Induction of compression wood in seedlings of Taiwan incense cedar (*Calocedrus macrolepis* var. *formosana*) during the mid-season growth pause

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**ABSTRACT.** Taiwan incense cedar, *Calocedrus macrolepis* var. *formosana*, is an endemic variety with excellent wood property. However the annual radial growth is slow and wood quality was frequently abating by the presence of compression wood. In this study, a mid-season growth pause was found in the branches as well as the seedlings. The effects of growth pause on compression wood formation were assessed. The seedlings of Taiwan incense cedar were induced to form compression wood by indole-3-acetic acid (IAA) as well as horizontally leaning in the growth pause. The results showed that although this growth pause decreased the vascular cambial activity in standing control seedlings, vascular cambium of all treated seedlings reacted to the induction and exhibited higher cambial activity in the expected compression wood forming side. The slow annual radial growth of Taiwan incense cedar was due to the tardy growing nature rather than the length of growing season. This nature may cause the slow presentation rate of compression wood observed in different treatments in Taiwan incense cedar.

**Keywords:** *Calocedrus macrolepis* var. *formosana*; Compression wood; Horizontally leaning; Indole-3-acetic acid; Vascular cambium.

**INTRODUCTION**

Mid-season growth pause was often revealed in temperate-zone species. It was believed contribute to optimize the growth condition of the tree (reviewed by Larson, 1994). The occurrence of growth pause might be related to the transition from earlywood to latewood or the physiology of cambium to react some unfavorable growth conditions which usually result in the formation of false ring (Larson, 1994). The influence of mid-season growth pause on the compression wood formation is not clear.

Compression wood occurs in the basal part of branches of coniferous trees and is often accompanied by significant eccentricity in the cross section. It is characterized by the presence of circular cell lumen, intercellular space, highly lignified layer in the cell wall of secondary tracheid (S2₇ layer) and spiral cavities (Timell, 1986). The formation of compression wood and the asymmetrical distribution of growth stresses increase the heterogeneity of the wood quality and consequently cause considerable disadvantages in wood processing, thus reducing its value (Mattheck and Kubler, 1995).

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Taiwan (Liao, 1976). The fragrant sawdust of this species has long been used for joss sticks and thus it was named Taiwan incense cedar. Taiwan incense cedar is distributed in hillsides of natural forests between 300 and 1,800 m in elevations (Lu et al., 1994). However, the annual radial growth is slower compared to two other important sister species, Taiwan yellow cypress (Chamaecyparis obtusa var. formosana) and Taiwan red cypress (C. formosensis) (Wang et al., 1987).

Most previous studies of compression wood formation focused on fast-growing tree species in the growing season. The purpose of the present study was to examine the role of IAA in compression wood formation in seedlings of Taiwan incense cedar during a growth pause period by monitoring the growth of cambium induced by IAA. Meanwhile, we also placed seedlings horizontally to investigate stimulation of compression wood formation with and without the presence of IAA. Based on cambial activity represented by cambium cell and differentiating xylem cell numbers as described by Timell (1986), cambium growth activities were carefully examined among the different test conditions. We hypothesized that the compression wood would form during the mid-season growth pause, especially in a tardy growing tree, and the compression wood formation should exist against the stress caused by leaning or application of IAA, or both.

MATERIALS AND METHODS

Seasonal changes in cambial activity

Seasonal changes in cambium cell activity in a mature tree of Taiwan incense cedar were measured. The tree was on the campus of National Taiwan University and its D.B.H. (diameter at breast height) was 110 cm and height is about 12 m. Samples were collected from the upper sides of branches on 15 March, 9 April, 7 May, 7 June, 5 July, 8 August, 9 October, and 6 November 2004.

Seedling selection

Thirty-nine 2-year-old seedlings of Taiwan incense cedar (Calocedrus macrolepis var. formosana) from the nursery of Chiayi Forest District Office of Taiwan Forestry Bureau in south western Taiwan (23°28’ N, 120°26’ E) were transplanted into a greenhouse at the Department of Forestry and Resource Conservation, National Taiwan University, Taipei (25°00’ N, 121°27’ E) on 5 May 2005. After a 1-month acclimation, the average height of seedlings was 106.9 ±13.3 (± SE) cm and average diameter of the trunks at 20 cm height was 7.45 ± 0.96 (± SE) mm.

