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ORIGINAL ARTICLE

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Negative gravitropism and growth stress in GA_3-treated branches of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*

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Abstract One of the roles of growth stress in branch shape formation was investigated using a weeping-type Japanese cherry, *Prunus spachiana*. Negative released strains, caused by longitudinal tensile growth stresses, were detected in the upper side of gibberellin A_3-treated (GA_3-treated) and control branches. The mean value of the released strain in the upper side of the treated branches was −0.104%, which was larger than the value (−0.067%) observed in the control branches. Both branches formed tension wood in the upper side of the xylem, and the treated branches formed tension wood near the pith as well. This suggested that the treated branches generated larger tensile growth stress from the early growth stages. The successive generation of growth stress from the early growth stages was considered to generate forces large enough to bend the branch upward.

Key words Negative gravitropism · Branch · Growth stress · Tension wood · Gibberellin A_3

Introduction

The reaction wood in the trunk of many gymnosperm trees generates large compressive growth stress in its axial direction, whereas angiosperm trees have large tensile growth stress.[1–6] In branches of Japanese cypress (*Chamaecyparis obtusa*), large compressive growth stress has been confirmed in compression wood formed in its lower side.[7] These phenomena show that the growth stress corresponds to the tree shape, but they do not predict the role of growth stress in the negative gravitropism of trees. To confirm this role, it is necessary to show that growth stress corresponds with formation of the tree shape during tree growth.

Nakamura et al. showed that a branch of a weeping-type Japanese cherry, *Prunus spachiana*, was transformed to upright growth by gibberellin A_3 (GA_3) treatment.[8] The weeping phenomenon is caused by insufficient mechanical support of the branch. Weeping occurs near the base of the branch where primary growth has ceased and is caused by the increase in weight accompanying elongation of the branch top. The weeping does not occur when GA_3 is applied to the terminal bud during budding; that is, a negative gravitropism appears in GA_3-treated branches.[8] The anatomical characteristics of tension wood generated by GA_3 treatment are the same as those of the tension wood induced by gravity stimulation.[9] The investigation by Nakamura et al. showed the role of tension wood in the negative gravitropism of trees.[8] Here we use the term "negative gravitropism" to describe the upward growth phenomenon resisting gravity in the wide sense, as mentioned by Mattheck.[10]

In our investigation, the growth stress generated in branches treated with GA_3 was compared with the stress in control branches. The role of the growth stress in the generation of negative gravitropism was also investigated experimentally.

Materials and methods

Plant material and treatment with GA_3

Current-year branches of 2-year-old grafts of *Prunus spachiana* Kitamura f. *spachiana* cv. *Plenarosea*, Yaebenishidare planted at the Tama Forest Science Garden of the Forestry and Forest Products Research Institute were used. Treatment with GA_3 began shortly after the outgrowth of the branches from the main shoot, when the branches were 10 mm long and had three unfolded leaves in

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the basal region. GA_3 was applied by the microdrop method to the apical bud of the branches four times weekly using a 10-μg dosage in a solution of 90% ethanol.[8] Control branches were treated with 90% ethanol only.

Measurement of growth stress

The released strain was used to evaluate growth stress because of the high correlation between the two measurements. The strain-gauge method was used in the conventional way.[11,12] Growth stresses of seven GA_3-treated branches and seven control branches were measured at the beginning of December, after growth had ceased for that year. The measuring points were set on a branch within the first 60 cm of its horizontal distance from the base, and the interval between strain gauges was 2–3 cm. For the GA_3-treated branches, the measuring points were set mainly near the base where the negative gravitropism was most remarkable. The bark and the cambial zone at the measuring points were carefully removed with a knife so as not to scratch the xylem surface; not all of the cambial zone was removed. The latter was carefully cleaned away with a knife-edge. Strain gauges 2 mm in length (Minebea B-FAE-2S-12-T11) were pasted on the outer surface of the secondary xylem in the longitudinal direction and connected to a strain meter (Kyowa UCAM-1A) by the three-wire method. The precision of the measurement was ±0.001%. The connected wires were supported to avoid weeping due to the weight of the wires. After taking the initial measurement, 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).

