Temporal water deficit and wood formation in Cryptomeria japonica

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Received September 6, 2002; accepted February 1, 2003; published online July 15, 2003

Summary Cell behavior in the cambium and developing xylem of 3-year-old Japanese cedar (Cryptomeria japonica D. Don.) trees, during and after an 11-day suspension of irrigation, was analyzed. Leaf xylem pressure potential and tangential strain of the stem surface were monitored throughout the experiment. Anatomical features and numbers of developing tracheids and cambial cells were observed in four trees, sampled on Days 0, 4, 8 and 11 after irrigation was suspended. Daytime xylem pressure potential decreased to –1.9 MPa on Day 7 and remained the same until irrigation was resumed on Day 11. The transverse dimensions of the tracheids, which began to form secondary walls, began to decrease on Day 4. The number of cells in the cambial zone and cell expansion zone decreased abruptly on Day 8. Tangentially aligned developing tracheids with collapsed cell walls were observed in samples harvested on Days 8 and 11. Secondary wall formation was recognized in these tracheids. After the resumption of irrigation, xylem pressure potential recovered rapidly to the same value as before the suspension of irrigation. Tangential strain increased within 30 min after the resumption of irrigation, and continued to increase until the onset of light the next day. Eighteen days after the resumption of irrigation, anatomical features of cells in the cambium and cell-expansion zone were similar to those observed before suspension of irrigation.

Keywords: developing tracheid, morphogenesis, xylem pressure potential.

Introduction The water status of a tree is an important factor determining xylem cell size of woody plants (Zahner 1968, Denne and Dodd 1981). Many studies have examined the relationship between tree water status and xylem development (Larson 1963, Shepherd 1964, Zahner et al. 1964, Sheriff and Whitehead 1984, Abe and Nakai 1999). A decreased frequency of cambial cell division and a reduction in the radial diameter of tracheids generally occur in conifers in response to water deficit. Larson (1994) suggested that the decline in the frequency of cambial cell division is followed by a reduction in the diameter of newly expanded xylem cells. A decline in tree water potential (Shepherd 1964) or a decrease in the internal concentrations of plant hormones (Larson 1963) has been suggested to cause the reduction in cell diameter. Previously, we found that cell diameter decreased during the early stage of water deficit, and was followed by a decrease in the number of cells produced (Abe and Nakai 1999). Based on these results, it was suggested that cell expansion is restricted by the decline in hydrostatic pressure during the early stage of water deficit, whereas the rate of cambial cell division declined during the later stages of water deficit.

This investigation was designed to study the processes underlying the restriction of cell expansion and the reduction of cell production in trees subjected to drought. We used young cloned trees of Japanese cedar (Cryptomeria japonica D. Don.) grown in a controlled environment cabinet. Anatomical features of developing tracheids and cambial cells were observed in trees every 4 days during the water deficit. Leaf water potential was monitored with a pressure chamber. Concurrently, the tangential strain of the stem surface was monitored with a strain gauge. The change in stem diameter reflects the sum total of changes in turgidity of cells in the cambial zone, developing xylem and phloem, and the growth of xylem and phloem (Klepper et al. 1971, Molz and Klepper 1973, Sheriff and Whitehead 1984, Herzog et al. 1995).

Materials and methods

Plant materials Twenty trees of 3-year-old Japanese cedar (height = 1.08 ± 0.06 m; stem diameter 0.2 m above the ground = 17 ± 2 mm) from one clone, planted in moist vermiculite in pots (300 mm in height, 200 mm in diameter), were placed in a controlled environment cabinet providing a day/night temperature of 23/20 ± 1 °C, humidity of 75 ± 1% and a 13-h photoperiod with a light flux of about 450 ± 4 µmol m–2 s–1, as measured with a light meter (LI-190SB, Li-Cor, Lincoln, NE) at the top of the tree. Trees were irrigated and fertilized with 1 liter of a nutrient solution (5:5:6 N,P,K), as described previously by Abe and Nakai (1999), between 1500 and 1600 h every 2 days.
Experimental design
After a 2-month acclimatization period, irrigation of all trees was suspended for 11 days. Four trees were cut on Days 0, 4, 8 and 11 after suspension of irrigation. Irrigation was resumed on Day 11 and continued for 18 days before the remaining trees were cut.

