Growth Rings in the Stem of *Thuja orientalis* L.

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Summary: During the winter dormancy period of 1998-99, the differentiation process of xylem and the formation of annual and pseudo-annual growth rings was studied in sections of the central stem of *Thuja orientalis* of different age (1–14 year old), starting from the top (the tip of leader shoot) towards the bottom. In the apical 1–2 cm portion of the one year-old leader shoot, only the protoxylem was formed by the end of vegetation. The protoxylem elements appeared first in 6 bundles than gradually merged into 2 semiquadrangular (triangular) bands (each containing 3 protoxylem bundels) around the pith. In this stem portion, the pith was cross-formed first and became gradually flattened at the lower stem parts, following the generally flattened shape of the stem and the respective facial and marginal position of leaves. A continuous xylem ring (with formation of metaxylem elements) apeared 3–5 cm below the shoot tip. In fact, it was the stem part where a "real" annual ring was formed by the end of vegetation. The first pseudo-annual rings were observed 16 cm below the top. The "regular" annual rings were completely continuous all around the stem, consisted of strongly flattened in radial direction thick-walled latewood tracheids and had a distinct border (demarcation line) at the end of the year. The "pseudo-annual" rings formed incomplete dark rings or semicircular bands within the earlywood. They were composed of tracheids with thick cell walls but somewhat wider radial diameter than those of the "real" annual rings, and the border between their outside margin and the next earlywood was less distinct.

In the xylem of two year old stem portion, the innermost central annual ring appeared not at the transition zone between the current and the former years of growth, but about 2 cm lower. Above that, only the thick-walled bundles of the former years protoxylem were found. Down the stem, the older sections showed similar features: the next annual ring appeared always somewhat lower than the borderzone of the given and the former years growth. The "pseudoannual" rings (or more correctly the growth rings) continued regularly to appear in the lower (older) sections of the stem as well. They were found untill the age of up to 14 years (the bottom of the studied plants). Their number was 3–4 per year first, than (from the 5th annual ring counted from the centre) decreased to 3, 2, 1, and in the youngest outer part of the xylem there was no pseudo-annual ring at all. The development of pseudo-annual rings was usually more marked on the thicker (more branched) than on the thinner side of stem. Stems older than 14 years were not studied.

Introduction

Thujas are among the most popular ornamental conifers in Hungary, widely planted in public parks and private gardens. They are, therefore, very important crops for our nurseries. In 1998, for example, the total number of seedlings and cloned transplants of *Thuja occidentalis, Th. orientalis* and *Th. plicata* grown in the nurseries (including all sizes and categories) was more than 1.5 million (OMMI, 1999).

In our earlier studies (Schmidt 1995; Schmidt et al, 1999) it was revealed that 6 *Thuja* cultivars, along with several other members of the *Cupressaceae* family, seemed to form more than one growth ring per year in the xylem. The aim of present studies was to investigate the mechanism of this phenomenon along with the growth pattern on the example of *Thuja orientalis.*

Materials and methods

During the winter dormancy period of 1998–99, the differentiation process of xylem and the formation of annual and pseudo-annual growth rings was studied in the sections of the central stem of Thuja orientalis of different age (1–14 year old), starting from the top (the tip of leader shoot) towards the bottom. Stem samples were taken from the one and two year old apical sections at 3–10 cm intervals and from the lower parts at 10–20 cm intervals, depending on the position and strength of the side branches and on the visible signs of the borderline (or, more correctly, borderzone) of the current and the former years of growth.

The samples were softened with cc. HF (hydrogene-fluorid-acid) for two weeks. After that the acid was washed out with tap water. The air was removed from the sections under vacuum.

Cross sections of 30–60 mm thickness were made from the samples with a freezing sledge microtome (from the lower more woody parts with sharp razor blade or with xylotome) and after staining with toluidine-blue they were examined and photographed under a transmission light microscope.

Results

Cross sections taken from the top towards the bottom of the strong one year old leader shoot are shown in *Figures 2–4*. They spectacularly illustrate that the different parts of this stem portion are in various phases of differentiation and lignification as follows:

The primary cortex and the leaf tissues are fully differentiated, and, like in the other scale-leafed members of the Cupressaceae family, are not clearly distinguishable from each other (Fahn, 1977, Fouda, 1996)

6 cm below the apex: Fully developed multilayered vascular cylinder, first signs for compression and degradation of primary cortex and leaf tissues.

