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ORIGINAL ARTICLE

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Seasonal and height-dependent fluctuation of starch and free glucose contents in moso bamboo (*Phyllostachys pubescens*) and its relation to attack by termites and decay fungi

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Abstract The potential of termite (*Coptotermes formosanus* Shiraki) and fungal attacks [*Fomitopsis palustris* (Berkeley et Curtis) Murrill (FFPRI 0507) and *Trametes versicolor* (L. ex Fr.) Quel (FFPRI 1030)] against moso bamboo (*Phyllostachys pubescens* Mazel) were evaluated with regard to the seasonal and height-dependent changes of the free glucose and starch contents, which were measured by a newly developed method. The free glucose contents were generally lower in autumn and winter than in spring and summer, whereas the lowest starch contents were obtained in August, and the contents increased almost linearly up to February and March. In terms of the height-dependent fluctuation, the free glucose contents tended to decrease as the sampling heights increased. There was no special correlation between the free glucose or starch contents and the consumption by termites, even though higher mortalities were obtained in the bamboo-fed termites than in the wood-fed termites. However, a positive correlation between the free glucose contents and mass losses of the samples at 4-m and 8-m heights from the bottom by the decay fungi was observed. For starch, no influence on fungal attack was found.

Key words Moso bamboo · Termites · Decay fungi · Starch and free-glucose contents · Seasonal fluctuation

Introduction

Bamboo grows widely in zones with a wet season ranging from the tropics to temperate areas, and is one of the most economically important raw materials. However, the production of bamboo culms has declined recently in Japan due to decreased use.[1] Even though bamboo had been used as a building material for centuries, changes in peoples' lifestyles and the architectural designs used to construct buildings throughout Japan likely have contributed to the decrease in bamboo usage. The result is a drastic increase of uncontrolled bamboo plantation forests. The phenomenon often results in local environmental problems, because bamboo grows fast and prevents the growth of other plants. The proper use of bamboo is important.

Building systems for use with bamboo are presently being developed in many Asian countries, due not only to bamboo's sustainability as a natural resource, but also its excellent flexibility and dynamic performance. For architectural applications, the durability of bamboo is a crucial consideration when it is used under conditions conducive to biological degradation because bamboo has little resistance to decay.[2] The biologically perishable properties of bamboo are assumed to be mainly caused by its high sugar and starch contents, which are excellent foods for fungi or insects. Some reports have indicated that the sugar and starch contents of bamboo depend on the season when it is cut, although the results vary widely among the reports.[3–5] Previous investigations mainly focused on the damage to bamboo caused by the attack of mold[4] or *Dinoderus minutus* Fabricius, a serious insect pest to bamboo.[3,6,7] Termites and decay fungi cause serious damage to building and construction materials in Japan. However, no detailed study on the biological degradation of bamboo by termites and decay fungi has been conducted to date. In this study, therefore, we evaluated the potential of termite and fungal attacks against moso bamboo (*Phyllostachys pubescens* Mazel) in correlation with the seasonal and height-dependent changes in the free glucose and starch contents, which were measured by a newly developed method.[8]

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Materials and methods

Materials

Samples of 3- to 5-year-old moso bamboo (*Phyllostachys pubescens* Mazel) culms were obtained from bamboo plantation forests in Yawata City, Kyoto Prefecture, every month from May 2002 to October 2003, in December 2003, and in February, March, and May 2004. The samples were obtained from a dense bamboo plantation forest in the first year (from May 2002 to April 2003) and from a thinned bamboo plantation forest in the second year (from May 2003 to May 2004). Three culms were harvested each month. The test samples were taken from the center sections of internodes located at 1 m, 4 m, and 8 m heights from the base. After wiping out the wax layer covering the epidermis on the outer surfaces with acetone, the segments were oven-dried at 65°C for 48 h, and kept in plastic containers with calcium chloride–based absorbents.

Free glucose and starch analyses

Preparation of sample

The "alkaline extraction – glucoamylase hydrolysis" method (AG method)[8] was used to determine the free glucose and starch contents. Bamboo specimens from the internodes were ground into a powder with an approximately 100-mesh particle size by a Wiley mill and a vibration mill. Three replicates were prepared for each sample. In a centrifuge tube, 500 mg of the bamboo powder was mixed with 10 ml of 0.5 N sodium hydroxide solution, and the mixture was sonicated for 30 min (5510J-DTH, Branson). After neutralization by 0.5 N acetic acid, the mixture was centrifuged for 10 min at 3000 rpm and the supernatant was recovered.

