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NOTE

# Diversities of decay resistance and *n*-hexane-extractive contents in seven half-sib families from plus trees in todomatsu (*Abies sachalinensis*)

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**Abstract** Todomatsu (*Abies sachalinensis*) is a commercial plantation species on Hokkaido Island, Japan. In the present study, to improve the decay resistance of todomatsu wood, seven families of 22-year-old todomatsu trees were investigated for family diversities in decay resistance of heartwoods against *Fomitopsis palustris* and *Trametes versicolor* and *n*-hexane-extractive contents in heartwoods. In addition, antifungal activity tests of *n*-hexane extracts were conducted for *F. palustris* and *T. versicolor*. Mean percentages of wood mass loss by *F. palustris* and *T. versicolor* were 18.2 and 10.5 %, respectively. Significant differences in mass loss and *n*-hexane-extractive contents were not found among seven families tested here. The mean value of *n*-hexane-extractive contents was 6.4 mg/g. By gas chromatography/mass spectrometry analysis, a main component of *n*-hexane extracts was suggested to be juvabione. Results of the antifungal activity test show that mycelial growth of *F. palustris* and *T. versicolor* was strongly inhibited over the concentration of 225 and 150 μg per disc, respectively. These concentrations corresponded to about 10.0 and 6.6 mg/g in wood. Thus, it is concluded that the todomatsu trees containing more than 10.0 mg/g of *n*-hexane extracts should be selected among the families for high decay resistance.

**Keywords** *Abies sachalinensis* · *n*-Hexane extracts · Decay resistance · Tree breeding

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## Introduction

Todomatsu (*Abies sachalinensis* Mast.) is naturally distributed in Hokkaido Island, Japan, and is one of the plantation species there [1, 2]. The wood of todomatsu has been used mainly for construction lumber [3]. However, durability of the wood is relatively poor as compared to that of other softwoods used for construction lumber [2].

In general, it is well known that extractives in wood are related to the decay resistance of the wood [4–8]. In todomatsu, *n*-hexane soluble fraction of ethanol extracts from the wood has antifungal activity against the following 6 white-rot fungi: *Lentinula edodes*, *Flammulina velutipes*, *Pleurotus ostreatus*, *Pholiota nameko*, *Grifola frondosa*, and *Pleurotus cornucopiae* [9]. In addition, juvabione, one of the sesquiterpenes, has been identified as a main antifungal compound in the extractives of todomatsu wood [10, 11]. Therefore, decay resistance of todomatsu wood is related to the *n*-hexane extracts, especially to the juvabione content in the wood.

The clonal and family diversities for decay resistance and extractive contents of wood have been investigated for several softwoods [5–7, 12–16]. Venäläinen et al. [13]

examined the degree of genetic determination in the decay resistance of *Larix sibirica* wood and its correlation to other wood traits. The results showed that the genetic determination appears to be stronger for decay resistance than for growth characteristics or heartwood formation, but weaker than for wood density of latewood formation. These results suggest that the decay resistance of wood can be improved by tree breeding. However, variations among families have not been clarified yet for decay resistance in todomatsu wood.

In the present study, variations of decay resistance of wood and *n*-hexane-extractive contents were examined for seven todomatsu plus tree families planted in Hokkaido, Japan, to estimate the possible improvement in the decay resistance of the wood by tree breeding. In addition, the relationship was also discussed between decay resistance of wood and extractive contents.

## Materials and methods

### Materials

Plus tree families of half-sib todomatsu (*A. sachalinensis*) were planted at the Hotsukan7 progeny test site, Tree Breeding Center, Forestry and Forest Products Research Institute (Fig. 1). This progeny test site included 80 plus tree families and two types of commercial seedlings. A random block design was used in the field. There were three blocks at the site. Thirty trees in each family were planted in two lines of a block with an initial planting density of 3,000 trees/ha. Forty-five trees from seven families (Fig. 1) were thinned in 2009 (22-year-old), and discs (10 cm in thickness) were collected at 1.3 m above the ground for conducting the following experiments. The stem diameter at 1.3 m above ground, air-dry density, and percentage of heartwood in the seven families used in this study are shown in Table 1.