Seedling treatments

Seedlings were subdivided into four groups. Group 1 seedlings were planted vertically as the control (Figure 1A). Group 2 seedlings were planted vertically and marked at 25 cm above the basal part for cambium cell investigation (Figure 1B). For IAA treatment, the outer bark with a little secondary phloem tissue was cut using a knife blade to form a tangential wound (0.2 cm in length × 1 cm in width), and then 1% IAA-lanolin (80 μL 5% IAA mixed with 400 mg melted lanolin) was directly applied to the wound site. The wound site was covered with aluminum foil and ventilated tape to protect the IAA from light (Figure 1B). Group 3 seedlings were laid horizontally to induce compression wood formation (Figure 1C). Group 4 seedlings were treated as group 2, but laid horizontally immediately after IAA application. In order to distinguish the compression wood induced by gravity, the IAA application site was carefully oriented so that it was on the upper side of the trunk (Figure 1D). The control group seedlings were harvested at 4 different times, i.e. days 0 (11 June 2005), 4 (15 June), 6 (17 June), and 13 (24 June), and three seedlings were harvested each time. The treatment groups were harvested after days 4, 6, and 13. Discs (1 cm in thickness) were collected from 20 cm above the basal part or 5 cm below the IAA application site of each seedling.

Cambium activity measurement

The cambium activity was measured by counting the numbers of vascular cambium and differentiating xylem cells in a single radial profile. Freshly cut discs (1 cm in thickness) were divided into two halves and immediately fixed in 1% glutaraldehyde (GA) in 0.1% sodium phosphate buffer (pH 7.0). Free hand sections from each disc were double-stained with 1% safranin O in a 50% ethanol solution and 1% fast green in a 95% ethanol solution. Under a light microscope (Leica Diaplan Microscope), cambium cells were counted. The counted cells included undifferentiated parenchymatous cells and differentiated xylem cells between the mature xylem and phloem. The cytoplasm of mature tracheids has already been degraded.
Cambium cell differentiation

To observe cell differentiation, freshly cut 1 × 1 × 2-mm cubes were fixed overnight in 2.5% GA in 0.1% sodium phosphate buffer (pH 7.0, 4°C). After washing with phosphate buffer, the cubes were post-fixed in 1% osmium tetroxide in 0.1% sodium phosphate buffer (pH 7.0, 4°C). The double-fixed samples were then dehydrated by a serial acetone treatment and embedded in Spurr’s resin as previously described (Spurr, 1969). An ultramicrotome (Ultracut E) was used to make a series of 1-μm sections; Stevenel’s blue was then used to stain the cells (Del Cerro et al., 1980). Cell walls of mature cells were light blue, and inner cell walls of differentiating cells were purple. The appearance of intercellular spaces and circular tracheids was characteristic markers of differentiating compression wood.

Statistical analysis

Cambium cell numbers of 24 radial cell files from three seedlings of each treatment are expressed as the mean ± SE. Means from different treated groups were compared by analysis of variance (ANOVA) (SAS vers. 9.0). Tukey’s honest significant difference was used to measure post hoc significant differences between the means.

RESULTS

Seasonal changes in cambial activity

Layers of cambium cells in branches of Taiwan incense cedar increased from March (four cell layers) to April (seven or eight cell layers), then decreased in May (four or five cell layers) and June (four to six cell layers), resumed growth in July (seven to eleven cell layers), and gradually decreased in October (six to nine cell layers) and November (four layers).

