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Effects of applied gibberellins and uniconazole-P on gravitropism and xylem formation in horizontally positioned *Fraxinus mandshurica* seedlings

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Abstract The present study deals with the effects of gibberellins (GA_3, GA_4) and uniconazole-P, an inhibitor of gibberellin biosynthesis, on negative gravitropism and xylem formation in the stems of horizontally positioned, 2-year-old *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. seedlings. Each growth regulator (100 µg) dissolved in 5 µl acetone (50%) was applied to the basal node of the current shoot on May 24, 1995. The same treatment was repeated five times weekly until June 28. Five seedlings were used for each treatment. The seedlings were positioned horizontally 24 h after the first application on May 25. Within 5 weeks the horizontal stem of control and GA-treated seedlings exhibited negative gravitropism. In contrast, the application of uniconazole-P inhibited negative gravitropic stem bending. The application of GAs increased the number of gelatinous fibers having thickened cell walls on the upper side of stems. The uniconazole-P application decreased xylem cell formation but did not inhibit the formation of gelatinous fibers. These results indicate that not only the differentiation of gelatinous fibers but also xylem increment is important in the negative gravitropism of horizontally positioned *F. mandshurica* seedlings. These results also suggest that GAs may be involved in xylem cell formation rather than the differentiation of gelatinous fibers in this species.

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Introduction

In leaning trees, radial growth is promoted on the upper or lower side of stems, shoots, and branches, forming a specialized xylem tissue referred to as reaction wood. The reaction wood of arboreal, dicotyledonous angiosperms is called tension wood, which forms gelatinous fibers and appears on the upper side of inclined stems. It is well known that both auxin and gibberellins (GAs) are involved in the cambial activity of woody plants.[1–6] In inclined woody angiosperms, various investigations have suggested that auxin deficiency on the upper side of stems causes differentiation of tension wood fibers.[7–10]

The role of GAs also is considered to be important in the formation of tension wood fibers in woody angiosperms. In the genus *Prunus*, various reports showed that GAs promote the formation of tension wood fibers. Denhard and Feucht[11] observed that the formation of tension wood-like fibers in vertical *Prunus avium* shoots was stimulated by GA application. Nakamura et al.,[12] Saotome et al.,[13] Nakamura,[14] and Baba et al.[15] reported that the application of GAs to growing shoots of drooping mutants of *P. spachiana* and *P. persica* caused resumption of vertical shoot growth and promoted the cell division of cambium and the formation of tension wood on the upper side of shoots. However, it is still obscure whether GAs regulate the differentiation of gelatinous fibers and cambial cell division in relation to negative gravitropism of stems in various species. Except in the genus *Prunus*, the effects of GA treatment on negative gravitropic bending or the formation of tension wood fibers are not always positive. For instance, the application of GA_3 along the upper side of horizontally positioned stems of *Acer rubrum* seedlings did not alter normal gravitropic reorientation.[7]

In the present study, preliminary experiments were performed on the effects of GA_3, GA_4, and uniconazole-P, a triazole-type plant growth retardant and an inhibitor of GA biosynthesis,[16] on negative gravitropism and the formation of tension wood fibers in horizontally positioned seedlings of *Fraxinus mandshurica* Rupr. var. *japonica* Maxim.

Materials and methods

Two-year-old *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. seedlings, grown in Iwamizawa, Hokkaido, Japan, were lifted from a nursery and transported to the nursery of the Department of Forestry Science, Tottori University, in October 1994. The seedlings were kept at about 5°C in a refrigerator until they were used. On May 12, 1995, each seedling was transplanted in vermiculite in a 7.0 × 7.0 × 19.5 cm paper pot and set in a greenhouse. On May 23, 1995, twenty seedlings with growing shoots were selected for their uniformity of size and development. The average stem length and diameter at 2.5 cm above the ground were 17.0 ± 0.4 cm and 3.22 ± 0.14 mm, respectively. The seedlings were divided into four groups. (1) five control plants treated with 5 µl 50% acetone; (2) five plants treated with 100 µg GA_3 in 5 µl 50% acetone; (3) five plants treated with 100 µg GA_4 in 5 µl 50% acetone; and (4) five plants treated with 100 µg uniconazole-P in 5 µl 50% acetone. Each acetone solution was applied to the basal node of the current shoot with a micropipette at the beginning of the experiment on May 24. The same treatment was repeated five times per week until June 28.