Free glucose analysis

To remove the inhibition of the alkaline extracts, 20–30 mg of activated charcoal (Nacalai Tesque) was added to the supernatant, with a contact time of 10–15 min. After centrifugation for 10 min at 3000 rpm, the glucose content of the supernatant (G1) was determined with the glucose oxidase reagent kit (Glucose B-Test, Wako) with a UV/Vis spectrometer (U-2001, Hitachi) at 505 nm.

Starch analysis

One milliliter of the supernatant was mixed with 1 ml of 0.1 M sodium acetate buffer (pH 4.8) containing glucoamylase from *Rhizopus* sp. (38.5 U/mg, Toyobo) and α-amylase from *Bacillus* sp. (1870 U/mg solid, Nacalai Tesque). The concentrations of glucoamylase and α-amylase were 4 mg/ml.[8] The solution was shaken for 2 h at 120 rpm at 40°C.[8] To remove the inhibition of the alkaline extracts, 20–30 mg of activated charcoal (Nacalai Tesque) was then added to the solution, with a contact time of 10–15 min. After centrifugation for 10 min at 3000 rpm, the glucose content of the supernatant (G2) was determined with the glucose oxidase reagent kit. The amount of starch in the bamboo was calculated as: (G2 − G1) × 0.9.[9]

Termite test

The termite tests were conducted according to the Japanese Industrial Standards (JIS) K-1571-2004. The bamboo samples were taken from the center sections of three internodes located at 1 m, 4 m, and 8 m from the base. Specimens (20 × 20 × thickness 4–10 mm) were prepared from each section. A specimen was placed in the center of the hard plaster bottom (thickness 5 mm) of the cylindrical container (8 cm in diameter and 6 cm in height), and 150 workers and 15 soldiers of *Coptotermes formosanus* Shiraki obtained from a laboratory colony maintained at 28° ± 2°C and >85% relative humidity (RH) in the dark (termite breeding room) were introduced into each container. The assembled containers were placed on dampened cotton pads to supply water to the specimens, and were kept for 21 days in a termite breeding room. At the end of the test, termite mortality was determined, and the mass loss of the test specimen after termite attack was calculated based on the differences in the initial and final oven-dried (60°C, 48 h) masses of the specimen. Three replicates were employed for each section. Sapwood blocks (20 × 20 × 10 mm) of Japanese red pine (*Pinus densiflora* Sieb. et Zucc) were used as controls.

Decay test

Bamboo culms harvested in June, August, October, and December 2002, in February, April, May, August, October, and December 2003, and in March 2004 were used for the decay tests. The test specimens were prepared by the same method as in the termite tests. The specimens were sterilized with gaseous ethylene oxide after measurement of their oven-dried masses (60°C, 48 h).

A monoculture decay test was conducted according to the Japanese Industrial Standards (JIS) K-1571-2004 with a brown-rot fungus, *Fomitopsis palustris* (Berkeley et Curtis) Murrill (FFPRI 0507) and a white-rot fungus, *Trametes versicolor* (L. ex Fr.) Quel (FFPRI 1030). A glass jar with 250 g of quartz sand (30–50 mesh) and 80 ml of nutrient solution containing 4.0% glucose, 0.3% peptone, and 1.5% malt extract for *T. versicolor* and half these concentrations for *F. palustris* was autoclaved, and then inoculated with 3 ml of liquid fungal suspension precultured in the nutrient solution containing 4.0% glucose, 0.3% peptone, and 1.5% malt extract. When the mycelial mat fully covered the medium, three specimens were placed on the mat surface with (*F. palustris*) or without (*T. versicolor*) a plastic net, and the jars were incubated at 26° ± 2°C for 12 weeks. Six replicates were tested for each section–fungus combination. Fungal attack was expressed as a percent mass loss, which was calculated by comparing the oven-dried masses before and after the test. Sapwood blocks (20 × 20 × 10 mm) of Japanese beech (*Fagus crenata* Blume) were used as controls.

