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Differences in β-thujaplicin content of wood between plantation- and naturally grown *Thujopsis dolabrata* var. *hondae* (hinokiasunaro) trees in Shimokita Peninsula, Aomori, Japan

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Abstract

β-Thujaplicin (hinokitiol) is an important extractive of *Thujopsis dolabrata* var. *hondae* (hinokiasunaro) that confers high durability to its wood. We investigated differences in β-thujaplicin content in wood from plantation- and naturally grown trees of *T. dolabrata* var. *hondae*, and also examined growth rate and wood density. A total of 29 trees were collected from two natural forests and a plantation forest in the Shimokita Peninsula, Aomori, Japan. β-Thujaplicin content was determined for each heartwood sample by gas chromatography. The content ranged from 0.29 to 3.67 mg/g (oven-dry weight basis). β-thujaplicin content of plantation-grown trees was significantly higher than for naturally grown trees, though with a large variation. The effect of radial growth rate on β-thujaplicin content was minimal. We conclude that *T. dolabrata* var. *hondae* wood from both plantation and natural forest has similar durability. The coefficient of variation of β-thujaplicin content in each stand was higher than for other wood properties. This degree of tree-to-tree variation in the trait suggests that promising production with more durable wood could be achieved by selecting *T. dolabrata* var. *hondae* trees containing a high content of β-thujaplicin for plantations.

Keywords: Between-tree variation, Heartwood extractives, Natural durability, *Thujopsis dolabrata* var. *hondae*, β-Thujaplicin

Introduction

Thujopsis dolabrata (L.f.) Siebold et Zucc. var. *hondae* Makino (hinokiasunaro in Japanese) is an essential forestry species in Aomori, the northern part of Japan, because of its valuable wood [1–4]. One of its most important characteristics is its high durability against fungi and termites, a property conferred by the presence of antifungal compounds including β-thujaplicin (hinokitiol) [2–4]. Thus, the wood of *T. dolabrata* var. *hondae* has been used in the construction of Japanese traditional shrines and temples, and for applications that require durability, such as sill beams.

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The situation regarding wood production from *T. dolabrata* var. *hondae* has been changing recently [5]. In Aomori, wood production of this species depends on natural forests managed since the 1800s by the selection thinning method to maintain this natural resource [1]. However, wood production from natural forests has been decreasing for the past several decades because of a decrease in natural resources [5]. However, an increase in the area of *T. dolabrata* var. *hondae* has been increasing recently. These plantations, in which *T. dolabrata* var. *hondae* exhibits faster growth rates than in the natural forests, will form the future supply of wood.

There are differences in wood quality between the faster-growing plantation trees and the slower-growing natural trees; the trees with faster growth rate generally contain a higher volume of juvenile wood, which raises concern for the quality of end products [6]. In a previous study [7], we showed that the differences in *T. dolabrata* var. *hondae* wood properties between plantation- and naturally grown trees tended to be small: there were no significant differences in basic density and compressive strength parallel to the grain; while the annual ring width of plantation-grown trees was double that of naturally grown trees. These results suggested that trees grown in plantations would produce wood similar to that obtained from naturally grown trees.

In plantation-grown *T. dolabrata* var. *hondae*, an appropriate level of β-thujaplicin is important since this affects the natural durability of the wood. However, few studies are available on the differences in extractive content between plantation- and naturally grown trees of this species. In studies on *Thuja plicata* (Western Redcedar), younger trees from second-growth trees had lower levels of tropolones (compounds related to the natural durability of wood in this species) than old-growth trees [8, 9]. It has also been reported in *T. plicata* that large tree-to-tree variability and radial variations exist in extractive content, including β-thujaplicin [8–13]. Ohira et al. [14] reported a provenance variation of β-thujaplicin in *T. dolabrata* trees from five stands; they did not mention tree-to-tree variation, and further information on extractive content variation is needed for *T. dolabrata*.