The seedlings were placed horizontally on May 25, 1995 at 24 h after the first treatment. To follow changes in stem bending, lateral-view photographs of each seedling were obtained at 0, 0.5, 1, 6, 13, 20, 27, and 34 days after horizontal displacement of stems. The angle of the basal node of the current shoot was determined in each photograph as indicated in Fig. 1. The seedlings were harvested on June 28, just 34 days after stem displacement, and the shoot lengths and stem diameters were measured in vertical and horizontal directions 2.5 cm above the ground level.

To determine the production of xylem cells, stem segments about 0.5 cm in length were taken 1 cm above the basal node of the current shoot and 1, 5, and 10 cm below the node. Each stem segment was fixed in FAA solution (formalin/acetic acid/ethanol/water, 5:5:60:30, v/v) for 24 h, rinsed in water, dehydrated in ethanol series, and then embedded in paraffin. Transverse sections 10–15 µm thick were cut on a sliding microtome, double-stained with safranin-fastgreen FCF, and mounted in Eukitt. For each section, the number of wood fibers in radial files in newly formed xylem were counted under an ordinary light microscope. The diameter, lumen width, and cell wall thickness of the wood fibers were measured in radial and tangential directions using a light microscope (Olympus BHS 323) in combination with a video micrometer (Olympus Flovel VM-31), of which the minimum limit for measurement is 0.27 µm, using cross sections taken 5 cm below the basal node of current shoots. About 70 fibers on the upper and lower sides of the stem were measured per section.

Results and discussion

The application of GA_3, GA_4, and uniconazole-P to the current shoots affected xylem formation and morphology of horizontally positioned *F. mandshurica* seedlings. Negative gravitropism of the seedlings was greatly influenced by the application of the GAs or uniconazole-P (Fig. 2). Figure 3 shows the pattern of change in the orientation of the horizontally positioned seedlings. During the experimental period, the rate of upward bending in the GA-treated seedlings was not different from that of control seedlings. In contrast, the application of uniconazole-P inhibited upward bending.

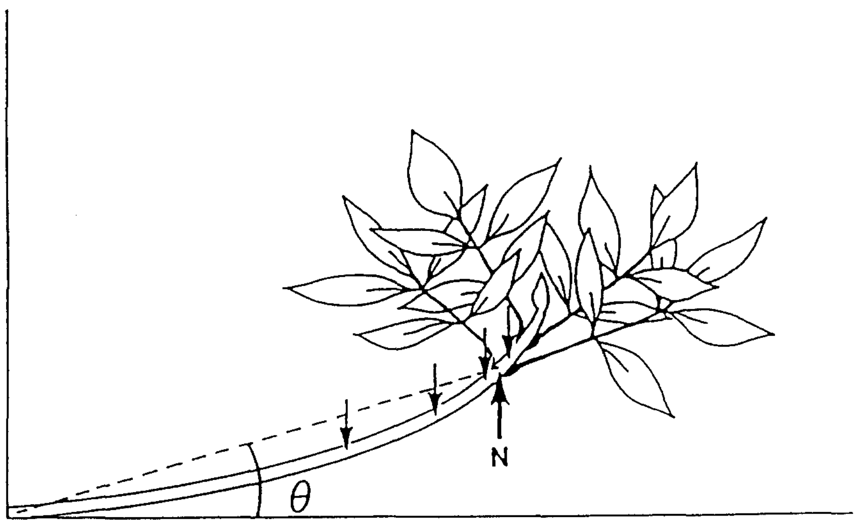


Fig. 1. How negative gravitropic stem bending was measured and where stem anatomy was assessed in horizontally positioned seedlings. The acute angle θ the between the horizontal line and the straight line segment from the stem base to the basal node (*N*, *large arrow*) of a current shoot was measured to determine the degree of upward stem bending. Stem segments for anatomical observation were taken 1 cm above the basal node (*N*) and at 1, 5, and 10 cm below (*small arrows*) the node in a 1-year-old stem

