Phototropic bending of non-elongating and radially growing woody stems results from asymmetrical xylem formation

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ABSTRACT

Active phototropic bending of non-elongating and radially growing portion of stems (woody stems) has not been previously documented, whereas negative gravitropic bending is well known. We found phototropic bending in woody stems and searched for the underlying mechanism. We inclined 1-year-old *Quercus crispula* Blume seedlings and unilaterally illuminated them from a horizontal direction perpendicular to (‘normal’ illumination) or parallel to (‘parallel’ illumination) the inclination azimuth. With normal illumination, active phototropic bending and xylem formation could be evaluated separately from the negative gravitropic response and vertical deflection resulting from the weight of the seedlings. One-year-old stems with normal illumination bent significantly, with asymmetrical xylem formation towards the illuminated upper surface and side of the stem, whereas those with parallel illumination showed non-significant lateral bending, with asymmetrical xylem formation only on the upper side. A mechanical model was built on the assumption that a bending moment resulted from the asymmetrical xylem formation during phototropic bending of the woody stems. The model fitted the relationship between the observed spatial distributions of the xylem and the observed lateral bending, and thus supported the hypothesis that phototropic bending of woody stems results from asymmetrical xylem formation, as such occurs during gravitropism.

Key-words: bending mechanism; gravitropism; growth stress; light environment; phototropism; reaction wood; stem reorientation; tension wood; tree.

INTRODUCTION

Stems of trees, shrubs and their seedlings are often inclined towards better light when horizontal asymmetry is present in the light environment, as such occurs during unilateral shading (Sierra-de-Grado et al. 1997), at forest edges (Loechle 1986), on forest floors under canopy gaps (Canham 1988; Cao 2001; Henry & Aarssen 2001), and on forest slopes (Ishii & Higashi 1997; Matsuzaki, Masumori & Tange 2006).

It is possible that this stem inclination results from active phototropic bending of non-elongating and radially growing portion of stems (‘woody stems’, henceforth), as well as phototropic orientation of elongating stems and deflection of the stems due to the weight of their main and lateral shoots. However, phototropic bending of woody stems has not been demonstrated, whereas negative gravitropic bending is well known (for a review, see Westing 1965; Wilson & Archer 1977; Timell 1986; Hejnowicz 1997). In a previous study, we showed that bending of woody stems of a deciduous oak species (*Quercus serrata* Thunb.) towards a light source in response to unilateral illumination could be attributed to active phototropic bending (Matsuzaki et al. 2006). However, that study could not exclude the possibility that the observed bending was caused solely by deflection of the stems due to the weight of shoots growing more vigorously at the illuminated side than at the opposite side.

The gravitropic bending and orientation of woody stems result from asymmetry in longitudinal growth stresses within the stem’s transverse section generated by asymmetrical formation of reaction wood and normal wood (Wilson & Archer 1977; Yamamoto, Yoshida & Okuyama 2002). Tension wood, the type of reaction wood formed by angiosperms, shrinks longitudinally and generates longitudinal tensile stresses during maturation of the xylem elements. Normal wood also attempts to shrink longitudinally and generates tensile stresses, but these are smaller than those generated by tension wood (Okuyama et al. 1990, 1994; Arakawa et al. 2003; Alméras, Thibaut & Gril 2005). Phototropism and gravitropism by elongating stems are thought to share a similar bending mechanism, namely differential longitudinal growth (Hangarter 1997). If active phototropic bending of non-elongating and radially growing woody stems exists, the phototropic bending is also likely to be achieved by means of asymmetrical xylem formation and development.

To examine whether an asymmetrical light environment causes active phototropic bending of woody stems, we inclined potted seedlings of an angiosperm tree species and provided unilateral illumination from a horizontal direction...
normal (perpendicular) to the inclination azimuth. With this treatment, active phototropic bending of the stems and the xylem formation expected to provide the bending mechanism can be evaluated separately from the negative gravitropic response and the vertical deflection due to the weight of the shoots, because the light stimulus in the treatment is perpendicular to the direction of gravity. The spatial distribution of the newly formed tension wood and of the normal wood in transverse stem sections were anatomically analysed to provide evidence for a possible bending mechanism. We also measured the torsional angle of the main stems, because torsion due to the asymmetrical load of the shoots might affect the apparent orientation and curvature of the stem and the apparent distribution of newly formed xylem, even though phototropic reorientation is expected to be caused mainly by active phototropic bending. We evaluated quantitative relationships between phototropic bending and stem anatomy, including xylem formation, to examine the contribution of xylem formation to the bending mechanism.

