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NOTE

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Estimating the equilibrium position by measuring growth stress in weeping branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*

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Abstract The equilibrium position of a current-year branch of the weeping Japanese cherry, *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*, was estimated by measuring the released strains of growth stresses. In current-year branches that were supported with wires to prevent weeping as soon as the branches first budded, tensile growth stresses on the upper side were smaller than those of the control branches. Gelatinous fibers were rarely found on the upper part of the cross section of the supported branches, whereas the control branches had many gelatinous fibers on the upper part. The upright orientation of the supported branches was closer to the equilibrium position than the weeping orientation of the control branches. The equilibrium position of the branches was thought to be in the initial bud direction, above the horizontal plane; and the weeping style of branch was not the preferred angular orientation for *P. spachiana*.

Key words *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea* · Growth stress · Equilibrium position

Introduction

When a branch is bent upward or downward, reaction wood forms to reorient the branch in the opposite direction by the large growth stresses generated in the reaction wood. Plagiogravitropism is an accepted concept for the formation of reaction wood in branches. Lateral shoots, unlike main shoots, have an equilibrium branch angle. It is thought that so long as a branch remains in its equilibrium position neither eccentric growth nor reaction wood develops. The equilibrium position is normally above the horizontal plane in the case of upright branches and below the horizontal plane in the case of weeping branches.[1,2]

In current-year branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea,* Nakamura et al.[3,4] revealed that the weeping phenomenon is caused by insufficient mechanical support for the increasing weight of the branch itself. We observed that gelatinous fibers are produced in the upper part of the weeping branches of *P. spachiana*.[5] From these preliminary results we predicted that the weeping orientation of the branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea* was not an equilibrium position.

Previous researchers[1,2] have estimated the equilibrium positions of branches from anatomical observations of reaction wood. On the other hand, the intensity of reaction wood can be determined also by the mechanical states. There is a high correlation between the released strain of growth stress and the intensity of reaction wood.[6–8] In compression wood, the longitudinally expansive released strain is positively correlated with the Klason lignin content, the microfibrillar angle, the darkness of the color, and the areas of the cell wall and intercellular space.[6] It is also negatively correlated with Young's modulus in the cell wall substance.[6] In tension wood, the longitudinally contractive released strain increases with the increase in α-cellulose content and its crystallinity.[7,8] It is negatively correlated with the Klason lignin content and the microfibrillar angle.[7,8] These relations apply to species that have no gelatinous fibers on the upper side of an inclined trunk.[7,8]

Some reports have used the released strain of growth stress to obtain a continuous and quantitative classification of wood samples.[9,10] Baillères et al.[9] characterized the tension wood of *Eucalyptus* species belonging to *Eucalyptus* family not characterized by gelatinous fibers by measuring the released strain of growth stress at the outer surface of secondary xylem on the stem. Baillères et al.[10] also used the released strain to determine a grade from normal wood to reaction wood in *Buxus sempervirens*, whose reaction wood resembled gymnosperm compression wood.[11] They found a clear correlation between the released strain and the lignin structure.

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We think that measuring the released strain of growth stress is a useful way to estimate the intensity of reaction wood. In this study we estimated the equilibrium position of the weeping branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea* from the released strain of growth stresses.

Materials and methods

Plant materials and support treatment

Current-year branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*, a weeping species of Japanese cherry, were supported with wires to maintain the initial branch angle seen at budding. The branch angles were 40°–60° from vertical. The wires were attached to the branch at the beginning of May, shortly after the outgrowth of the branch from the main shoot, when it grew to 10mm long and had three unfolded leaves in its basal region. The treatment was continued until the current growth stopped. During the growth period the direction of the shoot was maintained using additional wires (Fig. 1). Additional wires were attached before the shoot tip wept due to its own weight, at 1-week intervals. The excess wires were removed, and the remaining wires were rearranged with proper separations even during the growing season. After the current-year growth stopped, the wires were removed from the branches. Control branches were not supported. The sizes of the branches after the current-year growth stopped are shown in Table 1.

Measuring growth stress

To measure the released strain of growth stress, the strain-gauge method was used in the conventional way.[12,13] Growth stresses of each of three supported and control branches were measured at the beginning of December, after growth had stopped. The measuring points were on the upper and lower sides of each branch within 20cm of its base horizontally and with 4- to 5-cm intervals between strain gauges. The bark, phloem, and thin cambial zone at each measuring point were carefully removed with a knife so as not to scratch the outer surface of secondary xylem. Strain gauges 2mm in length (Minebea B-FAE-2S-12-T11) were glued lengthwise on the outer surface of the secondary xylem and connected to a strain meter (Kyowa UCAM-1A). The precision of the measurement was ± 0.001%. In the case of the supported branches, the initial balance of the strains was measured in the standing position after removing the guy wires. The growth stresses were released by cutting off the xylem with a thin knife, close to the edges on both sides of the strain gauge in the longitudinal direction.