Wood structure observations

The majority of secondary xylem of Taiwan incense cedar was composed of tracheids with a few scattered solitary axial parenchyma cells (Figure 2A). The cellular arrangement was plain and regular. The ray parenchyma was one to seven cells high, and was homogeneous and uniseriate (Figure 2B). Ray parenchyma cells were occasionally filled with starch grains, which appeared black with iodine staining (data not shown). Resin ducts were absent from the secondary xylem but were present in the bark. The dormant cambium zone of Taiwan incense cedar normally consisted of two or three layers of non-differentiated cells (Figure 2C). The secondary phloem consisted of repeated sequences of alternating cell types (in the order of sieve cells, parenchyma cells, sieve cells, phloem fibers) (Figure 2D). This particular cell arrangement was first described by Den Outer (1967) in Chamaecyparis pisifera.

In contrast to angular cells forming normal wood (Figure 3A), tracheids of compression wood were circular (Figure 3B). In addition, there was much great intercellular space among tracheid cells in compression wood. Severe compression wood also exhibited helical cavities in the inner cell walls (Figure 3).

Cambial activity

Cells of the cambium of standing control seedlings (group 1) decreased during the experimental period. At the beginning (day 0), seedlings had an average cell number of 7.29 ± 0.46 (± SE) per radial file, and cell numbers gradually decreased to 4.33 (± 0.44) during the 2 weeks of the experimental period (Figure 4).

Average numbers of cambial cells were about the same between the treated side and opposite side of IAA-treated standing seedlings on day 4 (group 2). The average
Cambial cell numbers were slightly higher on the IAA-treated side than on the opposite side on day 6, but the difference was not significant. At the end of the 2-week study period, a significant difference was observed between the IAA side and the opposite side (Figure 5A). For seedlings kept horizontal (group 3), cambial cell numbers decreased on the upper side of the stems in comparison with the control on day 4, but there was no difference between the upper and lower cambial cell numbers on day 6. At the end of the experiment (day 13), both sides of the treated seedlings had higher cambial activity than did control seedlings (Figure 5C).

**Cell Differentiation**

Control standing seedlings showed normal tracheid cells and no apparent morphological differences between days 0 (Figure 6A) and 13 (Figure 6B). Among the IAA-treated standing seedlings on day 13 (group 2), one of three seedlings exhibited round tracheids with intercellular spaces and a deeply stained area within S2 layer in cell wall on the IAA-application site (Figure 6C), but no helical cavities were observed. On the opposite side without IAA treatment, tracheids had the same morphology as those of the control (Figure 6D). Among horizontal seedlings (group 3), no compression wood was found on the upper side of the stems (Figure 6E). However, compression wood was found on the lower side of stems from two of three seedlings (Figure 6F). For example, cells marked numbers 1 and 2 are normal cambial layers and numbers 3 and 4 are cambial cells beginning to undergo radial enlargement. Cell numbers 5 and 6 were developing S1 layers that exhibited circular cell lumen and intercellular spaces.

Differentiating xylem cells of the IAA-treated upper side of horizontal seedlings (group 4) were developing an S2, layer, which is an early sign of compression wood formation (Figure 6G). However, one of three seedlings also exhibited compression wood on the lower side of IAA-treated seedlings. Differentiating compression wood of the lower side was not observed in the other two IAA-treated horizontal seedlings (Figure 6H).

**DISCUSSION**

There are two growing seasons in mature Taiwan incense cedar as determined by the cambial activity of
branches. The first growing season is between March and April, and the second one is July through October. A growth pause of Taiwan incense cedar was observed in the mid-season, May to June. The upright-growing seedlings in our experiment also showed a decline in cambial cell numbers in June (Figure 4). These observations were similar to the results of other coniferous species by Larson (1994). Factors affecting the growth pause were argued to be genetic control, climatic variation, or both (Larson, 1994). To produce compression wood not only in growth season but also in growth pause would be an advantage for conifers. That is, the growth stress could be continually generated until the stem retains its equilibrium position. We observed the compression wood formation of Taiwan incense cedar in early April wood before growth pause season and in July wood after growth pause season.

Figure 5. Cambial activity of seedlings undergoing different treatments (A) Group 2, seedlings treated with IAA; (B) Group 3, seedlings leaned horizontally; (C) Group 4, seedlings treated with IAA and leaned horizontally. Mean ± SE, n = 24. Within each group, means accompanied by the same letter are not significantly different at P < 0.01.