Statistical analysis

The average daily wood consumption per termite, the mean mortalities of the workers and soldiers after the termite

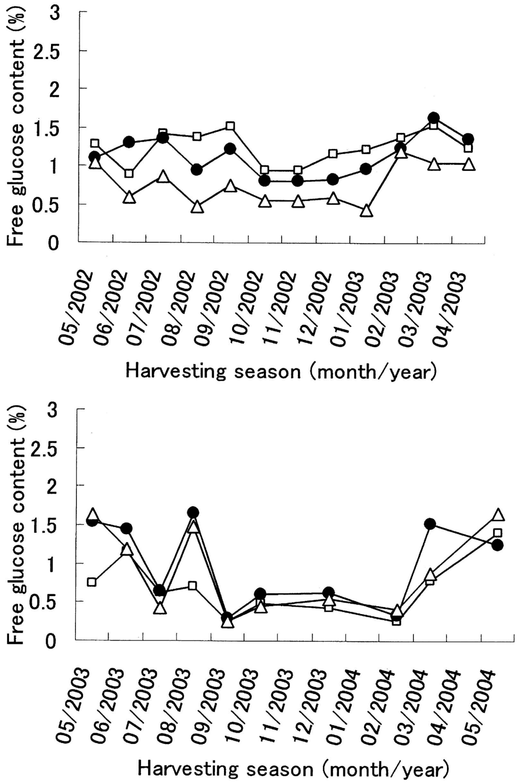

Fig. 1. Seasonal change of free glucose contents of moso bamboo samples obtained from 1-m, 4-m, and 8-m heights. *Upper*, 2002/03 year; *lower*, 2003/04 year; *squares*, 1 m; *circles*, 4 m; *triangles*, 8 m

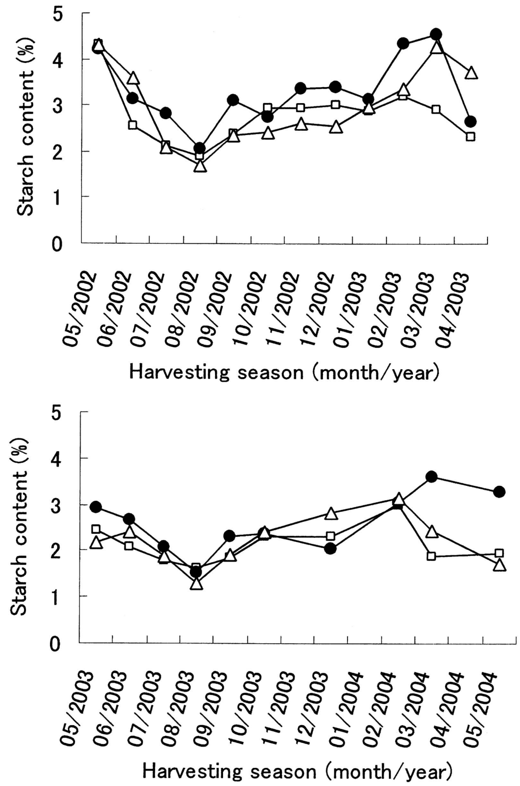

Fig. 2. Seasonal change of starch contents of moso bamboo samples obtained from 1-m, 4-m, and 8-m heights. *Upper*, 2002/03 year; *lower*, 2003/04 year; *squares*, 1 m; *circles*, 4 m; *triangles*, 8 m

tests, and the average mass losses in the specimens in the decay tests were subjected to statistical analyses with inerSTAT-a v1.3.

Results

Free glucose and starch contents

The free glucose and starch contents of the moso bamboo from May 2002 to May 2004 are shown in Figs. 1 and 2, respectively. As shown in Fig. 1, the free glucose content was generally low in autumn and winter and high in spring and summer. The contents were 0.44%–1.21% for 1 m, 0.60%–0.96% for 4 m, and 0.43%–0.59% for 8 m from October to January, and 0.70%–1.52% for 1 m, 0.64%–1.66% for 4 m, and 0.43–1.65% for 8 m from March to August. For the first year, compared with the specimens obtained from the 8-m height of the culms, the glucose content was significantly higher in the specimens from the lower sections of the culms (Tukey's test, $P < 0.01$). The average free glucose contents of the samples from the heights of 1, 4, and 8 m in the first year were 1.24%, 1.13%, and 0.76%, respectively. However, there was no special tendency regarding the height for the second year (average free glucose content: 1 m 0.68%; 4 m 0.99%; 8 m 0.90%).