Figure 1a Shoot tip of Thuja orientalis in January 1999, showing the shortening of internodes towards the top and the whirl of leaves at the apex.

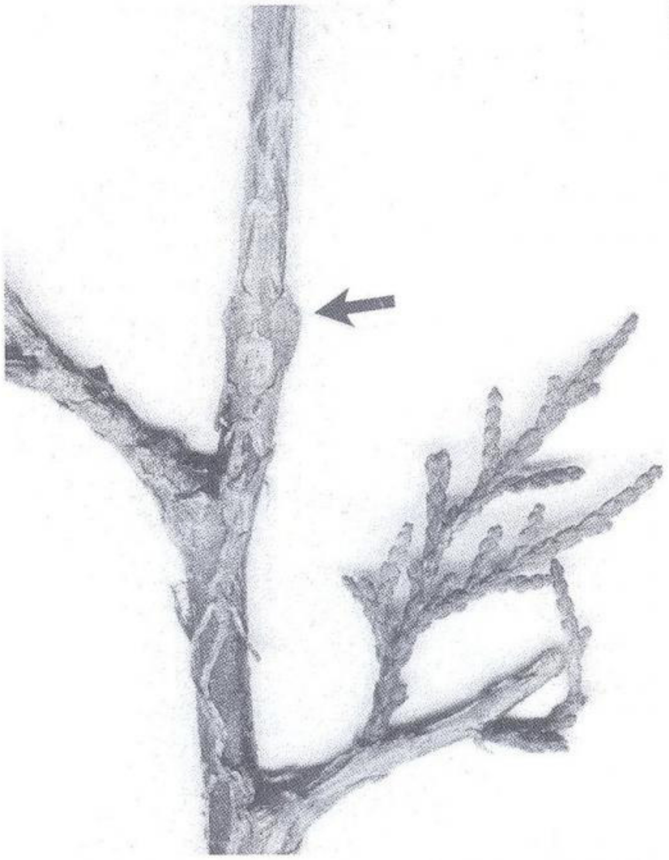

Figure 1b The lowest part of one year's growth and the upper part of two year's growth of Thuja orientalis stem. Arrow is indicating the borderline between the current and the former year's growth. Note the shortened internodes sections of central stem of Thuja orientalis at this zone and the abruptly elongated internodes below and above

Shoot apex (0.5 cm below the apex): Presence of primary tissues.

In this apical part of the stem the vascular system consists of 6 more or less distinct bundles, arranged in two semicircles (3-3 in each) around the flattened-cross-shape pith. The cells of pith are still in the process of division

2 cm below the apex: Formation of cambium ring and the vascular cylinder.

This stage is shown on *Fig. 2a-2b*. The pith is cross-shaped or flattened cross-shaped, changing alternately the direction of the "cross" according to the alternate position of leaf-pairs. On the periphery of the pith, the protoxylem elements are still in 6 bundles.

The vascular cylinder is quadrangular. Inside, the metaxylem consists of 2–16 layers of tracheid cells, surrounded by the dark continuous strip of cambium. The phloem contains one layer of thick-walled fibre cells.

As shown on *Fig 3.,* the vascular cylinder has strongly increased in diameter: the xylem is about 4 times wider (with 30–40 layers of tracheid cells), the phloem 3 times wider (with 4 clearly visible strips of phloem fibers) than it was just 4 cm upward (2 cm below the apex). The tissues of cortex and the leaves still are green in colour and (by enlargement and division of their cells) try to follow the increasing circumference of the stem. It is seen, however on the cross-section, that they are at the borderline of their capacity: many of the cells are crushed, disintegrated, and this whole tissue zone is starting to die.

