

THE INFLUENCE OF LABORATORY MAINTENANCE ON LIGNIN DEGRADATION  
BY *NASUTITERMES EXITIOSUS* (HILL)

L.J. Cookson

CSIRO Division of Chemical & Wood Technology

ABSTRACT

Two hardwoods, *Acer rubrum* L. and *Eucalyptus regnans* F.Muell., were labelled with  $^{14}\text{C}$  almost exclusively in their lignin components. The ability of termites from several colonies of *Nasutitermes exitiosus* (Hill) to degrade these lignins was found to decrease after about four months of laboratory maintenance. Termites maintained for six months did not retain radioactive lignin or its breakdown products in their bodies as long as freshly collected termites from the field. These differences may be due to changes in the bacterial flora of the termite gut. Laboratory maintained termites also had less workers/g of termites than fresh termites from the same colony. It is recommended that termites be used within three months of their collection from the field.

INTRODUCTION

Recently it was demonstrated that *Nasutitermes exitiosus* (Hill) could degrade small amounts of certain lignins (3,5,12). Estimates for the degradation of side chains in lignin ranged from 12-17% for maize and synthetic lignins (3) to 5-6% for hardwood lignins (5). These studies were made possible through the development of methods for the preparation of  $^{14}\text{C}$ -labelled lignins (7,15). Other lignin substrates and methods for lignin analysis often produced only ambiguous results in biodegradation studies (8,14).

The results for *N. exitiosus* were obtained from termites freshly collected from the field. However, *N. exitiosus* is often maintained in the laboratory for periods of up to eighteen months (13), although in practice

termites are used in our laboratories for pesticide bioassays within three months of collection (Creffield, pers. comm.). *N. exitiosus* and *Coptotermes acinaciformis* (Froggatt) do not form substitute reproductives (neotenics) in the laboratory. This compares with many species of Kalotermitidae, Termopsidae, *Heterotermes* and *Reticulitermes* which readily form neotenics and can therefore be bred in the laboratory, rather than only maintained (1).

It was considered that prolonged laboratory maintenance, especially in the absence of reproductive castes, could be detrimental to the vitality of *N. exitiosus*. This possibility was examined using  $^{14}\text{C}$ -lignin degradation as an indicator.

#### MATERIALS AND METHODS

##### i. PREPARATION OF $^{14}\text{C}$ -(LIGNIN)-LIGNOCELLULOSES

The lignin component of *Eucalyptus regnans* F.Muell. and *Acer rubrum* L. (red maple) was selectively labelled in small branchlets by infusing them with [ $3\text{'-}^{14}\text{C}$ (side chain)] cinnamic acid dissolved in distilled water. The branchlets were held upright in test tubes containing water and left to metabolize the cinnamic acid for seven days under an artificial light/dark cycle. They were then stripped of bark and leaves, sliced into 50% methanol, and extracted for 4 h. Four hourly extractions with 50%, 75%, and 100% methanol followed. The slices were dried ( $80^{\circ}\text{C}$ ), ground to pass a 60 mesh sieve, and the wood meal thoroughly extracted with water, ethanol and ethanol:benzene (1:1) (9).

*Eucalyptus* sp. are unusual in that not all of the polyphenolic extractives are removed by the above extraction procedure, therefore, a 0.5% NaOH extraction at  $98^{\circ}\text{C}$  for 1 h was used to remove these from *E. regnans* (4).

Analyses were performed on samples of the wood meals to determine the proportions of radioactivity located in the Klason lignin (10), polysaccharides (17), and aromatic amino acids (6). Esterified phenolic acids remaining in *A. rubrum* were removed from a sample using 1N NaOH and

collected by acidifying the hydrolysate to pH 2 and extracting with diethyl ether (11). The  $^{14}\text{C}$  present in the ether was then determined.

## ii. BIOASSAYS

A total of six different colonies of *N. exitiosus* were collected from Tallarook, Victoria, at various times during the year (Table 1). The termites were maintained as 50 g groups in the mound material of *Coptotermes lacteus* (Froggatt) contained in 1 litre jars (13).

TABLE 1

The influence of laboratory maintenance on caste numbers/g of *N. exitiosus* (mean of 5 replicates). W = workers. S = soldiers, N = nymphs.

Colony No.	Date collected	Period of laboratory maintenance (weeks)											
		0			7			10		16		51	
		W	S	N	W	S	N	W	S	W	S	W	S
1	4-11-81	256.4	11.8	0								193.2	32.3
2	26-3-82	212.4	34.6	8.6	200.6	41.8	12.6						
3	29-7-82												
4	1-10-82	258.4	1.2	0									
5	1-12-82	257.4	12.0	0									
7	12-3-83	229.0	12.4	0				215.6	13.8	197.8	11.0		

Termites (from several jars) from the same colony were removed after various periods of laboratory maintenance and placed in 1 g groups in Erlenmeyer flasks in order to determine their ability to degrade lignin. Each flask was sealed with a rubber bung (equipped with gassing ports) from which a vial was suspended that contained a saturated solution of  $\text{KH}_2\text{PO}_4$  to produce a relative humidity of 96% in the flask. Incubation was at  $26^\circ\text{C}$ . After three days, when all of the  $^{14}\text{C}$ -(lignin)-lignocelluloses that were offered had been eaten, moist vermiculite (250% M.C.) and a small block of *E. regnans* were added to each flask.

