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DISTRIBUTION AND QUANTIFICATION OF TENSION WOOD IN POPLAR SHOOTS

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Introduction

The presence of tension wood in a tree is a current defect on most species, especially in Poplar and Eucalyptus species. Being not detected by the users when tension wood development is still limited, it could become a major defect, prohibiting wood processing for more valuable utilizations. This is particularly true when the proportion of this tissue becomes important in a log, in comparison with normal wood tissue. Then, this defect is revealed by splits, wolly surface, warps, twists, etc., (CAMPREDON (1953), KOLLMAN & CÔTÉ (1968), ISEBRANDS & BENSEND (1972), WICKER (1979), CASTERA & MAHE (1992)).

Within the tree, tension wood formation plays an active role. It represents a high performance mechanism allowing the elaboration of proper species architecture and allows a development and a harmonious adaptation of the tree to its environment. It must not be considered as a defect but as a structure with characteristics particularly useful for the tree, (WARDROP (1956), SCURFIELD (1973), WILSON & ARCHER (1977), FISHER & al. (1981), KUBLER (1989)).

It seems accepted that a gravitational stimulus catched by a tree initiates the cambial formation of tension wood. This tissue is characterized by gelatinous fibres with an extra layer inside the lumen. During their maturation phase, these fibres show a more important axial contraction than normal fibres. The multiplication of these individual contractions acting asymmetrically will induce important tension forces, also called growth stresses, capable to modify spatially the orientations of the different axes. (JACOBS (1945), TRÉNARD & GUÉNEAU (1975), ARCHER (1976), MARIAUX & VITALIS-BRUN (1983), SUGIYAMA & al. (1993)).

The studies undertaken in the Forest Research Station of Gembloux aim to have a better understanding of tension wood formation in the tree as a reaction to a gravitational stimulus, particularly considering the dynamics of its formation, the distribution in the stem and the accurate quantification of the phenomenon. Different experimentations are under process.

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1. Experiments

1.1. *Aims of experiments*

The first aim is the study of the dynamics of gelatinous fibres formation which is typical of the tension wood of poplars, as well as the observation of generally connected phenomena such as shoot eccentricity and axes movements. Therefore all along the growing season artificially bended shoots are collected and microscopical observations are made at different levels along the shoot; this allows to quantify the evolution of tension wood formation.

The second and third aims respectively consider the effects of duration and intensity of the gravitational stimulus on the quantity of gelatinous fibres produced and on the distribution within shoot cross-section. Thus plants are leaning at different degrees (0°, 5°, 10° ,15° ,20°, 30°), and for different durations (6h, 12h, 1d, 2d, 4d, 8d, 16d, etc.).

The fourth aim tends to study the influence of tension wood on the global wood structure and on the primary and secondary shoot growth.

1.2. *Experimental material*

The experimental material is formed by shoot of the current year coming from cuttings of *P. euramericana* cv 'Ghoy' and *P. interamericana* cv 'Beaupré'. That choice presents several advantages (simplifying the observations and the interpretations of tension wood formation) such as:

- the use of a young clonal material;
- very little modifications induced by external influences;
- a perfect control of the environment in green house;
- a fast growing of poplar shoot;
- a high sensibility to the stimulus;
- the possibility to observe the complete shoot cutting by one microscopic cross-section;
- an easy display of tension wood area under stimulus;
- an easy treatment by image analysis system after staining.

Cuttings are placed in individual plant pots during May. The beginning of the growth appears one month later through the upper bud. The plants are kept in green house all along the growing season and the shoots are collected either during the season or when the growing season stop (at the beginning of November).

1.3. *Application of the stimulus*

Very little is know concerning the mechanism of perception of the gravitational stimulus. It is well know however, that any element inducing a loss of equilibrium in the setted-up architecture or even a very small modification of the orientation of the axis leads to the formation of a wooden structure, generally on the upper face of the stem, characterized by an usual higher stress level due to the formation of a proper tissue called tension wood.

It was thus decided to create artificially the best conditions for the formation of such a tissue by using a stimulus easily applied. Considering these elements and being practical, a gravitational stimulus artificially induced by sloping the shoot and its pot was selected. The growing shoot was fixed with elastics on a leaning stake. It results from this choice that:

- the stimulus is homogeneous on the whole shoot;
- it may easily be applied on each shoot individually;
- it may be reversed, modified or suppressed at any time;
- it is not influenced by other external conditions such as lighting, wind etc.;
- it remains stable wathever the duration is;
- such a stimulus was previously used by ROBARDS (1965,1966) with salix and by WARDROP (1956) with eucalyptus.

