Variation in xylem formation of *Viburnum odoratissimum* var. *awabuki*: growth strain and related anatomical features of branches exhibiting unusual eccentric growth

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Summary Growth strains (GSs) and growth eccentricity in the branches of *Viburnum odoratissimum* var. *awabuki* (K. Koch) Zabel were measured. A pronounced growth promotion occurred on the lower side of some branches. Although the GS of the branches was similar to that of normal wood, a larger GS was observed on the upper side of the branches. Thus, eccentric growth occurred on the side opposite to the larger GS. In addition, there was a strong negative relationship between spring-back bending and eccentric growth, indicating that eccentric growth largely precluded correction to the vertical position. To understand the function of eccentric growth on the lower side of the branches, we examined several anatomical features of the branches and found that (1) the cell walls of both sides lacked the gelatinous layer, (2) the microfibril angle measured by X-ray diffraction and polarizing light was small on both the upper and the lower sides and (3) the vessel number and the cell wall area did not change to a large extent. The anatomical features of the xylem did not differ obviously between the upper and the lower sides of the branches; however, the fibers were longer on the lower side than on the upper side. These results suggest that the growth stress pattern and formation of branch architecture in *V. odoratissimum* differ from those observed in other woody angiosperms.

Keywords: growth eccentricity, microfibril angle, reaction wood, spring-back strain.

Introduction

In trees, the pith may be located eccentrically in the trunk or the branch because the stem deviates from the vertical position or because of an uneven distribution of loading in a branch. Generally, the resulting pattern of eccentric growth differs between angiosperms and gymnosperms. Most woody angiosperms show a pronounced growth promotion on the upper side of the leaning stems and branches, whereas growth eccentricity occurs on the lower side in gymnosperms. Eccentric growth is usually associated with the formation of reaction wood (Tsoumis 1991). Commonly, there is a strong compressive stress on the lower side of a leaning trunk (compression wood) in conifers, whereas a high tensile stress occurs on the upper side (tension wood) in dicotyledonous trees (Onaka 1949, Timell 1986a, 1986b). However, growth eccentricity on the lower side in angiosperms has occasionally been reported. For example, Onaka (1949) and Yoshizawa et al. (1993) observed that *Buxus microphylla* Siebold & Zucc. exhibits a pronounced growth promotion on the lower side of the inclined stem and that its xylem has some anatomical features similar to those of compression wood. Furthermore, several studies have shown that the growth strain (GS) of *Buxus* is the same as that of compression wood (Yoshizawa et al. 1993, Baillères et al. 1997). Similarly, growth eccentricity of *Pseudowintera colorata* (Raoul) Dandy occurs on the lower side of the inclined branch and the lower-side tracheids have a large microfibril angle (MFA) similar to that found in gymnosperms, but *P. colorata* develops neither tension wood nor compression wood (Kucera and Philipson 1977, 1978, Meylan 1981).

We recently found that inclined branches of *Viburnum odoratissimum* var. *awabuki* (K. Koch) Zabel have a pronounced growth promotion on the lower side. *Viburnum odoratissimum* is a small tree (2–10 m high) with an open, multi-branched, rounded canopy. These special cases of growth eccentricity in woody angiosperms suggest that the xylem of these species possesses unusual features that are manifested during growth. In this study, we measured the growth eccentricity and the released surface GS in the trunk and the inclined branches of a *V. odoratissimum* tree. We also examined the anatomical features of the branches that are often related with growth stress, including gelatinous fiber, MFA, vessel number, cell wall area and fiber length.
Materials and methods

Plant material

A 12-year-old tree of *V. odoratissimum* grown in Uji campus (135°80' E and 34°81' N), Kyoto University, Japan, was studied. The tree was 6 m high with five branches in various orientations and many twigs growing out at the tips of the branches.