Fig. 1. Branches were supported with wires to keep the initial branch angle, thereby preventing weeping. The wires were attached shortly after the initial outgrowth of the branch from the main shoot

Table 1. Size of branches after the current-year growth stopped

Parameter	Supported[a]			Control[a]		
	1	2	3	1	2	3
Diameter at the base of branch with bark (mm)	4.2	4.0	3.8	4.5	4.0	3.6
Length of branch from base to top (cm)	50	50	51	52	50	48

[a]Three measurements were made for each parameter

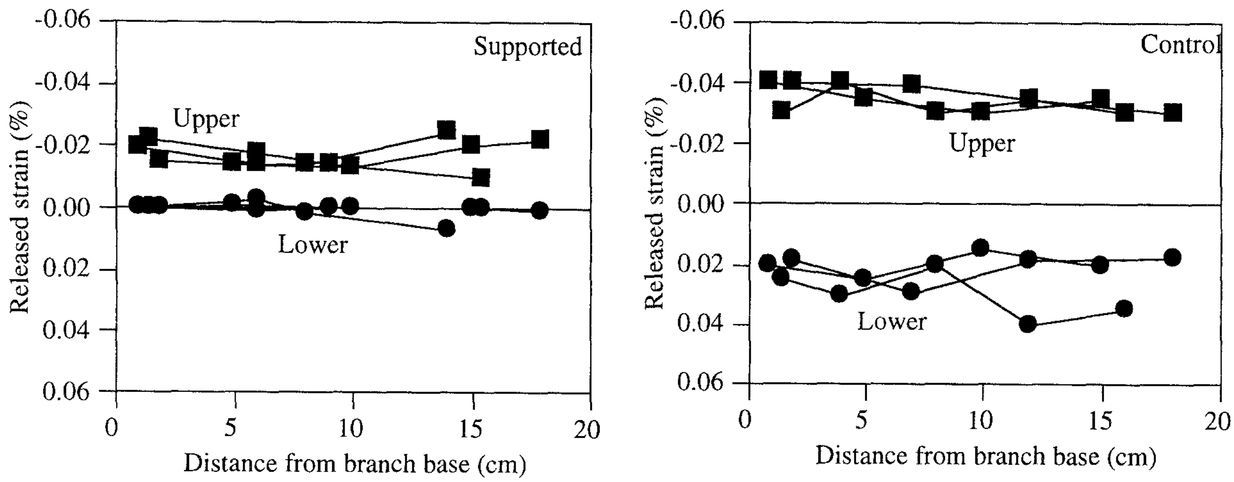

Fig. 2. Distribution of longitudinal released strains in the supported and control branches. All of the measurements are plotted. *Squares*, strain on the upper sides of the branch; *circles*, strain on the lower sides. The larger the negative released strain, the greater was the tensile growth

Microscopic observations

After measuring the released strain, small wood blocks were removed close to the measuring points and fixed with 50% ethanol for 1 month. Cross sections 15 μm thick were prepared for optical microscopic observation from the block samples. The microtome sections were dehydrated successively with an ethanol series and double-stained with safranine for 3 h and fast green for 10 min.

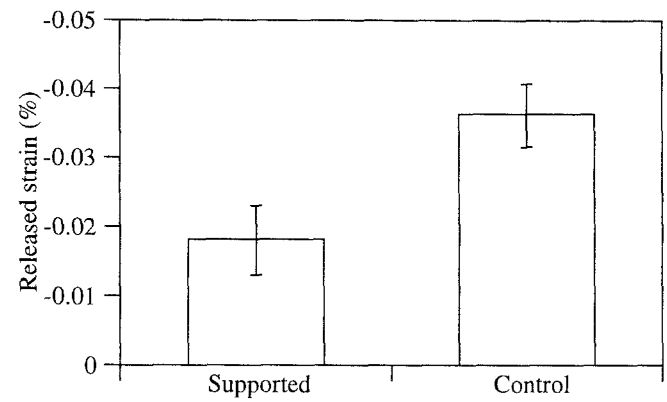

Fig. 3. Longitudinal released strain on the upper side of the supported (n = 13) and control (n = 12) branches. Mean values from tensile growth stress measurements are shown with the SD

Results and discussion

Released strain of growth stress

The branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea* weep because of their own weight. The phenomenon occurs because the elongation rate is greater than the thickening rate,[3] so the branches cannot support their increasing weight. The branches supported with wires did not weep and maintained an upright shoot direction, even after the guy wires were removed. During the dormant season the branches had enough rigidity to support their weight. It became clear that the weeping habit in this species was caused by a delay in the increase in bending rigidity.

