Response of the Termite, *Coptotermes formosanus* SHRIAKI to Extracts from Fungus-infected and Delignified Fungus-infected Woods

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Abstract——Pine (*Pinus densiflora* SIEB. et ZUCC.) and beech (*Faqus crenata* BLUME) wood meals which delignified or not delignified, were decayed by 4 species of white rot fungi, *Coriolus versicolor, Lenzites betulina, Pycnoporus coccineus* and *Ganoderma lucidum,* and 7 species of brown rot fungi, *Tyromyces palustris, Daedalea dickinsii, Lenzites trabea, Gloeophyllum saepiarium, Coniophora puteana, Serpula lacrymans* and *Lentinus lepideus.* Thereafter, a trail-following activity to the termite, *Coptotermes formosanus* in the ether extracts of these decayed wood meals was examined.

The extracts of the delignified pine wood meals decayed by the brown rot fungi other than *c. puteana* showed the trail-following activity. The extracts of the normal pine, the normal beech and the delignified beech wood meals which were rotted by *S. lacrymans,* also showed the trail-following activity. The wood meals decayed by white rot fungi were decomposed heavily, and a great deal of mycelia and fruit bodies were produced. Nevertheless, the extracts of both normal wood meals and delignified wood meals decayed by all the white rot fungi did not show the trail-following activity.

Introduction

Termites ingest the decayed wood and often live in the decayed wood. KOVOOR[1] examined the effect of the poplar wood decayed by 2 kinds of fungi to the termite *Microcerotermes edentatus,* and found that termites prefer decayed wood to sound wood. BECKER[2] reported on the preference to decayed wood by various termite species and the nutritional value of the infested wood.

ESENTHER *et al.*[3] found the fact the runways of *Reticulitermes* spp. on tree tend to go straight to decayed wood, and suggested that a gradient of sensitivity to the attractive materials might help termites find its food supply. They also found that the wood blocks invaded by *Lenzites trabea* PERS ex FR. attracted termites. SMYTHE *et al.*[4] found that a trail-following substance was extracted from the western white pine rotted by the fungus *Lenzites trabea* PERS. ex FR., and that the activity of the extract was approximately 20 times higher than that of the extract of the termite itself.

The present authors[5] reported previously, that termites prefer the brown rotted wood to the sound wood. It has been found by the present authors[6] that the

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extract of the brown rotted wood by the fungus, *Tyromyces palustris* MURR., *Daedalea dickinsii* YASUDA, *Lenzites trabea* PERS. ex FR. or *Serpula lacrymans* s. F. GRAY showed the trail-following activity to the termite, *Coptotermes formosanus* SHIRAKI.

However, some fungi (white rot fungi) other than brown rot fungi may also produce a trail-following substance. Therefore, in this experiment, the normal wood meals were decayed by 4 species of white rot fungi and the existence of the trail-following activity in the extracts of these decayed wood meals were examined. The delignified wood meals, which were decayed by 4 species of white rot fungi and 7 species of brown rot fungi, were also examined for the trail-following activity.

Materials and Methods

Test wood and test fungi

Pine (Akamatsu)--*Pinus densiflora* SIEB. et ZUCC. and beech (Buna)--*Fagus crenata* BLUME were used. The woods were chipped by a planer and offered for delignification or decay.

The tested fungi were as follows. White rot fungi--*Coriolus versicolor* (FR.) QUEL. (Cov), *Lenzites betulina* FR. (Lzb), *Pycnoporus coccineus* (FR.) BOND. et SING. (Pyc) and *Ganoderma lucidum* (FR.) KARST. (Gal). Brown rot fungi--*Tyromyces palustris* (BERK. et CURT.) MURR. (Typ), *Daedalea dickinsii* YASUDA (Dad), *Lenzites ttrabea* PERS. ex FR. (Lzt), *Gloeophyllum saepiarium* (FR.) KARST. (Gls), *Coniophora puteana* (SCHUM. ex FR.) KARST. (Cop), *Serpula lacrymans* (WULF.) S. F. GRAY (Sel) and *Lentinus lepideus* FR. (Lel).

