

## Influence of stem rot pathogen *Fomitiporia* sp. on “Sanbu-sugi” cultivar of the Japanese cedar *Cryptomeria japonica*

Yoshie Terashima

Received: 16 December 2011 / Accepted: 10 August 2012 / Published online: 24 August 2012  
© The Japan Wood Research Society 2012

**Abstract** An unidentified *Fomitiporia* sp. causes severe white-rot on stems of a cultivar “Sanbu-sugi” of the Japanese cedar, *Cryptomeria japonica*. The influence of the fungus on tree health and wood properties was determined. Bark from dead branches and xylem from living branches contained more glucose than bark from living branches and xylem from dead branches. Tree heights at which annual rings were disconnected were 2, 4 and 6 m at ages 37, 15 and 24 years old, respectively. The pH values of damaged parts were lower than those of non-damaged parts, and the damaged parts were clearly identified using bromocresol green solution. Weight loss of sapwood during 60 days of fungal degradation was 1.4 %, which was less than that by a saprophyte, *Trametes versicolor*. The amount of polyphenols in the heartwood from damaged tree stems was less than that from non-damaged stems. Degraded parts were less stiff than the non-degraded sapwood as measured with a wood-decay tester, Pilodyn. Our observations indicate that damaged stems are chemically and physically inferior to non-damaged stems.

**Keywords** Bromocresol green · Flavanol · *Fomitiporia punctata* · Pilodyn · Polyphenol

### Introduction

An unidentified white-rot fungus *Fomitiporia* sp. causes severe stem sap rot especially on a cultivar called “Sanbu-

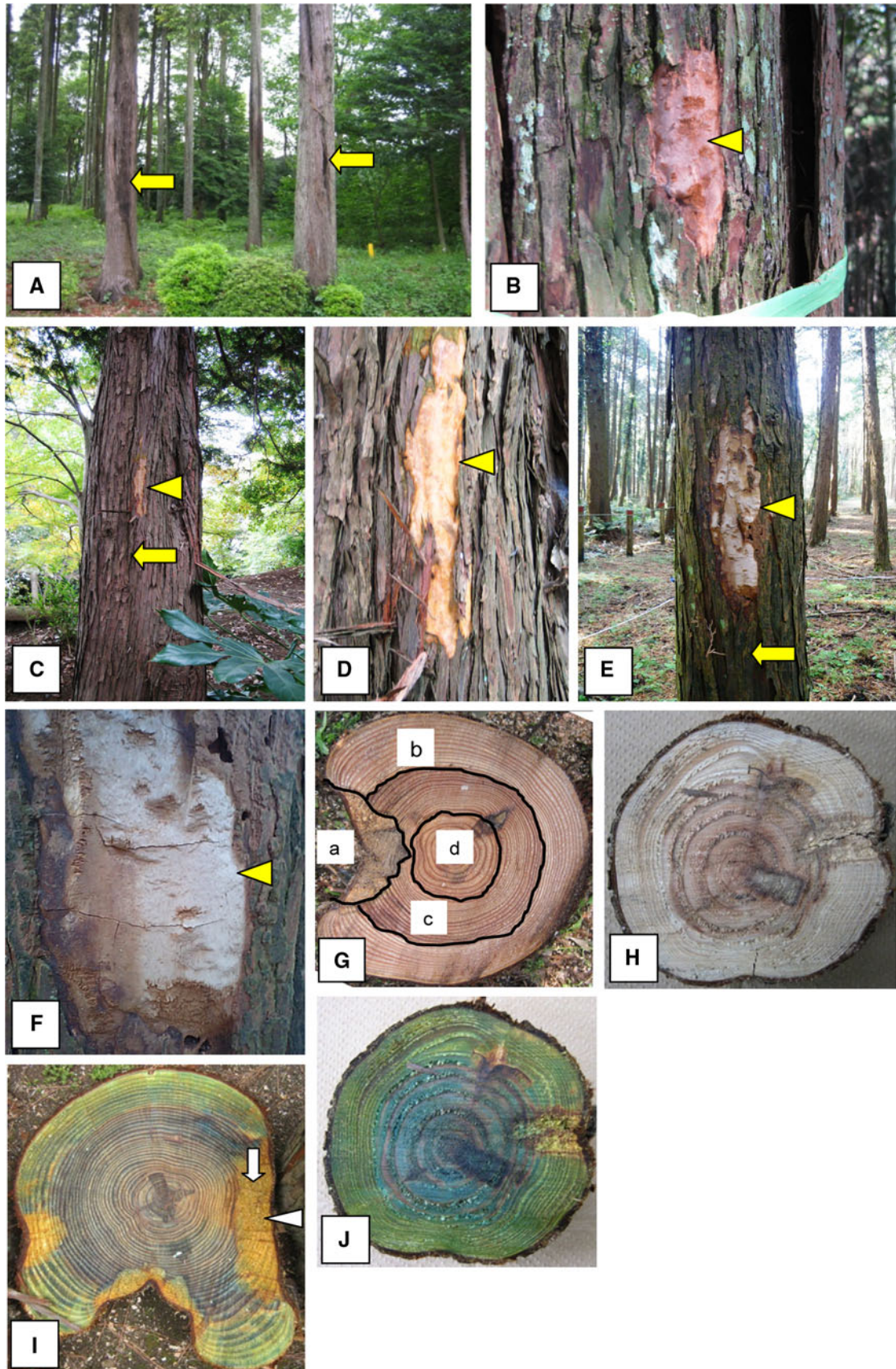
sugi” [1–3] of the Japanese cedar *Cryptomeria japonica* D. Don [4, 5] (Fig. 1A, B). Sanbu-sugi is a major lumber material in Chiba Prefecture. It was planted in 18 % of the prefectural area, and it occupies 45 % of the Sammu area [6]. This cultivar has beneficial characteristics: the wood is straight, non-tapered and round in cross section, and the color of the heartwood is beautiful pale rouge. Unfortunately, however, the tree is highly sensitive to this local sap rot disease, and 85 % of it has been damaged in Sammu area [6].