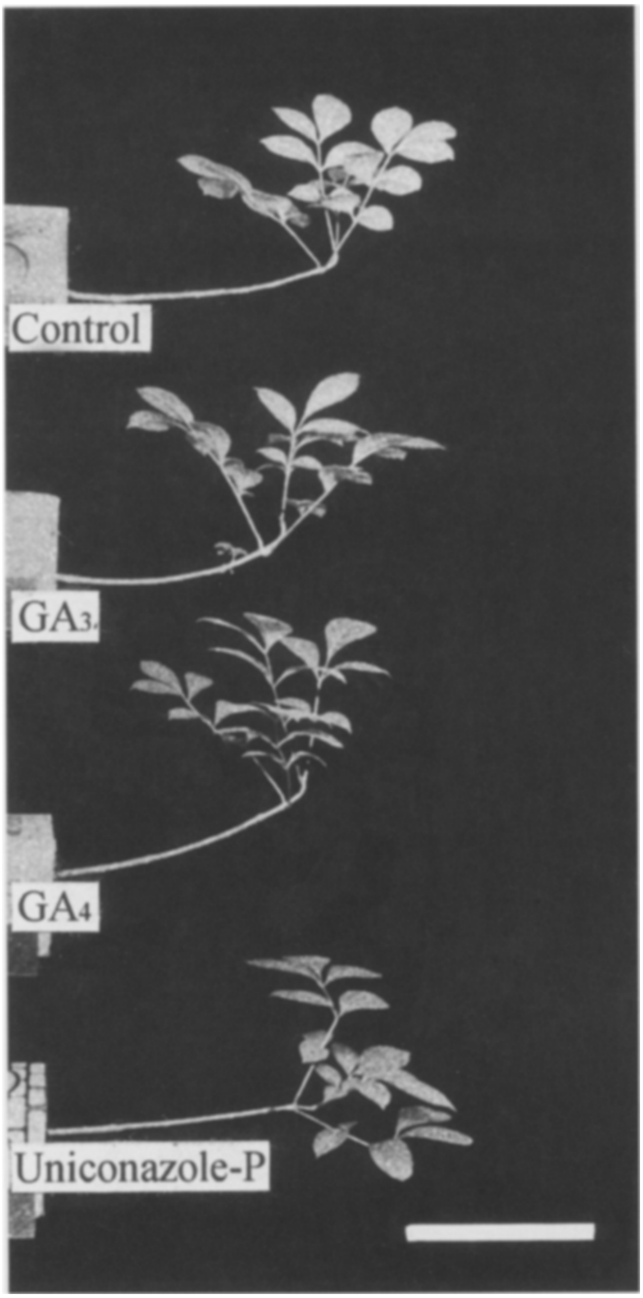


Fig. 2. Effects of applied GA_3, GA_4, and uniconazole-P on gravitropic bending in the stem of horizontally positioned seedlings on day 34. Note that the uniconazole-P-applied seedling shows almost no upward stem bending. The *horizontal bar* represents 10 cm

The application of GAs and uniconazole-P changed cambial activity and xylem anatomy. In comparison with the controls, both GA_3 and GA_4 increased xylem cell production not only on the upper side but also on the lower side 1 cm above the basal node of the current shoots (Fig. 4). The number of wood fibers on the upper side of 1-year-old stems 1 cm below the node also increased slightly after the application of both GA_3 and GA_4. The application of uniconazole-P significantly reduced the radial increment of xylem on the upper side 1 cm above the node and 10 cm below the node of horizontal stems (Fig. 4). The ratios of the cells numbers on the upper and lower sides at 1, 5, and 10 cm below the node in the GA_3-applied seedlings were constantly smaller than those of control, GA_4-applied, and uniconazole-P-applied seedlings (Fig. 5).

Prodhan et al.[17] have reported that the tension wood of *F. mandshurica* is not typical and is somewhat anomalous. However, the formation of tension wood, characterized by the presence of gelatinous fibers stained with fast green FCF, was observed on the upper side (Figs. 6, 7). The existence of cellulose in gelatinous fibers were also confirmed by the application of chlozinciodine solution to stem sections. The application of uniconazole-P also formed gelatinous fibers.

Application of GA_3 decreased the diameter of wood fibers on the upper side of the stems in the radial direction but not in the tangential direction comparied with that in the controls (Table 1). The application of GA_4 did not significantly affect the diameter of wood fibers on the upper side of the stems in the radial direction but increased those in the tangential direction. The uniconazole-P application did not affect the diameters of wood fibers on the upper side of the stems in both radial and tangential directions. On the lower side, the diameters of wood fibers in both radial and tangential directions increased after GAs application. The application of GA_4 and uniconazole-P did not affect the lumen width of wood fibers in either the radial or tangential direction. However, GA_3 application decreased the lumen width on the upper side in the radial direction but not in the tangential direction. The GAs increased lumen width on the lower side in the tangential direction.

