>
<th>H $^2$ (m)</th>
<th>LCr $^3$ (m)</th>
<th>LCr (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberry pine forest</td>
<td>1</td>
<td>78</td>
<td>31.1</td>
<td>28.0</td>
<td>10.0</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>80</td>
<td>32.7</td>
<td>27.0</td>
<td>9.5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>77</td>
<td>34.8</td>
<td>29.0</td>
<td>6.0</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>77</td>
<td>31.6</td>
<td>30.0</td>
<td>10.5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>78</td>
<td>31.7</td>
<td>29.2</td>
<td>6.7</td>
<td>23</td>
</tr>
<tr>
<td>Lichen pine forest</td>
<td>1</td>
<td>74</td>
<td>22.6</td>
<td>11.6</td>
<td>7.9</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>82</td>
<td>25.4</td>
<td>13.4</td>
<td>10.2</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>79</td>
<td>19.6</td>
<td>11.8</td>
<td>9.2</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>66</td>
<td>22.6</td>
<td>14.6</td>
<td>10.3</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>71</td>
<td>24.3</td>
<td>14.1</td>
<td>11.0</td>
<td>78</td>
</tr>
</tbody>
</table>

$^1$ DBH—diameter at the breast height; $^2$ H—tree height; $^3$ LCr—length of the living crown.

2.3. Cores Sampling and Measurement

Samples (cores) were taken within one tree at the height of 0.3 m and in the middle of three-meter sections from the trunk base to the crown (1.5 m, 4.5 m, 7.5 m and 10.5 m) to assess the heartwood formation process intensity depending on the C.A.

Cores were treated with 2,6-dichlorophenolindophenol [70] to identify the transition zone between sapwood and heartwood. As a result of this treatment, sapwood becomes blue, and heartwood, due to extractives, is coloured in pink (purple), which are well distinguishable visually and persist for a long time. In addition, a colour gradient from blue to violet is visible in the transition zone.

The treated cores were photographed using an ADF S645 stereomicroscope equipped with a 10 Mp camera. The fraction of core corresponding to HW (coloured in pink) was calculated on the obtained images using the ADF Image Capture image analysis program.

On each core, the number of annual rings in xylem (C.A.), number of annual rings in heartwood (HW), radial width for xylem (R(W)) and radial width in heartwood (R(HW)) were noted. Based on the data obtained, the cross-sectional area of xylem (S(W)), heartwood
area (S(HW)), sapwood area (S(SW)) and heartwood proportion (HW (%)) were calculated as follows:

\[ S(W) = \pi \times R(W); \]
\[ S(HW) = \pi \times R(HW); \]
\[ S(SW) = S(W) - S(HW); \]
\[ HW(\%) = \frac{R(HW)}{R(W)} \times 100. \]

The average annual ring width (Lring) was calculated for each core.

The rate of HW formation for a certain trunk section (0.3–1.5 m; 1.5–4.5 m; 4.5–7.5 m; 7.5–10.5 m) was calculated as the number of HW rings formed in this section/number of annual rings formed in this section):

\[ HW_{0.3-1.5} = \frac{(HW_{0.3} - HW_{1.5})}{(C.A._{0.3} - C.A._{1.5})}. \]

2.4. Sampling, Preparation and Microscopy Processing of the Transition Zone

Cores 5 mm in diameter at a height of 1.5 m from the trunk base were taken with a Pressler age borer in June 2021 from five model trees in each forest type. Immediately after extraction, the boundary between the wet (sapwood) and dry (heartwood) parts was marked on the cores with a pencil and photographed. A part of the core containing 10–15 annual rings, including heartwood and sapwood, was then immediately fixed with a 2.5% glutaraldehyde solution and placed in the cold. In the laboratory, wood samples were washed three times with phosphate buffer (pH 7.2–7.4) to remove glutaraldehyde and passed through an alcohols’ series 30°, 50°, 70°. In 70°C ethanol solution, the samples were then placed in a refrigerator, where they were stored at a temperature of +4 °C until the cross-section preparation.