Figure 2 shows the longitudinally released strains in the supported and the control branches. The negative released strain shows that tensile growth stress existed. Negative released strains were found on the upper sides of both the supported and the control branches. Therefore, the longitudinal tensile growth stresses were generated at those points. However, the values for the supported branches were smaller than those of the control branches.

Positive released strains were detected on the lower sides. The positive values of the control branches were greater than those of the supported branches, which were almost zero. These positive released strains were believed to be caused by the response to the weeping. As a result of weeping, the lower sides of the control branches were contracted by compressive bending stress. At the release of growth stress, the compressive stress conditions were released. Because the guy wires prevented the supported branches from moving from their initial orientation, the released strains on the lower sides of the supported branches were considered to be in nonstress conditions.

Figure 3 compares the mean values of the negative released strain on the upper side of supported and control branches. The value of the released strain in the supported branches was −0.017%, whereas for the control branches it was −0.035%. There was a significant difference between the supported and control branches according to the t-test ($P < 0.001$). The tensile growth stresses on the upper side of the supported branches were confirmed to be smaller than those of the control branches. This showed that branches of *P. spachiana* preferred the upright position, which was achieved with the support wires, preventing weeping.

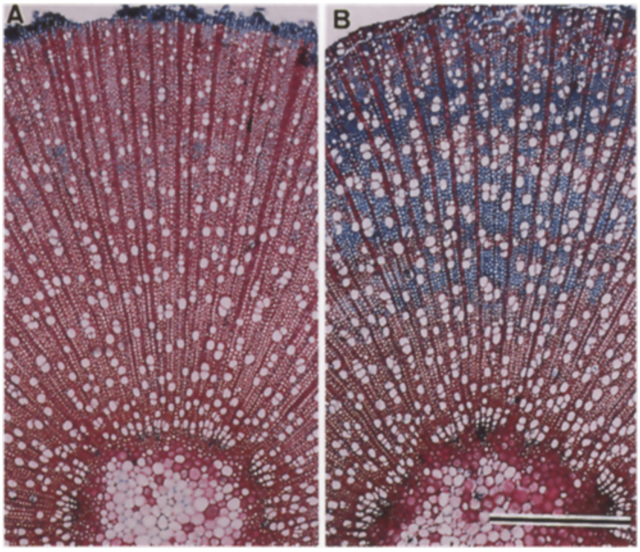

Fig. 4. Cross sections from the upper side of a supported branch (**A**) and a control branch (**B**) at the point where tensile growth stress was the largest in the dormant season. Sections were double-stained with safranine and fast green. The blue fibers are gelatinous fibers stained with fast green. Bar 0.5 mm

Microscopic observation

Tissues in both the upper and lower parts of the base of the branches were examined microscopically. The diameters of both supported and control branches without bark were almost same (3.09 ± 0.04 vs. 3.13 ± 0.08 mm; $P = 0.22$). There was no eccentric growth in either type of branch. Gelatinous fibers stained with fast green[14] were seen on the upper part of the control branches but were found only rarely on the upper part of the supported branches (Fig. 4). This result agreed with the finding of smaller tensile growth stresses on the upper side of the supported branches.

Gelatinous fibers were found in the outer regions of cross sections in the upper part of the control branches, but they were not found near the pith. Baba et al.[15] observed that the weeping branches of *P. spachiana* did not form gelatinous fibers during the initial growth stage. The branches of *P. spachiana* had an upright orientation at budding. The control branches began to weep with their increasing weight. During the later growth period, the control branches were thought not to be in the equilibrium position. Thus, gelatinous fibers were produced in the outer regions for reorientation. Gelatinous fibers rarely formed on the upper side of the supported branches because the supported branches remained near the equilibrium position.

Conclusions

Because the equilibrium position of a branch is the position in which no reaction wood forms, growth stresses are believed to be smallest at the equilibrium position. Growth stress is a useful method for estimating the equilibrium position of a branch. When a current-year branch was supported with wires to prevent weeping as soon as the branch first budded, gelatinous fibers rarely formed and tensile growth stresses on the upper side of the branch were smaller than those of the control branch. Therefore, the upright orientation of the supported branches was thought to be closer to the equilibrium position than the weeping orientation of the control branches. The equilibrium position of the branch of *P. spachiana* is the initial budding orientation, above the horizontal plane.

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