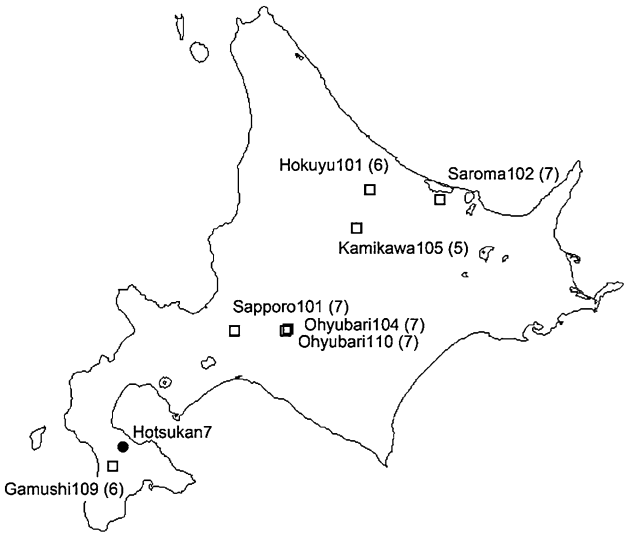


**Fig. 1** Location of progeny test sites used in the present study and location of mother trees. *Numbers* in *parentheses* after the family name of plus trees indicate the number of sample trees used in the present study

### Decay test

The decay test was conducted according to the Japanese Industrial Standards (JIS) K1571-2010 [17]. A brown-rot fungus (*Fomitopsis palustris*, FFPRI 0507) and a white-rot fungus (*Trametes versicolor*, FFPRI 1030), which were provided by the Forestry and Forest Products Research Institute, Tsukuba, Japan, were used in the present study. The fungi were precultured on potato-dextrose-agar (PDA) medium (Difco; Becton, Dickinson and Company, USA) in petri dishes (9 cm in diameter) at 26 ± 1 °C. Small clear specimens [20 (R) by 20 (T) by 10 (L) mm] were prepared from the heartwood of the discs. The specimens were oven-dried at 60 ± 2 °C for 48 h and then weighed. After measuring the drying weight, specimens were sterilized with propylene oxide for 2 days.

The medium, 100 ml (4 % glucose, 0.3 % peptone, 1.5 % malt extracts, and 2.0 % agar) in plastic bottles (9.5 cm in diameter, 850 ml in volume), was sterilized by an autoclave (HV-110, Hirayama, Japan) at 121 °C and 1.2 atm for 20 min. After cooling, the discs of mycelium were punched out using a cork borer (7 mm in diameter) from precultured mycelia, and they were inoculated at the center of the medium surface in plastic bottles. The fungus in plastic bottles was incubated at 26 ± 2 °C and 70 %

**Table 1** Stem diameter, air-dry density, and percentage of heartwood in seven todomatsu half-sib families

| Family | *n* | D (cm) | | AD (g/cm$^3$) | | Heartwood (%) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD |
| Hokuyu101 | 6 | 13.9 | 2.1 | 0.30 | 0.03 | 56 | 4 |
| Saroma102 | 7 | 13.3 | 1.6 | 0.28 | 0.01 | 58 | 7 |
| Kamikawa105 | 5 | 12.9 | 2.1 | 0.27 | 0.01 | 57 | 8 |
| Ohyubari104 | 7 | 15.8 | 2.7 | 0.29 | 0.01 | 58 | 4 |
| Ohyubari110 | 7 | 15.3 | 2.4 | 0.29 | 0.02 | 55 | 5 |
| Sapporo101 | 7 | 15.8 | 2.2 | 0.27 | 0.01 | 59 | 6 |
| Gamushi109 | 6 | 15.1 | 1.9 | 0.28 | 0.02 | 53 | 5 |
| Mean/total | 45 | 14.7 | 2.3 | 0.28 | 0.02 | 57 | 6 |
| Among family variations | | ns | | ns | | ns | |