The fungus was observed to cause stem rot on Sawara cypress *Chamaecyparis pisifera* (Sieb. & Zucc.) Endl. (Fig. 1C, D) and Hinoki cypress *Chamaecyparis obtusa* Sieb. & Zucc. (Fig. 1E, F). Imazeki [4] called this disease “Hi-akagare-sei-mizogusare-byo” in Japanese, stem sap rot. Aoshima et al. [5] identified the causal fungus as *Fomitiporia punctata* (Fr.) Murrill (*Phellinus punctatus*) and named the fungus from Chiba Prefecture, Japan “Chana-take-modoki” in Japanese. Recently, Hattori et al. [7, 8] morphologically and phylogenetically analyzed the preserved fruiting body and mycelia of this fungus, and reported that the causal organism was distinct from *F. punctata* [9–11] and was another species of the genus *Fomitiporia*. Ota et al. [12, 13] reported that the isolated fungus from *C. japonica* and that from stem rot of *C. pisifera* form a phylogenetically distinct clade from *F. punctata*, and discussed that the pathogens on these tree species were possibly conspecific.

Imazeki [4] found small pits, which were scars of dead and fallen branches, in the middle of the vertical axis of the affected parts and implied that the causal fungus invaded from these scars. Aoshima et al. [5] also observed that the causal fungus invaded through scars.

In earlier studies, stems of living trees of “Sanbu-sugi” were inoculated by the fungus and cankers were formed

Y. Terashima (✉)  
Iriomote Station, Tropical Biosphere Research Center,  
University of the Ryukyus, Uehara 870, Taketomi-cho,  
Yaeyama-gun, Okinawa 907-1541, Japan  
e-mail: yoshie@lab.u-ryukyu.ac.jp





◀ **Fig. 1** **A** “Hi-akagare-sei-mizogusare-byo” with cankers (*arrow*) on the “Sanbu-sugi” cultivar of *Cryptomeria japonica* by the causal *Fomitiporia* sp., Sammu, Chiba, **B** fruiting body (*arrowhead*) of the causal *Fomitiporia* sp. formed on the stem of “Sanbu-sugi”, **C** *Chamaecyparis pisifera* with an elongate canker (*arrow*) on the stem and fruiting body (*arrowhead*) of the causal *Fomitiporia* sp., Abiko, Chiba, **D** fruiting body (*arrowhead*) of the causal *Fomitiporia* sp. on the stem of *C. pisifera*, **E** *Chamaecyparis obtusa* with a canker (*arrow*) and fruiting body (*arrowhead*) of the causal *Fomitiporia* sp., Kisarazu, Chiba, **F** fruiting body (*arrowhead*) of the causal *Fomitiporia* sp. on the stem of *C. obtusa*, **G** schematic depiction of a stem disk from the “Sanbu-sugi” cultivar damaged by the causal *Fomitiporia* sp.: *a* degraded part, *b* non-degraded sapwood, *c* non-degraded outer heartwood, *d* non-degraded inner heartwood including pith. **H–J** Color changes of “Sanbu-sugi” wood disks soaked in the pH indicator, bromocresol green solution: **H** before soaking, **I** after soaking, the degraded part turned *yellow*; the non-degraded sapwood turned *green*; the outer heartwood turned *blue* and the inner heartwood turned *blue*; the *arrow* indicates the horizontal direction and the *arrowhead* the vertical direction for the Pilodyn test. **J** The same wood disk shown in **H** after soaking in bromocresol green solution

[14, 15]. Damage status of wood disks from the cultivar in the national forests was surveyed [16]. Although the hypothesis that invasion occurs through the scars of dead branches was based upon observations, the reasons why the fungus invades easily through the scars have not been clearly described. In addition, usability of the round logs from damaged trees has not been determined. The aims of this study are to (1) survey whether branch bases of dead branches are the possible gateway of fungal invasion; (2) survey at what ages and heights trees are attacked; (3) measure pH changes; (4) analyze secretion of anti-fungal compounds; (5) measure weight loss due to fungal degradation; and (6) examine stiffness of damaged wood.

## Materials and methods

### Tree and wood materials

For the study, wood materials were obtained from “Sanbu-sugi” cultivars grown at the Forestry Research Institute, Chiba Prefectural Agriculture and Forestry Research Center, Sammu, Chiba, Japan. The trees were planted in about 1965, and maintained in natural condition only with regular grass cutting until 5 years old and pruning until 10 years old.