GS measurement

Measurements were taken in September 2007. Released GS was measured in the trunk and in all the branches. Physiological conditions and measuring positions (A and B) of the trunk and the branches are shown in Figure 1. The branches were designated nos. 1–5 based on the length of the diameter. The measuring position A was about 30 cm from the base of the branch to avoid the influence of growth stress around the joint. The distance between A and B was five times greater than the diameter of the branch. After removing the bark at the measuring positions, 12 electrical resistance strain gauges (FLA-5-11-5LT, Tokyo Sokki Kenkyujo Co., Ltd., Japan) were glued with cyanoacrylate adhesive to the xylem in the longitudinal direction on the trunk and on the upper and the lower sides of the branches. Measurements were made using a portable digital strain meter (TDS500, Tokyo Sokki Kenkyujo Co., Ltd., Japan) with an 80-channel scanner.

After calibrating the strain gauges to zero, a cumulative measurement was made as follows: first, the twigs at the branch tips that induced bending of the branch were cut down, and the spring-back strains resulting from the removal of twigs (\( \delta e_1 \)) were measured on the standing tree; second, the branches were cut at branch–trunk junctions, and the spring-back strains (\( \delta e_2 = \delta e_1 + \delta e_3 \)) were re-measured; lastly, the released surface GSs (\( \delta e_4 \)) on the upper and the lower sides of the branches were measured as the dimensional change of the finally isolated wood portion relative to its initial length on the standing tree immediately, after they were released by cutting grooves about 3–5 mm deep with a handsaw on both sides of the strain gauges (Yoshida and Okuyama 2002). The value of \( \delta e_4 (\delta e = \delta e_1 + \delta e_2 + \delta e_3) \) is equal to the sum of the strain increment at various stages of load removal and stress release.

MFA measurement

Microfibril angles on the upper and the lower sides of the branches were measured using the X-ray diffraction (XRD) method. The samples [5(T) × 1(R) × 15(L) mm] were cut from the measuring positions where the GSs were measured. The air-dried samples were photographed with a flat-plate vacuum camera mounted on a Rigaku RU-200BH (Rigaku Co., Ltd., Japan) rotating anode X-ray generator, operated at 50 kV and 100 mA. Two-dimensional diagrams were recorded with Ni-filtered CuK radiation (\( \lambda = 0.15418 \) nm). The intensity curve of the reflection 200 was used to determine the MFA by the Cave T method (Cave 1966). Furthermore, to determine the MFA on a single cell wall, radial sections (1 \( \mu m \)) at position A of the branches were prepared because position A sustains larger bending moment with great eccentricity that should make orientation of microfibril remarkable and typical. The MFAs of fibers at 10 locations on each section were observed under crossed Nicols (crossed polarizers) using a polarizing light microscope (Orthoplan Leitz, Germany). The MFA was measured as the angle between the longitudinal axis of the cell at the maximal extinction position and the direction of the polarizer (Preston 1974).

Anatomical observation

Sample blocks [10–20(T) × 10–20(R) × 20(L) mm] were sawn from the upper and the lower sides at the measuring positions (A and B) of the branches. Strips (L: 10 mm) from these blocks were macerated with Schultze’s solution. Lengths of 50 fibers were measured with the aid of a light microscope (Olympus BX51, Olympus Co., Ltd., Japan) using image analysis software (Imagej 1.6.0_05, National Institutes of Health). On the other hand, cross sections, 10–20 \( \mu m \) thick were also sliced from these blocks and prepared for microscopic examination. The vessel number and the cell wall area were calculated using the same software. To determine the distribution of cellulose and lignin in the xylem of the branch, we examined the gelatinous layer (G-layer) in stained cross sections. The cross sections of the measuring positions on the branches, 20 \( \mu m \) in thickness, were stained with the mixed solution of zinc chloride and iodine. This color reaction can differentiate
lignified and non-lignified tissue in plants, with lignified cell walls being stained brown-yellow in color and non-lignified cell walls being stained blue-violet in color.