Figure 6. Observations of the differentiation of woody cells from seedlings subjected to different treatments for 13 days except for figure 6A which starts from day 0. (A-D, G, H) are micrographs from semithin sections, and (E, F) are micrographs from free hand section. (A) Cross-section from control group 1 seedlings on day 0. (B) Cross-section from control group 1 seedlings after 13 days. The IAA-treated side (C) and the opposite side (D) of upright-growing seedlings from group 2 seedlings. The upper side (E) and lower side (F) of horizontal group 3 seedlings. See text for more details. The upper side (G) and lower side (H) of IAA-treated horizontal group 4 seedlings. Bar = 10 μm. Arrowheads indicate the S2L layers. CZ, cambium zone; Dx, differentiating xylem; Mx, mature xylem; Ph, phloem; Xy, xylem.
Therefore, to examine cambial activity of reaction wood formation during the growth pause is needed.

Although upright-growing seedlings without treatment in group 1 showed decreased cambial activity, seedlings with treatments in groups 2, 3, and 4 showed either increase of cambial activity or presence of cell differentiation. In other words, the mid-season growth pause did not arrest the ability of cambium to form compression wood in seedlings with horizontal and/or IAA treatments (Figures 4, and 5). Formation of compression wood is associated with growth stress and thus causes stem curvature. Coutand et al. (2007) measured the radial growth and curvature change in poplar (Populus nigra × P. deltoides) seedlings after inclination at an angle of 45°, and found a significant correlation between curvature variation and radial growth. Poplars are angiosperms that usually produce tension wood, and thus anatomical observations of stems need to be further verified. In the present study, cambial activity was dynamic during the inclination induction treatment in the growth pause period, and the correlation of curvature variation and radial growth might not be linear as the origin of the coordinate graph is approached.

Indole-3 acetic acid is one of the most important plant hormones regulating many developmental events. The effect of IAA on the induction of compression wood has been demonstrated (reviewed in Timell, 1986). Onaka (1940) showed that applying 0.5% IAA to Pinus thumbergii induced 10 rows of typical compression wood in 18 days. In group 2, IAA-treated seedlings in our study showed significantly higher cambial activity on the IAA-application side than on the opposite side. Nevertheless, compression wood did not form in Taiwan incense cedar with IAA stimulation during 13 days of treatment, although 1% IAA treatment is considered to be high enough to induce compression wood. The slowness of compression wood formation probably is because of IAA treatment of seedlings occurred during the growth pause. According to Onaka (1940), compression wood extends 3–7 cm below the 1% IAA application site. Thus, the other possibility is that the polar transport of IAA in Taiwan incense cedar is so slow during the growth pause that only a small amount of IAA arrived in cambium area, and the concentration could only stimulate cell division. On the other hand, the slow cell division rate might be due to the character of tardy growing for xylem initial cells of Taiwan incense cedar.

In the inclination experiment in group 3 (Figure 1), cambial activity on the lower side increased by about 2-fold compared to upright-growing seedlings. The cell shape of young compression wood tracheids appears circular, but forms no helical cavities in the inner cell walls, it indicates that the differentiation of compression wood is not fast. Gravity is believed to be the most likely stimulus for producing compression wood (Timell, 1986). The inclination angle affects the downward bending moment which is theoretically in proportion to the stimulus due to gravity. Seedlings of Picea glauca were kept at 0°, 5°, 10°, 20°, 45°, and 90° by Yumoto et al. (1983) who found that the extent of compression wood was highly correlated with the inclination angle. However, formation of compression wood in the seedlings kept at angles of 20° and of 90° was about the same. A recent study by Yamashita et al. (2007) on Cryptomeria japonica seedlings kept at 0°, 10°, 20°, 30°, 40°, and 50° showed the threshold angle for induction of maximal compression wood is between 20° and 30°. An inclination angle of 90° was used in the Taiwan incense cedar study, but no compression wood was formed. In contrast to investigations focused on long-term induction of compression wood (Yumoto et al., 1982; Yamashita et al., 2007), our study on the short-term changes in cambium and xylem differentiation during compression wood induction found that it took 13 days to induce only three to four layers of differentiating compression wood tracheids. The production rate is slow when compared with the presentation time cited by Timell (1986). This suggests that a tardily growing cambium may react to a stimulus by gravity during the mid-season growth pause.