MATERIALS AND METHODS

Plant materials and treatments

One-year-old potted seedlings of Quercus crispula Blume, a deciduous oak species, were grown outdoors under natural conditions until the experimental treatments began. In April, when the seedlings had not yet flushed, they were moved into a shaded room, positioned so that the soil surface was inclined at 45° from the vertical, and horizontally illuminated using arrays of fluorescent tubes with a spectrum optimized for plant cultivation (Biolux-A; NEC, Tokyo, Japan) in one of two directions: perpendicular to (‘normal’ illumination) or parallel to (‘parallel’ illumination) a vertical plane running through the azimuth of the inclined main stem (Fig. 1a). The photosynthetic photon flux density at the average position of the apices of the main stems was about 120 μmol m−2 s−1, and the photoperiod was 16 h of light and 8 h of dark. The length of the main stem and the diameter at the base of the stem averaged 248 ± 35 mm and 7.4 ± 1.4 mm (mean ± SD), respectively. The seedlings were provided with sufficient irrigation. The minimum and maximum room temperatures during the treatment period were 7.9 and 36.4 °C, respectively. The minimum and maximum relative humidities during the treatment period were 7.9 and 36.4 °C, respectively. Five seedlings were provided with sufficient irrigation. The length of the main stem and the diameter at the base of the stem averaged 248 ± 35 mm and 7.4 ± 1.4 mm (mean ± SD), respectively. The seedlings were provided with sufficient irrigation. The minimum and maximum room temperatures during the treatment period were 7.9 and 36.4 °C, respectively. The minimum and maximum relative humidities during the treatment period were 7.9 and 36.4 °C, respectively. The seedlings were sampled at weeks 3, 5 and 11 for measurement of stem torsion and for analysis of xylem formation. The seedlings periodically flushed three times at most during the treatment period.

Measurement and analysis of stem form

The three-dimensional curvilinear form of the 1-year-old portion of the main stem, which had ceased elongation before the illumination treatments began, was measured from its base to its apex using a three-dimensional digitizer (Microscribe G2L; Immersion, San Jose, CA, USA). The coordinates along the stem axis were logged at intervals of about 4 mm, then the resulting model of the stem was rotated and translated into the XYZ Cartesian coordinate system defined in Fig. 1a,b.

We defined the stem portion that had elongated within a given growth flush as a growth unit (GU). Because a slight but abrupt difference in the orientation was often observed at the joint between two adjacent GUs, we modelled each GU as a separate curve. Most of the seedlings had three GUs within the 1-year-old stem. We termed the GUs basal, middle and apical. Some seedlings had only basal and apical GUs.

Principal components were computed for the coordinates composing each GU to standardize the axis direction. Subsequently, we represented the curvilinear form of the GU in a three-dimensional space by fitting two cubic functions that did not contain an inflection point within the interval occupied by the GU to the points that defined the GU using the first principal component as the predictor, and the second and third principal components as the target variables. For this analysis, we used the Solver function provided by Excel 2002 (Microsoft, Redmond, WA, USA). The
orientation and curvature were calculated using the first derivatives of the fitted function at a given point.

Reorientation of the 1-year-old stem was calculated as a decrease during the treatment period in the angle between a tangent to the apical end of the apical GU and a vector parallel to the positive y-axis, which was oriented towards the light source under normal illumination (Fig. 1a).