5–6 cm lower, as the stem is further increasing in diameter, the colour of cortex (seen from the outside) is turning first yellow and than brown, indicating that there are no more photosynthesising (living) cells in it and that a periderm has started to develop

15 cm below the apex (the upper third of the shoot):

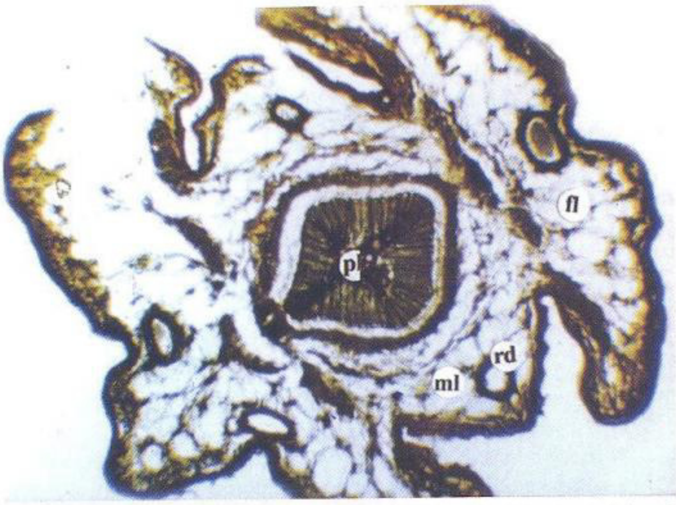

Figure 2a Cross-section from the middle of Thuja orientalis shoot, taken 2 cm below the apex, showing the first formation of interfascicular cambium and the cambium ring, initiating metaxylem elements towards inside and metaphloem outside. Note the cross shaped pith (= p), and the quadrangular central cylinder. (Obj. 3,2 x Oc. 3,2). (px=primary xylem, mx=metaxylem, rd=resin duct, ml=marginal leaf, fl=facial leaf)

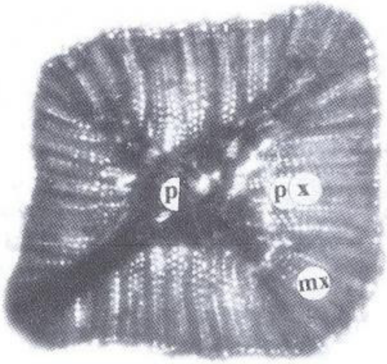

Figure 2b The quadrangular xylem at higher magnification (Obj. 10 x Oc. 3,2)

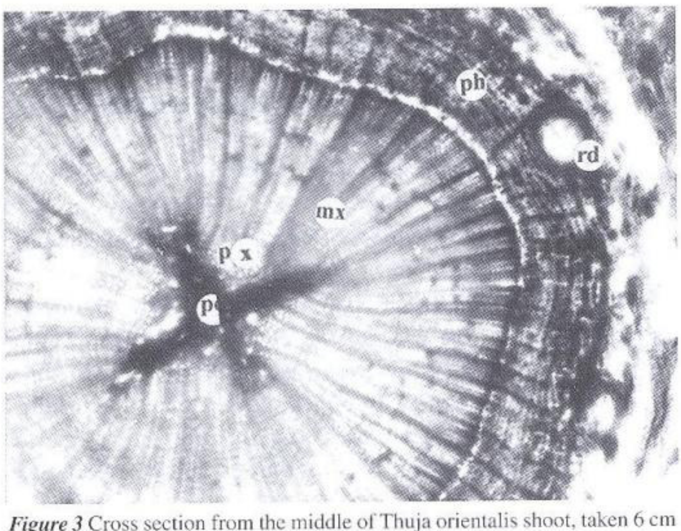

Figure 3 Cross section from the middle of Thuja orientalis shoot, taken 6 cm below the apex. (Obj. 10 x Oc. 3,2). Note that the xylem became cylindrical and about 2 times wider than on the former sample taken just 4 cm above. Abbreviations as Fig. 2