Metod of delignification

Pine and beech wood meals were extracted with ethyl alcohol-benzene (1:2) in a Soxhlets' extractor for 10 hrs. respectively. Twenty five gram of wood meal was placed in a flask containing 750 ml of distilled water, and treated with 10 g of sodium chlorite and 2 ml of acetic acid in the water bath at 70 to 80°C. Supplementary 10 g of sodium chlorite and 2 ml of acetic acid were added every one hour. This treatment was repeated 3 times in the case of pine wood meal and 2 times in the case of beech wood meal. The delignified wood meal was washed with cold water and acetone[7]. About 700 g of delignified pine wood meal and about 450 g of delignified beech wood meal were obtained.

Method of decay

About 20 g of the normal wood meal or the delignified wood meal was put into a cylindrical glass bottle (9 cm in diameter and 16 cm in height) containing 320 g of quartz sands (*ca.* 30 mesh) and 160 ml of nutrient solution. The bottles were autoclaved and inoculated with the test fungi. The decay period was 100 days (60

Table 1. Fungal species and wood meals.

Kind of decayed wood meal	Fungus	Wood meal		Decay period (days)
		Kind*	Weight (g)	
Cov (P)	*Coriolus vericolor* (FR.) QUÉL.	P (non-delignified)	80	100
Cov (F)		F (non-delignified)		
Cov (PD)		P (delignified)		
Cov (FD)		F (delignified)		
Lzb (P)	*Lenzites betulina* FR.	P (non-delignified)	80	100
Lzb (F)		F (non-delignified)		
Lzb (PD)		P (delignified)		
Lzb (FD)		F (delignified)		
Pyc (P)	*Pycnoporus coccineus* (FR.) BOND. et SING.	P (non-delignified)	80	100
Pyc (F)		F (non-delignified)		
Pyc (PD)		P (delignified)		
Pyc (FD)		F (delignified)		
Gal (P)	*Ganoderma lucidum* (FR.) KARST.	P (non-delignified)	80	100
Gal (F)		F (non-delignified)		
Gal (PD)		P (delignified)		
Gal (FD)		F (delignified)		
Typ (PD)	*Tyromyces palustris* (BERK. et CURT.) MURR.	P (delignified)	36	100
Dad (PD)	*Daedalea dickinsii* YASUDA	P (delignified)		
Lzt (PD)	*Lenzites trabea* PERS. ex FR.	P (delignified)		
Gls (PD)	*Gloeophyllum saepiarium* (FR.) KARST.	P (delignified)		
Cop (PD)	*Coniophora puteana* (SCHUM. ex FR.) KARST.	P (delignified)		
Sel (PD)	*Serpula lacrymans* (WULF.) S. F. GRAY	P (delignified)		
Lel (PD)	*Lentinus lepideus* FR.	P (delignified)		
Sel (P)	*Serpula lacrymans* (WULF.) S. F. GRAY	P (non-delignified)	100	60
Sel (F)		P (non-delignified)	100	
Sel (PD)		P (delignified)	80	
Sel (FD)		F (delignified)	80	

* P is pine wood meal and F is beech wood meal.

days in the case of *S. lacrymans*) (See Table 1.), and the incubator temperature was 28°C with the exception of *S. lacrymans* which was incubated at 20°C. The nutrient solution is composed of KH_2PO_4 (3.0 g), $MgSO_4 \cdot 7H_2O$ (2.0 g), peptone (5.0 g), malt extract (10.0 g), glucose (25.0 g) and distilled water (1,000 ml).

Decayed wood meals obtained are shown in Table 1. These decayed wood meals were extracted with ether in a Soxhlets' extractor for 8 hrs. The extracts were washed with water, dried over sodium sulfate and condensed.