To estimate the active phototropic bending of each GU, we calculated lateral bending using a local Cartesian coordinate system, the axial-lateral-dorsiventral (ALD) coordinate system shown in Fig. 1b. The magnitude of the lateral bending was represented by the change in curvature in the A–L plane. A curvilinear equation fitted to each GU was rotated around the A-axis using the torsional angle during the treatment period, which was measured as described further, so that the L-axis and D-axis represent those at the start of the treatment. This rotation corrects any overestimation of the active phototropic bending caused by torsion based on the approximation that each GU is rigid. Over- and underestimation of the active phototropic bending caused by the possible deflection resulting from the weight of the shoots subsequent to the torsion could not be completely eliminated, but the error could be reduced by this rotation. The difference between the tangent vector at the apical and basal ends of the curvilinear equation fitted to a given GU divided by the length of the fitted curve equalled the mean curvature vector of the fitted curve, which represents the magnitude and direction of the curvature. The norm of orthogonal projection of the mean curvature vector on the A–L plane with the sign of the L-component of the curvature denotes the lateral curvature with respect to the positive L-direction (i.e. a positive sign indicates a change towards the positive L-direction, whereas a negative sign indicates a change towards the negative L-direction). Changes in the lateral curvature during the treatment period were termed as the ‘lateral bending’ of each GU.

**Measurement of stem torsion**

To measure possible torsion of the main stem, the radial direction was marked by means of the pinning method (Kuroda & Shimaji 1984) at the start of the treatment and by incision with a knife at the time of sampling. In this approach, the uppermost surface of the stem in the radial direction, which corresponds to the positive D-direction in Fig. 1b, and a position 1 cm apical to the midpoint of the GU for the longitudinal position were marked with pins in each GU at the start of the treatment. At the time of sampling, the bark was incised longitudinally with a knife from the radial direction of the GU corresponding to the negative L-direction (Fig. 1b) at the same longitudinal positions as the pins. The sampled GU was cut transversely at the pinned positions, and the section was photographed under a stereomicroscope. The pinned position was recognized by the dark coloring of traumatic tissue. The angle between the line connecting the pinned position with the centroid of the pith and the line connecting the incision with the centroid of the whole section was measured using the ImageJ software (ver. 1.34, Rasband 1997–2005). If the GU did not twist during the treatment period, the angle is expected to be equal to 90°. The calculated deviation of the angle from 90° equalled the torsional angle.

**Analysis of xylem formation**

To analyse xylem formation during the treatment period, each pixel in digital images of the transverse sections was classified, and the spatial distribution of the pixels was analysed as follows. Samples containing the midpoint of each GU were incised as was done for the measurement of stem torsion, then the samples were fixed with formalin–acetic acid–alcohol, and embedded in Paraplast-Plus (Tyco Healthcare Group, Mansfield, MA, USA). A 15-µm-thick transverse section was obtained from the midpoint of each GU using a sliding microtome by means of the tape-transfer technique (Sahoo 1974; Sterchi & Eurell 1990). Tension wood was characterized by the existence of a gelatinous layer (the G-layer), which is the thick, cellulose-rich, innermost layer in the secondary wall, which contributes strongly to the generation of the large tensile stresses created by tension wood (Okuyama et al. 1990, 1994; Yamamoto 2004; Yamamoto et al. 2005). To distinguish between tension wood and normal wood, we double stained the sections with safranin–methyl blue and the lignified layer red.

Each section was photographed under a light microscope with a pixel size of 3.31 to 3.51 µm. The xylem formed in the current year and the xylem and the pith tissue formed in the preceding year were manually outlined based on the ring porosity of the tissue. Pixels classified as current-year xylem, which formed during the treatment period, were further classified into tension wood, normal wood or pores. In each image, at least 20 pixels in each class were manually marked and used as training data, and all the pixels previously classified as current-year xylem were subsequently classified by means of supervised classification with discriminant analysis using the LIA32 software (Yamamoto 2003).