The application of GAs increased the cell wall thickness of wood fibers on the upper side in both radial and tangential directions. The uniconazole-P application decreased the cell wall thickness on the lower side in the radial and tangential directions.

The application of GA_3 and GA_4 increased xylem cell formation and cell wall thickness of wood fibers on the upper side of stems in horizontally positioned *F. mandshurica* seedlings, indicating the importance of GAs in the formation of tension wood. In contrast, the application of uniconazole-P significantly inhibited negative gravitropic stem bending, decreased xylem cell formation in the radial direction on the upper side of stems, and reduced cell wall thickness in the radial and tangential directions on the lower side of stems. Uniconazole-P is an optical isomer of uniconazole, a triazole-type plant growth retardant, that inhibits GA biosynthesis.[16] Oshio et al.[18] have reported that uniconazole inhibits the oxidation of ent-kaurene to ent-kaurenoic acid, a direct precursor of GAs. The inhibitory effects of uniconazole-P on negative gravitropism are possibly related to the inhibition of GA biosynthesis, resulting in the reduction of xylem cell formation.

It is well known that GAs and auxin are involved in the control of cambial activity in woody plants.[1-6] The present results also indicate the importance of GAs in xylem cell formation of *F. mandshurica* seedlings. The effects of GAs on negative gravitropic response and reaction wood formation in woody plants has been observed to vary from having

Fig. 3. Changes in the degree of upward bending of horizontally positioned 1-year-old stems after the application of GA_3, GA_4, and uniconazole-P. Note that uniconazole-P application inhibited upward stem bending. *Vertical bars* represent standard errors (n = 5). Symbols with common letters on the same day are not different at the 5% level using LSD