Radial sections of 15–20 µm were made using a Frigomobil 1205 freezing microtome (Reichert–Jung, Heidelberg, Germany). The cross-sections were stained with 1% alcoholic safranin solution and 1% alcoholic alcian blue solution to determine the composition of tracheids pit membranes, iodine solution in potassium iodide to identify starch grains, and 4% acetocarmine solution to identify nuclei [71]. Each histochemical sample was performed in three separate sections. The studies were carried out using an AxiolImager A1 light microscope (Carl Zeiss, Jena, Germany) equipped with an ADFPRO003 camera (ADF Optics, Hangzhou, China). Photographs of the sections were obtained using the ADF Image Capture software (ADF Optics, Hangzhou, China).

2.5. Statistical Analyses

Sample sizes are denoted as n. All data in the diagrams appear as mean ± SD, where SD is the standard deviation. The results were statistically processed with PAST (version 4.0) and StatGraphics Plus (version 5.0) for Windows. Before starting the statistical analysis, raw data was initially tested for normality using the Shapiro–Wilk test, and the level of skewness was indicated. The significance of differences between variants was estimated by Mann–Whitney U-test. Different letters indicate a significant difference at \( p < 0.05 \). One-way analysis of variance (ANOVA) was performed to evaluate the impact of C.A. on the HW and SW distribution. The impact was characterized using the Pearson correlation and coefficient of determination (R²). The simple and multiple linear regression models were used to determine the dependence \( R(HW) \) on distribution using several tree characteristics (C.A., Lring and R(W)) as predictor variables. The models were evaluated based on the coefficient of determination (R²), standard error of estimate (SEE).

All assays were performed at the Core Facility of the Karelian Research Centre of RAS.
3. Results

3.1. Spatial Distribution of Heartwood in Scots Pine Trees Trunks Growing in Blueberry and Lichen Pine Stands

To assess how heartwood is distributed in tree trunks from different forest types, we took samples at different trunk heights from 70–80-year-old trees in lichen pine and blueberry pine forest. Due to the restrictions on the reserve territory, we could not cut trees and could obtain data on the heartwood distribution only from selected cores. Cores were taken up to a height of 7.5 m; the 4.5 m mark was approximately at the lower crown border, and the 7.5 m mark was in the central crown part in the lichen pine forest. Cores were taken up to a height of 10.5 m, i.e., in the lower third of the trunk in the blueberry pine forest.

Heartwood was detected up to a height of 4.5 m in the lichen pine forest, but it was not found at the height of 7.5 m in this forest type. Heartwood was present in all samples taken in blueberry pine trees. The C.A. of the samples, wood radius, and various parameters SW and HW are shown in Figure 3. The most significant differences were found for HW% on the cross-cut. This parameter varied from 2.8% to 16.3% in the lower trunk part (0.3–4.5 m) in the lichen pine forest, while it ranged from 29.5% to 57.9% in the blueberry pine forest.

Figure 3. Cont.
The HW formation rate in blueberry pine forest was, on average, 0.54 annual rings per year. Its maximum values were observed at a trunk height of 1.5 to 4.5 m (1 annual ring per year), and the minimum from 4.5 to 10.5 m (0.32 annual rings per year on average). The HW formation rate in model trees averaged 0.56 annual rings per year in a lichen pine forest. At the same time, its values were also high (0.68 annual rings per year) at a trunk height of 1.5–4.5 m.

### 3.2. Validation of the Models Developed for the Investigated Forest Types

Previously, several authors have developed models that predict the number of rings in heartwood and the heartwood radius depending on various parameters [4,38,39,45]. We checked how our data, obtained in two types of forests, fitted into the models proposed (Tables 5–7). High $R^2$ coefficients and low errors values were obtained for all models describing the relationship between the number of rings in heartwood and the C.A. in a particular sample. The lower $R^2$ coefficient values for the blueberry pine forest can be explained by the limited data set on the cambial age, which varied from 54 to 77 years in the blueberry pine forest (Figure 3a). The cambial age of the samples varied from 16 to 76 years (Figure 3a) in the lichen pine forest, which was considered the reason for the higher $R^2$ values for this forest type. Models predicting heartwood radius showed low $R^2$ values and high error values for the data set.

Table 5. Models developed for lingonberry pine forest [45] describe the dependence of the number of heartwood rings as a function of cambium age for Scots pine and general statistics for lichen and blueberry pine forests.