**Evaluation of the contribution of xylem formation to phototropic bending**

To assess the contribution of xylem formation to phototropic bending, each GU was approximated as a straight beam and was opposed by a flexural rigidity composed of the moment of inertia of the cross-sectional area.
Phototropism of woody stems by asymmetrical xylem formation

(1) and a modulus of elasticity (E), the resultant lateral curvature \( \kappa \) is calculated using Eqn 1:

\[
\kappa = \frac{M}{EI}
\]

(1)

\( I \) and \( M \) were calculated using Eqs 2 and 3, respectively, with the centroid of the xylem and the pith formed in the current and preceding year (excluding pores) used as the origin and \( x \) as the L-coordinate in Fig. 1b at the start of the treatment:

\[
I = \frac{\sum_{i=1}^{n_{TW}} x_{i,TW}^2 + \sum_{i=1}^{n_{NW}} x_{i,NW}^2 + \sum_{k=1}^{n_{PRCD}} x_{k,PRCD}^2}{n_{TW} + n_{NW} + n_{PRCD}}
\]

(2)

\[
M = \sigma C = \sigma \frac{\sum_{i=1}^{n_{TW}} x_{i,TW} + \sum_{i=1}^{n_{NW}} x_{i,NW}}{n_{TW} + n_{NW}}
\]

(3)

where \( x_{TW}, x_{NW} \) and \( x_{PRCD} \) are the L-coordinates (Fig. 1b) of the pixels classified into tension wood formed in the current year, normal wood formed in the current year, and xylem and pith formed in the preceding year, respectively; \( n_{TW}, n_{NW} \) and \( n_{PRCD} \) are the number of the pixels classified as tension wood formed in the current year, normal wood formed in the current year, and xylem and pith formed in the preceding year, respectively; \( \alpha \) is a weighting factor used for the pixels classified as tension wood, because tension wood generates larger tensile stresses than normal wood; and \( \sigma \) is a coefficient representing stress.

Substituting Eqn 3 for Eqn 1 gives Eqn 4:

\[
\kappa = \frac{\sigma C}{EI}
\]

(4)

Equation 4 means that \( \kappa \) is proportional to the \( CI/I \) value. Approximating \( \kappa \) at the start of the treatment to be zero, we could estimate the lateral bending (i.e. the change in lateral curvature) during the treatment period using \( CI/I \) calculated from the spatial distribution of xylem. We optimized \( \alpha \) to maximize the coefficient of determination in the prediction of the lateral bending as a function of \( CI/I \) by means of linear regression, and evaluated the contribution of xylem formation to the lateral bending and the relative contributions of tension wood and normal wood. The optimization was performed using the data for week 11, when the maturation strain is expected to be fully generated. The data for both treatments were used in the optimization, because the bending mechanism is expected to be common irrespective of treatment.

Unless otherwise noted, the analyses in the present study were performed using the R software (ver. 2.3.1, R Development Core Team 2006) or the ImageJ software (Rasband 1997–2005).

RESULTS

Stem form and stem torsion

The apical ends of the 1-year-old stems under normal illumination reoriented significantly towards the positive Y-direction, in the direction of the light source, whereas those under parallel illumination exhibited no reorientation with respect to the Y-direction (Fig. 2). The reorientation under normal illumination started between weeks 2 and 3, when the first flushing from the apex of the 1-year-old stems was almost finished.

The torsional angles of the GUs composing the 1-year-old stems did not differ significantly between treatments, among sampling weeks or among the longitudinal positions of the GUs (\( P > 0.05; \) three-way analysis of variance (ANOVA) with random effects of individual seedlings; Fig. 3).

The GUs composing the 1-year-old stems exhibited significant lateral bending (\( P < 0.01 \)) towards the illuminated side under normal illumination but not under parallel illumination (Fig. 4). The magnitude of the bending did not differ significantly among the GU positions (\( P > 0.05 \)) based on two-way ANOVA with random effects of the individual seedlings (Fig. 4).

Xylem formation

Blue-stained xylem (i.e. tension wood) that formed during the treatment period was distributed continuously along both the upper side and the illuminated side of the sections obtained from the 1-year-old GUs under normal illumination (\( x_c, \) Fig. 5a), but was distributed only along the upper side of the stem of the sections under parallel illumination (\( x_c, \) Fig. 5b). The blue-stained xylem at both the upper and the illuminated sides under normal illumination contained the G-layer and did not differ in anatomical characteristics between these two sides, providing further evidence that both tissues were tension wood.

Eccentricity of xylem formation in each transverse section was quantitatively evaluated using the deviation of
DISCUSSION

Existence of phototropic bending in woody stems

The apical ends of the 1-year-old stems reoriented towards the light source under normal illumination (Fig. 2). The GUs composing the 1-year-old stems contributed equally to this reorientation, irrespective of their position (Fig. 4). The 1-year-old stems had finished elongation in the preceding year, and were thus non-elongating and were growing only radially. Our results thus reveal phototropic reorientation of the non-elongating and radially growing portion of stem (woody stem).

