SEASONAL RHYTHMS OF XYLEM GROWTH MEASURED BY THE WOUNding METHOD AND WITH A BAND-DENDROMETER: AN INSTANCE OF CHAMAECYPARIS OBTUSA

by

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SUMMARY

The pinning method for the measurement of xylem growth was modified for easier application. Trunks of Chamaecyparis obtusa were monthly incised with a knife instead of a thin needle. Two years later, xylem blocks including wounded areas were harvested. For comparison, circumferential increases of the same trees were measured with a band-dendrometer. Measurements from the wounding method indicated a tendency for cambial cell production to accelerate twice a year, around April and August. Circumferential increase measured with the band-dendrometer differed from radial growth measured from wounding. Circumferential increase was very small around August and continued after the cessation of cell production. The climatic data near the plantation suggested that the circumferential size of the trunk probably is affected by the physical shrinkage of trunks because of water shortage during drought season and trunk swelling following precipitation. Circumferential increments did not reflect the seasonal rhythms of xylem growth. Therefore, for detailed information on radial growth within a season, the wounding method is recommended.

Key words: Xylem growth, pinning method, wounding method, band-dendrometer, drought shrinkage, Chamaecyparis obtusa.

INTRODUCTION

In the phenological researches on trees, there is a demand to know the growth rhythms of trunk-xylem during a season (Worbes 1995), as well as shoot and leaf growth. As a convenient method to measure xylem growth, band-dendrometers (Liming 1957; Palmer & Ogden 1983) have been used (Katsch et al. 1992; Kiyono et al. 1984; Komiyama et al. 1985). This non-destructive method is suitable for long-term measurements. However, there are disadvantages to this method. Physical shrinkage of trunks occurs during drought and cold season (Kozlowski 1972; Hinckley & Bruckerhoff 1975). Therefore, circumferential measurement with a band-dendrometer does not seem to be suitable for providing detailed short-term information on the growth rhythms of xylem.
As a precise method to measure xylem growth, the ‘pinning method’ was proposed by Wolter (1968), and subsequently modified by Kuroda (1986). This method is based on the wound reaction of cambial cells differing from that of enlarging and wall-thickening cells (Yoshimura et al. 1981a, b; Nobuchi et al. 1995). Unfortunately, this method has not become popular because it can be difficult to obtain good sections from the center of tiny wound tissues. To make this method easier, incisions with a knife, rather than a thin needle, were made on plantation-grown trunks of Hinoki cypress (Chamaecyparis obtusa Endl.). These same trees were fitted with band-dendrometers, so that data on circumferential growth could be compared with the data for radial growth obtained from the wounding method. The data from the two methods are discussed in correlation with the climatic data near the plantation.

MATERIALS AND METHODS

Wounding, dendrometer equipment and sampling

In the 28-year-old plantation of Hinoki cypress (Chamaecyparis obtusa Endl.) in Shiga Prefecture, two sample trees (Trees A and B in Fig. 2–4) were selected from the upper and lower area of a slope. DBHs were 10.4 cm and 15.8 cm, respectively. Stainless-steel band-dendrometers (Liming 1957), thickness 0.15 mm and width 6.05 mm, were placed at breast height of the trees on April 25, 1989. Prior to installing the band, the outer bark under the band was slightly removed to make the band smoothly contact the trunk surface. Incisions, 5 cm long and deep enough to reach the xylem, were made 15 cm below the dendrometers with a 18 mm width disposable blade.

Values on the band-dendrometers were read at one-month intervals until June 1991, except for the dormant season, January and February. On the same dates, incisions were made spaced laterally c. 10 mm from the previous wounds. On August 8, 1991, wounded areas were harvested with a chisel. Xylem blocks (c. 15 × 20 × 20 mm) were fixed in FAA (formaldehyde : acetic acid : 50% ethanol = 5 : 5 : 90 v/v).