### Influences of fungal degradation on trees

#### *Chemical analyses of living and dead branches*

To survey a possibility that the causal fungus invades through bases of dead branches, 4 kinds of branch materials were analyzed, specifically, bark and xylem from living

and dead branches ( $n = 3$ ). Branches with living leaves and those with dead leaves in living trees were separately cut. Twigs with leaves and bark were removed from both kinds of branches, and inner xylem was obtained. The wood components ( $\alpha$ -cellulose, hemicelluloses and mixtures of lignin and others), organic solvent extracts, glucose, and flavanols were determined. The materials were milled into meal and sieved into particles from 0.18 to 0.44 mm (the same size hereafter). To calculate the contents of hemicelluloses, the content of  $\alpha$ -cellulose (JAPAN TAPPI No. 60, 2000) was subtracted from that of holocellulose (JAPAN TAPPI No. 65, 2000). Contents of mixtures of lignin and others and organic solvent extracts were determined by JAPAN TAPPI No. 61 (2000) and No. 63 (2000), respectively.

To determine the amount of glucose, 100 ml distilled water was added to 2 g of meal. The suspension was boiled for 3 h, and filtered through a glass filter (pore size 10–16  $\mu\text{m}$ ). Distilled water was added to the hot-water extract solution to a total volume of 150 ml. The glucose in the solution was determined by the enzymatic method with F-Kit for D-glucose (Roche Diagnostics, Tokyo, Japan) by measuring absorbance at 340 nm using a double beam spectrophotometer (U-2900, Hitachi High-Technologies Co., Tokyo, Japan).

The meal (1 g) was extracted with 70 % (v/v) acetone under agitation for 8 h, and the suspension was paper-filtered. Total amounts of flavanols were determined according to the vanillin-HCl method [17]. The calibration curve was prepared using catechin as a standard. Thirty millilitre of vanillin reagent [4 % (v/v) in methanol] and then 15 ml conc. HCl were added to 5 ml of the extract solution in a 50 ml-volumetric flask wrapped in aluminum foil, and the solution was left still for 15 min at  $20 \pm 2$  °C. Absorbance at 500 nm of the solution was measured.

### *Surveying ages and heights of damaged trees*

To determine the ages and heights of the damaged trees by the fungus, 2 living trees (tree 1 and 2) with cankers were fell down and logged into 2-m lengths. The widths of the annual rings on the cross sections near the base of each log were measured from the center to 4 crosswise directions. Both trees were 42 years old.

### Influences of fungal degradation on wood

#### *pH values of damaged wood*

Four kinds of wood meals (5 g each) from (a) degraded part, (b) non-degraded sapwood, (c) outer heartwood and (d) inner heartwood including pith of a damaged stem in Fig. 1G were separately suspended in 10-times the weight

**Table 1** List of fungal mycelia used

Isolate no.	Scientific name	Original name/number	Location	Host	Isolated section
P-1	<i>Fomitiporia</i> sp.	MAFF420042	Chiba, Chiba	Unknown	Basidiospores
P-2	<i>Fomitiporia</i> sp.	MAFF420111, Pa46f	Matsuo, Chiba	<i>Cryptomeria japonica</i>	Basidiospores
P-3	<i>Fomitiporia</i> sp.	MAFF420112, Pa46 m	Tohgane, Chiba	<i>C. japonica</i>	Degraded wood
P-6	<i>Fomitiporia</i> sp.		Sanbu, Chiba	<i>C. japonica</i>	Decomposed sapwood
P-7	<i>Fomitiporia</i> sp.		Sanbu, Chiba	<i>C. japonica</i>	Decomposed sapwood
P-21	<i>Fomitiporia</i> sp.	WD-984	Meguro, Tokyo	Unknown	Decomposed wood
P-26	<i>Fomitiporia</i> sp.	National Institute of Fruit Tree Science (NIFTS) C5	Tukuba, Ibaragi	<i>Chamaecyparis pisifera</i>	Decomposed sapwood
P-27	<i>Fomitiporia</i> sp.	NIFTS C6	Tukuba, Ibaragi	<i>C. pisifera</i>	Decomposed sapwood
420002	<i>Trametes versicolor</i>	MAFF420002, FFPRI 1030 (Kawara-take)	Shimada, Shizuoka	Unknown	Basidiospores

of distilled water. pH value of each suspension was measured with a pH meter (F-23, Horiba Ltd., Kyoto, Japan).

To examine pH changes of damaged wood disks by color changes, 3 kinds of acid-based indicators were used: bromocresol green (BCG) with pH range of 3.8–5.4 and color change from yellow to bluish green, bromophenol blue (BPB) with pH range of 3.0–4.6 and color change from yellow to purple, and methyl red (MR) with pH range of 4.4–6.2 and color change from red to yellow. BCG (0.04 g), BPB (0.1 g), and MR (0.2 g) were diluted with each 20 ml ethanol, and volume was made up to 100 ml with distilled water, respectively. These original solutions were diluted with distilled water to 1/10 and 1/100. Sliced wood disks with degraded parts (ca. 3 cm length and 20 cm in diameter, Fig. 1H) were soaked in the original and diluted solutions separately and color changes of the disks were observed, respectively.