Bioassay method

The termite, *Coptotermes formosanus* SHIRAKI which was reared at Wood Research Institute was used for the bioassay of the trail-following.

The condensed extract (the original solution) was diluted with ether to a tenth (1/10), a hundredth ($1/10^2$), a thousandth concentration ($1/10^3$),... by volume successively. The concentration of the original solution was determined by evaporating the solvent contained in a portion (9.4×10^{-3} ml) of the original solution. The original solution and the diluted solutions were used for the bioassay. A sample (9.4×10^{-3} ml) was taken from each of the original solution and the diluted solutions by means of a glass capillary tube, and streaked along a 10 cm diameter circle on the ground surface of a glass plate (15 cm × 15 cm × 0.5 cm). A worker termite was placed at the center of the circle. The aggregate time (in seconds) for which the termite followed the circumference within 60 seconds after reaching the circumference of the circle was recorded. This test was repeated three times with a new termite. The evaluation and judgement standard on the trail-following activity is shown in the Table 2.

Table 2. Evaluation and judgement standards on the trail-following activity.

Mark	Judgement standard
O	We judged the extract had a trail-following activity in the following case i), ii) or iii). i) One or more of the 3 termites walked on the circumference for a long time (30...60 sec.). ii) Two or more of the 3 termites walked on the circumference (15...30 sec.). iii) Only one of the 3 termites walked on the circumference (15...30 sec.), and the total time of the other two termites is 15 sec. or more.
X	We judged the extract had no trail-following activity in the case other than above.
XX	We judged the extract had a repellent activity especially in the case that more than 2 termites turned back in flurry as soon as they reached the circumference of a circle, and repeated this action several times and finally went quickly acroos the circumference.

Results and Discussion

Weight losses (%) of the wood meals by the delignification were about 28% in pine and about 20% in beech wood meal, coinciding well with the data of UDA *et al.*[8]

Table 3 shows the weight of mycelia produced in the decay process, the weight of the remained wood meal and weight loss (%) by decay.

All of 4 species of white rot fungi produced a great deal of mycelia. Among others, the weight of the mycelium of Lzb(FD) was 45.3 g and that of Pyc(FD) was 41.6 g, indicating that more than a half of the initial weight (80 g) of the wood meal

Table 3. Weights of mycelia and weight losses of wood meals.

Kind of decayed wood meal	Initial weight (g) of wood meal[a]	Weight (g) after decay		Weight loss[c] (%)
		Fungus itself (g)	wood meal[b] (g)	
Cov (P)	80	11.6	56.9	28.9
Cov (F)		15.5	52.5	34.4
Cov (PD)		21.5	14.3	82.1
Cov (FD)		29.3	21.1	73.6
Lzb (P)	80	8.0	48.6	39.3
Lzb (F)		18.7	32.2	59.8
Lzb (PD)		32.6	0.7	99.1
Lzb (FD)		45.3	1.6	98.0
Pyc (P)	80	11.5	28.8	64.0
Pyc (F)		22.2	11.2	86.0
Pyc (PD)		27.0	1.2	98.5
Pyc (FD)		41.6	1.5	98.1
Gal (P)	80	11.9	47.4	40.8
Gal (F)		15.6	31.9	60.1
Gal (PD)		12.5	18.3	77.1
Gal (FD)		38.9	8.7	89.1
Typ (PD)	36	4.9	18.5	48.6
Dad (PD)		9.4	13.5	62.5
Lzt (PD)		(0)	25.6	28.9
Gls (PD)		(0)	26.8	25.6
Cop (PD)		(0)	33.0	8.3
Sel (PD)		(0)	22.5	37.5
Lel (PD)		(0)	26.1	27.5
Sel (P)	100	(0)	87.0	13.0
Sel (F)	100	(0)	96.5	3.5
Sel (PD)	80	(0)	53.8	32.8
Sel (FD)	80	(0)	71.5	10.6

c) : Culculated from $c=(a-b)/a\times 100$ (%)

(0) : We judged the weight of this fungus slight although it was impossible to separate clearly the fungs itself from the decayed wood meal.

was converted into the mycelium. In the case of Gal(PD), one fruit body was produced in one cylindrical glass bottle and two fruit bodies appeared in another one.