The present study aimed to clarify the differences in β-thujaplicin content between plantation- and naturally grown *T. dolabrata* var. *hondae* trees. We also evaluated tree-to-tree variation in β-thujaplicin content and the relationship between β-thujaplicin content and annual ring width or basic density.

Materials and methods

Materials

Sample trees were collected from three different national forests in the Shimokita Peninsula, Aomori, Japan: two natural forests (stands A and B) and a plantation (stand P). Stands A and B had been managed by selection cutting on a 30-year rotation cycle with regeneration. Stand P was established in 1914 with an initial density of 4200 trees/ha using planting stocks. Sample trees were felled in 2010 from stands A and B, and in 2009 for stand P. Disks (10 cm in thickness) were selected randomly and collected from the basal parts (about 10–20 cm above the ground) in 2010. The numbers of sample trees were 9, 10, and 10 for stands A, B, and P, respectively. The number of annual ring and radius of each disk is shown in Table 1.

β-Thujaplicin content

Sample preparation and acetone extraction

β-Thujaplicin content was determined for each sampled tree according to the method of DeBell et al. [15]. Heartwood samples within 10 cm of the pith were used for the following extraction and analyses. Each sample disk was cut into several small blocks to have heartwood within 10 cm from the pith. Since the heartwood/sapwood boundary was clearly visible, sapwood could easily be excluded. The sample blocks were milled using a hammer crusher (NH-34S, Sansho Industry, Japan) and a rotary mill (P-14,FRITSCH,Germany). Then, the sample was passed through a 0.180–0.355 mm mesh sieve. Moisture content was measured for each wood meal sample to determine oven-dried weights of extraction samples.

Table 1 Statistical values for annual ring number, radius, acetone extractives and β-thujaplicin contents in sample trees

Stand	Annual ring number		Radius (cm)		Acetone extractives content (mg/g)		β-Thujaplicin content (mg/g)	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Natural								
A	163.6	21.8	18.4	30.4	18.92	24.5	1.01	57.4
($n=9$)	(125–240)		(10.9–29.1)		(12.06–26.33)		(0.50–2.49)	
B	145.5	24.5	15.0	28.7	17.80	13.8	0.71	52.1
($n=10$)	(84–225)		(9.8–25.5)		(13.93–21.38)		(0.29–1.28)	
Plantation								
P	79.0	10.9	14.4	23.3	19.84	29.7	1.64	61.0
($n=10$)	(62–87)		(8.6–20.3)		(10.92–29.53)		(0.33–3.67)	
All	128.2	36.3	15.8	29.7	18.85	23.9	1.12	70.5
	(62–240)		(8.6–29.1)		(10.92–29.53)		(0.29–3.67)	
t-test	**		ns		ns			*

Values in parentheses are minimum and maximum values

n, number of sample trees; CV, coefficient of variation

**, *, and ns indicate significant differences ($p < 0.01$ and $p < 0.05$) and no significant difference ($p > 0.05$) between the stands by the *t*-test, respectively. The *t*-test was conducted to detect the differences between natural (combined data of stands A and B) and plantation stands

For cold-acetone extraction, air-dried wood meal (3 g) was put into a 300-mL Erlenmeyer flask. Then, 54 mL of acetone and 6 mL of an internal standard solution (3,4,5-trimethoxyphenol in acetone at a concentration of 0.35 mg/mL) were added. The flask was left for 16 h at room temperature (20–25 °C). The cold-acetone extract was filtered and transferred to an eggplant-shaped flask where the solvent was evaporated off by a rotary evaporator (N-1100, EYELA, Tokyo, Japan). The extract was moved into a small glass vial and dried in a vacuum desiccator with an aspirator. The content of cold-acetone extractives (hereinafter referred to as acetone extractives) was calculated by dividing the weight of the extract by the dry weight of the wood meal. After that, all samples were stored in a freezer at − 20 °C. Acetone extraction was repeated three times for each sample.