The starch contents of the specimens showed more clear seasonal fluctuation than the free glucose contents (Fig. 2). The lowest starch contents were obtained in August (first year: 1 m 1.89%; 4 m 2.05%; 8 m 1.70%; second year: 1 m 1.61%; 4 m 1.53%; 8 m 1.30%), and the contents increased almost linearly up to February (first year: 1 m 3.20%; 4 m 4.34%; 8 m 3.35%; second year: 1 m 2.97%; 4 m 3.04%; 8 m 3.14%) and March (first year: 1 m 2.91%; 4 m 4.56%; 8 m 4.28%; second year: 1 m 1.90%; 4 m 3.61%; 8 m 2.44%). Unlike the free glucose contents; there was no special tendency for the starch contents regarding the height of the sample collection. On average, the starch contents of the samples at the heights of 1, 4, and 8 m in the first year were 2.79%, 3.29%, and 3.00%, respectively, and in the second year were 2.12%, 2.58%, and 2.22%, respectively.

Termite resistance

As shown in Fig. 3, no special seasonal fluctuation was observed in daily wood consumption per termite. However, compared with the specimens from 1-m culm height, the yearly average consumptions were significantly lower in the specimens from the upper culms sections (Tukey's test, $P < 0.01$). The yearly average consumptions in the specimens from the 1-m height section in the first and second year were 0.11 mg/day per worker, while the values in the specimens from the 4-m and 8-m sections in the first year were 0.09 mg/day per worker and 0.05 mg/day per worker, and in the second year were 0.09 mg/day per worker and 0.07 mg/day per worker, respectively. On average, a termite worker consumed sapwood of Japanese red pine (control) at the rate of 0.09 mg/day per worker. The mortalities of the workers and soldiers at the end of the termite tests are shown in Fig. 4. The yearly average mortalities of the termites fed on moso bamboo were significantly higher than those of the control samples (Tukey's test: worker $P < 0.01$; soldier $P < 0.01$). No seasonal and height-dependent tendencies were observed. The yearly average mortalities of the workers that were fed on the 1-m, 4-m, and 8-m height samples were 27.2%, 27.2%, and 30.9% in the first year and 43.6%, 33.2%, and 33.3% in the second year, respectively. In the control, the respective yearly average mortalities were 10.7% and 11.3%. For the soldiers, the mortalities were 46.3%, 49.4%, and 60.2% in the first year and 63.8%, 62.8%, and 69.6% in the second year, respectively. The soldiers fed on control samples showed 13.7% and 14.7% mortalities in the first and second years, respectively.