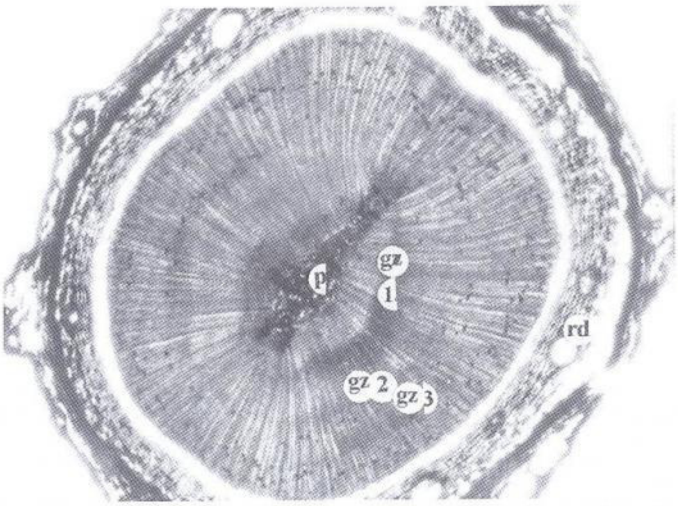

Figure 4 Cross section of Thuja orientalis shoot, taken 15 cm below the apex. (the upper third of this year's growth). Note the multi-layer phloem, the numerous resin ducts (=rd) in the cortex and in the phloem, and the presence of growth zones (=gz 1, 2, 3) (pseudo-annual rings) in the xylem. Other abbreviations as on fig. 2. (Obj. 3,2 x Oc. 3,2)

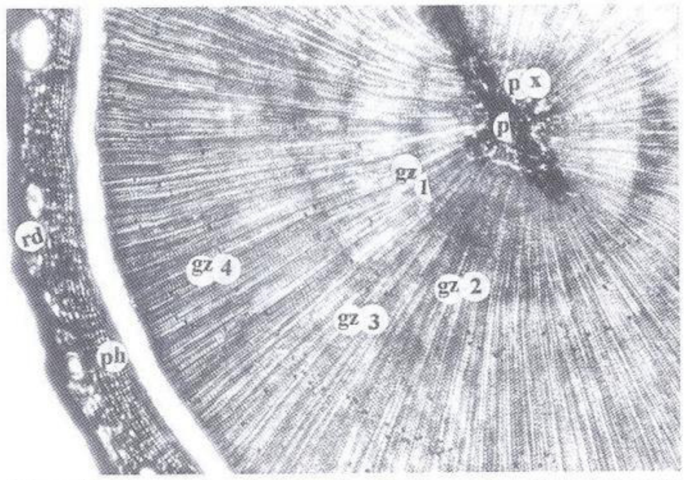

Figure 5a Cross section of Thuja orientalis shoot, taken 45 cm below the apex, where is the borderzone between the former and the current year's growth. No sign of real annual ring in the middle, but there are 3-4 dark coloured growth zones in the current year's xylem. (Obj. 3,2 x Oc. 3,2).(p=pith, gz=growth zones 1-2-3-4-etc., px=primary xylem, x=metaxylem, rd=resin duct, ph=phloem)

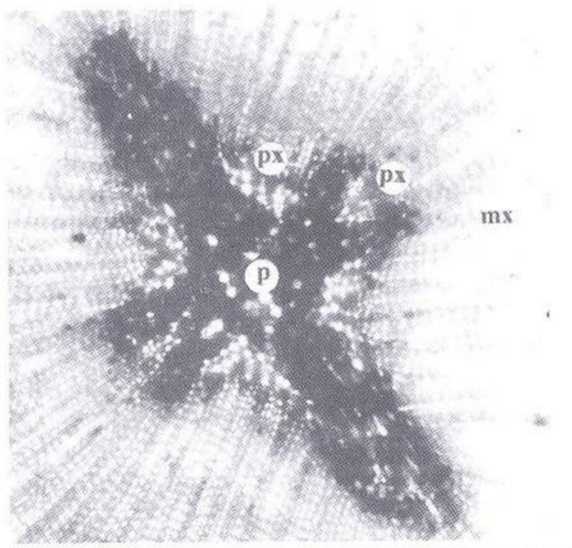

Figure 5b A closer view of the cross-shape the 6 bundles of protoxylem and the triangular beginnings of metaxylem from the former year. (Obj. 10 x Oc. 3,2)