Gas chromatography

Acetone extract (2 mg), 100 μL of pyridine and 150 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (Wako, Japan) were put into a reaction vial. The vial was capped and placed in an oil bath at 70 °C for 30 min. The trimethylsilylated samples were analyzed using a gas chromatograph (HP-6890 series GC system, Agilent, USA) with a flame ionization detector and a capillary column (DB-1, Agilent, USA; 30 m, 0.25 mm i.d., 0.25 μm film thickness). Column oven temperature was held at 150 °C for 5 min, raised at 5 °C/min up to 200 °C, then held at 200 °C for 5 min. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. Injection mode was splitless, and the injection pot and the detector were set at 250 °C. The β-thujaplicin was identified by comparing the retention time of β-thujaplicin standard (Kanto Kagaku, Japan) with that of extractive sample in the gas chromatogram. The ratio of the peak area of β-thujaplicin to that of internal standard was used to determine the β-thujaplicin content. β-Thujaplicin content was expressed as a percentage of wood dry weight.

Annual ring width and basic density

A pith-to-bark strip (1 cm in thickness) was cut from a sample disk to measure ring width and basic density [7]. A cross-sectional image of the strip was acquired using a scanner with a resolution of 600 dpi. Annual ring width was measured for each 5th annual ring from the pith to 10 cm and toward the bark using the open source image analysis software ImageJ [16]. Each strip was then cut at 1-cm intervals from the pith to the bark for measuring basic density, measured by the water displacement method [17].

Results

Mean concentrations of β-thujaplicin for stands A, B, and P were 1.01, 0.71 and 1.64 mg/g, respectively (Table 1). Mean concentrations of acetone extractives and β-thujaplicin were compared between plantation (stand P) and natural stands (combined data from stands A and B) by the *t*-test. No significant difference in acetone extractives was found, whereas β-thujaplicin content in stand P was significantly higher than that in wood from the natural forests. The coefficient of variation for β-thujaplicin content ranged from 52 to 61% in each stand.

Figure 1 shows the relationships between acetone extractives or β-thujaplicin content and annual ring width or basic density. The content of acetone extractives was not significantly related to annual ring width, while a relationship was observed with basic density in stands A and B. Annual ring width did not correlate with β-thujaplicin significantly in stand P ($r = 0.250$) and stands A and B ($r = 0.271$) when the correlation was calculated separately. Significant correlation coefficients were observed between acetone extractives and β-thujaplicin content for stand P (Fig. 1). When these relationships were analyzed separately for each stand, the correlation coefficient and slope of the linear regression of stand P was higher than those of stands A and B.

Discussion

In the present study examining β-thujaplicin content in *T. dolabrata* var. *hondae* trees grown in Aomori, Japan, we found concentrations ranging from 0.29 to 3.67 mg/g (oven-dry weight basis) for both plantation- and naturally grown trees. Ohira et al. [14] reported that β-thujaplicin content in naturally grown *T. dolabrata* trees ranged from 0.03 to 0.18 mg/g from five different stands, but the method of measurement differed.

The β-thujaplicin content of plantation-grown trees was higher than for naturally grown trees, though with a large variation. This may be caused by the degradation of extractives over time within tree [18], resulting in relatively lower amount of β-thujaplicin in naturally grown trees with older heartwood. However, previous reports in *T. plicata* revealed that the differences in tropolone content between second- and old-growth trees were mainly caused by the presence of radial variation in content of extractives [8, 10–12], especially due to effects of cambial age [11]. However, the presence of an age effect on β-thujaplicin content as well as radial and axial variations could not be evaluated. Further research is needed to clarify the within-tree variation of β-thujaplicin content in *T. dolabrata* var. *hondae.* Although the reason why plantation tree had higher β-thujaplicin content was