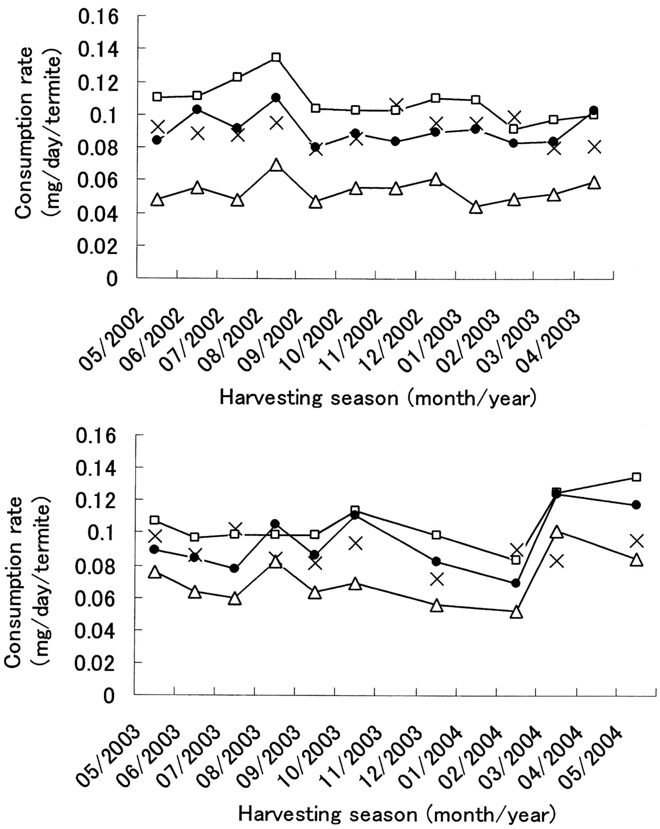

Fig. 3. Daily consumption rates of moso bamboo samples obtained from 1-m, 4-m, and 8-m heights by *Coptotermes formosanus*. *Upper*, 2003/04 year; *lower*, 2003/04 year; *squares*, 1 m; *circles*, 4 m; *triangles*, 8 m; *crosses*, control

Decay resistance

The results of the decay tests are shown in Table 1. On average, the moso bamboo showed significantly lower mass losses after exposure to both *Fomitopsis palustris* and *Trametes versicolor* than Japanese beech (Tukey's test, $P < 0.01$). In particular, the mass losses of the bamboo samples attacked by *T. versicolor* were a third less than that of the control. The average mass losses in the specimens from the 1-m, 4-m, and 8-m heights in the first year after exposure to *F. palustris* were 10.58%, 10.58%, and 9.74%, respectively, and in the second year were 6.22%, 8.49%, and 8.08%, respectively. *Trametes versicolor* consumed 11.49%, 11.45%, and 11.24% of the respective moso bamboo samples in the first year, and 10.08%, 11.87%, and 13.02% in the second year after 12 weeks. The average mass losses in sapwood of Japanese beech in the first year by *F. palustris* and *T. versicolor* were 30.96% and 45.77%,

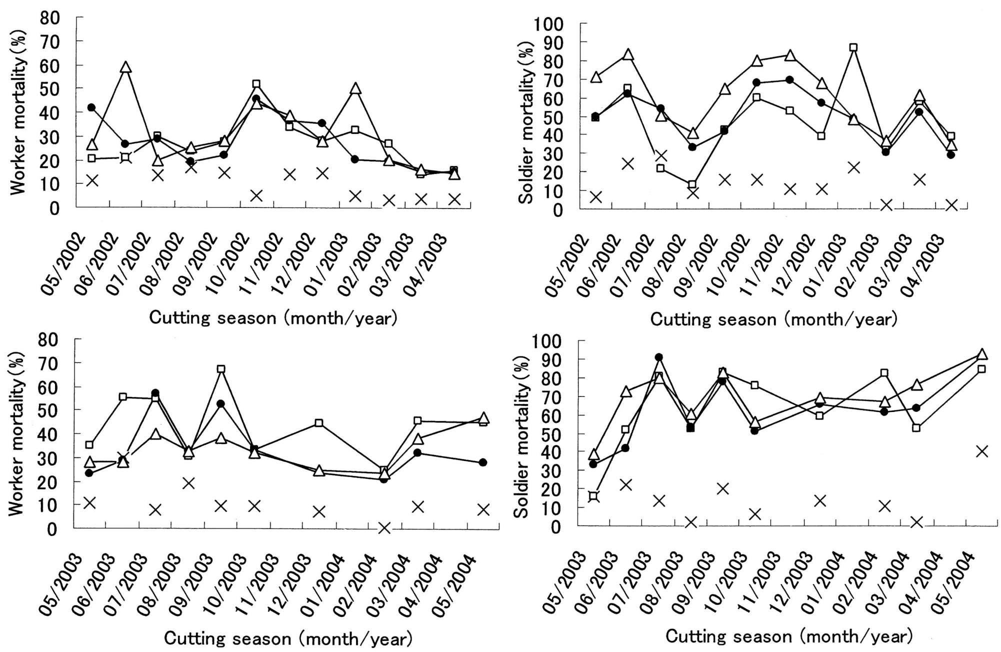

Fig. 4. Mortality of *Coptotermes formosanus* fed on moso bamboo samples obtained from 1-m, 4-m, and 8-m heights after 3 weeks. *Left*, workers; *right*, soldiers; *upper*, 2002/03 year; *lower*, 2003/04 year; *squares*, 1 m; *circles*, 4 m; *triangles*, 8 m; *crosses*, control