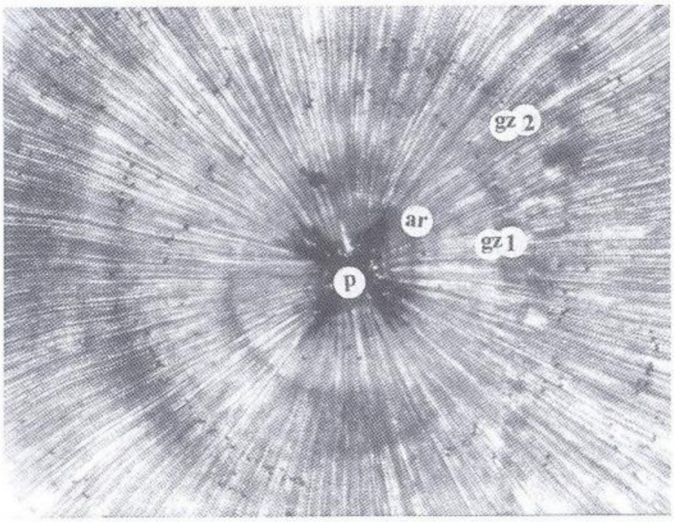

Figure 6 Cross section from two years old part of Thuja orientalis leader shoot taken 2 cm below the transition zone 47 cm below the apex. Note the similarities of the central tissue region (the near-apex part of the former year's shoot) with the analogue tissues shown in Fig. 2. (Obj. 3,2 x Oc. 3,2). ar=annual ring, other abbreviations as on Fig. 5

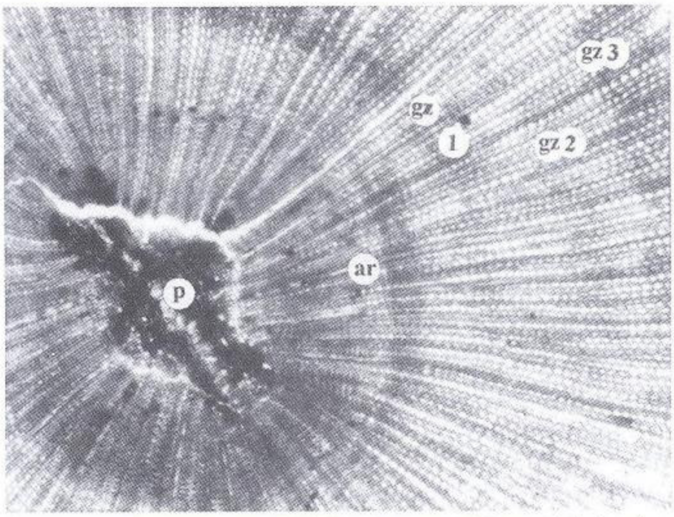

Figure 7 Cross section from the middle of two years old part of Thuja orientalis leader shoot taken 6 cm below the transition zone (51 cm below the apex). (Obj. 10 x Oc. 3,2). Note the differences between the "true" annual ring and the "pseudo-annual rings" (growth zones). Abbreviations as in Fig. 5-6

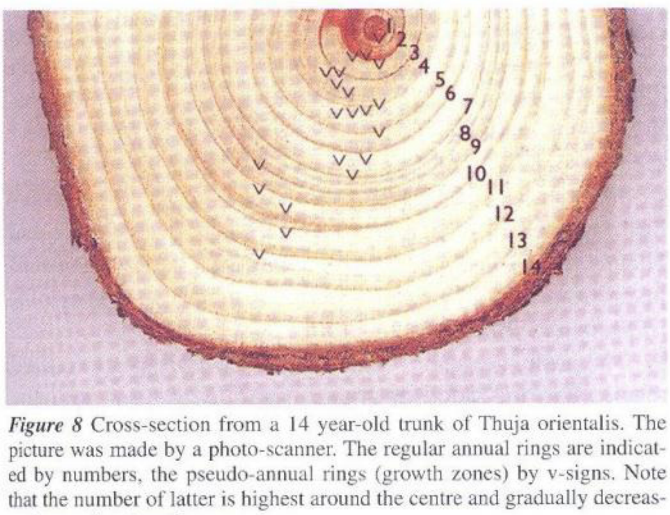

Figure 8 Cross-section from a 14 year-old trunk of Thuja orientalis. The picture was made by a photo-scanner. The regular annual rings are indicated by numbers, the pseudo-annual rings (growth zones) by v-signs. Note that the number of latter is highest around the centre and gradually decreases (to zero) outwards.