**Results and discussion**

*Growth eccentricity and GS of the trunk and branches*

Except for branch no. 1, which exhibited no growth eccentricity, the other branches showed pronounced growth promotion on the lower side, and the pattern of growth eccentricity did not change along their length (Table 1). Although growth eccentricity on the lower side of some angiosperms species has been found (Fisher and Stevenson 1981), their growth stress was not measured.

The spring-back strains and released surface GSs on the upper and the lower sides of the branches are given in Table 1. Except for the thinnest branch no. 1, where little spring-back strain occurred after cutting off the twigs, for all other branches, all or most of the spring-back strains were caused by excising the twigs. In the following discussion the total spring-back strain ($s_2$) will be considered. The spring-back strain was contractile on the upper side and extensive on the lower side of most branches. This is the expected response to the suppression of the gravity load that, in the standing tree, tends to bend the branch downward and compress it along its length. A different pattern was noted at the base of branch no. 3A that not only produced a contraction on both sides, but also a higher contraction on the lower side.

The large amounts of spring-back strain observed in the branches raise a few methodological issues. First, if the released surface strain of the branch had been only measured under the fallen condition, the results of the released strain would not reflect the real GS. Second, permanent strains induced in the temporary stages as the result of large spring-back strain may produce artifacts relative to the final GS measurement. The mechanical properties of wood extracted from the branch may be significantly influenced by the order of pre-cutting operations. Ideally, specimens should be cut directly from the standing tree by first releasing the growth stress, thus ensuring that no overload is applied in later manipulations.

On the other hand, the released GS ranged from $-706$ to $433 \mu \text{m}$. The values in most case were negative (contraction), corresponding to a low tensile growth stress typical of normal wood. For the trunk, the stress value was moderate and similar on both sides, indicating stable vertical growth of the whole tree. For the branches, the GS differed between the upper and the lower sides at all positions, and various patterns were observed.

In branch no. 1, the upper side produced little GS (positive or negative) while the lower side contracted. In branch no. 2, there were positive GSs (corresponding to a compressive growth stress) on the lower side. The same pattern was found in several branches of other *V. odoratissimum* trees.

<table>
<thead>
<tr>
<th>Trunk and branch no.</th>
<th>$R_1$ (mm)</th>
<th>$R_2$ (mm)</th>
<th>After cutting down the twigs ($e_1 = \delta e_1 + \delta e_2$)</th>
<th>After cutting down the branch ($e_2 = \delta e_1 + \delta e_2$)</th>
<th>Released GS (mm/m)</th>
<th>Upper</th>
<th>Lower</th>
<th>Upper</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunk A</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch B</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch 1A</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch 1B</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
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</tr>
<tr>
<td>Branch 3A</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch 3B</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
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<tr>
<td>Branch 4A</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
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</tr>
<tr>
<td>Branch 4B</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch 5A</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
<tr>
<td>Branch 5B</td>
<td>100</td>
<td>55</td>
<td>–918</td>
<td>–918</td>
<td>–589</td>
<td>444</td>
<td>–589</td>
<td>444</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1. Spring-back strains and GSs on the trunk and branches.*
This distribution of GS is rather unusual in angiosperms but has been observed in some species (Clair et al. 2006). Branch no. 3 exhibited a little positive GS at the lower side of the top position (3B), which could be partly explained by a permanent strain caused by the large spring-back. Branches 4 and 5 produced a contraction on both sides, corresponding to a tensile growth stress, with a higher value on the upper side. This is the usual pattern of an inclined stem counteracting the effect of gravity. The results of GS show that there was no regular distribution of GS with eccentric growth.