Light microscopy and measurement of xylem growth

Fixed blocks were washed with tap water for one day. Transverse sections 30 μm thick were cut with a sliding microtome and stained with 1% Nile-blue. Each wound tissue was photographed under a light microscope. Radial growth was measured on the photographs. The site of cambium at the wounding date was estimated based on the criterion of the pinning method (Kuroda 1986), e.g., an abrupt increase of radial rows of tracheids (see Fig. 1b & c) as a result of the radial division of cambial initials replacing destroyed cells. This increase occurs on the outer margin of wound tissue in conifers (Fig. 1). Radial growth by the wounding date (Fig. 1a, Gr) was indicated as averaged tracheid numbers counted adjacent to the wound tissue on both sides, from the preceding ring’s growth ring boundary to the assumed site of cambium at the time of wounding (Fig. 1a, broken line; 1b & c). Not only cell production by the cambium, but radial growth in ‘width’ was measured to compare with the circumferential increment measured in mm with the band-dendrometer.
To calculate the proportional radial growth by each wounding date in one growth ring, tracheid numbers of annual rings were counted radially. Counts were made and averaged on three radial rows at normally growing sites (Fig. 1a, AR) on both sides of wound tissues. The width of annual rings also were measured at the same sites.

Precipitation, sunshine in hours and temperature near the plantation were extracted from monthly reports of climatic data in Shiga Prefecture (1989, 1990). The climatic data were examined for correlation with the seasonal rhythms of radial growth and circumferential increments.

RESULTS AND DISCUSSION

Estimation of radial growth with incision wounds

Because longitudinal incisions make longer wound tissue, locating suitable areas for sectioning and measuring was much easier than for very tiny pinning wounds. From May to July, wound tissues appeared spindle- to diamond-shaped in cross-sections (Fig. 1a). Increase in radial rows of tracheids, which looks like a palm of the hand in cross section (Fig. 1b, arrows; 1c), was detectable around the cambial side margin of wound tissue (Fig. 1a, arrow; 1b). This feature indicates the site of the cambium at the wounding dates as described in reports of the pinning method (Yoshimura et al. 1981a, b; Kuroda 1986). An increase of tracheid files occurs to repair the destroyed cambium (Yoshimura et al. 1981b). The broken line in Figure 1a was judged to have been the site of cambium at the wounding date. A callus-like cluster of parenchyma cells (Fig. 1b, Pa), which originally was proposed to be the site of the cambium at the time of wounding (Wolter 1968), is a little inside from the site defined by Kuroda's criterion given above. Pinning and incision both destroy enlarging immature tracheids and result in tangential splits (Fig. 1c) in the enlarging zone, the most fragile tissue (Yoshimura et al. 1981b). Callus-like cells are formed in such gaps as the result of abnormal ray cell proliferation (Kuroda 1986). In the period of latewood formation from August onwards, wound tissue changed to be bow-shaped in cross section (Yoshimura et al. 1981b) because the immature cell zone now is radially narrower that it is early in the growing season. The increasing point of tracheid files also could be found in this season.

Annual ring boundaries became wavy at the wound sites (Fig. 1a) because of the tangential crack in the xylem caused by wounding, and because of a local increase or decrease in tracheid production after wounding. Reactivation of tracheid production occurred in some cases after autumn wounding. Such abnormal cambial activity may occur because of a hormonal unbalance caused by wounding. Wavy annual-ring borders were restricted to wound sites, and did not affect the radial growth measurement because tracheid numbers produced before wounding were normal (Fig. 1a, Gr), and because annual ring width was measured at normally growing sites away from the area around wound tissues (Fig. 1a, AR).

A strong push and/or rough downward cut of the knife blade makes big cracks in the xylem and induces large wound tissue. This makes it difficult to estimate the site of cambium at the time of wounding because of the prolonged disturbance in cell divisions. Thin blades are recommended to avoid wider destruction of xylem. The spacing
between each wound must be wide enough to avoid the effect of neighboring wounds. We recommend more than several centimeters between incisions, especially in actively growing trees. Because the incision also affects tracheid production in the year after wounding, repeated wounding on the same site, or just above or below should be avoided in the following year.

**Seasonal rhythms of radial growth assumed by wounding method and circumferential increase measured with the band-dendrometer**

Annual ring width variations depend on the sites, even at the same height of a tree (Fig. 2). To exclude such local variation, cumulative growth curves were graphed as the proportional growth against the total number of cells in one annual ring (Fig. 3).
Fig. 2. Annual ring width at the wounded sites of tree A and B (measured after harvests in 1991).

Fig. 3. Cumulative proportional growth of xylem measured by the wounding method (cell number, width) and with band-dendrometers (circumference).
Fig. 4. Growth rate of xylem based on wounding method and band-dendrometer. Rates at the date of wounding were calculated from the increments about one month preceding the date of wounding.