<table>
<thead>
<tr>
<th>Studied Stand</th>
<th>Model</th>
<th>Slope</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>$R^2$</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichen pine forest</td>
<td>$SW = 0.36 \times \text{C.A.} + 11.88$</td>
<td>0.91</td>
<td>0.02</td>
<td>49.8</td>
<td>&lt;0.001</td>
<td>0.90</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>$HW = 0.64 \times \text{C.A.} - 11.74$</td>
<td>1.12</td>
<td>0.04</td>
<td>29.6</td>
<td>&lt;0.001</td>
<td>0.95</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>$\sqrt{(HW)} = 0.95 \times \sqrt{(\text{C.A.})} - 2.45$</td>
<td>1.01</td>
<td>0.04</td>
<td>25.7</td>
<td>&lt;0.001</td>
<td>0.90</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>$Lg(HW) = 1.61 \times Lg(\text{C.A.}) - 1.55$</td>
<td>0.88</td>
<td>0.04</td>
<td>23.6</td>
<td>&lt;0.001</td>
<td>0.94</td>
<td>3.1</td>
</tr>
<tr>
<td>Blueberry pine forest</td>
<td>$SW = 0.36 \times \text{C.A.} + 11.88$</td>
<td>0.96</td>
<td>0.02</td>
<td>61.8</td>
<td>&lt;0.001</td>
<td>0.54</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>$HW = 0.64 \times \text{C.A.} - 11.74$</td>
<td>1.03</td>
<td>0.02</td>
<td>50.4</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>$\sqrt{(HW)} = 0.95 \times \sqrt{(\text{C.A.})} - 2.45$</td>
<td>0.94</td>
<td>0.02</td>
<td>49.7</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>$Lg(HW) = 1.61 \times Lg(\text{C.A.}) - 1.55$</td>
<td>0.81</td>
<td>0.02</td>
<td>47.6</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Figure 3. Characteristics of the xylem generally, sapwood and heartwood on sampled cores from pine trees. (a) Number of annual rings in the xylem (C.A.). (b) Radial width for xylem (R(W)). (c) The number of annual rings in sapwood (SW). (d) Sapwood area (S(SW)). (e) Number of annual rings in heartwood (HW). (f) Radial width in heartwood (R(HW)). (g) Heartwood proportion (HW (%)). Red boxes relate to the lichen pine forest, green boxes relate to the blueberry pine forest. The x-axis indicates the heights at which the cores were taken. Different letters indicate a significant difference at $p < 0.05$. 

Characteristics of the xylem generally, sapwood and heartwood on sampled cores from pine trees. (a) Number of annual rings in the xylem (C.A.). (b) Radial width for xylem (R(W)). (c) The number of annual rings in sapwood (SW). (d) Sapwood area (S(SW)). (e) Number of annual rings in heartwood (HW). (f) Radial width in heartwood (R(HW)). (g) Heartwood proportion (HW (%)).
Table 6. Models developed for pine trees [4,38,39] describe the number of heartwood rings as a function of cambium age for Scots pine and general statistics for lichen and blueberry pine forests.

<table>
<thead>
<tr>
<th>Studied Stand</th>
<th>Model</th>
<th>Slop</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>R^2</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichen pine forest</td>
<td>(\sqrt{\text{HW}} = \sqrt{\text{C.A.}} - 3) [38]</td>
<td>0.95</td>
<td>0.03</td>
<td>28</td>
<td>&lt;0.001</td>
<td>0.95</td>
<td>2.8</td>
</tr>
<tr>
<td>Blueberry pine forest</td>
<td>0.89</td>
<td>0.02</td>
<td>48</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Lichen pine forest</td>
<td>(\text{HW} = 0.0021 \times \text{C.A.}^2 + 0.3232 \times \text{C.A.} - 4.8297) [4]</td>
<td>0.94</td>
<td>0.04</td>
<td>26.71</td>
<td>&lt;0.001</td>
<td>0.95</td>
<td>2.9</td>
</tr>
<tr>
<td>Blueberry pine forest</td>
<td>0.87</td>
<td>0.02</td>
<td>48.6</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Lichen pine forest</td>
<td>(\text{HW} = 0.669 \times \text{C.A.} - 13.650) [39]</td>
<td>1.11</td>
<td>0.04</td>
<td>30.3</td>
<td>&lt;0.001</td>
<td>0.95</td>
<td>3.0</td>
</tr>
<tr>
<td>Blueberry pine forest</td>
<td>1.03</td>
<td>0.02</td>
<td>49.8</td>
<td>&lt;0.001</td>
<td>0.65</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. The model developed for lingonberry pine forest [45] describes the heartwood radius as a function of cambium age, \(L_{\text{ring}}\) and \(R(\text{W})\) for Scots pine and general statistics for lichen and blueberry pine forests.