Full decay of primary cortex, development of periderm, appearance of the first pseudo-annual rings (growth rings).

As shown on *Fig. 5.,* the vascular cylinder has further strongly increased in diameter: it has doubled it size as compared to the former sample *(Fig. 4.)* taken 10 cm above.

Tissues of the primary cortex and leaves died entirely. Below, a true periderm is protecting the stem, consisting of flattened, dark brown cork cells. The residues of resin ducts in the leaves are still visible, but there are new resin ducts within the phloem too, formed probably by schyzogene way. This is the stem portion, where the first "pseudo-annual rings" or (perhaps more correctly) "growth zones" are observed in the xylem. They form dark semicircular bands (or almost complete rings) within the earlywood, mostly in the inner parts of xylem. They are usually incomplete in circumference, composed of tracheids with thicker cell walls and somewhat wider radial diameter than those of the "real" annual rings, and the border between their outside margin and the next earlywood (with larger and thinner-walled tracheids again) is not distinct.

45 cm below the apex (the borderzone between the current and the former year's growth):

According to the outside morphological signs (a whirl of densely arranged scales indicating the end of the former year, and than a starting section of this years growth with abruptly increasing internodes, *(Fig. 1b,* on page 24*)* this is the place where the one and the two-year-old stem sections meet. Theoretically, the annual ring of the former year should appear at, or slightly below, this point. Practically, however, there is no sign of a 2nd year's annual ring in the middle, just the dark coloured pith and the flattered triangles of the starting metaxylem-formation and 6 spot-like traces of protoxylem bundles around it. So practically the whole xylem is consisting of current year's tissues, with clearly visible sections of growth zones arranged semicircularly or almost circularly in the earlywood. The inner zones are darker and the outer ones thinner and lighter which shows a kind of correlation with the stronger state of side shoots that developed earlier at the basal part and to that of smaller side shoots developing later towards the apical part of the central leader shoot.

47 cm below the apex (2 cm below the borderline between the current and the former year's growth):

An incompletely developed annual ring in the middle, and pseudo-annual rings in the current year's xylem as before.

As seen on *Fig 6.,* the middle part of this stem section contains a cross-shaped pith surrounded by 4 triangular sections of former year's metaxylem. The outer sides of these triangles are forming a vaguely rectangular "annual ring" which is, however, not complete. There are breaks at the four corners of the rectangle, where metaxylem formation is none or minimal. In the inner side of the triangular metaxylem-section, the 6 darker bundles of protoxylem (4 at the 4 inner corners of the triangles and 2 at the shorter edges of the cross-shaped pith) are still visible. If we compare this central part of *Fig. 6* with *Fig. 2a-2b,* a striking similarity is observed between the almost closed young rectangular vascular cylinder 2 cm below the apex, and the almost closed rectangular "annual ring" formed in the middle of the xylem 2 cm below the borderline between the former and the current year's growth.

Outside the rectangular (incomplete) annual ring, there are 2–3 dark coloured "pseudo-annual rings" (growth zones) in the current year's xylem.

50 cm below the apex and 5 cm below the border of current and former year's growth:

The 2nd years annual ring is fully developed in the middle, "pseudo-annual rings" in the current year's xylem as before.

The lower (older) parts of the stem. Going down the stem, the same phenomenon repeated from year to year: the next annual ring appeared not at but always 2–4 cm below the transition zone and the xylem contained growth zones up to the 13th year. It is well illustrated on *Fig. 8,* showing a scanned image from the cross-section of 14 year-old trunk. The annual rings appear as marked continuous circular lines, while the growth zones are seen as lighter and somewhat interrupted lines, forming usually 1/3, 1/2 or 3/4 of circles on the stronger side of trunk. The largest number of pseudo-annual rings (growth zones) was formed in the innermost side of trunk, until the 4th annual ring. Proceeding outwards, the number of pseudo-annual rings is gradually decreasing: first to 3, than 2, 1, and finally, between the 13th and 14th annual ring, to zero.