Torsion and possible subsequent deflection of the stem, as well as active phototropic bending during the treatment period, would have contributed to this phototropic reorientation. However, because the torsional angle did not differ significantly between the treatments (Fig. 3), torsion was not the major factor in the observed phototropic reorientation. Phototropic reorientation of woody stems thus appears to result mainly from active phototropic bending.

The bending mechanism in phototropism of woody stems is based on the spatial distribution of the xylem

The gravitropic uprighting and orientation in woody stems of angiosperms are due to a bending moment generated by asymmetrical xylem formation towards the upper side of the inclined stems (Wilson & Archer 1977; Yamamoto et al. 2002). The bending moment is caused by asymmetrical distribution of longitudinal shrinkage that results from maturation of asymmetrically distributed tension wood and normal wood.

The eccentric formation of tension wood and normal wood towards the upper side of the inclined stems during

![Figure 3. Torsional angle of the growth units (GUs) composing the 1-year-old stems during the treatment period. Torsion in which upper sides of the GUs rotate towards the positive L-direction in Fig. 1b is expressed as a positive value. Vertical bars denote SD. *, significant difference (P < 0.05; three-way analysis of variance (ANOVA) with random effects of the individual seedlings).](image)

![Figure 4. Lateral bending of the growth units (GUs) composing the 1-year-old stems after 11 weeks of treatment. Positive values represent bending towards the positive L-direction in Fig. 1b. Vertical bars denote SD. *, significant difference (P < 0.05, t-test) between treatments for GUs at a given position. No results differed significantly among GU positions [P > 0.05; two-way analysis of variance (ANOVA) with random effects of the individual seedlings]. Data for the seedlings sampled at week 11 are shown.](image)
the treatment period (Figs 5b & 6) under parallel illumination corresponds to the known response of xylem formation through gravitropism. This suggests that the GUs in this treatment were sustaining the inclined orientation or were bending upright through gravitropism. Under normal illumination, the tension wood and normal wood formed more along the upper side of the inclined stems (Figs 5a & 6) than along the lower side, which can also be attributed to xylem formation in response to gravitropism.

However, under normal illumination, vigorous formation of tension wood and of normal wood was also observed on the illuminated side of the stem (Fig. 5a), with the centroids of these tissues deviated in the direction of the illumination (Fig. 6). The biased formation of tension wood and normal wood towards the illuminated side causes an asymmetrical distribution of longitudinal tensile stresses and generates a bending moment towards the illuminated side. The contribution of the phototropic lateral bending of the GUs towards the illuminated side (Fig. 4) to the observed phototropic reorientation (Fig. 2) appears to result from asymmetrical xylem formation. The asymmetry in xylem formation at week 3 (Fig. 6a,d), prior to the point at which

**Figure 5.** Light-micrographs of typical transverse sections obtained from the growth units (GUs) composing 1-year-old stems under (a) normal illumination and (b) parallel illumination. The right side of the micrographs corresponds to the positive L-direction (Fig. 1b) and the top to the positive D-direction at the start of the treatment. The incision at the bark corresponds to the negative L-direction (Fig. 1b) when sampled. Bars represent 1 mm. The sections were double stained with safranin-astra blue. xc, xylem formed in the current year (i.e. during the treatment period); xp, xylem formed in the preceding year; pi, pith.

**Figure 6.** Eccentricity in the formation of tension wood and normal wood (TW + NW) and of tension wood alone (TW) during the treatment period in the growth units (GUs) composing 1-year-old stems. The eccentricity was evaluated using the deviation of the centroids from the origin, which is defined as the centroids of the xylem and pith including pores that formed in the preceding year. The positions of the centroids were computed from the coordinates of pixels classified into the corresponding category. The horizontal and vertical axes represent lateral and dorsiventral deviation, respectively, which correspond to the L-axis and D-axis (Fig. 1b). The lateral and dorsiventral directions are that at the start of the treatment, which are defined by that at the end of the treatment and the torsional angle during the treatment period for each GU. Deviations were standardized by dividing the value by the mean radius of the xylem and pith that formed in the preceding year.