*D* diameter at 1.3 m above ground, *AD* air-dry density, *SD* standard deviation, *ns* no significance

relative humidity. After the mycelia covered the medium surface in the plastic bottles, the sterilized three small clear specimens were put on the medium in a plastic bottle and incubated at 26 ± 2 °C and 70 % relative humidity for 12 weeks. In the case of *F. palustris*, a plastic net (60 mm in diameter, 1 mm in thickness, and 4 mm in grid size) was placed between the specimen and the mycelium. Two plastic bottles of each fungus were prepared for the specimens from a tree. After 12 weeks of incubation, the specimens were collected from the plastic bottles, and the mycelia on the specimens were removed using a small brush or tweezers. The decayed specimens were air-dried for 24 h and then oven-dried at 60 ± 2 °C for 48 h. The percentage of mass loss was calculated by dividing the decay weight loss by the initial weight of the specimen.

### GC/MS analysis of *n*-hexane extracts and quantification of putative juvabione contents

Wood meal (0.180–0.355 mm mesh size) was obtained from the heartwood of the discs. Air-dried wood meal (5–10 g) from each tree was put in a 300 ml Erlenmeyer flask, and then 10 ml *n*-hexane per 1 g of air-dried wood meal was added to the flask. For extraction, the flasks containing wood meal and *n*-hexane were agitated for 24 h by a shaker (NR-150, Taitec, Japan). Extraction was repeated three times. The solvent was evaporated using a rotary evaporator (N-1100, EYELA, Japan), and then the *n*-hexane extracts were dried in a desiccator in vacuo using a vacuum pump (GCD-136X, ULVAC).

The *n*-hexane extracts of todomatsu wood were analyzed by gas chromatography/mass spectrometry (GC/MS). The analysis was performed on TRACE DSQ (Thermo Fisher Scientific, USA) equipped with a capillary column (DB-1, 15 m by 0.25 mm with 0.25 μm film, Agilent, USA). Column oven temperature was set at 50 °C for 3 min (hold), programmed from 50 to 250 °C at 20 °C/min, and then held at 250 °C for 3 min. Helium was used as a carrier gas at a constant flow of 0.2 ml/min. Split injection (1:100 in split ratio) at a temperature of 280 °C and constant pressure at 82.8 kPa were applied. Other operating parameters were as follows: ionization voltage at 70 eV, transfer line temperature at 280 °C, and scan range from *m/z* 50 to 650.

Putative juvabione in *n*-hexane extracts from todomatsu wood was quantified by gas chromatography (GC). For quantification, *n*-hexane extracts were dissolved in 2 ml *n*-hexane with 0.5 % methyl *n*-octanoate as an internal standard. Quantification was performed on an HP6890 Series GC System (Agilent) equipped with a capillary column (DB-1, 15 m by 0.25 mm with 0.25 μm film, Agilent). Column oven temperature; type and flow rate of carrier gas; and injection mode, temperature, and pressure were the same as in the GC/MS analysis. Putative juvabione content was calculated according to the effective carbon number as follows [18]:

$$c_j = \frac{w_{is}}{w_w} \times \frac{PA_j}{PA_{is}} \times \frac{ECN_{is}}{ECN_j} \times \frac{m_j}{m_{is}} \quad (1)$$

where $c_j$ is the putative juvabione content in wood (mg/g), $w_{is}$ is the amount of internal standard (mg), $w_w$ is the oven-dry weight of todomatsu wood meal (g), $PA_j$ is the peak area of putative juvabione, $PA_{is}$ is the peak area of internal standard, $ECN_{is}$ is the effective carbon number of internal standard (methyl *n*-octanoate = 7.0), $ECN_j$ is the effective carbon number of juvabione (=12.9), $m_j$ is the molecular mass of juvabione, and $m_{is}$ is the molecular mass of internal standard.

The other unknown compound content in wood was calculated with the equivalent amount of internal standard as follows:

$$c_{uc} = \frac{w_{is}}{w_w} \times \frac{PA_{uc}}{PA_{is}} \quad (2)$$

where $c_{uc}$ is the unknown compound content in wood (mg/g), $PA_{uc}$ is the peak area of unknown compound.