On the normal wood meal and the delignified wood meal of the same wood species decayed by the same fungus, the weight of the mycelium produced in the latter was heavier than that in the former, and the weight loss (%) of the latter was also clearly higher than that of the former.

On the normal wood meal and the delignified wood meal decayed by the same

fungus (in spite of the differene of wood species), the weights of the mycelia produced in the latter were heavier than those in the former except for the case of Gal, and the weight losses of the latter were also clearly higher than that of the former.

Therefore, as for white rot fungi, it seems that the delignification promotes the production of the mycelia and fruit bodies and the deterioration of the wood meals.

The weights of the mycelia of 7 species of brown rot fungi produced by decay were only slight with the exception that Typ(PD) was 4.9 g and Dad(PD) was 9.4 g. The weight losses (%) of 7 kinds of the delignified wood meals were not so high as those of the delignified pine and beech wood meals rotted by white rot fungi, and especially those of Cop(PD) was considerably low.

On the fungus Sel, the weight loss of the delignified wood meal was clearly higher than that of the normal wood meal in the same wood species.

It is clear that there is a tendency that the white rot fungi prefer beech wood to pine wood, the brown rot fungi do pine wood to beech wood, and both fungi prefer the delignified wood to the normal wood.

Table 4 shows the results of the bioassay. Regarding the wood meals decayed by brown rot fungi, the extracts of the delignified wood meals Typ(PD) and Dad (PD) had a trail-following activity to the termite at the concentrations of above 10^{-5} g, and those of Lzt(PD), Gls(PD) and Sel(PD) had also the activity at the concentrations of above 10^{-4} g. Moreover, those of Lel(PD) had also the activity only at the order of 10^{-5} g, but that of Cop(PD) showed no trail-following activity. The fact that the extract of Cop(PD) had no activity may have some connection with the low weight loss(%) of the wood meal Cop(PD). When these results are compared with Table 5 which had been already mentioned by the present authors[6], it is noteworthy that Typ(PD) and Dad (PD) gained the activity even at the lower concentration (10^{-5} g). Moreover, the extract of Gls(PD) gained newly the trail-following activity at the concentrations of above 10^{-4} g and that of Lel(PD) also gained newly the activity only at the order of 10^{-5} g.

On the other hand, the extracts of Sel(P), Sel(PD) and Sel(FD) had the trail-following activity at the concentrations of above 10^{-5} g, and that of Sel(F) showed the activity at the concentrations of above 10^{-4} g. As for the fungus Sel, therefore, the influence of the delignification upon the production of the trail-following substance seems to be negligible in the case of pine wood, and only a little in the case of beech wood.

Thus, in the case of brown rot fungi, it would be possible to say that the production of a trail-following substance in the decayed wood meal is often promoted by delignification.

Table 4. Response to the ether extract of the decayed wood meal.