Discussion

The results show that the histological structure of one year old central shoot of *Thuja orientalis* resembles the structure of an actively growing shoot even during the period of winter dormancy: The stem near the apex is in the primary stage of differentiation, with 6 vascular bundles and half-developed pith, primary cortex and leaf tissues. The cambium has not formed a continuous ring yet. As known from the literature (*Haraszty,* 1979; *Esau,* 1977) during this primary stage of stem differentiation the histological structure of woody plants resembles that of the herbaceous plants. The strange thing is only that the stem part of *Thuja orientalis,* the sample was not in the process of active growth (and hence continuous differentiation), but in the state of winter dormancy. It would have been supposed, therefore, to consist of stabilised (consolidated) tissues, with fully lignified xylem ending with an annual ring around, like all the "normal" woody plants of temperate zone do at the end of vegetation.

A possible explanation to this anomaly is given by Schmidt and Batiz (1999). Studying the shoot growth on 16 temperate-zone members of *Cupressaceae* family (belonging to genera *Calocedrus, Chamaecyparis, Cupressus, Juniperus, Thuja*) they found that (unlike the "normal" temperate-zone woody plants) these species do not finish their yearly growth by the end of June, but continue to grow (although at a slower rate) until October or sometimes to early December. In the autumn, therefore, they do not "complete" their yearly shoot development, but just "cease to grow on".

As shown on *Fig. 1 a* (on page 24), the apical part of *Thuja orientalis* do not end with a real bud either. The internodes between the scale-like leaves are getting shorter towards the top and finally, a whirl of leaves stays at the end. The next years growth will start from here by simple elongation of internodes first, and than by formation of new leaves *(Fig. 1 b).*

The lower position of the second year's annual ring from the point where the last year's growth ended is caused probably by the same phenomenon. The apex was not ripe by the end of vegetation and the stem did not contain a vascular cylinder here yet, just vascular bundles, with no interfascicular cambium between them. The cambium ring developing at this position next spring, started to initiate and build the vascular cylinder belonging to the given (second) year.

The "pseudo-annual rings" (growth zones) begin to appear in the xylem at about the upper half of the first year's growth. Going even lower, they become more and more definite. Their number shows some relationship with the number of the side branches (or pairs of branches) that have developed above them. It is suggested that the development of these growth rings is in interaction with the multiple branching system of *Thujas,* producing ramifications of up to the 5th degree within one single year. (*Schmidt,* 2000).

Greguss (1955) made detailed xylotomical studies on conifers including *Thuja orientalis.* The investigations were carried out on longitudinal radial and tangential sections and on cross sections of xylem, taken from the old trunks. The author does not mention the presence of pseudo-annual rings, probably it was not a subject of his investigations or (according to his microphotos) they were no more present in the investigated old xylem.

In the xylotomic part of his doctoral-dissertation with another member of the *Cupressaceae* family *X Cupressocyparis leylandii* Orlóci (1996) does not mention any pseudo-annual ring either.

The general plant anatomical works, like the books of *Esau* (1969, 1977), *Fahn* (1977) or that of *Haraszty* (1979) do not speak about any irregular annual ring formation at the *Cupressaceae* family, neither do the general dendrological works dealing with conifers (*Krüssmann,* 1985, *Bean,* 1976-80, *Den Ouden,* 1978, etc.).

Recent publications, Parker and Johnson (1987) dealt with the leader growth of young *Thuja plicata* plants, *Briand* et al. (1993) made a detailed study of the influence of age and growth rate on radial anatomy of *Thuja occidentalis* trees of different ages on the Niagara Escarpment, Ontario, Canada. In the same area, *Larson* et al (1994) reported on the radically sectored pathways in the xylem of *Thuja occidentalis.*

In Hungary, *Felhősné* (1993) made a comparative histological study of leaves of scale-leaf evergreens, and *Fouda* (1996) described the anatomical characteristics of juvenile and adult shoots associated with rooting ability of *X Cupressocyparis leylandii* cuttings.

The present research proved that growth rings are formed regularly in the wood of *Thuja orientalis* at least until the age of 13 years. Further studies are needed, to clarify the possible interactions between the growth dynamics, branching pattern and the formation of growth rings.

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