Growth curves based on width were only slightly different from those based on the number of cells. This slight difference must be due to immature tracheids that enlarged after wounding. In contrast, the circumferential increments (data from the band-dendrometer) and the radial growth (data from wounding) were very different from one another.

To try to explain the cause of the different results for the two methods, growth rate per day was compared (Fig. 4). Radial growth, as measured by the wounding method, was activated twice a year, around April and August. In contrast, the rate of circumferential increase, as measured by the band-dendrometer, accelerated once a year from April to June, was remarkably low during the summer months of July and August, and slightly accelerated around September. The termination of circumferential increase...
Fig. 5. Precipitation, sunshine, and temperature near the sampling field in Shiga Prefecture in 1989 and 1990.

was more than a month later than the termination of cell production by the cambium. Radial enlargement of latewood tracheids is very slight, and so seems an unlikely explanation for the continued circumferential increase measured by the band-dendrometer.

In various tree species, water columns in xylem are under strong tension (lower than \(-1.5\) MPa) during the summer due to active transpiration and insufficient water supply (Scholander et al. 1965; Morikawa & Sato 1976). Such high tension causes physical shrinkage of trunk diameter in both conifers and dicotyledons (Holmes & Shim 1968; Hinckley & Bruckerhoff 1975; Lassoie 1973). Kozlowski (1972) described shrunken stems expanding minutes after irrigation. Shrinkage of trunks during midwinter is also known (Kozlowski 1972), and apparent negative growth and the consequent slipping out of place of band-dendrometers has been reported (Komiyama et al. 1985). In spite of the evidence indicating that circumferential measurements by band-dendrometers include effects of physical shrinkage and swelling of trunks, such problems seem to have been underestimated. Figure 5 describes the climatic data near the plantation (monthly report of climatic data; Shiga Prefecture 1989, 1990). June to the beginning of July is the rainy season in Japan. Precipitation in August 1990 was abnormally low. After the dry and hot period, usually precipitation increases in September because of typhoons. The circumferential increment (Fig. 4) was very small during this summer when radial growth was active. This suggests that physical shrink-
age of trunks during summer was unexpectedly large. The dry summer in 1990 might have caused the large discrepancies between radial growth and circumferential increment curves (Fig. 3, 4). Slight recovery around September and much delayed termination of circumferential increase after the actual cessation of cambial activities seem to reflect the lessening of trunk shrinkage because of reduced transpiration and adequate soil moisture in autumn.

The observations on *C. obtusa* reported here demonstrated that the increase of trunk circumferences as measured by band-dendrometers did not reflect the real process of xylem growth. Some studies using band-dendrometers found that circumferential increments correlate with precipitation (Komiyama et al. 1985; Ninomiya et al. 1987). It is doubtful whether these increments correlate with biological growth. Thus, band-dendrometers should not be used to assess seasonal growth rhythms of trees, although they are useful for long-term measurements of tree growth.

There are no serious technical difficulties with the wounding method. One caution is that the callus-like area in the wound tissue (Fig. 1b, PA) should not be used as a marker for the site of the cambium at wounding. In genera that form traumatic resin canals, e.g., *Tsuga, Abies, Larix*, and some of the Dipterocarpaceae (Shiokura 1980), these canals are useful indicators. Application of the wounding method to other conifers and to dicotyledons also is possible (Yoshimura et al. 1981a, b; Kuroda & Shimaji 1984; Kuroda 1986). For dicotyledons, the criteria for determining the location of the cambium at the time of wounding are different from those for conifers. Immature cells around the wound differentiate into abnormal parenchymatous cells. In addition, wall residue of immature cells crushed by wounding can be observed as a thick radial line on cross section. From these features, the site of the cambium at the time of wounding is assumed to be the cambial-side end of cell-wall residue in the outer part of the abnormal cell area (Kuroda & Shimaji 1984; Kuroda 1986). We suggest that additional studies examining phenology together with data on xylem growth studied by the wounding method will reveal information on the seasonal rhythms of tree growth and its correlation with environmental factors. Additional comparisons on the data from the wounding method and band-dendrometer are needed for both conifers and dicotyledons. The degree of differences between data from the two measuring methods is unknown for other species, and for other climatic regimes.

REFERENCES