Water potential
Xylem pressure potential of current-year leaves of all trees was measured with a pressure chamber (3000-1411, Soil-moisture Equipment, Santa Barbara, CA) at 0700 to 0800 h, 1400 to 1500 h and 2000 to 2100 h. Three measurement values were averaged for each tree at each time.

Tangential strain of the stem surface
Tangential strain of the stem surface was monitored with a strain gauge in 10 trees (gauge length = 10 mm, accuracy = 1 µst; Tokyo Sokki, Tokyo, Japan). Strain gauges were glued with cyanoacrylate adhesive to the surface of the inner bark after removing the outer bark with a steel knife (Nakai and Abe 1997, 1998, Abe and Nakai 1999). The stem area around the strain gauges was covered with Vaseline to prevent dehydration. The tangential strain output values were stored in a data acquisition controller (DE-1200, NEC Sanei, Tokyo, Japan) set to a measurement speed of 20 ms integral time. The interval of data acquisition was set to 10 min. In this study, we discuss only the pattern of diurnal change over the course of about 2 weeks, because of the durability of the strain gauge, the relaxation of the bonding interface, and the decline in the response of the gauge caused by traumatic tissue development around the strain gauge.

Microscopy
For microscopic observation of cambial cells and developing xylem cells, small blocks including cambium and developing xylem and phloem from the stems of cut trees, were fixed in 3% glutaraldehyde. The blocks were immersed in a graded ethanol and propylene oxide series, and embedded with epoxy resin. Transverse sections, 3 µm thick, were cut with a glass knife. All sections were stained with safranin and gentian violet, and observed with an optical microscope (BIOPHOT, Nikon, Tokyo, Japan) and a polarizing light microscope (OPTIPHOT-POL, Nikon).

Image analysis
The image analysis method used has been described previously by Abe et al. (1997). Monotone photographs of light microscope and polarizing light microscope views were taken. The negatives were scanned at 2400 dpi with 256 color grades (Cool Scan 2; Nikon, Tokyo, Japan), and images were analyzed with the public domain NIH Image program 1.56 (Wayne Rasband, U.S. National Institutes of Health). Lumen area was measured with the polarizing microscope for each tracheid at which birefringence was first observed, relative to the cambium, for about 50 tracheids in each sample. The accuracy of this method was 0.11 µm.

Cell numbers in the cambial and cell-expansion zones
Cell numbers in the cambial and cell-expansion zones were counted for about 50 radial files of tracheids in each sample. Because it was difficult to identify the boundary between the differentiating xylem and phloem in the cambial zone, we defined the second cell from the clearly identifiable phloem cells with thick walls, in the direction of the xylem, as the first differentiating xylem cell in the cambial zone (Abe et al. 1995). The number of tracheids between the first differentiating tracheid and a tracheid that was beginning to form the secondary wall in a radial file, was defined as the number of cells in the cambial and cell-expansion zones. The first cell in the radial file to form a secondary wall was identified from its birefringence as seen with the polarizing microscope.

Results
Xylem pressure potential
Figure 1 shows the change in xylem pressure potential during the experiment. The highest pressure potentials were measured at 0700 to 0800 h every day until the resumption of irrigation. The lowest values in a day were measured at 1400 to 1500 h until Day 4 after the last irrigation, with similar values at 1400 to 1500 h and at 2000 to 2100 h after Day 5. Xylem pressure potential at 1400 to 1500 h and 2000 to 2100 h decreased to about −1.9 MPa by Day 7 after the last irrigation, and did not change much until the resumption of irrigation on Day 11. Xylem water potential at 0700 to 0800 h continued to decrease during the suspension of irrigation, reaching −1.8 MPa by Day 11.

