# Study of differences in hardiness, phenology and stem characters for beech with and without red heartwood

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Omslag: Beech forest, photo taken by Lars Rytter

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## **Summary**

In order to test whether red heartwood in mature beech might be caused by frost hardiness problems, 15 trees with, and 15 trees with no, red heartwood were selected by means of bore cores taken at a height of 4 metres in a 100-year-old beech stand in southern Sweden.

Frost hardiness was tested on three occasions during autumn 1999 by the electrolyte-conductivity method (EC). Vital, current-year leafless shoots from the lower part of the crown were used. In addition, diameter, total height, height to live crown base, and branch-free length of stem were assessed for all 30 trees. Phenology in spring (bud burst) and autumn (leaf discoloration), together with different stem characters, such as number of branches, branch thickness, apical main stem problems, crown type and the proportion of the crown damaged by drought, were also classified.

The EC was only significant on one of the test dates, when reduced electrolyte leakage for trees with red heartwood was exhibited. No differences in phenology were found among heartwood types. Thus, there were no indications that trees with red heartwood were less frost hardy than those with no red heartwood. Neither did the various tree characters differ between the two heartwood types. However, the results should be interpreted with caution since they were based on a limited number of trees from only one stand.

# Introduction and objective

Red heartwood is a common discoloration defect in beechwood. It is mainly found in diameters greater than 40 cm, ie, at an age of 80–100 years or more, and has a high adverse impact on the economic value. It is therefore of great economical interest to get to know the causes behind the damage.

The red heartwood in beech is believed to be caused by a physiological process involving oxidation of phenols in older, less healthy wood. Low moisture conditions together with certain enzymes and oxygen are required for initiation of the process (Zycha, 1948; Dietrichs, 1964; Torelli, 1984).

The physiological activity and thus the water content inside a tree both diminish continuously with age. The ability to limit the consequences of external injury is less in this inner-wood zone than in the more active outer zone. For smaller wounds on the outer side of the stem, the ingress of air through the wood is inhibited by the formation of tyloses in the vessels. For larger wounds, such as deep stem cracks and large broken branches that create a connection to the inner sections of the wood, there is less chance of effectively preventing oxygen from penetrating deeper into the wood. Red heartwood substances are then developed by the oxidization of phenols in the wood and red heartwood is formed (Jensen & Pagh; 1999).

Since the area of the inner inactive zone increases with the diameter of the tree, the probability of red heartwood developing increases over time (Larsen, 1943; Racz, 1961).

In a study by Kiss (1971) it was reported that trees that shed their leaves later had a higher volume production but also a higher frequency of false heartwood associated with frost damage. Older studies also suggested that red heartwood was mainly caused by poor frost hardiness (Rohde, 1933; Nilsson & Johnsson, 1944) and this does not conflict with the theory outlined above. If this is true, it emphasizes the importance of using material that is well adapted to the climate of the plantation site.

The main objective of this study was to evaluate whether red heartwood might be caused by frost-hardiness problems. However, other possible causes for the emergence of red heartwood, such as different stem characters, are also included in the investigation.

## Material and methods

Approximately 100 core samples were taken at a height of 4 m in an indigenous beech stand in southern Sweden in summer 1997 (Table 1). After thorough inspection of each core, 20 trees with red heartwood (RH) were identified. For tests of differences in autumn frost hardiness, phenology and stem characters, 15 trees with the most extensive RH formation were selected, together with 15 additional trees with no trace of RH. The latter trees were as close to the RH trees as possible in the selected stand in order to minimize the influence of different environments.

Table 1. Stand information.

Lat., Long., Alt.	55° 56', 13° 19', 100 m a.s.l.
Area	2 ha
Total age	100 years
Stems/ha	180
Mean diameter	46 cm
Mean height	24 m
Soil texture	Clay
Soil moisture	Mesic
Vegetation	Grass type

Frost hardiness was measured by electrolyte leakage after controlled freezing (Deans et al. 1995). The technique is based on measurements of electrical conductivity (EC) from sap diffused from damaged tissue. A high level of leakage gives high conductivity, indicating badly damaged tissue. This method was previously found to be useable for ranking the sensitivity to chilling of different beech genotypes (Stener, et al., 2002a).