2. Determination and quantification technique

2.1. Determination of gelatinous fibres

The technique here after described allows, thanks to a differential colouring, to display areas of tension wood on thin cross-sections, realized in one year old poplar shoots. This technique is also convenient for the observation of gelatinous fibres of mature trees.

The possibility to display gelatinous fibers, is based on the purely cellulosic nature of the inner layer, (called « G layer ») of the secondary wall. The double colouring « Safranin - Astra blue » allows to clearly distinguish between tension wood tissues and normal wood tisues on cross-section. Safranin is a specific colouring of lignified tissue used for permanent mounting. It stains all walls inlayed by lignin red. Astra blue is a specific colouring of cristallin cellulose found in G layer, staining this layer blue.

Due to the fact that G layer is relatively thicker than the remaining of the fiber wall (middle lamella, primary wall and S1 & S2 layers of secondary wall in the case of poplar), this double colouring is very effective for microscopical observations. At low magnifications, tension wood areas appear blue on cross-section and normal wood areas red. At higher magnifications, there is a good contrast between blue coloured G layer and the red coloured remaining fibre wall. This differential staining is effective enough to detect a single G fiber in a normal wood area and vice versa.

2.2. Quantification by image analysis

The quantification of tension wood and other parameters of a microscopic shoot section, are realized by using a KONTRON image analysis system, linked to an optical microscope or to a stereomicroscope with a black and white video camera. The use of an image analysis system requires very high quality, thin and homogeneous sections as far as their colouring is concerned. The high accurate level of measurements as well as their fast realization are major advantages for the study of the distribution and the quantification of tension wood.

The methodology uses a digitalization process in grey levels of coloured microscopic sections. These digital images allow the treatment by computer. The discrimination of grey levels between different areas of tension wood, normal wood, pith, etc., allow to separate these objects. Consequently, it is possible to measure their different morphological parameters. These measurements may be applied to different angular sectors. A similar approach may be used at macroscopical scale to discs cut from mature trees if a staining as, for instance, zinc chloro-iodide (HERZBERG method) clearly shows tension wood areas.

These measurements repeated on sequential sections collected along the shoot axis allow to quantify the volume of tension wood and to realize a tridimensional representation within a given shoot.

3. First results on the study of a *P. euramericana* cv 'Ghoy' shoot

As an illustration, the results from the study of a shoot of P. "Ghoy" are presented here after. It concerns a one year old naturally bended dwon shoot, 1.20 m long, collected on the field in November. The shoot was cut in portions of 10 cm long; on each one, a microscopical cross-section coloured by safranin - astra blue was performed at the bottom and measured by the image analysis procedure. Table 1 indicates, for each section, the values of various parameters.

If each portion is assimilated to cylinders, the total shoot volume is 93.9 cm^3, the wood volume without pith is 84.1 cm^3 and the tension wood volume is 21.6 cm^3. Thus the tension wood volume accounts for 23.0 % of the total shoot volume or 25.6 % of the shoot volume without pith. It clearly shows that the pith surface grows significantly along the shoot axis. On an other hand, the proportion of tension wood in one section strongly varies and may reach 34,0 % in surface (Fig 1).

Table 1. Analysis of *P. euramericana* cv 'Ghoy' shoot

Section number	Section level (cm)	Total area (mm2)	Total area without pith (mm2)	Pith area (mm2)	Tension wood area (mm2)	Proportion of tension wood area (%)	Proportion of tension wood area without pith (%)
12	111	29.9	16.7	13.2	1.7	5.7	10.2
11	101	40.1	24.8	15.3	6.9	17.3	28.0
10	91	46.3	31.3	15.0	7.0	15.0	22.2
9	81	47.4	35.6	11.8	13.4	28.3	37.6
8	71	53.0	42.2	10.8	12.0	22.6	28.4
7	61	55.8	46.8	9.0	13.2	23.5	28.1
6	51	67.8	60.8	7.0	10.3	15.2	17.0
5	41	77.8	71.3	6.5	17.2	22.1	24.1
4	31	86.6	81.6	5.0	21.6	25.0	26.5
3	21	114.1	111.4	2.7	38.2	33.5	34.3
2	11	144.4	143.6	0.8	36.4	25.2	25.3
1	3	175.8	175.1	0.7	37.9	21.5	21.6
mean value		78.2	70.1	8.1	18.0	21.2	25.3