Xylem pressure potential recovered rapidly after the resumption of irrigation, reaching about −1.2 MPa at 2000 to 2100 h on Day 11, and about −0.4 MPa at 0700 to 0800 h the next day. Six days after the resumption of irrigation, xylem pressure potential recovered to the value before the suspension of irrigation.
Tangential strain of the stem surface

The tangential strain of stems can be converted to the change in stem diameter; the stems have a completely circular cross section. Tangential strain showed a regular diurnal fluctuation (Figure 2), increasing immediately after the end of the light period, and decreasing immediately after the onset of light. Tangential strain increased within 30 min after the resumption of irrigation, and continued to increase until the onset of light the next day.

Vascular development

Samples harvested on Day 0 contained 8–14 cell layers in the cambium and cell-expansion zones (Figure 3). The appearance of these cells was similar to that of cells normally observed during a period of active growth. In the samples collected at Days 8 and 11 after the last irrigation, several layers of developing tracheids with birefringent cell walls had collapsed (Figures 4a and 4b). These tracheids, which appeared to have expanded and become distorted, were observed in three of four trees at each harvest on Days 8 and 11.

In samples harvested 18 days after the resumption of irrigation, cells in the cambial and cell-expansion zones had shapes similar to samples harvested on Day 0 (Figure 5). In the zone of xylem secondary wall formation, distorted tracheids were tangentially aligned in a band of three to five cells. There were about 18 tracheids between the distorted tracheids and the first differentiating xylem cell.

Figure 6 shows the lumen area of each tracheid at which birefringence, relative to the cambium, was first observed with the polarizing microscope. Birefringence indicates the existence of a secondary wall, and the secondary wall deposition

Figure 2. Changes in the tangential strain of the stem surface. Arrow indicates resumption of irrigation on Day 11.

Figure 3. Light micrograph of the transverse section of the cambium, developing xylem and phloem cells on Day 0 (control). Abbreviations: Xy = xylem; Ph = phloem; and Ca = cambium. Bar = 50 µm.

Figure 4. Bright field (a) and polarizing light (b) micrographs of transverse sections of the cambium, developing xylem and phloem cells on Day 8. Arrows indicate distorted cells with birefringence. Abbreviations: Xy = xylem; Ph = phloem; and Ca = cambium. Bar = 50 µm.

Figure 5. Light micrographs of transverse sections of cambium and developing xylem cells, 18 days after resumption of irrigation. Arrow indicates distorted birefringent cells. Abbreviation: Xy = xylem. Bar = 50 µm.
terminates the expansion of the tracheids (Abe et al. 1995, 1997). The tracheid at which birefringence was first observed was in the early stage of secondary wall deposition. The lumen area of these tracheids began to decrease by Day 4 after irrigation was suspended.

The mean number of cells in the cambial and cell-expansion zones decreased from 10.9 to 10.1 between Days 0 and 4, and abruptly decreased to 6.6 on Day 8 (Figure 7). Eighteen days after the resumption of irrigation, values had recovered to those observed before the suspension of irrigation.

**Discussion**

**Water deficit and cell expansion**

Several layers of distorted developing xylem cells were observed in the samples collected on Days 8 and 11 after irrigation was suspended, indicating that the tracheids could not maintain their turgidity after cell expansion, when the pressure potential was below –1.9 MPa. This phenomenon can be explained as follows. At the onset of drought, xylem cells derived from cambial cells expanded by absorbing water, mainly during the night. The pressure potential of the apoplastic water surrounding the expanded cells decreased abruptly after the onset of the photoperiod, and during severe water deficit, it became lower than the osmotic potential of the expanded cells. As a result, newly expanded cells lost turgor even though they had formed secondary walls.