### Antifungal activity of extracts

*n*-Hexane extracts were redissolved in *n*-hexane and sterilized using a membrane filter (Millex-FG, Millipore, Germany) for evaluating the antifungal activity. Each paper disc (8 mm in diameter, 1.5 mm in thickness, and 30 mg in weight, ADVANTEC, Japan) was impregnated with sterilized extract solution. Paper discs containing *n*-hexane extracts of nine concentrations (20, 30, 50, 100, 150, 225, 300, 450, and 3,000 μg per disc) were prepared to evaluate the effect of the concentration of the extracts on antifungal activity. The *n*-hexane-extractive content on paper disc was converted to the *n*-hexane-extractive content (mg/g) in wood according to the following equation:

$$c_w = \frac{c_p}{w_p} \times \frac{d_w}{d_p} \quad (3)$$

where $c_w$ (mg/g) is the *n*-hexane-extractive content in wood, $c_p$ (μg/disc) is the *n*-hexane-extractive content in paper disc, $w_p$ (mg) is the air-dry weight of paper disc, $d_w$ and $d_p$ are the air-dry density of wood and paper disc, respectively. As the results, *n*-hexane-extractive contents on paper disc were corresponded to 0.9, 1.3, 2.2, 4.4, 6.6, 10.0, 13.3, 20.0, and 133.3 mg/g in wood.

Precultured *F. palustris* and *T. versicolor* mycelial discs, which were prepared in the same way as in the decay test, were inoculated on the center of a petri dish (9 cm in diameter) with PDA medium (Difco; Becton, Dickinson

and Company) at 26 ± 1 °C. Four to five paper discs containing various concentrations of *n*-hexane extracts were circumferentially placed on the medium in the petri dishes (Fig. 2). When the mycelium reached the paper discs, the incubation was terminated. The antifungal test was repeated five times. Antifungal activity was evaluated using the following three grades: ++, the activity was found in all paper discs tested; + the activity was found in more than half of the paper discs tested; and −, no activity was found (Fig. 2).

Data analysis

A one-way analysis of variance (ANOVA) test was conducted using commercial software (Excel 2010, Microsoft) to detect the differences among families in mass loss after the decay test, *n*-hexane extracts, and juvabione contents.

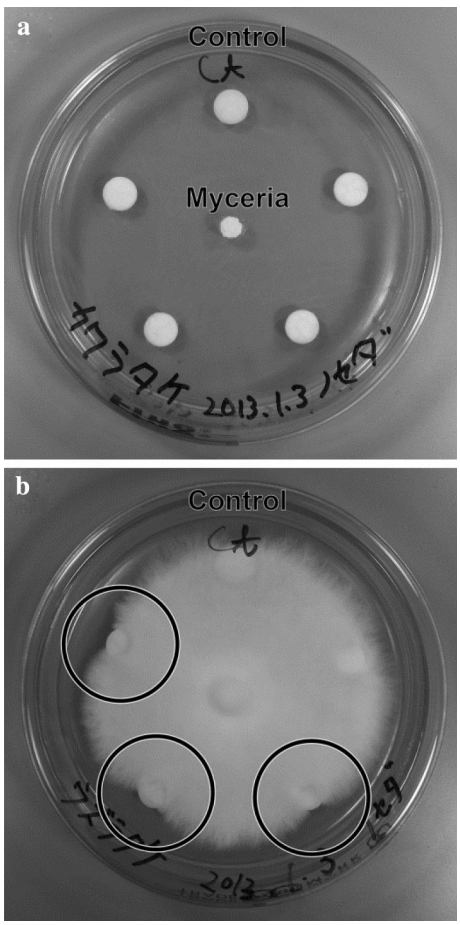


**Fig. 2** Photographs of antifungal activity test using a paper disc containing various concentrations of *n*-hexane extracts. A mycelial disc of *Fomitopsis palustris* or *Trametes versicolor* was inoculated on the center of the potato-dextrose-agar medium in a 9 cm petri dish, and paper discs (8 mm in diameter) containing various concentrations (20–3,000 μg per disc) of *n*-hexane extracts were placed around the mycelial disc (**a**). *Circles* indicate inhibition of mycelial growth of *F. palustris* after 8 days of inoculation by *n*-hexane extracts (**b**)

## Results

Table 2 shows the mean values of mass loss after the decay test in each family. In the decay of *F. palustris*, maximum and minimum values of the mean percentage of mass loss were obtained in Ohyubari104 (23.9 %) and Sapporo101 (13.1 %), respectively. However, those after the decay of *T. versicolor* were obtained in Kamikawa105 (12.3 %) and Gamushi109 (8.5 %), respectively. However, in both fungi, significant differences were not found among different families.