#### Weight loss of sapwood pieces due to fungal degradation

Mycelia of 8 isolates of the causal *Fomitiporia* sp. and those of one isolate of a white-rot fungus *Trametes versicolor* were used for the experiment (Table 1). The mycelia of P-6 and P-7 were obtained by isolating the degraded sapwood just under the bark where the fruiting bodies of the fungus developed.

Quartersawn pieces of sapwood (20 × 20 × 10 mm) were prepared from non-damaged wood. The wood pieces were oven-dried at 60 °C for more than 48 h to constant weights, and then soaked in distilled water for 24 h. Silica sand was placed in a polypropylene bottle (80 mm in diameter, 110 mm in height) to 50 mm height and was soaked with potato dextrose broth (Tanabe Seiyaku Hanbai Co., Ltd., Osaka, Japan) medium. The sterilized wood

pieces ( $n = 3$ ) were put onto the mycelial colony of the objective fungus by attaching the annual rings to the colony surface. The wood pieces were degraded at 26 °C for 60 days before collecting them. The mycelia were removed from the wood pieces, and the pieces were oven-dried at 60 °C and weighed.

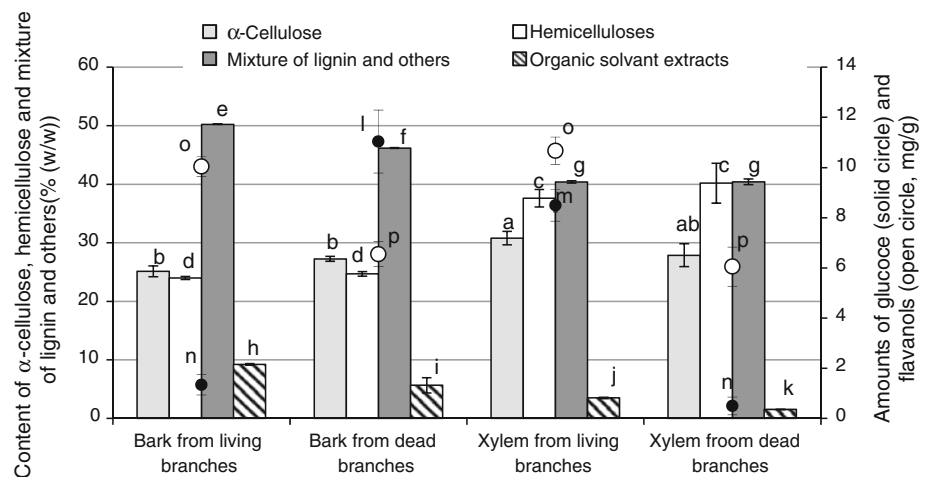
#### Amounts of degradation-derived glucose and secreted antifungal compounds in damaged wood disks

The amounts of glucose derived from polysaccharide degradation, and of antifungal compounds, polyphenols and flavanols in wood powder were determined in the 4 parts (Fig. 1G), degraded part (a), non-degraded sapwood (b), outer heartwood (c), and inner heartwood (d) from the damaged stem, and 2 parts, sapwood and heartwood from the non-damaged stem. Sample extracts in 20 ml of 70 % (v/v) acetone were used for quantitative measurements. The amount of polyphenols as total phenolics was determined according to the Folin–Ciocalteu assay [18]. The calibration curve was made using catechin as a standard. One millilitre of the extract was introduced into a test tube, and 1 ml of Folin–Ciocalteu's reagent and 5 ml of 20 % (w/v) sodium carbonate were added to it. The mixture was filled up to 10 ml with distilled water and mixed. The mixture was allowed to stand for 1 h, and its absorbance at 765 nm was measured. The amounts of flavanols and glucose were determined as described above.

#### Stiffness of damaged sapwood

A damaged tree was felled down, logged, and sliced into ca. 30 mm-length discs. The stiffness of the degraded part and the co-existing non-degraded part in the disk was

**Fig. 2** Contents of  $\alpha$ -cellulose, hemicelluloses, mixtures of lignin and others, organic solvent extracts, glucose and flavanols in bark and xylem from living and dead branch materials of the “Sanbu-sugi” cultivar of *Cryptomeria japonica*. Mixture of lignin and others was determined by JAPAN TAPPI No. 61 (2000). Bars indicate standard deviations. Letters indicate the significant differences among the 4 kinds of branch materials for respective chemicals ( $p < 0.05$ )



measured with the wood decay tester, Pilodyn 6J Standard (Proceq Co., Ltd., Schwerzenbach, Switzerland). The maximal inserted spring length of the Pilodyn, which indicates minimal stiffness of wood decay, is 40 mm; lower lengths indicate greater stiffness of damage. The measurements were done in the horizontal (arrow in Fig. 1I) and vertical (arrowhead in Fig. 1I) directions.

#### Statistics

The obtained data were analyzed statistically with Sheffe's *F* test ( $p < 0.05$ ) or Student's *t* test ( $p < 0.05$ ).