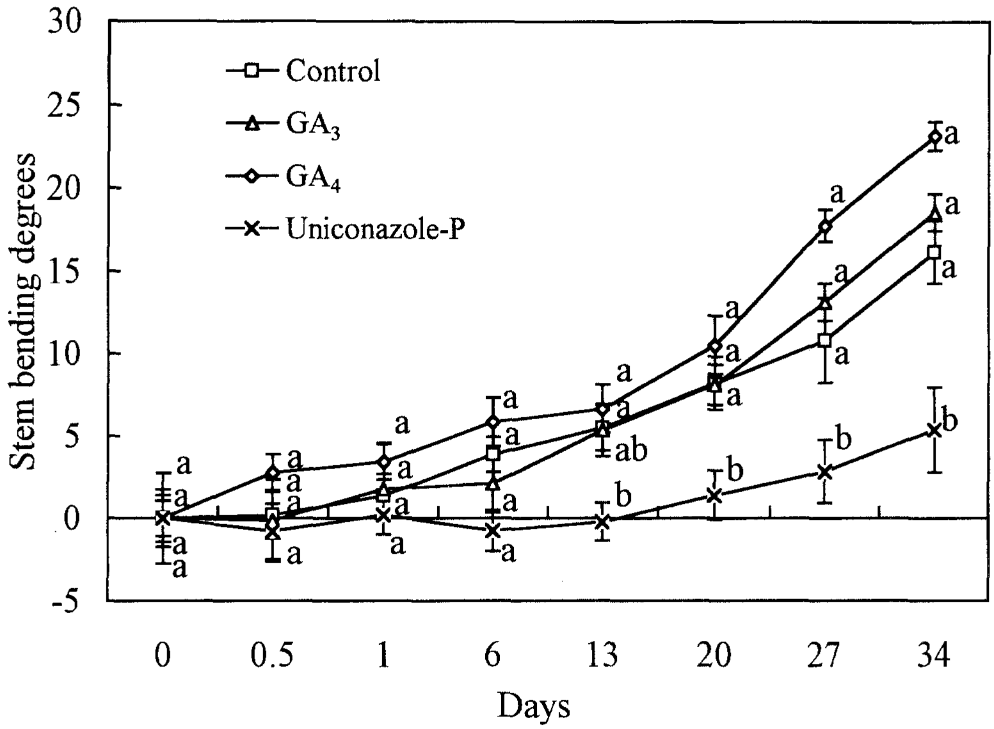
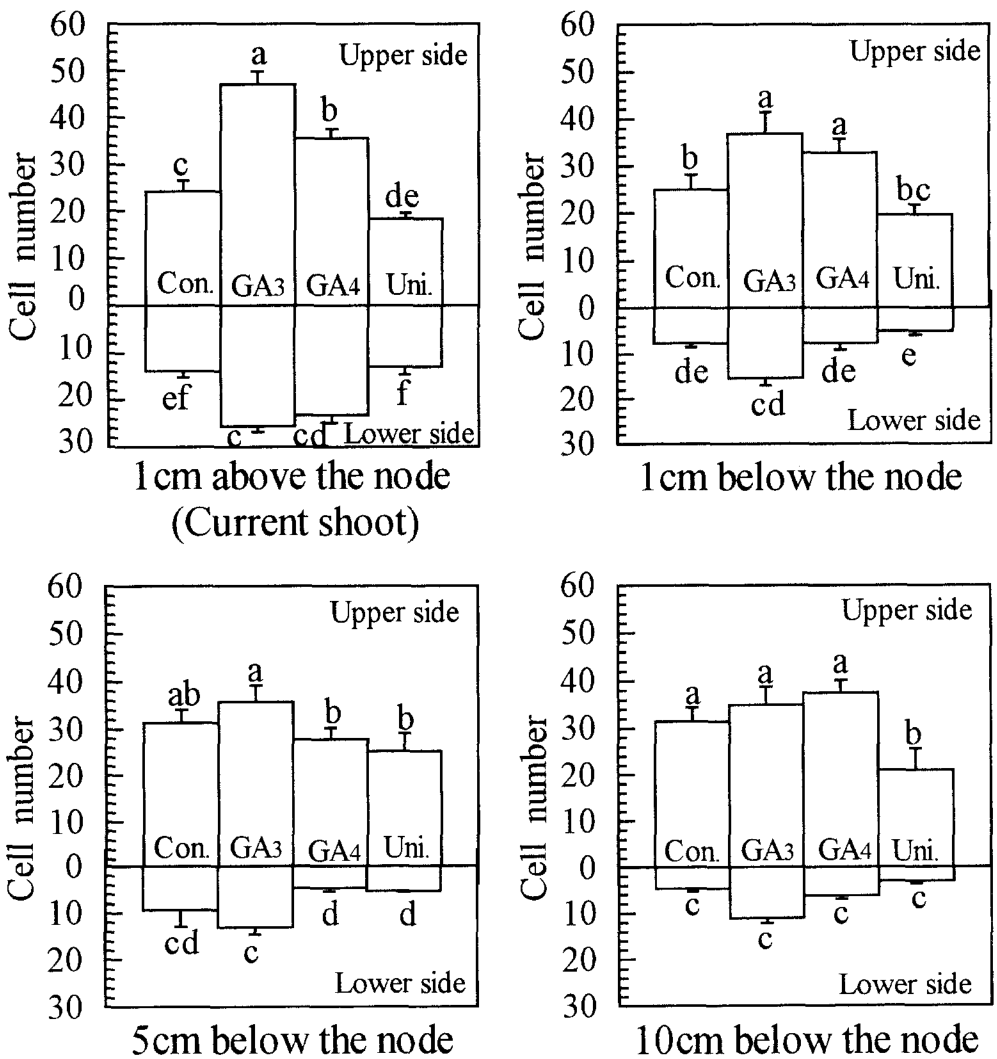


Fig. 4. Effects of applied GA_3, GA_4, and uniconazole-P on radial number of wood fibers on the upper and lower sides of horizontally positioned seedlings. Stem samples were harvested 34 days after stress initiation. Note that uniconazole-P application inhibited production of xylem cells on the upper side 10 cm below the node. Vertical bars represent standard errors (n = 5). Columns with common letters within figures are not different at the 5% level using LSD