To clarify the biomechanical advantage of eccentric growth, we examined the relationship between the downward-bending trend, quantified by the spring-back bending \( (e_{s2_{\text{lower}}} - e_{s2_{\text{upper}}}) \) and the eccentricity (ratio of \( R_2 \) to \( R_1 \)) of the branch. As shown in Figure 2, there was a negative relationship \( (R^2 = 0.78) \) between both factors (except for the thinnest branch no. 1). This suggests that eccentric growth, which appears to be a common feature of this species, resulted in a disadvantage for upright movement or stabilization of the branch orientation so that it had to be somewhat reduced when the bending load became higher. Moreover, a weak relationship \( (R^2 = 0.47) \) between the downward-bending trend and the upright movement of the branch against bending, quantified by \( (e_{r_{\text{lower}}} - e_{r_{\text{upper}}}) \), is shown in Figure 3, indicating that the GS of the branches partially counteracts the effect of gravity. The observed deviations can be explained as the biomechanical requirement of each branch. For instance, at positions 2B, 4A and 3B, the higher GS differences indicate a stronger upward trend, whereas the unusual spring-back strain pattern observed at position 3A is consistent with the downward movement produced by the GS asymmetry.

We found that, although eccentric growth occurred on the lower side of the branches, the larger GS on the upper side counteracted the gravity effect. Because the branches of *V. odoratissimum* formed neither tension wood nor compression wood, they were able to produce GS variations together with eccentricity reduction sufficient to fulfill the biomechanical requirements. In this study, the values of GS were limited because the measurements were made at a single time on one tree, so it is necessary to measure more trees to further obtain conclusive results.

**Anatomical features of V. odoratissimum**

Figure 4 shows a cross section of branch no. 4A, and a pronounced growth promotion occurred on the lower side of this branch. This pattern of growth eccentricity resembles...
that of compression wood in gymnosperms. However, a
dark zone, which often appears in compression wood,
was not observed. The growth rings on the lower side were
more distinct than those on the upper side. These features
also occurred on other branches. We expected that the anato-
mical features similar to those in reaction wood would
form on the lower side of the branches during eccentric
growth. Commonly, tension wood in angiosperms is char-
acterized by anatomical features such as a G-layer of the
secondary wall (IAWA Committee 1964), small MFAs
(Sugiyama et al. 1993, Okuyama et al. 1994, Yamamoto
1998) and reduced vessel frequency (Baba et al. 1996).
Although not all these characteristics are always present
(Onaka 1949, Okuyama et al. 1990, 1994, Yoshida et al.
2000), they represent the main factors affecting the tree’s
ability to sustain high tensile stress. We, therefore, exam-
inied the G-layer, MFA, vessel number, cell wall area and
fiber length of the branch in an attempt to understand the
functions of eccentric growth on the lower sides of the
branches.

The stained cross sections were observed to determine the
distribution of G-layer in the xylem. As shown in Figure 5
(branch no. 4A), the sections showed little blue-violet color
staining in the cell walls of the upper and the lower sides,
indicating that the cell walls were lignified and there was
no G-layer. Another four species in the genus *Viburnum*
also have no G-layer (Onaka 1949) but the eccentric growth
pattern of them is unclear. Observations of the cell wall of
branch no. 4A by polarizing light microscope with 10-μm-
thick cross section (Figure 6) revealed the typical structure
of the secondary wall, and the presence of the inner layer
(S3) on both sides was confirmed. Similar results were
obtained with the other branches.

Okuyama et al. (1990, 1994) and Yoshizawa et al. (2000)
have pointed out that the tensile GS increases with decreas-
ing MFA on the leaning stem in some tension woods that

Figure 5. Cross sections of the branch (no. 4A) stained with the
mixed solution of zinc chloride and iodine. The cell walls are
lignified and there are no G-layers on either side. A color version
of this figure is available as Supplementary Data at *Tree
Physiology* Online.

Figure 6. The cell wall structure of the branch (no. 4A) under
the polarizing light microscope. The inner layer (S3) of second-
ary wall (arrowhead) was confirmed. A color version of this
figure is available as Supplementary Data at *Tree Physiology*
Online.
have no G-layer. Therefore, the MFAs were successively measured by XRD and polarizing light (Table 2). Mean MFA was 5–11° based on XRD and the MFAs of the base position A for the branches were about 11–14° based on polarizing light. There was little difference in the MFAs of the upper and the lower sides of the branches, indicating that the MFA did not change with eccentric growth in V. odoratissimum.