## Results

### Influence of fungal degradation on trees

#### Chemical characteristics of living and dead branch materials

Figure 2 shows the contents of  $\alpha$ -cellulose, hemicelluloses, mixtures of lignin and others, which were residues treated by JAPAN TAPPI No. 61 (2000) and organic solvent extracts, and amounts of glucose and flavanols in the bark and xylem from living and dead branch materials. Bark from both living and dead branches contained more mixtures of lignin and others and organic solvent extracts than xylem. Xylem from both living and dead branches contained significantly more  $\alpha$ -cellulose and hemicelluloses than bark. The bark from dead branch materials and xylem from living branches contained significantly more glucose than the bark from living branches and dead xylem from dead branches. The bark and xylem from living branches contained significantly more flavanols than the dead ones.

### Ages and heights of damaged trees

Figure 3 shows the annual rings measured from the center to four crosswise directions on the cross sections of stems. The numbers of the annual rings on the faces of disks from tree 1 were 42, 38, 34, 28, 24, 21, 17, and 13 in that order from the tree base. Annual rings seized forming at the 10th ring on the first disk (Fig. 3A) and at the 16th ring on the third disk (Fig. 3B). The numbers of the annual rings on the faces of disks from tree 2 were 42, 39, 36, 30, 25, 22, 19, 16 and 9 from the base. Annual rings disconnected at the 37th ring on the first disk (Fig. 3C). The height of the second and third disks of tree 1 and first disk of tree 2 were 4, 6 and 2 m, and the rings were disconnected at the ages of 15, 24, and 37 years old, respectively.

### Influence of fungal degradation on wood

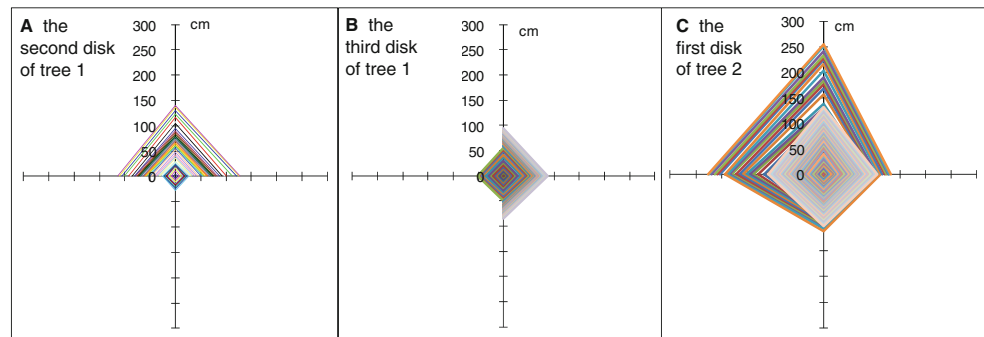
#### pH values of damaged wood

The pH values of the degraded part, non-damaged sapwood, outer heartwood and inner heartwood were 4.5, 5.7, 6.7 and 6.9, respectively. Color changes were most obvious with 1/100 BCG solution. Color of the damaged part, sapwood, and outer and inner heartwood turned to yellow, green, blue and blue, respectively (Fig. 1I, J).

#### Weight loss of sapwood due to fungal degradation

Percentages of weight loss of the sapwood pieces degraded by mycelia of the 8 fungal isolates are shown in Fig. 4. The percentage of weight loss by *T. versicolor* (6.0 %) was significantly greater compared with those isolates of *Fomitiporia* sp. (1.4 % on average). The ability of the saprophyte *T. versicolor* to degrade sapwood was greater than that of the causal fungus.

**Fig. 3** Radar graphs showing annual rings measured from the center to four cardinal directions on the base of 2 m-long logs from two trees of the “Sanbu-sugi” cultivar of *Cryptomeria japonica*. The 10th and 16th annual rings were seized in the second (A) and third (B) disks of tree 1, respectively, and in the 37th ring on the first disk (C) of tree 2



#### Amounts of glucose and secreted antifungal compounds in damaged wood

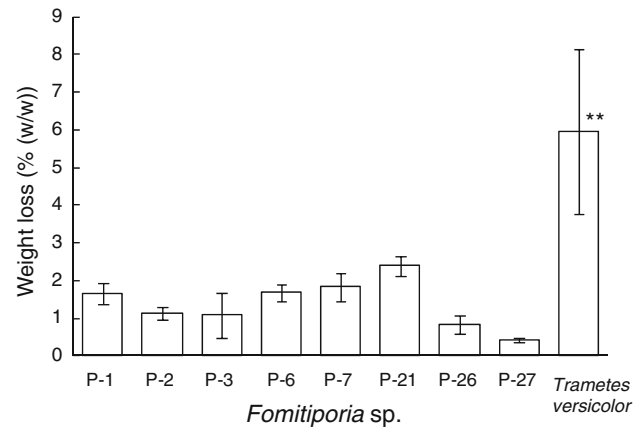
There were no statistically significant differences in glucose amounts among the 6 kinds of samples (Fig. 5). Amounts of flavanols were higher in the degraded parts and in the heartwood from damaged and non-damaged stems than those in the sapwood from damaged and non-damaged stems. Amount of polyphenols was lower in the heartwood from damaged stems than that in the heartwood from non-damaged stems.