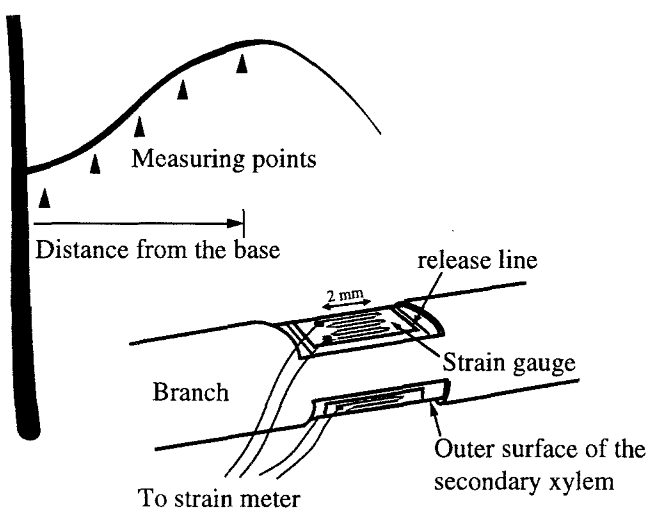

Fig. 1. Measurement of growth stress by the strain-gauge method and the measuring points. The bark at the measuring positions was removed, and the outer surface of the secondary xylem was exposed. The strain gauges, 2 mm in length, were pasted on the measuring position in the longitudinal direction connected with the strain meter. The growth stresses were released by cutting off the xylem completely near both ends of each strain gauge longitudinally

Tissue observation

To confirm the presence of tension wood, crosscut sections 15 μm thick (for optical microscopic observation) were prepared from the block samples close to the measuring points. After measuring the released strain, small blocks were taken and fixed with 50% ethanol for 1 month. The microtomed section was dehydrated with an ethanol series and double-stained with safranine and fast green. The area of the tension wood region estimated by microscopic observation was measured by an image analyzer (Zeiss, KS-400).

Results and discussion

Growth stress

Figure 2 shows the results of a longitudinally released strain on a GA_3-treated branch and a control branch. The negative released strain represents the tensile growth stress observed. The largest negative strains were found about 10 cm horizontally away from the base of the branch. Thus, the largest tensile growth stresses were generated at those points. The bending moment due to the self-weight was also largest at those points because the control branch began to weep. The branch shape is related to the balance of the bending moment due to the self-weight and that due to the growth stress induced. When the downward bending moment due to self-weight exceeds the upward bending moment due to the growth stress, the branch weeps.

Negative released strains were also detected in the upper side of the control branches. The values were smaller than for the treated branches. The released strains in the lower side of the control branch showed a positive value induced by the compressive stress there. Compressive growth stress occurs almost exclusively in gymnosperms, although it has been reported that members of the genus *Buxus* form reaction wood resembling those of gymnosperm compression wood.[13,14] Instead of developing tensile growth stresses, compressive growth stresses have been measured in *Buxus* wood.[15] In the case of *Prunus spachiana*, it was thought that these positive released strains were produced as a response to weeping. The lower sides of the control branches were compressed because the tensile growth stress in the upper side of the branch could not generate an upward bending moment of enough magnitude to exceed that due to its weight.

The GA_3-treated branches showed a larger negative released strain in the upper side. Therefore, the larger tensile growth stress was believed to be generated by the GA_3 treatment. Large negative released strains were measured even in the lower side of the treated branches. However, this negative strain did not come from the tension wood, because no gelatinous fibers were found there by microscopic observation. The large tensile growth stress in the upper side pulled the branch upward, so the lower side of