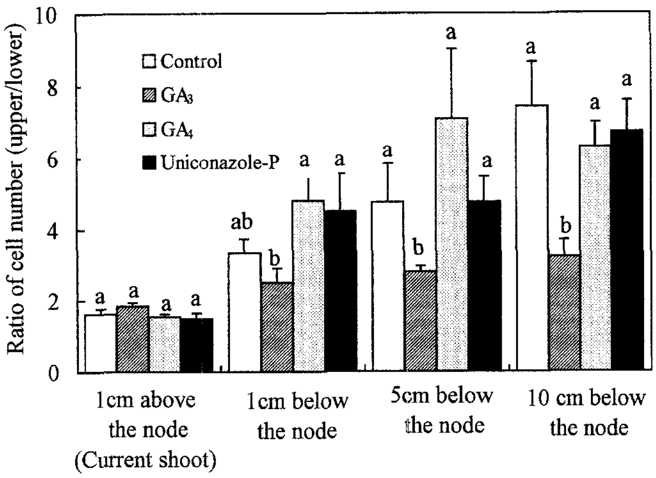


Fig. 5. Effects of applied GA_3, GA_4, and uniconazole-P on the ratio of the number of wood fibers on the upper and lower sides of horizontally positioned seedlings. Note the decreased ratios at all positions below the node in 1-year-old stems of GA_3-treated seedlings. *Vertical bars* represent standard errors ($n = 5$). Columns with common letters within measured portions are not different at the 5% level using lSD

no effect to acceleration. In coniferous species, exogenous GA_3 enhanced branch hyponasty by promoting compression wood formation on the lower side of branches in decapitated *Cupressus arizonica* seedlings.[19] In woody angiosperms, GA_3 applied to the stem surface of upright *Acer rubrum* seedlings stimulated cambial activity but did not cause the formation of gelatinous fibers.[8] Cronshaw and Morey[7] also reported that GA_3 applied along the upper side of horizontal stems of *Acer rubrum* did not affect normal gravitropic reorientation or the formation of gelatinous fibers. In contrast, the formation of tension wood-like fibers in vertical *Prunus avium* shoots was stimulated by GA application.[11] Nakamura et al.,[12] Baba et al.,[15] Saotome et al.,[13] and Nakamura[14] demonstrated that the application of GA_3 and GA_1 to growing shoots of the drooping mutants of *Prunus spachiana* and *P. persica* restored vertical shoot growth and promoted the formation of gelatinous fibers on the upper side of drooping shoots.

In the present study, the application of uniconazole-P to the horizontal stems also supports the importance of GAs in negative gravitropism and xylem formation in horizontally