Table 1. Percentage mass losses of moso bamboo samples from heights of 1, 4, and 8 m after 12-week exposure to decay fungi *Fomitopsis palustris* and *Trametes versicolor*

Harvest	*Trametes versicolor*			*Fomitopsis palustris*		
	1 m	4 m	8 m	1 m	4 m	8 m
June 2002	10.59 ± 2.07	12.26 ± 0.83	11.03 ± 1.22	10.52 ± 1.12	11.04 ± 0.36	9.91 ± 0.81
August 2002	9.18 ± 0.39	9.17 ± 0.69	8.98 ± 2.34	10.84 ± 1.37	10.71 ± 1.02	9.59 ± 1.54
October 2002	10.59 ± 1.81	9.65 ± 0.57	9.29 ± 0.95	9.85 ± 0.26	9.36 ± 0.65	7.72 ± 0.71
December 2002	13.68 ± 1.62	11.18 ± 0.51	11.76 ± 1.79	11.40 ± 2.14	9.87 ± 0.27	9.18 ± 0.73
February 2003	13.5 ± 1.14	13.36 ± 1.31	13.26 ± 2.42	10.82 ± 0.18	11.39 ± 1.12	10.73 ± 0.70
April 2003	11.42 ± 0.71	13.10 ± 1.05	13.13 ± 1.97	10.06 ± 1.12	11.09 ± 1.27	11.32 ± 1.57
Control		45.77 ± 2.56			30.96 ± 1.64	
May 2003	12.19 ± 4.35	13.11 ± 1.89	13.78 ± 3.53	9.34 ± 2.74	10.91 ± 2.01	10.64 ± 0.85
August 2003	9.87 ± 2.04	11.80 ± 0.90	15.18 ± 1.53	4.53 ± 1.56	8.55 ± 0.53	8.99 ± 1.78
October 2003	9.34 ± 1.73	10.67 ± 0.61	11.89 ± 0.60	6.74 ± 1.75	7.38 ± 2.10	6.36 ± 1.48
December 2003	9.57 ± 1.56	10.15 ± 2.30	11.48 ± 2.00	4.90 ± 0.71	5.88 ± 2.57	6.66 ± 2.17
March 2004	9.41 ± 1.54	13.63 ± 2.12	12.77 ± 3.11	5.59 ± 1.47	9.72 ± 2.34	7.76 ± 1.34
Control		40.9 ± 3.78			25.49 ± 2.67	
Average	10.85 ± 1.64	11.64 ± 1.58	12.05 ± 1.86	8.60 ± 2.62	9.63 ± 1.75	8.99 ± 1.67
Control[a]		43.36 ± 3.4			28.22 ± 3.86	

[a] Mean of control values

respectively, and in the second year were 28.22% and 43.36%, respectively.

Discussion

Previous studies have shown that the amount of free sugar in moso bamboo increases in spring.[5,10] In this study, the free glucose contents were generally lower in autumn and winter than in spring and summer. For starch, it was reported that the contents in ma bamboo (*Phyllostachys bambusoides*) are very low from July to September and show a maximum value in May.[3] In the present investigation, the starch contents in moso bamboo were lower in summer than in winter and spring. These results were well in accordance with the results of previous studies.[3–5,10] It has long been believed that the best season to cut bamboo is autumn, because that is when the free glucose and starch contents are low.[4] Considering the free glucose and starch contents obtained in this study, the best cutting season is most likely to be late summer and early autumn, which supports this traditional belief.

Regarding the sampling height, it was reported that the starch contents were higher in the upper section of the culms.[11] However, in this study there was no height-dependent tendency. For the free glucose contents, the contents tended to decrease as the sampling height increased only in the first year, while no special tendency was observed in the second year. In the present investigation, the samples were obtained from a dense bamboo plantation forest in the first year and from a thinned bamboo plantation forest in the second year. In addition, test samples were prepared from the center sections of the internodes located at heights of 1, 4, and 8 m with an average overall height of 14.15 m. More research is needed to clarify the relationship between the glucose and starch contents and the sampling heights.