Cambial activities of leaning seedling with IAA treatment in group 4 increased as shown in Figure 5. However, the effects were lower than single treatment either by IAA or leaning. Onaka (1940) applied 0.5% IAA to the upper side of leaning seedlings of Pinus thumbergii, and found that compression wood was not only on the lower side of the stem, but also on the upper side. Cambial activity also occurred on the upper and lower sides of Taiwan incense cedar when leaning seedlings were treated with IAA on the upper side, but the cambial activity on the lower side significantly decreased compared to the same side of leaning seedlings without IAA treatment. A possible explanation is that a counteraction between the upper and lower sides of the cambium arrested the growth of the lower side. That is, the upward bending stimulus from the lower side probably offset the IAA stimulus from the upper side. The mechanical stimulus was therefore not as strong as the single-treated groups (group 2 and group 3). Besides, compression wood formation on the lower side of stems or branches is often accompanied with opposite wood with growth suppression, i.e. extremely small growth rings (Timell, 1986). In group 4 seedlings, the IAA-induced reaction wood side is the gravity-induced opposite wood side, and vice versa. Therefore, the growth suppression probably offset the induction of cambial activity in both the upper and lower sides of stems of group 4 seedlings. In Figure 5B, the lower cell numbers on the upper side were shown after 4 days’ treatment, speculating that the asymmetric distribution of auxin with a lower IAA level on the upper side might affect cell division rate (Haga and Lino, 2006).

Only a little radial increment of wood in Taiwan incense cedar is produced annually (Wang et al., 1987). The possible reasons might be the short growing season or the slow growth rate. Our cambial activity data showed that
the cambium growth period is quite long at about 6 month compared to those of boreal and temperate species (Larson, 1994; Deslauriers et al., 2003), and we speculated that the slow radial wood formation probably influences Taiwan incense cedar growth, and interrupted growth between the late spring and early summer growing period also cannot be eliminated compared to other species. A phenological investigation would help understand the nature of cambial activity of Taiwan incense cedar further.

In this study, we examined tardily growing cambium exposed to IAA treatment during the mid-season growth pause. We specially focused on cambial activity and cell differentiation in the growth pause season. Investigating the factors affecting the mid-season growth pause of vascular cambium will help clarify the growth phenomena of vascular cambium. The vascular cambium of slowly growing tree species has received less attention than those of fast growing ones (Downes et al., 1993; Tsai et al., 1994; Deslauriers et al., 2003), and we speculated that the formation and structure of the compression wood cells induced by artificial inclination on young trees of *Picea glauca*. I. Time course of the compression wood formation following inclination. Res. Bull. Coll. Exp. For. Hokkaido. Univ. 39: 137-162.


LITERATURE CITED


台灣肖楠苗木於生長季生長暫止時誘導其產生抗壓材

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台灣肖楠為台灣特有變種，具有良好的木材性質，然而其年徑生長量短少，且抗壓材的形成經常影響木材品質。台灣肖楠苗木主幹與枝條一樣，具有生長季生長暫止現象。本研究之目的在於研究此生長暫止對抗壓材形成的影響，實驗中，以施加 indole-3-acetic acid (IAA) 以及水平放倒之方式誘導抗壓材形成。結果顯示，即使生長暫止減弱了整體維管束形成層活力，各處理組對於誘導皆有反應，即預期產生抗壓材側的維管束形成層活力較對應側高。此外，台灣肖楠年徑生長量短少歸因於生長緩慢的特性而非生長季長短，該特性可能造成各處理組形成抗壓材之反應速率緩慢。

關鍵詞：台灣肖楠；抗壓材；水平放倒；indole-3-acetic acid；維管束形成層。