The mean value of *n*-hexane-extractive contents in all trees was 6.4 mg/g (Table 3). The highest and the lowest contents were 7.4 mg/g for Ohyubari104 and Sapporo101 and 5.1 mg/g for Saroma102, respectively. No significant difference in *n*-hexane-extractive contents was observed among different families.

In the results of GC/MS analysis of the *n*-hexane extracts, an internal standard, methyl *n*-octanoate, was detected at the retention time of 5.14 min, and two major peaks with retention times of 10.66 and 10.83 min were obtained from the total ion chromatogram (Fig. 3). The mass spectrum of the compound at the retention time 10.66 min was similar to that of juvabione reported by Yoneyama et al. [10], suggesting that juvabione was also contained in the *n*-hexane extracts from todomatsu wood used in the present study. The highest and lowest putative juvabione contents were obtained in Gamushi109 (3.0 mg/g) and Saroma102 (1.6 mg/g), respectively (Table 3). However, there was no significant difference among

**Table 2** Percentage of mass loss after the decay test by *Fomitopsis palustris* and *Trametes versicolor*

| Family | *Fomitopsis palustris* | | | *Trametes versicolor* | | |
|---|---|---|---|---|---|---|
| | *n* | Mean (%) | SD (%) | *n* | Mean (%) | SD (%) |
| Hokuyu101 | 6 | 14.1 | 11.5 | 6 | 10.8 | 4.2 |
| Saroma102 | 7 | 17.2 | 5.6 | 7 | 11.2 | 4.1 |
| Kamikawa105 | 5 | 22.9 | 10.6 | 5 | 12.3 | 2.0 |
| Ohyubari104 | 7 | 23.9 | 2.0 | 7 | 9.0 | 2.0 |
| Ohyubari110 | 7 | 20.4 | 9.5 | 7 | 12.0 | 3.2 |
| Sapporo101 | 6 | 13.1 | 7.5 | 7 | 10.1 | 4.6 |
| Gamushi109 | 6 | 15.7 | 8.0 | 6 | 8.5 | 4.0 |
| Mean/total | 44 | 18.2 | 8.6 | 45 | 10.5 | 3.6 |
| Among family variations | | ns | | | ns | |

*n* number of specimen, *SD* standard deviation, *ns* no significance

**Table 3** *n*-Hexane-extractive, putative juvabione, and unknown compound contents in seven todomatsu half-sib families

| Family | *n* | *n*-Hexane extracts (mg/g) | | Putative juvabione (mg/g) | | Unknown compound (mg/g) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD |
| Hokuyu101 | 6 | 5.2 | 2.9 | 1.9 | 1.2 | 0.24 | 0.13 |
| Saroma102 | 7 | 5.1 | 2.2 | 1.6 | 1.1 | 0.25 | 0.25 |
| Kamikawa105 | 5 | 6.2 | 1.6 | 2.5 | 0.9 | 0.17 | 0.05 |
| Ohyubari104 | 7 | 7.4 | 4.4 | 2.8 | 2.2 | 0.33 | 0.24 |
| Ohyubari110 | 7 | 6.3 | 1.3 | 2.1 | 0.9 | 0.45 | 0.29 |
| Sapporo101 | 7 | 7.4 | 3.6 | 2.7 | 1.6 | 0.32 | 0.13 |
| Gamushi109 | 6 | 6.8 | 3.8 | 3.0 | 1.5 | 0.28 | 0.17 |
| Mean/total | 45 | 6.4 | 3.0 | 2.4 | 1.4 | 0.29 | 0.09 |
| Among family variations | | ns | | ns | | ns | |

The unknown compound contents were presented as the equivalent amount of internal standard