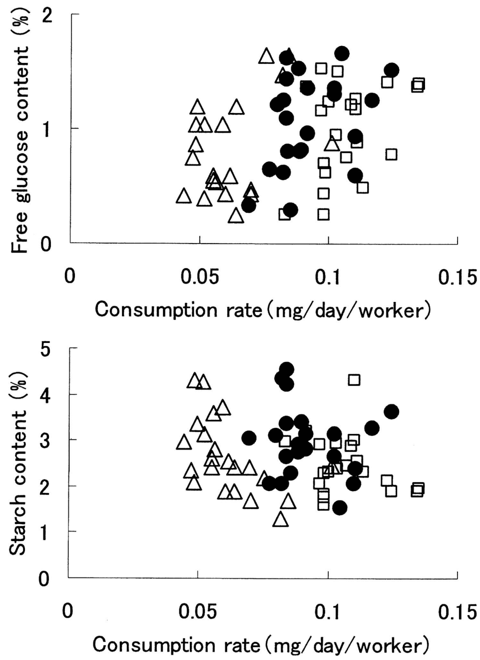

Fig. 5. Plots of free glucose (*upper*) and starch contents (*lower*) of moso bamboo samples against daily consumption by *Coptotermes formosanus*. Heights of samples indicated by: *squares*, 1 m; *circles*, 4 m; *triangles*, 8 m

Previous investigations into the damage by the insect *Dinoderus minutus* Fabricius, which is a serious pest of bamboo, showed that the damage by the pest increased with the starch contents in the ma bamboo culms.[3] However in our study, there was no special correlation between the free glucose or starch contents and the wood consumption rate by termites (Fig. 5). Kanai et al.[12] found that feeding on starch and sugars had a detrimental effect on the protozoan fauna of *Coptotermes formosanus* workers. In addition, it was reported that workers of the lower termite, *Mastotermes darwiniensis* Frogatt, lost their large-sized symbiotic protozoa in the hindgut by the forced feeding of starch.[13] Yoshimura et al.[14] also reported that feeding on low molecular weight celluloses had a detrimental effect on the large-sized protozoa of *C. formosanus*. These results may indicate the negative effect of starch on the feeding activity of termites. As shown in Fig. 6, the starch contents of the bamboo had no influence on worker mortality in this study, although the mortalities of the *C. formosanus* workers that fed on moso bamboo were significantly higher than those fed on Japanese red pine. The effect of glucose and starch in bamboo on the feeding activity of termites will be considered in further research.

Extractives or physical properties of samples are other possible factors affecting the termite feeding activity. Nishina[15] extracted an antimicrobial component, a quinone derivative, from moso bamboo culms, and Quitain et al.[16] also extracted antimicrobials and antioxidants, an ethoxyquin, a sesquiterpene, a cyclohexanone derivative, hydroquinone, benzoquinone, and hydroxycinnamic acid, from moso bamboo. These compounds may affect the feeding activity of termites by upsetting the microbial community in their gut, which is indispensable for termite survival. In our previous study, termite attack against moso bamboo was closely related to the surface roughness of the material.[17] Doi et al.[18] measured the surface hardness of steamed boards using a Brinell hardness tester and contradicted the effect of the surface hardness of wood on the termite activity. Compared with the specimens from the 1-m-high culms, the consumptions were significantly lower in the specimens from the upper sections of the culms. In this study, termites attacked only the radial inner sections of the samples. Parenchyma cells in bamboo are highly dense at the inner culm, and sclerenchymatous fibers, bundle sheath, are highly distributed at the outer culm.[19] Suzuki[20] reported that sclerenchymatous fibers increased in density with height. These trends clearly indicate that mechanical strengths such as the bending strength, shear strength, and Young's modulus are increased from the inner culm to the outer culm, and from the lower sections of the culms to the upper sections.[20] From these experimental results, it is suggested that the difference in anatomical structure and/or physical properties of bamboo also affect termite feeding activity.