Glerum (1970) reported distortion of tangential walls of tracheids of 2-year-old seedlings of *Pinus resinosa* Ait. and *Picea glauca* (Moench) Voss grown in soil with a water potential of about –1.5 MPa. Barnett (1976) reported distorted tracheids in small trees of *Pinus radiata* D. Don. grown under conditions of water deficit. These tracheids had a secondary wall. Barnett concluded that the collapse occurred during severe water deficit. The structures of the distorted tracheids observed in our study were similar to those reported by Glerum (1970) and Barnett (1976). Consecutive collections of samples confirmed that the tracheids were distorted after cell expansion in response to decreasing water potential, even when they had formed a secondary wall.

**Water deficit and the productivity of cambial cells**

On Day 4 after irrigation was suspended, cell expansion was restricted (Figure 6) and the number of cells in the cambium and the cell-expansion zone decreased slightly (10.9 to 10.1) (Figure 7). We conclude that cell expansion was inhibited directly by the decrease in xylem pressure potential during the early stage of water deficit. Thereafter, cell production was inhibited by physiological factors such as the decline in cambial cell activity (cf. Abe and Nakai 1999).

Hsiao (1973) gave many examples of a reduction in cell enlargement and speculated that, in general, cell division is less inhibited than cell enlargement during water deficit. He also suggested that time-course studies monitoring water potential and the number and size of cells in the meristematic and cell enlargement zones may provide more insight into the effects of water stress on cell expansion and division. Our results confirm this speculation.

**Recovery of cambial cells**

Cambial cells and developing xylem cells recovered their productivity 18 days after severe water deficit. Tangential strain started to increase within 30 min of the resumption of ir-
rigation, suggesting that irrigation water was taken up immediately as irrigation commenced and that the pressure potential of apoplastic water around the cambial cells increased rapidly. Diurnal fluctuation in stem diameter was mainly caused by fluctuation in cell size in the cambium and the developing xylem and phloem (Klepper et al. 1971, Molz and Klepper 1973). This suggests that cell turgidity, including that of cells produced until Day 4 after the suspension of irrigation, recovered with the increase in pressure potential of apoplastic water following the resumption of irrigation. However, distorted cells that had already deposited a secondary wall were unable to regain their original shape because of the rigidity of the cell wall (Figure 5).

In trees harvested 18 days after the resumption of irrigation, about 18 cells were present between the cambial cells and the last formed distorted cells, with more than six cells in the cambium and cell-expansion zone (Figure 5). Less than 12 cells were produced during the 18 days after the resumption of irrigation, which is less than 0.66 cells produced per day. About 1.5 cells were produced per day in well-watered Japanese cedar trees of the same size and of the same clone in our previous study (Abe and Nakai 1999). Kubo (1985) reported that 1–2 cells were produced per day in 18- and 20-year-old Japanese cedar trees, which suggests that, in our study, cambial cell productivity did not recover rapidly after the end of the water deficit. In 5-m tall radiata pine trees, after a 1-month suspension of irrigation, pressure potential did not increase until 24 h after re-watering (Sheriff and Whitehead 1984). The recovery process may depend on differences in the duration of water deficit. More frequent sampling is needed to analyze the recovery process of cambial cells after the resumption of irrigation.

In conclusion, we believe this to be the first study of the decline and recovery of cells in the cambium and developing xylem during a drought followed by irrigation, with continuous sampling and monitoring of water conditions and stem turgidity. In response to water deficit, cell expansion was inhibited physically at the early stage, and cell division was inhibited physiologically at a later stage. During recovery from water deficit, cell turgidity recovered rapidly, whereas cell division recovered slowly.

Acknowledgments

We thank Mrs. F. Endo and I. Sakamaki for taking care of the trees, Dr. A. Nagao and Ms. M. Fukui for their suggestions regarding cloning of Japanese cedar and Dr. T. Jones for his linguistic suggestions.

References