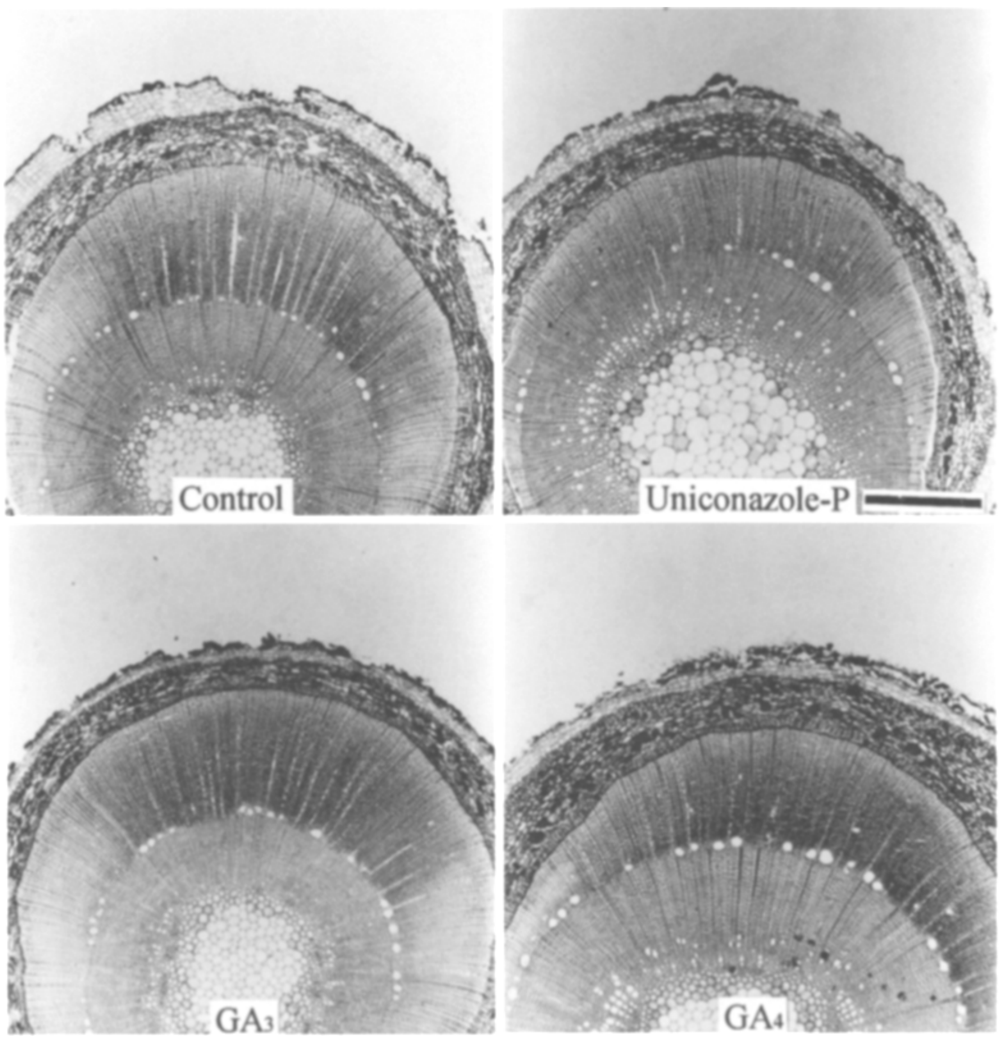


Fig. 6. Transverse sections of wood fibers on the upper side of the stem 5 cm below the basal node of current shoots. Stem samples were harvested 34 days after the horizontal displacement of stems. Note that uniconazole-P forms gelatinous fibers. The *bar* represents 500 μm