The relationship between the free sugar content in bamboo and mold growth on the surface was reported in

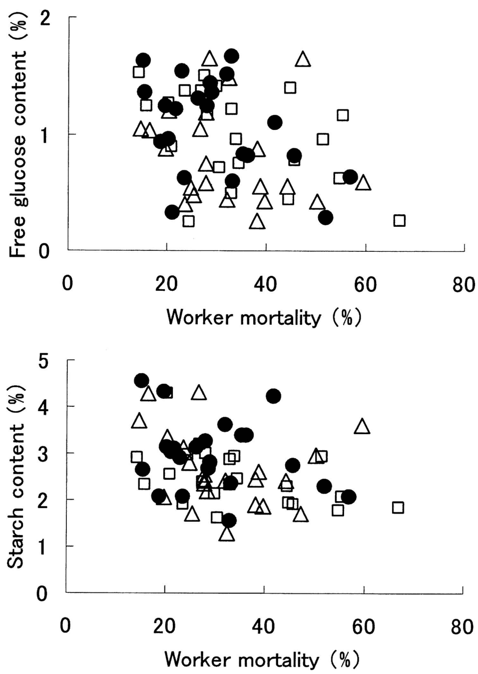

Fig. 6. Plots of free glucose (*upper*) and starch contents (*lower*) of moso bamboo samples against the mortality of *Coptotermes formosanus* workers

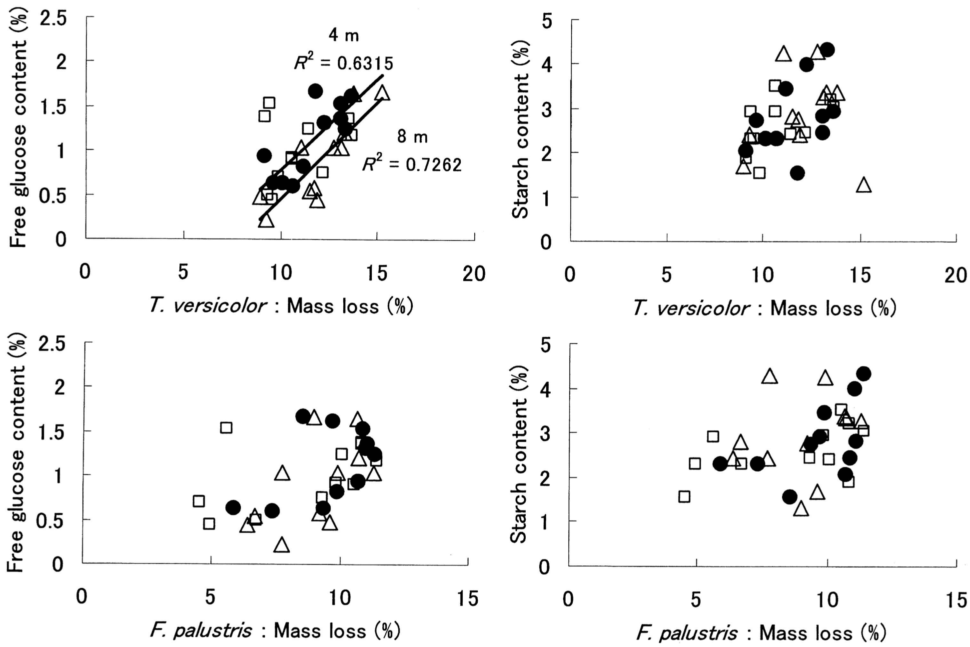

Fig. 7. Plots of free glucose (*left*) and starch (*right*) contents of moso bamboo samples against mass losses after 12-week exposure to the decay fungi *Fomitopsis palustris* (*lower*) and *Trametes versicolor* (*upper*)

previous studies.[21,22] These reports showed that the damage by mold attack increased with the free sugar content in the ma bamboo and moso bamboo culms. In this study, there was a positive correlation between the free glucose contents and mass losses in the specimens from the 4-m and 8-m high culms in decay tests (Fig. 7), particularly in the case of *T. versicolor* (4 m $r^2 = 0.63$; 8 m $r^2 = 0.73$). For starch, no influence on fungal attack was observed. Therefore, it can be concluded that the glucose content of moso bamboo enhances the growth of decay fungi. The moso bamboo showed lower mass losses after exposure to both *F. palustris* and *T. versicolor* than the sapwood of Japanese beech. Some antimicrobial compounds in moso bamboo were reported as described above.[15,16] This result may suggest that these compounds have protective effects against decay fungi.

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