<table>
<thead>
<tr>
<th>Studied Stand</th>
<th>Slope</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>R^2</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R(HW) = -37.0691 + 0.3746 \times \text{C.A.} + 4.8351 \times L_{\text{ring}} + 0.5593 \times R(\text{W}))</td>
<td>0.96</td>
<td>0.18</td>
<td>5.3</td>
<td>&lt;0.001</td>
<td>0.53</td>
<td>34.3</td>
</tr>
<tr>
<td>Lichen pine forest</td>
<td>0.79</td>
<td>0.02</td>
<td>40.3</td>
<td>&lt;0.001</td>
<td>0.34</td>
<td>8.7</td>
</tr>
<tr>
<td>Blueberry pine forest</td>
<td>0.84</td>
<td>0.04</td>
<td>23.1</td>
<td>&lt;0.001</td>
<td>0.79</td>
<td>17.25</td>
</tr>
</tbody>
</table>

3.3. Anatomical Characteristics of the Transition Zone

We studied radial sections of the transition zone from cores taken at the height of 1.5 m from 70–80-year-old model trees, growing in two forest types. In the sections, we noted the position of the following marker events detected by histochemical tests:

1. A change in the colour of the tori of tracheids bordered pits. The bordered pits’ tori were stained with alcian blue in the sapwood, indicating cellulose and pectins in their composition. In heartwood, tori were stained with safranin in a dirty pink colour due to the deposition of extractives and lignin on the pit membrane.

2. Disappearance of starch grains from the ray parenchyma cells and parenchyma sheath of axial resin canals.

3. Disappearance of nuclei from radial and axial parenchyma cells.

The change in the colour of the bordered pits’ tori and the nuclei disappearance in parenchyma cells occurred very consistently in all the trees studied, regardless of the growing conditions. In this case, the chemical properties of the pit membranes of the tracheids changed in the ring (or part of the growth ring) located closer to the trunk periphery. We observed the nuclei disappearance closer to the trunk centre, either in the layer of earlywood of the same growth ring or in the previous growth ring. Thus, these marker events on the cross-sections were located at a distance of 1–2 annual rings from each other (Figures 4 and 5).

We did not notice any regularity in the starch disappearance from the ray parenchyma cells and the parenchyma sheath of axial resin canals. Regardless of the forest type, starch ceased to be detected in the radial sections, both in the same annual rings in which other marker events were localized and in other sapwood parts (data not shown). In individual trees growing in different forest types, starch was not detected.
Therefore, some authors consider the formation of heartwood as an active developmental program [33, 48, 74].

The pipe model theory generally accepted today relates the sapwood area to the required water conductivity to maintain leaf area [72]. In turn, Bamber suggested that the maintenance of sapwood at an optimal level to provide the crown with water is achieved by transforming unused sapwood into heartwood [73]. Therefore, some authors consider the formation of heartwood as an active developmental program [33, 48, 74].

It is known that in habitats with poor and dry soils, reduced productivity leads to the formation of narrower growth rings in comparison with more favourable conditions.