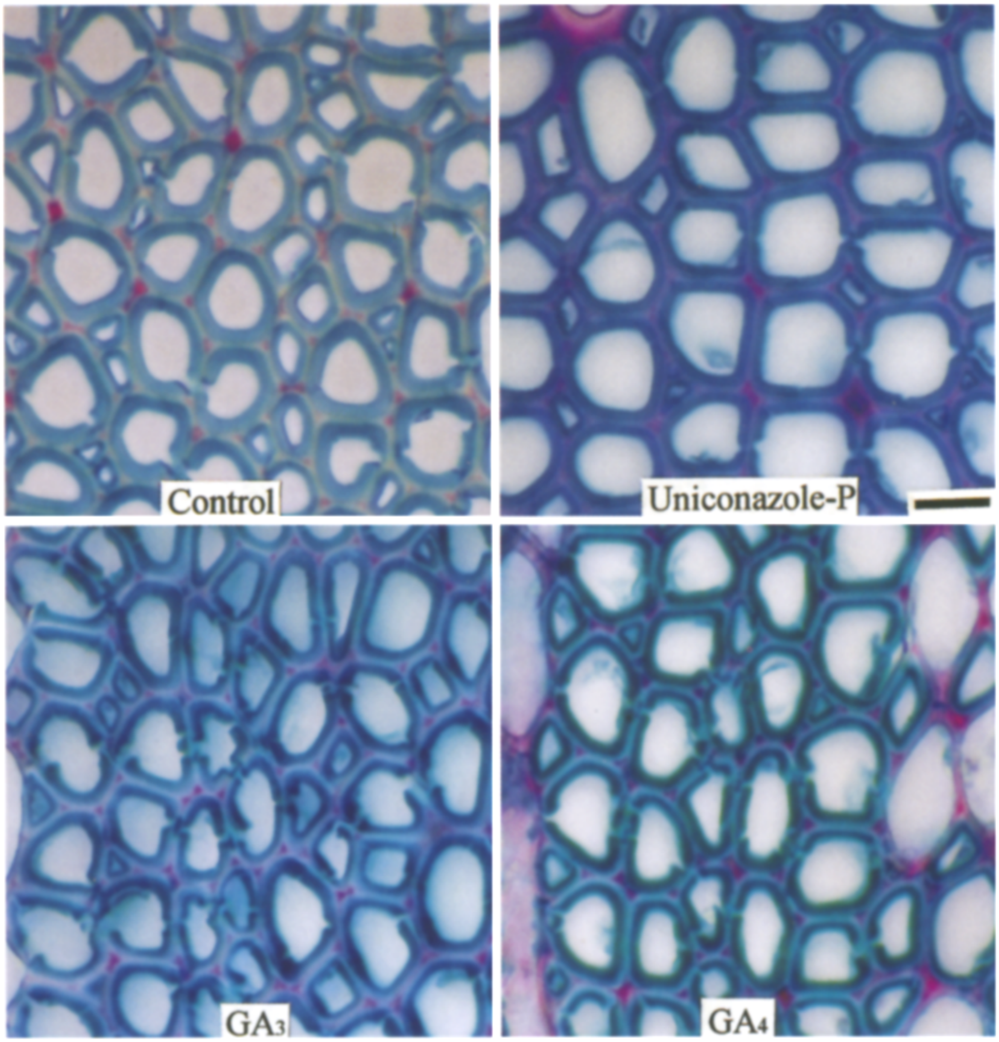


Fig. 7. Transverse sections of wood fibers on the upper side of the stem 5 cm below the basal node of current shoots. Stem samples were harvested 34 days after the horizontal displacement of stems. Note that uniconazole-P forms gelatinous fibers. The *bar* represents 10 μm

Table 1. Effects of GA_3, GA_4, and uniconazole-P on various parameters of wood fibers in radial and tangential directions 5 cm below the basal node of current shoots in seedlings

Parameter	Result (μm)			
	Control	GA_3	GA_4	Uniconazole-P
Radial direction				
Cell diameter				
Upper	15.0 ± 1.5^{a}	13.0 ± 0.7^{bc}	14.7 ± 0.6^{ab}	13.5 ± 1.2^{ab}
Lower	11.5 ± 0.8^{cd}	14.2 ± 0.7^{ab}	14.2 ± 2.2^{ab}	11.1 ± 1.3^{d}
Lumen width				
Upper	11.1 ± 1.5^{ab}	7.7 ± 0.6^{d}	10.2 ± 0.6^{bc}	10.0 ± 0.9^{abc}
Lower	9.2 ± 0.7^{cd}	12.0 ± 0.8^{a}	12.4 ± 1.9^{a}	9.9 ± 1.5^{bc}
Cell wall thickness				
Upper	1.9 ± 0.3^{c}	2.7 ± 0.4^{a}	2.2 ± 0.1^{b}	1.7 ± 0.2^{c}
Lower	1.2 ± 0.2^{d}	1.1 ± 0.1^{a}	0.9 ± 0.2^{d}	0.6 ± 0.1^{e}
Tangential direction				
Cell diameter				
Upper	11.2 ± 1.0^{d}	11.5 ± 0.9^{cd}	12.9 ± 0.6^{bc}	12.0 ± 1.2^{bcd}
Lower	13.1 ± 1.4^{b}	15.1 ± 0.7^{a}	16.0 ± 0.8^{b}	12.4 ± 0.9^{bcd}
Lumen width				
Upper	7.5 ± 1.2^{cd}	6.2 ± 0.8^{d}	8.5 ± 0.5^{c}	8.4 ± 1.0^{c}
Lower	10.6 ± 1.7^{b}	12.6 ± 0.8^{a}	13.7 ± 0.6^{a}	10.9 ± 0.9^{b}
Cell wall thickness				
Upper	1.9 ± 0.3^{c}	2.7 ± 0.3^{a}	2.2 ± 0.2^{b}	1.8 ± 0.3^{c}
Lower	1.2 ± 0.2^{d}	1.3 ± 0.1^{d}	1.2 ± 0.2^{d}	0.8 ± 0.1^{e}

Values are means with standard errors (n = 5).

$^{a-e}$Means with common letters within parameters are not different at the 5% level LSD positioned *F. mandshurica* seedlings. However, uniconazole-P application did not inhibit the formation of gelatinous fibers on the upper side of horizontal stems.

The application of uniconazole-P inhibited upward stem bending through the reduction of xylem cell production in the basal portions of horizontally positioned stems, despite the appearance of gelatinous fibers on the upper side. These results indicate that not only the differentiation of gelatinous fibers but also the increment of them to a certain number is important in negative gravitropic stem bending in horizontally positioned *F. mandshurica* seedlings. It is difficult to explain the exact roles of GAs in the negative gravitropism in this study, because there is no information about endogenous levels of GAs. It is suggested that GAs are involved in xylem cell production rather than in the differentiation of gelatinous fibers. Further investigations are needed to clarify the role of gibberellins in gravitropic responses and the formation of gelatinous fibers in woody angiosperms.

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