*n* number of trees, *SD* standard deviation, *ns* no significance

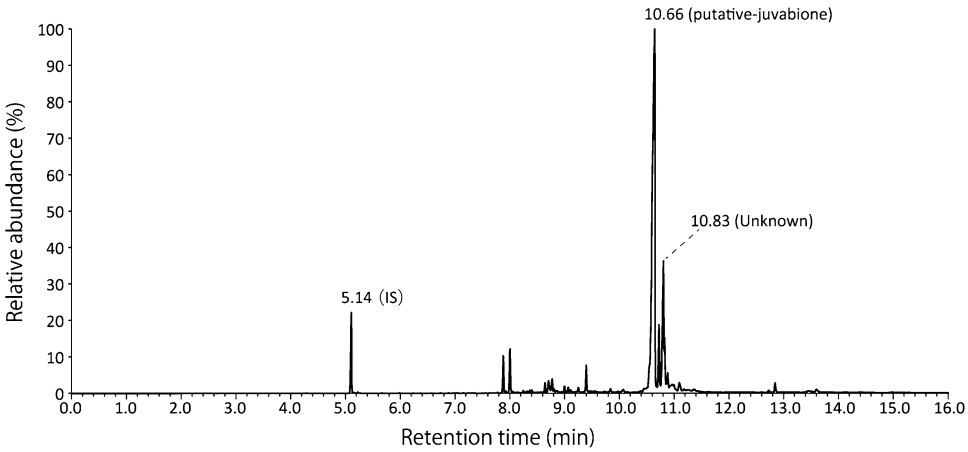


**Fig. 3** Total ion chromatogram in the gas chromatography/mass spectrometry analysis of *n*-hexane extracts from heartwood of Ohyubari104 family. Methyl *n*-octanoate as an internal standard (IS) was detected at the retention time of 5.14 min

families. On the other hand, the compound at the retention time 10.83 min was not identified by GC/MS analysis. Ohyubari110 showed the highest contents of this unknown compound, and Kamikawa105 showed the lowest contents of it. As like putative juvabione contents, the unknown compound contents also indicated no significant difference among families (Table 3).

Antifungal activity of *n*-hexane extracts was observed against both *F. palustris* and *T. versicolor* (Table 4). Mycelial growth of *F. palustris* and *T. versicolor* was strongly depressed above the concentration of 225 μg per disc and 150 μg per disc, respectively.

**Table 4** Antifungal activity of *n*-hexane extracts against *Fomitopsis palustris* and *Trametes versicolor*

| Concentration of *n*-hexane extracts (μg per disc) | *F. palustris* | *T. versicolor* |
|---|---|---|
| 20 | − | − |
| 30 | − | − |
| 50 | − | − |
| 100 | − | + |
| 150 | + | ++ |
| 225 | ++ | ++ |
| 300 | ++ | ++ |
| 450 | ++ | ++ |
| 3,000 | ++ | ++ |

++ antifungal activity was found in all paper discs tested, + antifungal activity was found in more than half the paper discs tested, − no antifungal activity of paper discs was found

## Discussion

In the present study, variations in mass loss of wood specimens by decay test and *n*-hexane-extractive contents were investigated for seven half-sib families of plus trees in 22-year-old todomatsu. The results show that significant differences among families were not found in all examined factors (Tables 2, 3). Venäläinen et al. [13] examined the mass loss of wood specimens from 53 clones in 25-year-old *Larix sibirica* by a brown-rot fungus, *Coniophora puteana*.