### 4. Discussion

Sapwood and heartwood represent two parts of trunk wood fundamentally different in their properties and functions. Sapwood is located at the trunk periphery and actively participates in the water transport to the crown. The pipe model theory generally accepted today relates the sapwood area to the required water conductivity to maintain leaf area [72]. In turn, Bamber suggested that the maintenance of sapwood at an optimal level to provide the crown with water is achieved by transforming unused sapwood into heartwood [73]. Therefore, some authors consider the formation of heartwood as an active developmental program [33, 48, 74].
Several studies have concluded that Scots pine can compensate for low radial growth and maintain sapwood proportion at the required level due to the later transition of sapwood to heartwood in dry soil conditions [75,76]. Dominant trees were selected in each forest type to exclude the influence of cenotic status on the studied parameters. At the same time, the selected trees in the two forest types differed significantly in height and DBH but had similar L(Cr) and Lring. For this, we did not see significant differences in the number of sapwood rings and the sapwood area in the lower part of tree trunks from lichen and blueberry pine forests. The differences observed at heights of 4.5 m and 7.5 m are obviously due to the trunks’ taper in the lichen pine forest. Additionally, our data did not demonstrate differences in the number of heartwood rings at a certain age between trees growing in a dry lichen pine forest and a wet blueberry pine forest. Following the conclusions of other authors, the number of heartwood rings in Scots pine trees correlates with the cambium age at each trunk height [4,38,39].

The fact that internal developmental mechanisms regulate the transition of sapwood to heartwood is also indicated by the constancy in the width of the transition zone in three different types of forest—lichen and blueberry pine forests (in this work) and lingonberry pine forest [45]. Changes in the chemical composition of pit membranes (associated with the water transport cessation) and parenchyma cell death occurred within 1–2 neighbouring annual rings, both in dry lichen pine forests and in moist blueberry pine forests. Of all the processes we studied, the least predictable was the starch disappearance from living parenchyma cells. This fact is associated with the intensive use of starch during the period of active cambial growth when we took our samples [77]. Thus, under the conditions of Karelia, the lifespan of the parenchyma cells is up to 45 years in the trunks of 70–80-year-old Scotch pine trees, regardless of the growing conditions [45]. Similar values of the lifespan of parenchyma cells for Scots pine trees at the age of 70–80 years were obtained by other authors [57,64,78].

Indicators such as heartwood radius (R(HW) and heartwood proportion (HW (%)) were significantly different in the two forest types. This is consistent with the data of other researchers who studied similar indicators in pine forests with different productivity [42,43]. The relationship between heartwood formation and growth of Scots pine trees has been previously demonstrated by Ojansuu and Maltamo [79]. They showed that both the HW radius and HW cross-sectional area increase with the tree size. Vanninen et al. [80] also concluded that the heartwood proportion in the total tree biomass depends on the tree height. This parameter did not differ for Scots pine trees of the same height from different forest types. In a later publication, it was clarified that the HW proportion correlates with tree size only in dominant trees, for which the size is proportional to the age. Trees of the same age but differing in size due to competition for light showed no difference in the heartwood contribution to total biomass [81]. As for other woody species, Krause and Gagnon reported that the number of annual rings in heartwood was lower in trees of black spruce growing on dry plots than in those found on wet plots [82]. Thulasidas and Bhat [83] found significant differences in HW (%) and R(HW) in teak trees between wet and dry sites in India, with the wet sites presenting larger heartwood diameters.

In contrast, Pérez and Kanninen [84] and Crespo et al. [85] reported, for studies conducted in Costa Rica and Ecuador, respectively, significantly greater HW (%) in teak plantations growing at dry sites. However, previously developed models linking the HW radius with cambium age, annual ring width, and tree height showed high SEE values associated with a small sample size on our dataset. Based on the data obtained in this work and earlier work [45], we have drawn a diagram of the HW distribution along the trunk of Scots pine trees of 70–80 years of age in three different types of forest—lichen, blueberry and lingonberry pine forests in South Karelia (Figure 6).

All the data presented in this article were obtained in different forest types within a small area with similar climatic characteristics. Some works have shown the influence of climate on the heartwood formation in various pine species and other woody plants [86–90]. However, we could not find similar works for Scots pine.
were significantly higher in the blueberry pine forest than in the lichen pine forest. The average tree height (h) is given, and a red line indicates the height of the lower crown border. The radius of the trunk cross-sections at a given height and heartwood radius is proportional to the R(W) and the R(HW), respectively.

Our results show that it is necessary to consider the forest type to avoid misinterpretation of the results obtained and highlight the climate’s influence while studying the heartwood formation features in Scots pine in territories with different climates. Other conditions, for example, soil fertility and moisture, can be similar.