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significant reorientation becomes apparent (Fig. 2), also indicates that the asymmetrical xylem formation is not the result but rather the cause of the phototropic bending. The asymmetrical xylem formation towards the lower side of the stem that was observed in some GUs under parallel illumination at weeks 5 and 11 (Fig. 6b,c,e,f) is a response that caused the main stem to incline more, and can also be explained by phototropism. Because the lower side of the main stems was illuminated under parallel illumination (Fig. 1a), phototropism would act to increase the inclination of the main stem. If the main stems reoriented towards a photogravitropic equilibrium angle, determined by the balance between the responses to the phototropic and gravitropic stimuli that is known to occur in elongating stems (Iino 2001; Galland 2002) and that has been suggested to occur in non-elongating and radially growing portion of stems (Matsuzaki et al. 2006), the main stems would incline more when the initial inclinations were less than the photogravitropic equilibrium angle. Because the array of fluorescent tubes used as the light source was set at almost the same height as the main stems, the limited area of illumination would result in less irradiation of apical part of some large seedlings. Such a light environment might have induced phototropic bending of the stem towards greater inclination under both normal and parallel illumination. The spatial distributions of the xylem types suggest that asymmetrical xylem formation is the mechanism responsible for the bending of non-elongating and radially growing portion of stems (woody stems) for phototropism as well as for gravitropism.

**Asymmetrical xylem formation appears to be responsible for phototropic bending of woody stems, according to the mechanical model**

We evaluated the quantitative relationships between stem anatomy and phototropic bending of woody stems using a mechanical model, which assumed that asymmetrical xylem formation is responsible for the phototrophic bending. The significant linear relationship between the estimates of lateral bending ($C/I$) computed from the spatial distribution of xylem, and the observed lateral bending with the non-significant deviation of the intercept from the origin at week 11 (Fig. 7c) indicates that the active phototropic bending was due to a bending moment generated by the asymmetry in xylem formation ($C$) and the resulting asymmetry in the longitudinal tensile strain. Because the weighting factor assigned to the pixels classified as tension wood ($a$), optimized based on the data for week 11, was greater than one, this observation is compatible with the larger longitudinal maturation strain and consequent longitudinal tensile stresses that occur in tension wood containing a G-layer than in the normal wood (Okuyama et al. 1990, 1994; Arakawa et al. 2003; Alméras et al. 2005). With the data for weeks 3 and 5, the linear relationships between the lateral bending and $C/I$ were weak and the regression line optimized for week 11 overestimated the lateral bending (Fig. 7a,b). These may be attributed to incomplete maturation of the current-year xylem, and thus to a reduced generation of longitudinal maturation strain. The close fit between the mechanical model and the observed results indicates that phototropic bending of woody stems results mainly from the asymmetrical formation of tension wood and normal wood.

**Possible role of phototropic bending of woody stems in the development of tree architecture**

Phototropism has been considered to have little effect on the development of tree architecture because this development can be roughly reconstructed using the growth, branching and death of shoots in response to the local light environment, which determines gross photosynthetic production by the shoots (e.g. Takenaka 1994). In addition, phototropism has been shown to have little effect on the orientation of elongating stems in canopies of some evergreen oak species (Koike 1989). However, the active phototropic bending of woody stems that was demonstrated in the present study, combined with gravitropic bending and apical dominance, may also play a significant role in controlling or maintaining the orientation of woody stems to maximize photosynthesis under spatially heterogeneous light environments.

**ACKNOWLEDGMENTS**

We thank the Experimental Station at Tanashi of the University of Tokyo for providing the facilities and the assistance during our study. We also thank Dr. T. Hogetsu (the University of Tokyo) and Dr. R. Funada (Tokyo University of Agriculture and Technology) for their instructive discussion of our study, and Dr. D. Kusumoto (Utsunomiya University) and Dr. M. Yoshida (Nagoya University) for their help with wood anatomy. J.M. is a research fellow of Japan Society for the Promotion of Science.
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