#### Stiffness of damaged wood

As shown in Fig. 6, the stiffness of the degraded and non-degraded parts of wood disks with cankers was measured by the Pilodyn from the horizontal (A) and vertical (B) directions. The degraded parts showed significantly lower stiffness than the non-degraded parts irrespectively of the direction of measurement. The stiffness from the horizontal directions was less than that from the vertical directions.

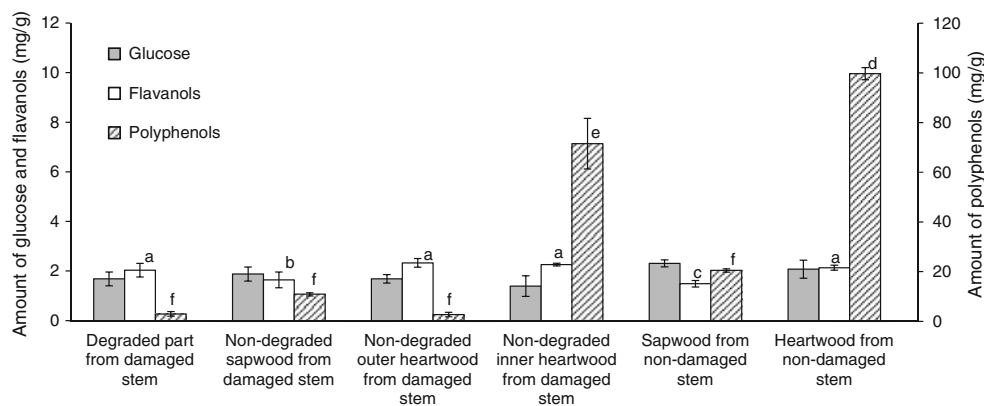
#### Discussion

To survey possibilities that bases of dead branches comprised the invasion gateway for *Fomitiporia* sp., chemical components were determined in the bark and xylem from living and dead branch materials of “Sanbu-sugi”. Bark contained significantly more mixtures of lignin and others and organic solvent extracts than xylem (Fig. 2). When bark is treated by JAPAN TAPPI No. 61 (2000), polyphenols and suberin were mainly determined as well as lignin [19]. Kofujita et al. [20] reported that bark has to be treated with 1 % NaOH after ethanol–benzene treatment to determine true lignin content, and *C. japonica* bark contains 27 % of acid insoluble lignin. Lignin is a defensive substance against penetration of invasive pathogens [21]. In addition, polyphenols are known as resistant substances when plants are under stress from a fungal invasion



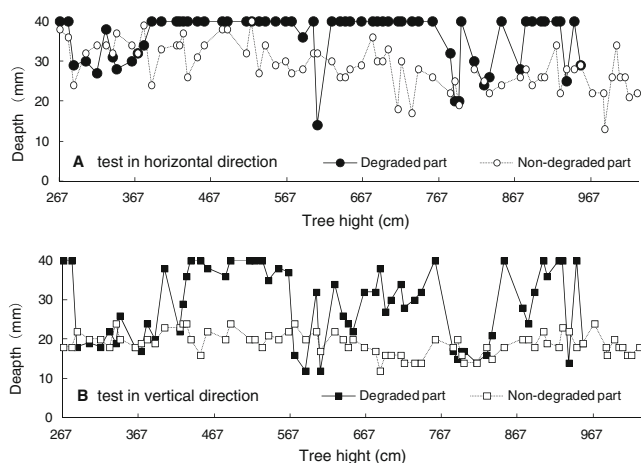
**Fig. 4** Weight loss in sapwood pieces from the “Sanbu-sugi” cultivar of *Cryptomeria japonica*, degraded by 8 isolates of the causal *Fomitiporia* sp. and one isolate of *Trametes versicolor*. The wood pieces were degraded by the fungi at 26 °C for 60 days. Bars indicate standard deviations and the asterisks indicate significant difference ( $p < 0.01$ )

[22, 23], and suberin itself is an antifungal substance [24, 25]. Furthermore, organic solvent extracts usually contain antifungal substances [21]. The bark from dead branches and xylem from living branches contained more flavanols than the bark from living branches and xylem from dead branches (Fig. 2). As flavanols were found in high concentrations in resistant cultivar in apple trees, levels of flavanols screen for resistance and susceptibility to fungal infection [26]. The xylem contained more  $\alpha$ -cellulose and hemicelluloses than the bark (Fig. 2). Cellulose and hemicelluloses are available to fungi as a major carbon source [21]. It might be suspected that the causal fungus invaded through sapwood of branch bases; branch bases were not protected with antifungal substances in the bark of living branches and sapwood was exposed at the base of dead and fallen branches. The bark from dead branches and xylem from living branches contained significantly more glucose than the bark from living branches and xylem from dead branches; it is considered that the glucose is derived from cellulose and hemicellulose degraded by *Fomitiporia* sp. This observation may imply that glucose in the bark from dead branches and xylem



**Fig. 5** Amounts of glucose, flavanols and polyphenols in four kinds of wood: degraded part, apparently non-degraded sapwood, outer heartwood and inner heartwood including pith, from damaged stems of the “Sanbu-sugi” cultivar of *Cryptomeria japonica*, and two kinds of wood: sapwood and heartwood, from non-damaged stems.