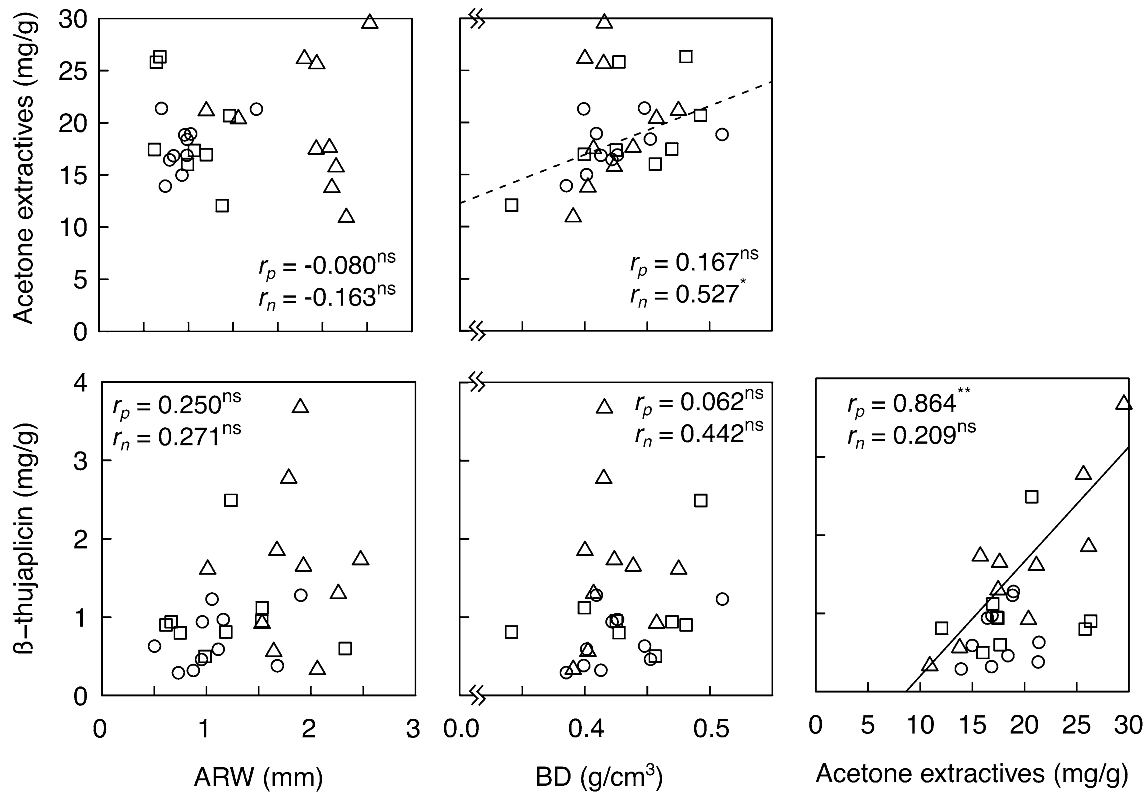

Fig. 1 Relationships between acetone extractives or β-thujaplicin contents and annual ring width (ARW) or basic density (BD). Square, circle, and triangle indicate stands A, B, and P, respectively. Solid, dashed, and dotted and dashed lines indicate the regression lines of whole sample ($n=29$), natural [combined data of stands A and B ($n=19$)], and plantation stands [P ($n=10$)], respectively. The "r_p and r_n" are the correlation coefficients of plantation [P ($n=10$)] and natural [combined data of stands A and B ($n=19$)] stands, respectively, all sampled trees ($n=29$). **, *, and ns indicate that the correlation coefficients are significant ($p<0.01$ and $p<0.05$) and not significant ($p>0.05$), respectively. Solid and dashed indicate the regression lines of plantation and natural stands, respectively

unclear, our results suggest that wood from plantation-grown *T. dolabrata* var. *hondae* would possess similar β-thujaplicin content to those from natural forests.