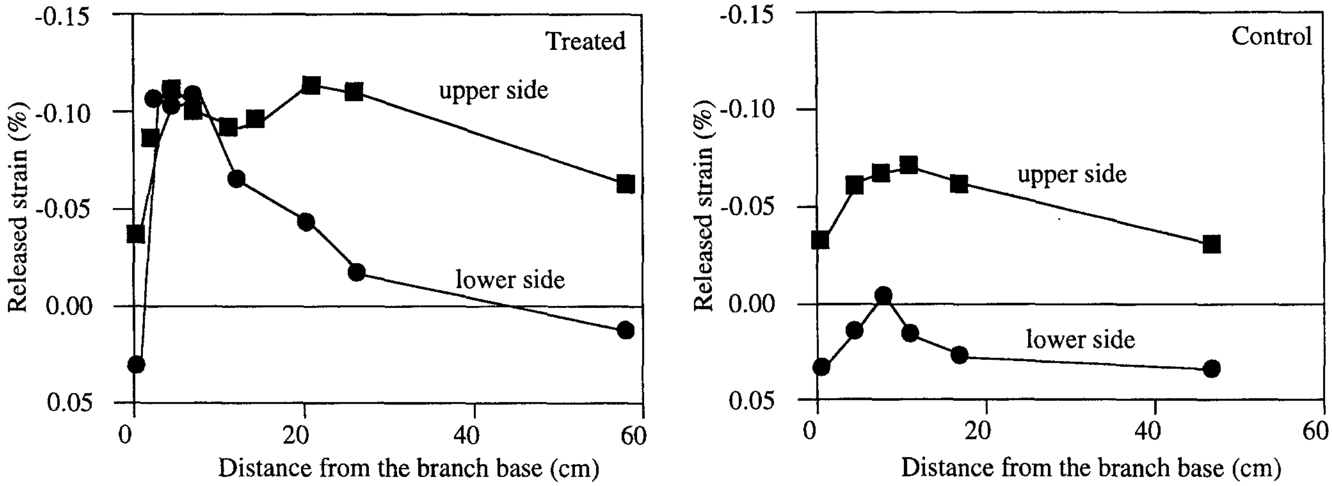

Fig. 2. Distributions of longitudinal released strains in the GA_3-treated branches and the control branches. *Squares*, strain in the upper sides of the branch; *circles*, strain in the lower sides. The larger the negative released strain, the larger is the tensile growth. Shown here are one pair of results; the other results showed similar patterns

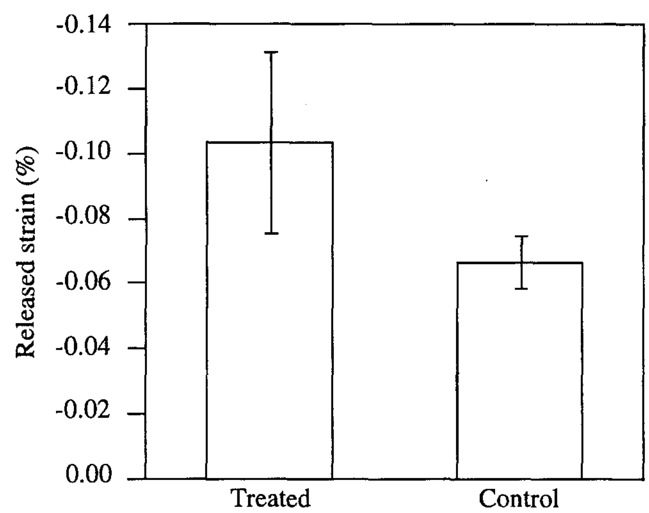

Fig. 3. Effect of GA_3 treatment on the longitudinal released strain in the upper side of the branch. Mean (±SD) values from measurements 10 cm apart horizontally from the base of the branch are shown ($n = 7$)

the branch was in a tensile stress condition as a response to the tensile stress in the upper side. The large negative strain in the lower side of the treated branch indicated that there was a large negative gravitropism induced by the tensile growth stress in the tension wood generated by GA_3 treatment.

Figure 3 shows the mean value of the largest negative released strain in the upper side near the base of the branch from treated and control branches. The value of the released strain in the branches treated with GA_3 was −0.104%, whereas for the control branches it was −0.067%. There was a significant difference between the GA_3-treated and the control cases, as shown by the *t*-test ($P = 0.006$). It was confirmed that the large tensile growth stress in the upper side of the treated branch was induced by the GA_3 treatment.

Tissue observation

The tissue at the site where the largest negative released strain was examined microscopically (Fig. 4). The diameter of the xylem of GA_3-treated branches was larger than that of the control branches (0.78 ± 0.05 vs. 0.61 ± 0.06 cm; $P < 0.001$). The upper sides of treated and control branches had tension wood stained with fast green,[16] and the area of tension wood of the GA_3-treated branches was larger than that of the control branches (0.18 ± 0.03 vs. 0.03 ± 0.02 cm^2; $P < 0.001$). This observation coincided with the larger tensile growth stresses in the upper side of the treated branches. This species had a gelatinous fiber classified into II–III types according to Onaka.[17]

The control branches did not form tension wood near the pith in the upper side of the branches, whereas the treated ones did have tension wood near the pith. This indicated that the control branches did not create tension wood at the initial growth stage when the secondary growth started, but the treated branches formed tension wood from the beginning of the secondary growth. Baba et al. observed that control branches of *Prunus spachiana* cannot form tension wood and suggested that GA_3-treated branches do not weep owing to the formation of tension wood.[9] They arrived at their result after observations at the initial growth stage, but our observation during the dormant season revealed that even the control branches formed tension wood in the later growth period. It was clear that the control weeping cherry tree did not form tension wood at the initial growth stage but at a later period, whereas the treated branches formed tension wood from the beginning of growth.