Vessel number and cell wall area were similar between the upper and the lower sides (Table 2), whereas fiber length was greater on the lower side than on the upper side except in the trunk and the youngest branch (Figure 7). Fiber length generally decreases due to increasing frequency of anticlinal division during eccentric growth. Although fiber can improve the bending strength of pulp (Wimmer et al. 2002), its role in the mechanical standing of solid wood, like in a branch, remains to be investigated. According to the results of anatomical observation, other remarkable anatomical features of the cell wall associated with eccentric growth were not confirmed.

In conclusion, V. odoratissimum exhibited eccentric growth on the sides of branches opposite to the larger GS, which is not common in woody angiosperms. No anatomical features associated with eccentric growth were identified in a comparative study of the upper and the lower sides of the branches, although the fibers were longer on the lower side of the branches than on the upper side. Growth promotion associated with a tensile GS on the lower side competes with the higher tension on the upper side, thereby preventing correction of leaning stems and downward-bending branches. The existence of a mechanical function of the eccentric growth was thus not clearly identified: only when a significant compressive strain is generated on the lower side (which was observed here in just one case) would the eccentric growth positively contribute to the upright movement. For such species with shrub-like forms (Wilson 1997), the

<table>
<thead>
<tr>
<th>Trunk and branch no.</th>
<th>MFA (°)</th>
<th>Vessel (no./mm²)</th>
<th>Cell wall area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>Trunk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.0</td>
<td>(13.8 ± 0.6)</td>
<td>48.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
<td>47.8</td>
</tr>
<tr>
<td>B</td>
<td>6.3</td>
<td>4.8</td>
<td>46.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53.1</td>
</tr>
<tr>
<td>Branch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>9.1</td>
<td>11.1</td>
<td>59.2</td>
</tr>
<tr>
<td></td>
<td>(12.8 ± 0.7)</td>
<td>(13.5 ± 1.0)</td>
<td>59.4</td>
</tr>
<tr>
<td>1B</td>
<td>9.5</td>
<td>5.4</td>
<td>65.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>65.5</td>
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<tr>
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<td>7.0</td>
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<td></td>
<td></td>
<td></td>
<td>43.1</td>
</tr>
<tr>
<td>2B</td>
<td>4.3</td>
<td>7.7</td>
<td>50.3</td>
</tr>
<tr>
<td></td>
<td>(12.1 ± 0.6)</td>
<td>(13.5 ± 0.7)</td>
<td>49.6</td>
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<tr>
<td>3A</td>
<td>8.1</td>
<td>9.0</td>
<td>57.3</td>
</tr>
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<td></td>
<td>(13.6 ± 0.7)</td>
<td>(13.0 ± 0.9)</td>
<td>44.6</td>
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<tr>
<td>3B</td>
<td>8.4</td>
<td>8.5</td>
<td>52.0</td>
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<td></td>
<td></td>
<td></td>
<td>47.4</td>
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<tr>
<td>4A</td>
<td>5.2</td>
<td>7.2</td>
<td>51.1</td>
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<tr>
<td></td>
<td>(12.9 ± 0.6)</td>
<td>(11.5 ± 0.4)</td>
<td>40.2</td>
</tr>
<tr>
<td>4B</td>
<td>8.6</td>
<td>7.8</td>
<td>46.7</td>
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<td>51.1</td>
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<td>37.0</td>
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<tr>
<td></td>
<td>(12.4 ± 0.6)</td>
<td>(14.8 ± 0.7)</td>
<td>40.4</td>
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<td>5B</td>
<td>9.2</td>
<td>6.1</td>
<td>50.7</td>
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</table>

(*) MFA by polarizing light microscope.
progressive downward bending of the branches may be advantageous by allowing the branches to grow quasi-horizontally and serve as the support for many vertical twigs. We conclude that the generation of GS in *V. odoratissimum* contributes to the stabilization of its branch architecture.

**Supplementary Data**

Supplementary data for this article are available at *Tree Physiology* Online.

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