Kind of decayed wood meal	Weight in the original solution[a] (g)	Weight (order) in the original solution and its diluted solution[b] (g) and the results of the response[c]					
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Cov (P)	1.12×10^{-4}	—	X	X	X	X	X
Cov (F)	2.75×10^{-4}	—	X	X	X	X	X
Cov (PD)	2.13×10^{-4}	—	X	X	X	X	X
Cov (FD)	1.77×10^{-4}	—	X	X	X	X	X
Lzb (P)	1.43×10^{-4}	—	X	X	X	X	X
Lzb (F)	2.77×10^{-4}	—	X	X	X	X	X
Lzb (PD)	2.80×10^{-4}	—	X	X	X	X	X
Lzb (FD)	2.59×10^{-4}	—	X	X	X	X	X
Pyc (P)	1.83×10^{-4}	—	X	X	X	X	X
Pyc (F)	2.73×10^{-4}	—	X	X	X	X	X
Pyc (PD)	1.76×10^{-4}	—	X	X	X	X	X
Pyc (FD)	2.74×10^{-4}	—	X	X	X	X	X
Gal (P)	2.23×10^{-4}	—	X	X	X	X	X
Gal (F)	1.55×10^{-4}	—	X	X	X	X	X
Gal (PD)	3.29×10^{-4}	—	X	X	X	X	X
Gal (FD)	1.80×10^{-4}	—	X	X	X	X	X
Typ (PD)	2.02×10^{-3}	O	O	O	X	X	X
Dad (PD)	2.51×10^{-3}	O	O	O	X	X	X
Lzt (PD)	2.29×10^{-3}	O	O	X	X	X	X
Gls (PD)	1.43×10^{-3}	O	O	X	X	X	X
Cop (PD)	2.10×10^{-3}	XX	XX	X	X	X	X
Sel (PD)	3.01×10^{-3}	O	O	X	X	X	X
Lel (PD)	2.83×10^{-3}	XX	X	O	X	X	X
Sel (P)	1.70×10^{-3}	O	O	O	X	X	X
Sel (F)	1.03×10^{-3}	O	O	X	X	X	X
Sel (PD)	1.78×10^{-3}	O	O	O	X	X	X
Sel (FD)	1.34×10^{-3}	O	O	O	X	X	X

a) Each sample was taken up with a capillary glass tube from the original solution of the decayed wood meals and used for the bioassay.

b) Each sample was taken up with a capillary glass tube from the solution prepared by diluting the original solution to a 1/10, $1/10^2$, $1/10^3$ concentration... by volume and used for the bioassay.

c) O: Trail following activity. X: No trial following activity.
XX: Repellent activity. —: Bioassay was not performed.

The extract of Cop(PD) showed the repellent activity at the concentrations of above 10^{-4} g, and that of Lel(PD) also had the repellent activity only at the order of 10^{-3} g. However, when compared with the data of Table 5 which were

Table 5*. Response to the extract of the decayed wood blocks.

Kind of decayed wood blocks[a)]	Weight (order) in the original solution and its diluted solution[b)] (g) and the results of the response[c)]				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Typ blocks	O	O	X	X	—
Dad blocks	O	O	X	X	—
Lzt blocks	O	O	O	X	X
Gls blocks	X	X	X	X	X
Cop blocks	—	XX	XX	X	X
Sel blocks	—	O	O	X	X
Lel blocks	XX	XX	X	X	—

* Summarized from the Table 2 of the preceding paper[6)].

a) Each sample was taken up with a capillary glass tube from the original solution of 25 decayed wood blocks and used for the bioassay.

b), c) See Table 4.

already mentioned by the present authors[6)], the termite responded only at the higher concentrations. In the case of the delignified wood meal, therefore, it seems that the repellent substance was not produced so much as in the case of the normal wood meal, but the further investigations are needed on this problem.

As for the white rot fungi, all the extracts of the wood meals, whether delignified or not, showed no trail-following or no repellent activity. It was the best record that one termite walked on the circumference of the extract of Lzb(PD) at the order 10^{-5} g for 9 sec., and it was the second record that 2 termites walked on those of the extracts of Cov(P) and Pyc(F) at the order of 10^{-5} g for 5 sec respectively. The other termites walked only 3 sec. at most in any other extracts. Therefore, it seems that a trail-following substance was not produced in the case of white rot fungi.

Consequently, in the case of the wood meals decayed by brown rot fungi, a trail-following activity may be apt to appear by delignification, but not in white rot fungi. This may be due to the difference of the decay mechanism between white rot fungi and brown rot fungi.

Further investigations are needed to resolve the problems on the producing mechanism of a trail-following substance in the decayed wood.

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