The schematic depiction of a damaged wood disk is shown in Fig. 1G. Bars indicate standard deviations. Letters indicate significant differences among the six kinds of samples for respective compounds ( $p < 0.05$ )



**Fig. 6** Stiffness of damaged sapwood of the “Sanbu-sugi” cultivar of *Cryptomeria japonica* by the causal *Fomitiporia* sp. measured in horizontal (A) and vertical (B) directions with a wood decay tester, Pilodyn. The vertical and horizontal directions are shown in Fig. 11

from living branches accelerated mycelial growth of the causal fungus. It is known empirically that many wood-decaying fungi penetrate into the stem via injuries in which xylem has been exposed [25]. As dead branches of “Sanbu-sugi” naturally and easily fall off retaining the bases of branches on stems [27, 28], the bases expose the xylem to the fungus. Based on the results of chemical analyses of the branches and on the biological characteristics of “Sanbu-sugi”, the remaining bases of dead branches provide possible open gates for the fungus to invade.

Pathogenic micro-organisms in the xylem of trees are divided into 6 groups including wood decaying fungi [29]. On the basis of colonization strategies, wood decaying fungi are further classified into 5 types: heart rot, active pathogenesis, specialized opportunism, desiccation tolerance, and unspecialized opportunism [22, 29]. As the

causal fungus is not clearly aggressive and initially colonizes at only functionally compromised sapwood associated with major wounds, *Fomitiporia* sp., therefore, is considered to belong to the specialized opportunism type.

Aoshima et al. [5] described that the causal fungus invaded from scars of dead branches to a part of tree cambium through sapwood, and killed the cambium. Dead cambium stopped producing new cells, contrary to non-damaged cambium which continued producing new cells to cover the damaged part. To explain the localization of decay and discoloration in injured sapwood through the active formation of 4 barriers, the concept called CODIT (compartmentalization of decay in trees) [23] has been used. The walls in trees limit the spread of fungi; wall 1 impedes vertical spread, wall 2 impedes inward radial spread, wall 3 impedes tangential spread, and wall 4 is the barrier zone made of anti-fungal substances, occurring at the junction of injured wood and wood newly developed by the vascular cambium [23]. Given that the causal *Fomitiporia* sp. kills the cambium, the CODIT model is not applicable to “Hi-akagare-sei-mizogusare-byo”.

“Sanbu-sugi” cultivar of *Cryptomeria japonica* has been one of the most economically important trees used for lumber in Japan. The tree heights where annual rings disconnected were 4 and 6 m in tree 1 and 2 m in tree 2, and tree ages were 15 and 24 years old in tree 1 and 37 years old in tree 2, respectively (Fig. 3). Imazeki [4] reported on the prevention of the disease by early artificial pruning. In the Japanese Agricultural Standard for round logs [30], the standard length for log production of *C. japonica* is 1.9–4.3 m. Our results show that at heights of 2, 4 and 6 m, damage concerned the first, second, and third 2 m-round logs, respectively. These logs which had to be discriminated against were supposed to be the most economically valuable compared with the upper parts of the trees.



The non-degraded outer and inner heartwood from the damaged stems secreted less polyphenols than the heartwood from non-damaged stems (Fig. 5). Although the weight loss due to the causal fungus was significantly lower than that due to *T. versicolor* (Fig. 4), the degraded parts were less stiff than the non-degraded sapwood (Fig. 6). Based on these findings, it is concluded that the damaged stems with degraded parts are chemically and physically inferior to the non-damaged stems.

**Acknowledgments** Many thanks go to Dr. Seiji Ohara in Forest and Forestry Products Research Institute for the instruction of polyphenol analyses, Dr. Hitoshi Nakamura in National Institute Fruit Tree Science for providing the fungal isolates, and the staff of the Forest Research Institute, Chiba Prefectural Agriculture and Forest Research Center for providing useful information on “Sanbu-sugi”. I thank Dr. Tsutomu Hattori in Kansai Research Center, Forest and Forestry products Research Institute, Dr. Norio Sahashi in Forest and Forestry products Research Institute, and Dr. Azusa Fujiie for helpful comments on the manuscript.