Assessment of EC was planned to start in autumn 1998 and to continue in spring and autumn 1999. However, 1998 was a mast year, resulting in difficulties in finding vital test material. Consequently, no studies were made either in autumn 1998 or in spring 1999. The EC study was carried out on only three occasions in autumn 1999: 29 September, 13 October and 27 October.

On each of these dates, three vital, current-year shoots having an approximate length of 10 cm were collected from the lower part of the main crown, ie, at a height of 10–14 m, from each of the 30 selected trees. This was accomplished by using a ladder and a 10-m-long pruning pole. No shoots were taken from adventious branches. Since a gradual development of hardiness along the stem has been reported for *Picea mariana* and *Picea abies* seedlings (Colombo et al., 1995; Hultén, 1980), the importance of collecting the shoots from roughly the same relative height of the trees on each date was underlined. Since this was given priority, the number of branches cut down on each occasion was restricted. Each shoot was sprayed with de-ionized water before being placed in three Oasis blocks, each measuring  $40 \times 30$  cm (Fig. 1). Oasis (Smithers-Oasis, Denmark) is a material that has a high water-absorption capacity and is normally used for flower decorations.

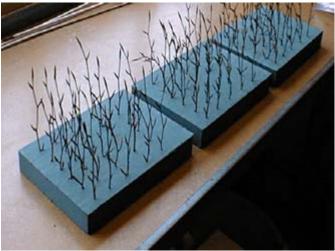


Figure 1.
Oasis blocks used for freezing shoots to different test temperatures.

Two of these Oasis blocks were frozen to two different test temperatures in two computer-controlled freezers. On each occasion, the temperatures were chosen in such a way that moderate to severe damage was induced. A third Oasis block representing the control was stored at 4°C during the freezing of the others. Each freezer was cooled at a rate of 10°C per hour to 2°C and thereafter at 5°C per hour until the pre-set freezing temperature was reached. The minimum test temperature was maintained for 3 hours and then the temperature was increased by 10°C per hour to 2°C (Deans et al., 1995).

After freezing, each shoot was rinsed by being sprayed with de-ionized water. Next, 2–5 cm of the lower part of the shoot was trimmed and thrown away. From the remaining part, a 1-cm-long bottom piece, approximately 3 mm thick, was cut off and immersed in a polypropylene scintillation vial containing 15 ml of ultra-pure water. The vials were placed in darkness on a shaker in a cold-storage room at 4°C for 3 days, after which the conductivity was measured at room temperature using a temperature-compensating probe (ATI, Orion model 130, USA). Each sample was killed by autoclaving for two hours at 121°C and being shaken for 24 hours at 4°C, after which the conductivity of the water was measured a second time.

The relative electrolyte leakage (REL) was estimated as the relation between the conductivity before and after autoclaving for frozen vs. unfrozen samples and calculated as the Index of injury according to Flint et al. (1967):

$$I_{t} = (REL_{F} - REL_{C}) \cdot 100 / (1 - (REL_{C}/100)), \text{ where}$$

 $I_t = Index of injury, \%$ 

 $REL_{F} = (EC_{F} / EC_{FA}) \cdot 100$   $REL_{F} = (EC_{F} / EC_{FA}) \cdot 100$ 

 $REL_C = (EC_C / EC_{CA}) \cdot 100$ 

EC<sub>F</sub> = Electrolyte conductivity of the frozen sample

 $EC_{EA}$  = Electrolyte conductivity of the frozen sample after autoclaving

EC<sub>C</sub> = Electrolyte conductivity of the control

EC<sub>CA</sub> = Electrolyte conductivity of the control after autoclaving

Spring phenology was classified on one occasion (6 May) by a 1–7 bud-burst index:

- 1 = Dormant bud
- 2 = Buds swollen and elongated
- 3 = Buds begin to burst when the first green shows
- 4 = Folded and hairy leaves begin to appear
- 5 = Individually visible folded and hairy leaves
- 6 = Leaves unfolded, still fan shaped and pale scales present
- 7 = Leaves unfolded, smooth and bright.