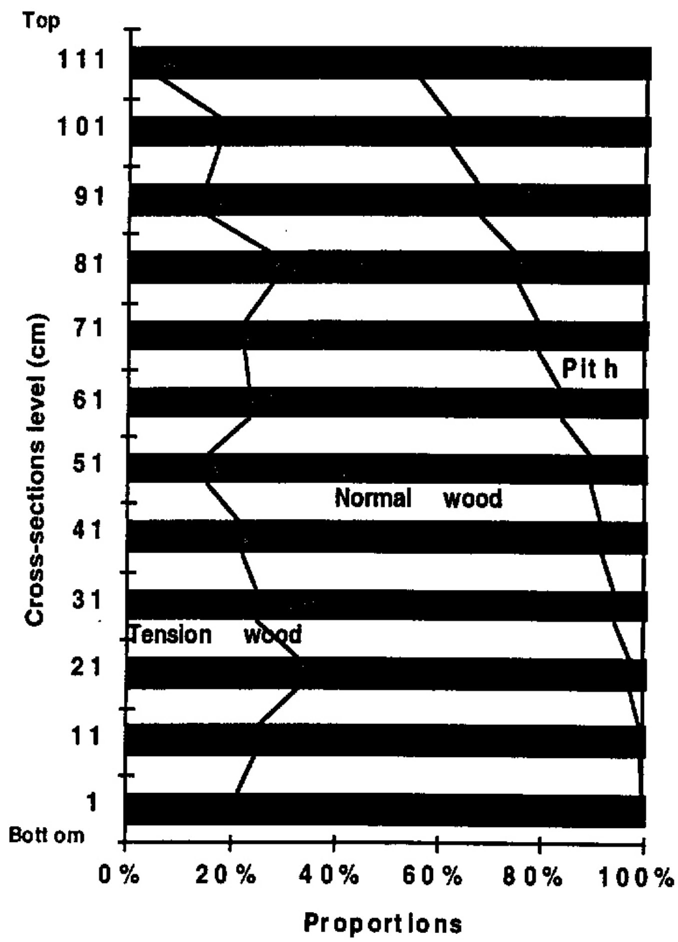

Figure 1. Proportion of tension wood, normal wood and pith per height level

The sections analysis per angular sectors of 15° (Fig 2), shows the shoot eccentricity. Excepted for the first level, corresponding to the bottom of the plant, a more important growing appears on the upper face of the shoot. In the higher levels the eccentricity is not yet quite visible, due to the fact that the sections are mainly composed of pith.

The angular distribution of tension wood indicates a preferential direction along which this tissue was formed. That direction remains rather identical at each level. Figure 3, clearly displays that the preferential direction of tension wood is not corresponding to the leaning orientation at the time of the collection on the field. It exists a shifting of 45° between the eccentricity orientation and the one of the sectors where tension wood was mainly produced. This seems to indicate that although two phemomena answer to a same stimulus their mechanism of reaction remain however independent.

Finally, as shown in Figure 4, it appears that the tension wood distribution on each side of a preferential orientation is not symmetrical. On one side, there is a sharp transition between normal and tension wood, while on the other side, the proportion of tension wood per sector gradually decreases. In considering the 21 cm height level, this leads to observe sectors composed of nearly 45 % of gelatinous fibres on the under face of the shoot and some sectors nearly without tension wood at the upper face of the shoot. It must be still noted that tension wood distribution becomes more and more erratic higher along the shoot.