One reason for the control branches weeping, although they form tension wood, is because of the timing of the

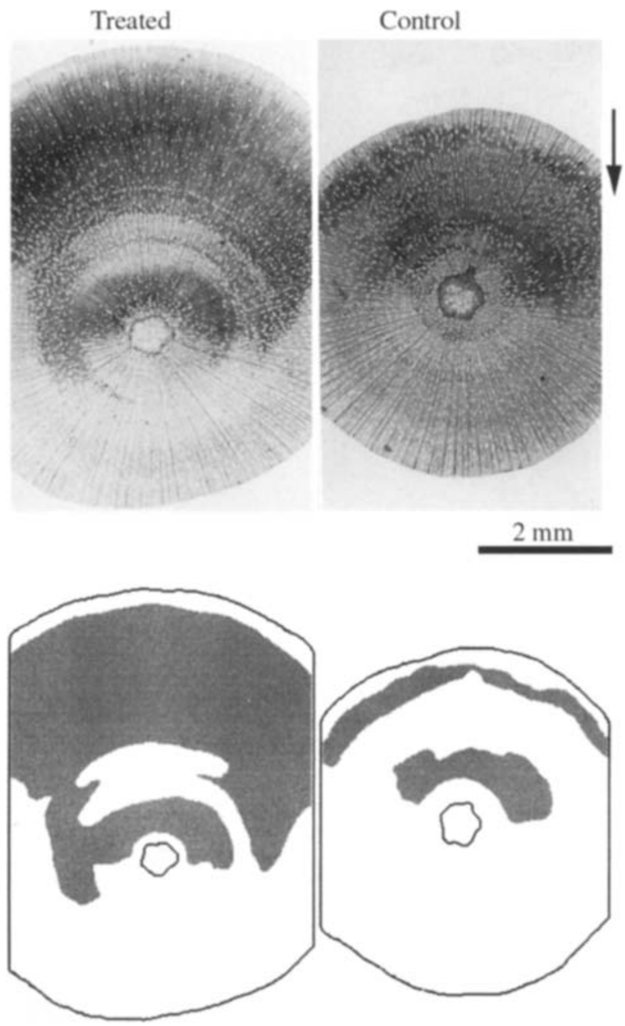

Fig. 4. Cross section of a GA_3-treated branch and a control branch from a position 10 cm from the base of the branches during the dormant season when the current growth stops. The *arrow* represents the direction of gravity. Sections were double-stained with safranine and fast green. **Top** The dark regions represent tension wood stained with fast green. **Bottom** Tension wood is the shaded region

tension wood formation. The control branches wept largely because the tension wood was not generated when secondary growth started. The weeping branch could not return to the original position even though tension wood formed later because the growth stress in the tension wood of the control branches was not large enough to bend the already thickened branch upward. Consequently, the control branches took the weeping shape even when tension wood was formed during the late growth stage.

On the other hand, for the GA_3-treated branches there was tension wood and tensile growth stress from the initial growth stage when the secondary growth started. The treated branch grew up without weeping, as the tensile growth stress supported the increasing self-weight from the slender to the thickened branch. Moreover, the accelerated formation of tension wood in the upper side of the treated branch enlarged the total upward bending moment.

Conclusion

Branches of weeping-type *Prunus spachiana* were transformed to the upright-type by GA_3 treatment. The mechanism of negative gravitropism was investigated from a physical point of view. Both control and treated branches formed tension wood at the upper side, but only treated branches formed tension wood from the early growth stage when the secondary growth started. The treated branches gave larger released strains than the control ones in the longitudinal direction. We believe that the treated branches generated a larger tensile growth stress in the longitudinal direction from the early stages of growth. The successive generation of larger growth stresses in the branch induced the negative gravitropism. We conclude that the tree shape is controlled by the large growth stress generated in the reaction wood.

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