Assignment to a certain class of bud development was done if at least 50% of the buds in the upper part of the crown had reached this stage (Wang & Tigerstedt, 1993). Autumn phenology was scored on three occasions, 14 Oct, 20 Oct and 28 Oct, by observation of the proportion of discolored leaves (Wang, Tigerstedt, 1993). This classification was done in the middle of the crown, since the uppermost parts of the tops of most of the trees had been damaged by drought.

Furthermore, tree characters such as diameter at breast height, total height, height to live crown base and branch-free length of stem were measured for all 30 trees. In addition, branch numbers, branch thickness, apical main stem problems, crown type and the proportion of the crown damaged by drought were classified on a scale of 1–5 (the higher the number, the more favourable the trait). The classification of crown type was based upon the mean angle of the branches, where index 1 represented crowns with branches growing perpendicular to the stem and index 5 represented crowns with branches growing vertically, parallel to the stem.

The temperature was recorded every hour, one metre above the ground in the central part of the stand by means of a miniature temperature logger (Tinytag Plus, INTAB Interface-Teknik AB, Sweden). The logger was protected from direct solar radiation by being placed inside a perforated tin, wrapped in aluminum.

## **Calculations**

The statistical analysis of all traits was performed by PROC GLM (SAS, 1996) and was based upon individual observations. The stand was divided into four blocks prior to analysis and the following model was used:

$$Y_{ijk} = \mu + b_i + hw_j + e_{ijk},$$
  
where,

 $Y_{iik}$  = Observation ijk

 $\mu$  = Mean value

b<sub>i</sub> = Fix effect of block i

hw; = Fix effect of trees with or without red heartwood

 $e_{ijk}$  = Random error term for observation ijk, with expected mean 0 and

variance  $\sigma^2$ 

As the records from the phenology scores and the tree quality characters did not display normal distribution, they were transformed within each block to normal-score values prior to statistical analysis (Gianola & Norton, 1981). The  $I_t$ -values exhibited approximately normal distribution and, since arcsin transformations did not change the model predictions, these traits were kept untransformed. All the means presented in the figures and tables are based upon original, untransformed values.

Correlation coefficients were estimated as Pearson correlations based on individual observations (SAS, 1996).

## Results

## Phenology

The mean, minimum and maximum day—night temperatures in the beech stand in autumn 1999 are presented in Fig. 2. Autumn that year was very mild and the minimum day—night temperature never fell below zero during the study period.

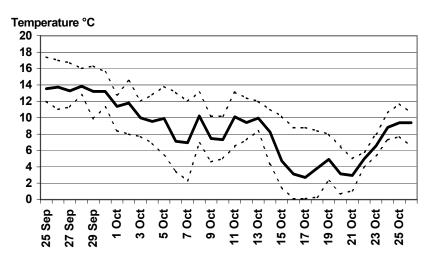


Figure 2.

Mean, min and max temperatures in the beech stand in autumn 1999.

No significant differences in phenology in either autumn or spring were found among trees with or without RH (Fig. 3).

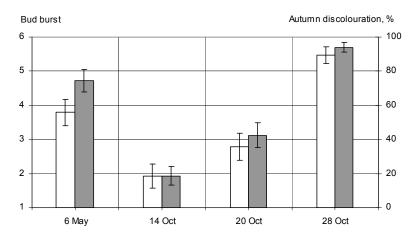


Figure 3.

Mean phenology and standard error on different dates. White bars represent means for trees with no red heartwood and grey bars means for trees with red heartwood. The left y-axis refers to the classification of bud burst in May and the right y-axis to the three classifications of leaf discoloration in October.