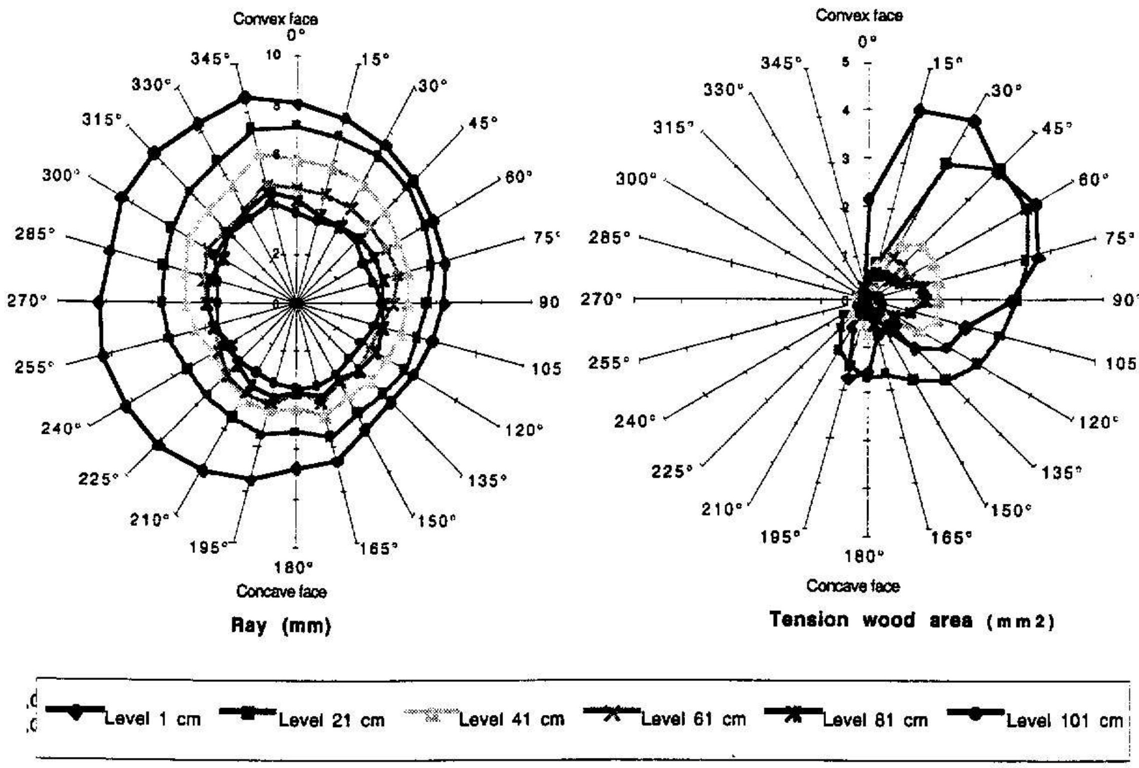

Figure 2. Angular distribution of radius and surfaces of tension wood

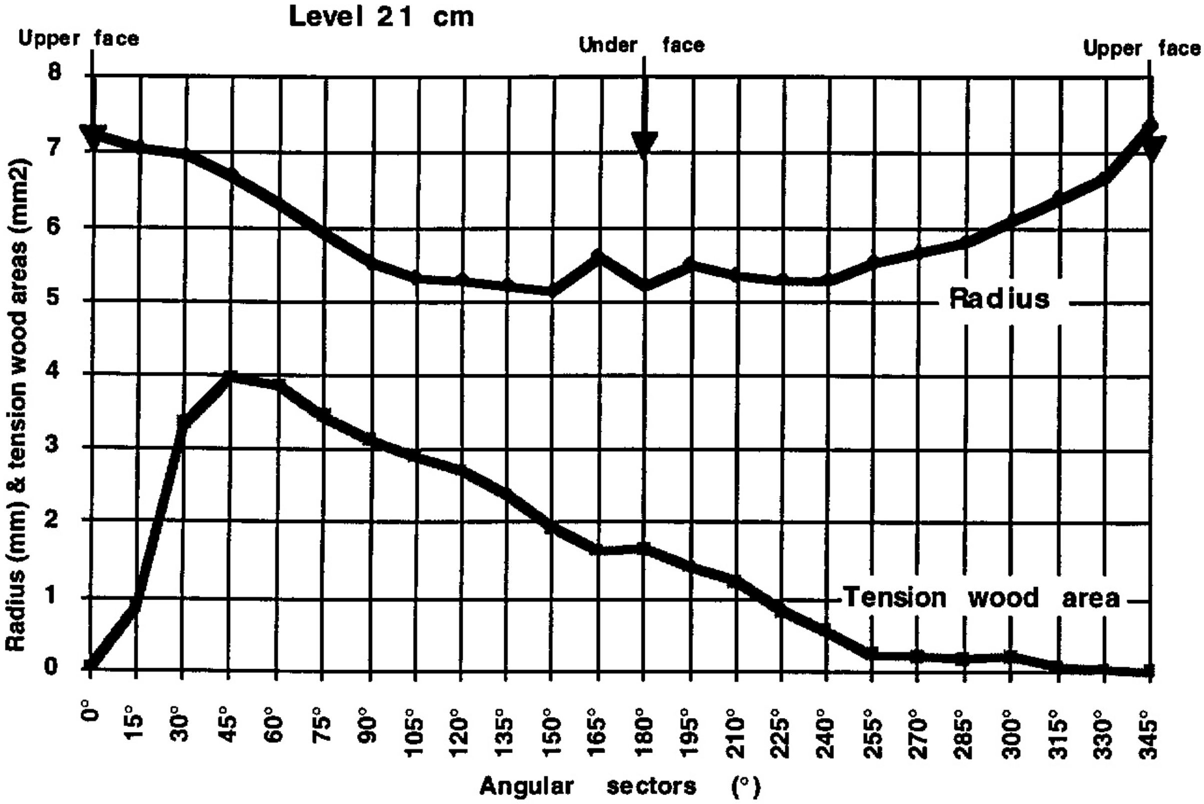

Figure 3. Representation of shifting between eccentricity and tension wood

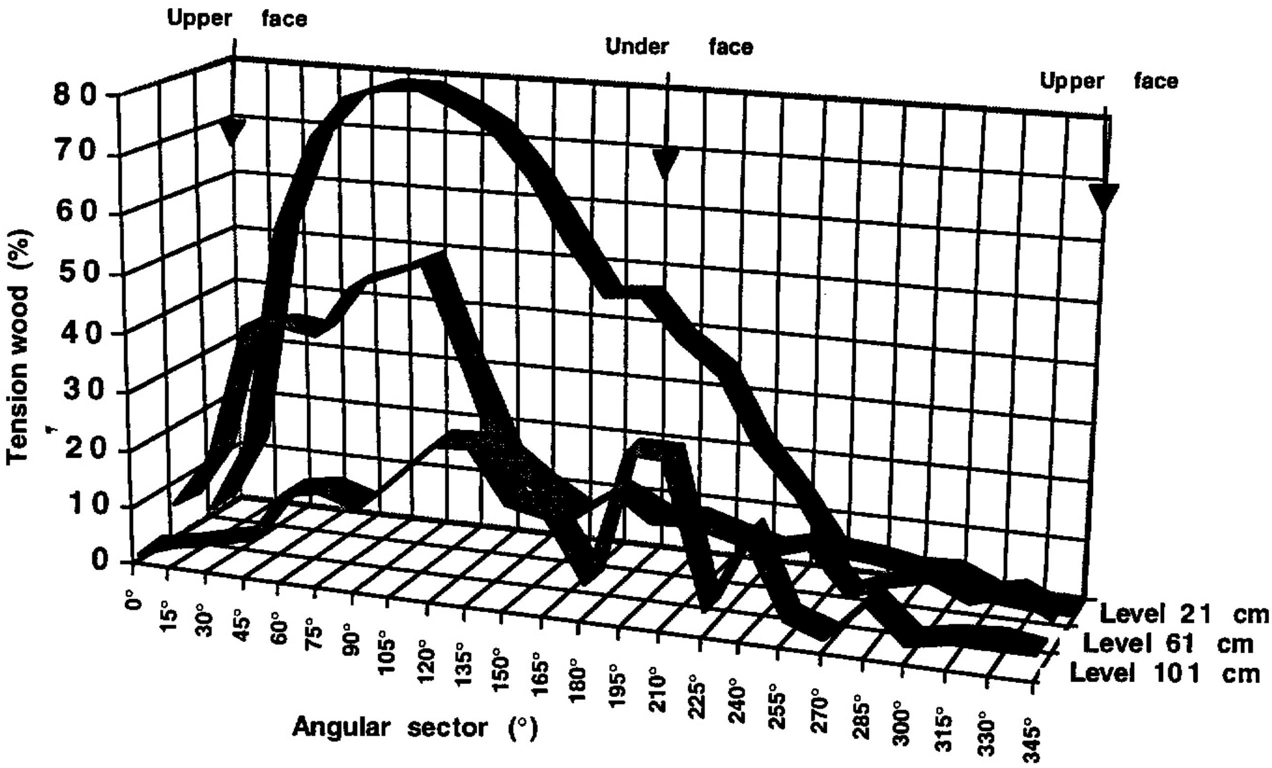

Figure 4. Proportion of tension wood per angular sectors in the lower, medium and upper parts of the shoot

4. Conclusion

The young shoots of *P. euramericana* cv 'Ghoy' and *P. interamericana* cv 'Beaupré' form an ideal raw-material for studying the distribution and the quantification of tension wood, due to a high sensibility to a stimulus and a fast growing.

The choice of an artificial gravitational stimulus easily produce a homogeneous tension wood tissue all along the shoot. The safranin-astra blue colouring technique and the use of an image analysis system allow to estimate quickly and with a high accuracy the quantity of gelatinous fibres produced.

Its clearly appears from the examination of a given shoot from the field that tension wood formation and stem eccentricity are two independent phemonena even if they react to a same stimulus. On the other hand, the quantity of tension wood produced could be relatively important and is oriented allong a preferential direction.

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