## References

- Okizumi H (1993) Clone analysis of collected Sugi-cutting cultivars from the Sanbu area, Chiba prefecture, by the multilocus genotypes of twelve isozyme loci. *J Jpn For Soc* 75(5):398–404
- Yoshimaru H, Kodaira T, Endo R, Fukushima S, Kawahara H, Okuizumi H (1995) Clone analyses of Sugi cutting-cultivars from the Sanbu area using RAPD markers (in Japanese). *Theses of the 106th annual meeting of the Jap For Soc*, pp 253–254
- Kondo T, Watanabe A (2005) Relationships between two Sugi local varieties, Sanbu-sugi and Nyukawa-sugi, and plus trees revealed by SSR markers (in Japanese). *Theses of the 56th annual meeting of the Jap For Soc*, pp 139–140
- Imazeki R (1960) A new disease of Sanbu-sugi, “Hi-akagaresei-mizogusare-byo” and its ecological prevention (in Japanese). *Forest Pest News* 9:230–235
- Aoshima K, Hayashi Y, Yonebayashi H, Kondo H (1964) “Hi-akagaresei-mizogusare-byo” on Sanbu-sugi (in Japanese). *Theses of the 91th annual meeting of the Japanese Forest Soc*, pp 394–397
- Chiba Prefecture HP; <http://www.pref.chiba.lg.jp/lab-nourin/nourin/sanmusugi.html>. Accessed 31 March 2012
- Hattori T, Terashima Y, Suzuki T (2009) Pathogen of “Hi-akagaresei-mizo-gusare-byo” of *Cryptomeria japonica* (in Japanese). *Database of the Annual Meeting of For Soc*, vol 120, p 835
- Hattori T, Ota Y, Nakamura H, Terashima Y (2010) Morphological characters of “Cha-ana-take-modoki” (in Japanese). *Database of the Annual Meeting of For Soc*, vol 121, p 703
- Wagner T, Fischer M (2002) Proceedings towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. *Mycologia* 94(6):998–1016
- Cony D, Sara HF, Gerardo R, Gabriel C (2007) *Fomitiporia punctata* (Basidiomycota, Hymenochaetales) and its presumed taxonomic synonyms in America: taxonomy and phylogeny of some species from tropical/subtropical areas. *Mycologia* 99(5):733–752
- Dai YC, Due BK, Cony D (2008) A new species of *Fomitiporia* (Hymenochaetaceae, Basidiomycota) from China based on morphological and molecular characters. *Mycol Res* 112:375–380
- Ota Y, Hattori T, Nakamura H, Terashima Y (2009) Relationship among *Fomitiporia* species, the trunk rot pathogens on Japanese cedar, Sawara cypress and Nashi pear in Japan. *The Abstract of the 2009 KSPP Fall Meeting and the 1st Japan-Korea Joint Symposium*, p 96
- Ota Y, Hattori T, Nakamura H, Terashima Y (2010) Relationship among pathogens cause trunk rots on Japanese umbrella-tree, Japanese cedar, and leaf atrophy on Nashi pear (in Japanese). *Database of the Annual Meeting of Jap For Soc*, vol 121, p 704
- Hayashi Y, Kobayashi T, Abe T, Aoshima K (1980) Inoculation experiment of *Fuscoporia punctata* (Fr.) Cunn. to Sanbusugi, a variety of Japanese cedar, *Cryptomeria japonica* (in Japanese). *Theses of the 91th annual meeting of the Japanese Forest Soc*, pp 385–386
- Abe Y, Hattori T (1991) Artificial inoculation of *Phellinus punctatus* into six cultivars of Japanese cedar (in Japanese). *Theses of the 102th annual meeting of the Japanese Forest Soc*, pp 323–324
- Ishii M, Shiizu K, Sakata M (1985) “Hi-akagaresei-mizogusare-byo” of Sanbu-sugi (in Japanese). *Tokyo Eirinnyoku Gyomu Kenkyu Happyo Shu* 17:41–48
- Broadhurst RB, Jones WT (1978) Analysis of condensed tannins using acidified vanillin. *J Sci Food Agric* 29(9):788–794
- Julkunen-Titto R (1985) Phenolic constituents on the leaves of northern willows. *Methods for the analysis of certain phenolics*. *J Agric Food Chem* 33:213–217
- Sogo M (1971) Studies on the bark lignin and bark phenolic compounds (in Japanese). *Memoirs Fac Agric Kagawa Univ* 25:1–72
- Kofujita H, Etyu K, Ota M (1999) Characterization of the major components in bark from five Japanese tree species for chemical utilization. *Wood Sci Technol* 33:223–228
- Cook RC, Rayner ADM (1984) *Ecology of saprotrophic fungi*. Longman, New York
- Rayner ADM, Boddy L (1988) *Fungal decomposition of wood*. Wiley, Chichester
- Schwarze FWM, Englels J, Mattheck k (1999) *Fungal strategies in trees*. Springer, New York
- Rioux D, Ouellette GB (1991) Barrier zone formation in host and nonhost trees inoculated with *Ophiostoma ulmi*. 1. Anatomy and histochemistry. *Canadian J Bot* 69(9):2055–2073
- Murata M, Yamada T, Matsuda Y, Ito S (2011) Comparison of hyphal growth and defense responses in four Fagaceae species inoculated with *Raffaelea quercivora* (in Japanese). *Res Bull Tokyo Univ For* 125:11–21
- Picinelli A, Dapena E, Mangas JJ (1995) Polyphenolic pattern in apple tree leaves in relation to scab resistance: a preliminary study. *J Agric Food Chem* 43:2273–2278
- Kawana A, Aiba Y, Endo T (1967) Varieties of Sugi in Sanbu area (1) Variety development and morphological characteristics (in Japanese). *Theses of the 78th annual meeting of the Jap For Soc*, pp 156–158
- Aonuma K (1993) Reconsidering Sanbu-sugi (in Japanese). *Green Kikaku*
- Pearce RB (1996) Tansley Review No. 87 Antimicrobial defenses in the wood of living trees. *New Phytol* 132:203–233
- Japanese Ministry of Agriculture, Forestry and Fisheries (2007) Japanese Agricultural Standard for round logs. [http://www.maff.go.jp/j/jas/jas\\_kikaku/pdf/kikaku\\_55.pdf](http://www.maff.go.jp/j/jas/jas_kikaku/pdf/kikaku_55.pdf). Accessed 31 March 2012