### Electrolyte leakage

Mean leakage expressed as the Index of injury (I<sub>t</sub> values) is presented in Fig. 4 for trees with and without RH on the different test dates. There were considerable differences in leakage between the two test temperatures on certain dates. As expected, leakage increased with decreasing test temperature.

There were no significant differences in leakage among trees with and without RH for any test date at a given test temperature, with the exception of 13 Oct for test temperature –25°C (Table 2, Fig. 4). In this test, leakage was less for trees with RH, indicating higher frost hardiness compared to trees without RH.

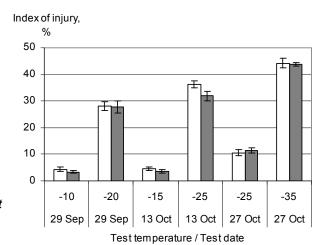


Figure 4.
Mean Index of injury (%) and standard errors for trees with no RH (white bar) and trees with RH (grey bar) for different test temperatures on different test dates.

Table 2. Significance levels (P > F) from the analysis of variance for Index of injury. Bold figures are significant (p < 0.05).

Date	Temp	df			P>F	P>F		
	°C	Block	Heart wood	Bl. x Heartw.	Block	Heart- wood	Bl. x Heartw.	
29 Sep	-10	3	1	3	0.684	0.134	0.772	
29 Sep	-20	3	1	3	0.007	0.384	0.556	
13 Oct	<b>–15</b>	3	1	3	0.947	0.305	0.824	
13 Oct	-25	3	1	3	0.265	0.030	0.332	
27 Oct	-25	3	1	3	0.266	0.735	0.349	
27 Oct	-35	3	1	3	0.041	0.902	0.212	

On average, the size of the RH discoloration in relation to the whole length of the core sample (representing the tree radius) was 20%, varying between 8 and 32%. When the central pith was missing, the missing core length was approximated. After deletion from the analysis of the four trees with the lowest discoloration percentages, the average changed to 23%, varying between 15 and 32%. New evaluations based on this reduced number of trees did not change the results in any way. Correlations between  $I_t$  and the proportion of RH in the core length for the 15 trees in which RH had been found were weak (r< -0.22) and not significant.

Correlations between I<sub>t</sub> and autumn phenology were in general negative and weak (Table 3). This negative trend indicated that trees with a high proportion of leaf discoloration were more frost hardy than those with a low proportion of discoloration.

Table 3. Correlations between autumn phenology observed on different dates and Index of injury for different test dates and temperatures. Bold figures are significant (p < 0.05).

Phenology	Test date and test temperature (°C)						
Dates	29 Sep		13 Oct	13 Oct		27 Oct	
	<b>–10</b>	-20	<b>–15</b>	-25	<b>–25</b>	<b>–</b> 35	
14 Oct	-0.272	-0.467	-0.189	-0.221	-0.047	-0.071	
20 Oct	-0.201	-0.460	-0.122	-0.330	0.078	-0.125	
28 Oct	-0.032	-0.206	-0.022	-0.306	0.272	-0.113	

#### Tree characters

There were no significant differences in tree characters between the two heartwood types (Table 4).

Table 4. Means for trees with (RH) and with no (No RH) red heartwood, and significance levels (P > F) from the analysis of variance for different tree characters. Bold figures are significant (p < 0.05). Block and block x RH effects were zero for the quality traits, since they were transformed to normal score values within each block.

Trait	Mean			P>F		
	Total	RH	No RH	Block	RH	Bl. x RH
Diameter at b.r.h., mm	463	473	452	0.087	0.353	0.934
Total height, m	23.9	24.1	23.8	0.009	0.596	0.972
Height to green crown, m	4.2	4.2	4.2		0.854	
Steml. without branch., m	2.5	2.8	2.1		0.215	
Branch thickness	2.79	2.67	2.93		0.446	
Branch number	2.86	2.73	3.00		0.064	
Apical stem problems	2.41	2.33	2.50		0.570	
Crown type	3.62	3.73	3.50		0.188	
Crown drought damage, %	18	14.0	22.0		0.131	

### **Discussion**

### Electrolyte leakage

Adaptation of growth initiation and cessation and, hence, frost hardiness in boreal climates is vital for survival and fitness. In a study of 70 species in North America it was found that hardiness of all species and provenances was related to the average minimum temperatures on the sites, indicating that cold resistance is essential for the distribution of plant species (Sakai & Weiser, 1973).