It has been shown that large tree-to-tree variations in content of extractives occur due to genetic variation [10–13]. In the present study, the coefficients of variation for β-thujaplicin content were >50% among individuals within the three stands. These values were higher than those for basic density (>5%) and compressive strength parallel to grain (up to 16%) in the same samples of this study [7], indicating that considerable tree-to-tree variation exists for this trait. Thus, there would be a large degree of genetic variation in β-thujaplicin content, which is favor for selecting superior genotypes in a tree breeding of this species. Genetic control of extractive content would also be important for tree breeding programs, and strong genetic control of the production of heartwood extractive was reported [19]. In a breeding program of *T. plicata* in Canada, Russel and Daniels [13] reported that tropolone content was a trait which genetically varied and was moderately heritable (0.24–0.55 of narrow-sense heritability). It is also suggested, therefore, that improving durability and reducing its variability can be achieved by selecting superior *T. dolabrata* var. *hondae* trees in tree breeding programs.

The relationship of β-thujaplicin content to other wood properties as well as growth rate is important for suggesting practical implications of *T. dolabrata* plantation management. The correlation coefficients between annual ring width and β-thujaplicin were not significant, when the relationships were calculated separately between plantation and natural stands. In second-growth *T. plicata*, growth rate had no influence on tropolone content [11]. Taylor et al. [19] also reported that extractive content was not affected by silvicultural treatment, such as thinning and fertilization in *T. plicata*. Our result also supports these findings about relationship between extractive contents and silvicultural treatments [19]. In addition, there was no significant correlation between wood basic density and β-thujaplicin. Therefore, our results suggest that radial growth rate and basic density seem to be independent of β-thujaplicin content in *T. dolabrata* var. *hondae*.

There was a relatively strong relationship ($r = 0.864$, $p < 0.01$) between acetone extractives and β-thujaplicin content in plantation-grown trees, which is consistent with earlier researches: the significant relationship between β-thujaplicin content and ethanol–benzene extractive content in *T. plicata* [11, 19]. On the other hand, the correlation was not significant in naturally grown trees. In addition, the regression slope differed between plantation and natural stands: slope for the plantation stand was steeper than that for natural forests. This result indicates that younger trees from plantation forest might have a higher ratio of β-thujaplicin to total extractives than the trees from natural forests, and the ratio varies between naturally grown trees. We could not find an explanation for this based on our data set. However, a possible reason may be variation in the ratio of β-thujaplicin to total tropolone content or extractive content. For example, Daniels and Russel [12] reported that the ratio of β-thujaplicin to γ-thujaplicin differed between two provenances, while the ratio showed no radial variation in *T. plicata* [10]. This fact supports the presence of tree-to-tree variability in the ratio of β-thujaplicin to total extractives in *T. dolabrata* var. *hondae*. Further research is needed to investigate the tree-to-tree variation and/or the difference in content of other tropolones between plantation- and naturally grown trees in *T. dolabrata* var. *hondae*.

Conclusions

We investigated the β-thujaplicin content of *T. dolabrata* var. *hondae* (hinokiasunaro) wood from plantation- and naturally grown forests. We demonstrated that the β-thujaplicin content difference was minimal between plantation- and naturally grown trees, and the effect of radial growth rate on β-thujaplicin content was minimal. It is suggested, therefore, that wood of this species from plantation-grown trees would be expected to possess almost the same natural durability compared to that from naturally grown trees. Moreover, tree-to-tree variation of β-thujaplicin content suggests that *T. dolabrata* var. *hondae* plantations grown from trees selected for high β-thujaplicin content would provide wood with improved natural durability.

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Authors' contributions
JT and YT designed this study, analyzed the data, and were major contribution to writing the manuscript. FI have drafted the work, contributed to interpretation of data and assisted in the preparation of the manuscript. HS, JO, KI and SY contributed to interpretation of data and preparation of the manuscript. All authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

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