5. Conclusions

We studied the heartwood formation features in Scots pine in two forest types. For the study, dominant trees were selected that grew in a blueberry pine forest on rich and moist soil and in a lichen pine forest on poor and dry soil. The plots were located in the middle taiga subzone at a 22 km distance from each other and therefore did not differ in climatic conditions. The data obtained showed that, in both forest types, the number of heartwood rings depended on the cambium age, which indicates a predominantly internal regulation of this process. We have also studied the patterns of parenchyma cell death for the first time using three cytological markers under different conditions. We showed that sapwood into heartwood transformation occurs in the same way in all studied forest types. This fact also indicates that the transformation of sapwood into heartwood is a developmental program.

At the same time, the heartwood radius and heartwood area on the cross-sections were significantly higher in the blueberry pine forest than in the lichen pine forest. The model developed earlier to predict the heartwood radius, taking into account the C.A., the width of the annual ring and the wood radius, showed a high error due to the small amount of data. Further research is needed to develop workable models for Scots pine heartwood width in a wide range of conditions, including trees growing in different forest types and climates.

Author Contributions: Conceptualization, N.A.G.; methodology, T.V.T., N.A.G. and S.A.M.; formal analysis, T.V.T., N.A.G., S.A.M., K.M.N., E.V.M. and N.V.G.; writing—original draft preparation, T.V.T.; visualization, T.V.T. and N.A.G.; funding acquisition, N.A.G. All authors have read and agreed to the published version of the manuscript.

Funding: The research was supported by the Russian Science Foundation, grant number 21-14-00204.
Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We warmly thank the staff of the Strict Nature Reserve “Kivach” for organizing the fieldwork in the territory. We thank Maria A. Ershova, Olga V. Chirva, Vladimir A. Kharitonov, Ivan V. Romashkin, Nikita V. Afoshin, Aleksandra A. Serkova, Diana S. Ivanova, Ludmila I. Semenova, and the specialists from the Analytic Laboratory of the Forest Research Institute of the Karelian Research Centre of RAS for their technical assistance in sampling and carrying out anatomical and chemical analyses within the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the study’s design, in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References
57. Saranpaa, P. Plastids and Glycolipids in the Stemwood of Pinus sylvestris L. Trees 1988, 2, 180–187. [CrossRef]
58. Bergstrom, B. Chemical and Structural Changes during Heartwood Formation in Pinus Sylvestris. Forestry 2003, 76, 45–53. [CrossRef]
70. Saranpaa, P. Plastids and Glycolipids in the Stemwood of Pinus sylvestris L. Trees 1988, 2, 180–187. [CrossRef]
71. Rigling, A.; Bräker, O.; Schneiter, G.; Schweingruber, F. Intra-Annual Tree-Ring Parameters Indicating Differences in Drought Stress of Pinus sylvestris Forests within the Érico-Pinion in the Valais (Switzerland). Plant Ecol. 2002, 163, 105–121. [CrossRef]
72. Sterck, F.J.; Zweifel, R.; Sass-Klaassen, U.; Chowdhury, Q. Persisting Soil Drought Reduces Leaf Specific Conductivity in Scots Pine (Pinus sylvestris) and Pusbesent Oak (Quercus pubescent). Tree Physiol. 2008, 28, 529–536. [CrossRef]
76. Rigling, A.; Bräker, O.; Schneiter, G.; Schweingruber, F. Intra-Annual Tree-Ring Parameters Indicating Differences in Drought Stress of Pinus sylvestris Forests within the Érico-Pinion in the Valais (Switzerland). Plant Ecol. 2002, 163, 105–121. [CrossRef]
77. Sterck, F.J.; Zweifel, R.; Sass-Klaassen, U.; Chowdhury, Q. Persisting Soil Drought Reduces Leaf Specific Conductivity in Scots Pine (Pinus sylvestris) and Pusbesent Oak (Quercus pubescent). Tree Physiol. 2008, 28, 529–536. [CrossRef]
83. Thulasidas, P.K.; Bhat, K.M. Log Characteristics and Sawn Timber Recovery of Home-Garden Teak from Wet and Dry Localities of Kerala, India. Small-Scale For. 2009, 8, 15–24. [CrossRef]