In a frost hardiness study in southern Sweden by Stener et al. (2002a) significant differences in the Index of injury (leakage) for different beech genotypes were found in both spring and autumn, making ranking in hardiness possible. Significant differences in bud burst have been reported as well (Stener et al., 2002a, 2002b; von Wuehlisch et al., 1995a, b). Autumn phenology in terms of the proportion of leaf discoloration was found to be significant for provenances (Stener et al., 2002b). Notice that the hardiness method and the phenology scores used in the studies by Stener et al. (2002a, 2002b) were designed in the same way as in the present one.

In this study there were no differences in either spring or autumn phenology among trees with or without RH (Fig. 3). The EC test was unlike that expected, indicating that trees with RH were no more sensitive or even less sensitive, to freezing temperatures than trees with no RH (Table 2, Fig. 4). It is debatable whether the results would have been the same if trees with a higher degree of RH damage had been found. The correlations between leakage and proportion of core sample with RH were weak and not significant. However, for all 6 tests (3 dates x 2 temperatures), the correlations were negative (r < -0.22), indicating that leakage was higher for trees with a smaller proportion of RH. There were clearly no indications that trees with RH were less frost hardy then those with no RH.

The classification of trees into the two RH groups was of course crucial for the result. To reduce this risk of misclassification, all core samples were taken at a height of 4 m, since the distribution of the RH along the stem is usually shaped like a cigar, with the largest diameter a few metres from the base, then progressively narrowing towards the end. For instance, Junker (1936) stated that the largest diameter of the RH was found at a point 20–30% along the length of the stem, whereas Sachsse (1991) asserted that it could be at a point 50% along the length. Another more unusual RH formation which, in cross-sectional form, is formed more like a star along the border with the sapwood, has its maximum diameter at the base of the tree and therefore decreases with increasing stem height (Sachsse, 1991).

One might ask if the poor differentiation between the two heartwood groups was caused by errors that can be referred to the EC method itself. This is unlikely, however. The EC method was apparently reliable, since the general trends in leakage were logical (Fig. 4):  $I_{\rm t}$  (leakage) increased with decreasing test temperature and decreased over time, ie, the later in autumn it was, the higher became the degree of frost hardiness. This concurred with results from two other similar studies of hardiness in beech (Stener, 2002a and 2002b).

The limited number of trees and the number of shoots collected per tree reduces the scope for drawing any general conclusions. The aim was to investigate a number of mature beech stands. However, since it was impossible for us to get permission from the landowners to take core samples, owing to the very real risk of damage being done to the trees, only one stand was available for the study.

#### Stem characters

The following stem/crown characters have previously been linked to the development of RH in beech: Age and diameter (Larsen, 1943; Racz, 1961; Hupfeld et al., 1997; Soil-water conditions (Juncker, 1936; Larsen, 1937; Racz, 1961); Exterior stem damage and branch breakage (Racz, 1961; Shigo, 1986); Crown type/branch angle (Meilby & Jensen, 1993; Jensen & Pagh, 1999) and, lastly, Leaf defoliation (Jörgensen & Bergstedt, 1996). None of the stem characters examined in the present study showed any differences between trees with or with no RH (Table 4). This was not in accordance with the studies mentioned above.

#### **Conclusions**

- 1. There were no indications that trees with red heartwood were less frost hardy then those with no red heartwood.
- 2. No differences among various tree characters were found between the two heartwood types.

The results should be interpreted with caution, since they were based on a very limited number of trees sourced from only one stand.

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