The results show that mean mass loss from decay ranged from 30 to 46 %, and the clonal repeatability of the mass loss was 0.41. They reported that genetic control over decay resistance appeared to be stronger than over growth characteristics (0.22–0.26 in clonal repeatability), yet weaker than that over wood density (0.57 in clonal repeatability). However, when wood specimens from 20 trees in 75-year-old *Larix laricina* were decayed by *Gloeophyllum trabeum* and *Postia placenta*, mean mass loss by decay ranged from 30 to 63 % and from 30 to 58 %, respectively [5], suggesting that the deviation values of mass loss were high. In the present study, mass loss by decay ranged from 13.1 to 23.9 % in *F. palustris* and from 8.5 to 12.3 % in *T. versicolor* (Table 2). Due to the large deviations, significant differences among the seven todomatsu families tested in the present study were not found by ANOVA test (Table 2). On the other hand, Gierlinger et al. [7] reported that in 106 trees from 39-year-old *Larix decidua* var. *decidua*, *L. decidua* var. *sudetica*, *Larix kaempferi*, and *L.* × *eurolepsis*, variations were not found among trees in hot-water-extract content but were found in acetone-extract contents, total phenolic compound contents, and lignin contents. However, as shown in Table 3, significant differences among the seven todomatsu families tested in the present study were not found in *n*-hexane-extractive contents by ANOVA test.

It has been reported that antifungal compounds were found in ethanol and *n*-hexane extracts of todomatsu wood [9, 11]. Yoneyama et al. [9] reported that *n*-hexane-soluble fraction of ethanol extracts from todomatsu wood showed antifungal activity against six white-rot fungi. In addition, juvabione was identified as a main antifungal compound in the *n*-hexane-soluble fraction of ethanol extracts from todomatsu wood [10]. In fact, juvabione had antifungal activity against two *Fusarium* spp., two *Pythium* spp., and *Lepista sordida* [11]. Therefore, decay resistance of todomatsu wood is considered to be closely related to juvabione contents. However, in the present study, as shown in Table 3, significant variations among families were not found in putative juvabione content.

Table 5 shows the relationship between the percentage of wood mass loss and air-dry density, *n*-hexane-extractive content, putative juvabione content or unknown compound content. In *T. versicolor*, relatively high negative correlation coefficients were found between the percentage of mass loss and *n*-hexane-extractive content or putative juvabione content, suggesting that decay resistance against *T. versicolor* increases with an increase in *n*-hexane-extractive and putative juvabione contents in seven todomatsu half-sib families. In *F. palustris*, on the other hand, the correlations coefficients were weak between the mass loss and *n*-hexane-extractive content or putative juvabione content. In addition, as shown in Table 4, mycelial growth of *F. palustris* and *T. versicolor* was strongly inhibited at concentrations of more than 225 and 150 μg per disc, respectively. These results suggest that *F. palustris* has stronger resistance to toxicity of *n*-hexane extracts and/or putative juvabione than *T. versicolor*. Concentrations of 225 and 150 μg per disc of *n*-hexane-extractive content in paper discs corresponded to approximately 10.0 and 6.6 mg/g, respectively, in wood. In the present study, the highest *n*-hexane-extractive content (7.4 mg/g) was found in the Ohyubari104 and Sapporo101 families (Table 3). Therefore, trees containing more than 10.0 mg/g of *n*-hexane-extractive content might possess high decay resistance in todomatsu families.

**Table 5** Correlation coefficients between wood mass loss and *n*-hexane-extractive, putative juvabione, or unknown compound contents in seven todomatsu half-sib families

| | ML in *Fomitopsis palustris* | ML in *Trametes versicolor* |
|---|---|---|
| Air-dry density | −0.104 (0.824) | −0.088 (0.851) |
| *n*-Hexane-extractive content | 0.189 (0.685) | −0.535 (0.216) |
| Putative juvabione content | 0.173 (0.711) | −0.640 (0.122) |
| Unknown compound content | 0.083 (0.860) | −0.092 (0.844) |

Values in parentheses indicate probability. Correlation coefficients were determined using mean values of the percentage of wood mass loss and the contents in each half-sib family

*ML* mass loss

## Conclusion

In the present study, to estimate the possible improvement in the decay resistance, variations of decay resistance of wood and *n*-hexane-extractive contents were examined in seven todomatsu plus tree families. Although there was no significant difference in decay resistance and *n*-hexane-extractive content among the seven families, the test of antifungal activity of *n*-hexane extracts indicated that the concentration for high decay resistance might need more than 10.0 mg/g of the extracts in wood. Therefore, it is concluded that the todomatsu trees containing more than 10.0 mg/g of *n*-hexane extract should be selected among the families for high decay resistance.

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