The degradation of the  $^{14}\text{C}$ -(lignin)-lignocelluloses by *N. exitiosus* was determined by finding the percentage of  $^{14}\text{C}$  offered to the termites that was converted to  $^{14}\text{CO}_2$  after fourteen days. The flasks were flushed with

air every 2-4 days (100 ml/min. for 15 min.) and the CO<sub>2</sub> collected directly into ethanolamine-containing scintillation fluid (15).

## RESULTS

The amount of radioactivity located in the non-lignin components of *A. rubrum* and *E. regnans* was less than 2% and 1% respectively (Table 2). Degradations above these figures indicate lignin degradation.

The caste ratios were determined for three colonies of *N. exitiosus* after laboratory maintenance (Table 1). The number of workers/g of termites decreased with laboratory maintenance.

TABLE 2

Distribution of radioactivity in extracted <sup>14</sup>C-(lignin)-lignocelluloses (mean of 3 replicates)

Lignocellulose	Klason lignin	Klason filtrate	Radioactivity (%)				Total non-lignin
			Polysaccharides	Aromatic amino acids	Bound phenolic acids		
<i>E. regnans</i>	78.0	14.2	0.4	0.5	0	0.9	
<i>A. rubrum</i>	81.6	12.3	0.3	0.3	1.2	1.8	

The survival in the flasks of workers from two colonies maintained for seven and sixteen weeks was 94.3% and 92.2% respectively, which was similar to the survival of freshly collected workers. However, the population maintained for fifty-one weeks had a significantly lower survival (83.5%) compared to fresh termites (94.4%).

Termites that had been previously maintained in the laboratory for periods ranging from 4 to 12 months, in each case had a significantly lower lignin degrading ability compared to freshly collected termites (Tables 3 and 4). One colony (No. 5) was sampled monthly for six months following its collection from the field. After maintenance periods of 1, 2 and 3 months, degradation was as great as when the termites were first collected. At least 7% of the label was degraded, of which at least 5%

represented lignin degradation. After one month of laboratory maintenance there was actually a slight increase in lignin degradation (95% significant). After maintenance periods (colony No. 5) of 4-6 months there was a progressive decline in lignin degrading ability. Termites maintained for six months degraded 3.10% of the label, of which at least 1.1% was lignin degradation, i.e. a 78% decrease from the lignin degradation by freshly collected termites.

TABLE 3

Influence of laboratory maintenance of *N. exitiosus* on its ability to degrade  $^{14}\text{C}$ -(lignin)-*A. rubrum*. Mean (%) of  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  over 14 days (5 replicates)

Colony No.	Period of laboratory maintenance (months)						
	0	1	2	3	4	5	6
5	7.01*	8.34	7.30	7.49	6.05	4.18	3.10
7	7.29		7.24		5.11		3.82

\* 3 replicates

TABLE 4

Influence of laboratory maintenance of *N. exitiosus* on its ability to degrade  $^{14}\text{C}$ -lignin. Mean (%) of  $^{14}\text{C}$  released as  $^{14}\text{CO}_2$  over 14 days (5 replicates)

Colony No.	<i>A. rubrum</i>			<i>E. regnans</i>				
	Period of lab. maint. (mths)	0	8	12	Period of lab. maint. (mths)	0	6	12
Several	6.91**				5.05***			
2							1.05	
3				2.72				2.04*
4		4.60						

\* 3 replicates

\*\* mean of 5 colonies

\*\*\* mean of 3 colonies

Populations of *N. exitiosus* that had been maintained for six months, were found not to contain  $^{14}\text{C}$  in their bodies after they had fed for fourteen days on  $^{14}\text{C}$ -(lignin)-*E. regnans*. This compares to a similar bioassay using freshly collected termites. After fourteen days the fresh termites contained a mean of 48.7 dpm/10 workers. This represented about 2.0% of the total original  $^{14}\text{C}$  that had been added as food.

#### DISCUSSION

The number of workers/g of termites tended to decrease for *N. exitiosus* after laboratory maintenance, due to a change of instar-range distribution as workers developed and the early instars were not replaced. Presumably this would not occur in laboratory bred termites due to the production of eggs by reproductives.

As lignin degradation by *N. exitiosus* requires the presence of endosymbiotic bacteria (Cookson, unpubl. data), the decrease in lignin degradation after prolonged laboratory maintenance suggests that the gut flora has changed. After a long period of laboratory maintenance, *Anocanthotermes* sp. (16) and *Reticulitermes flavipes* (Kollar) (2) contained differences in the relative abundance of their gut bacteria compared to freshly collected termites from the field.

If some gut bacteria in fresh termites are able to assimilate  $^{14}\text{C}$ -lignin breakdown products, an alteration of the gut flora could also explain the lack of radioactivity found in six month laboratory maintained termites after day 14 of the bioassays. On the other hand, it is possible that trophallactic exchange or coprophagic behaviour was less frequent in the six month maintained termites, factors which would recirculate food in freshly collected termites.

Previous reports have shown that methane production (19) and nitrogen fixation (18) by termites decreased after they were introduced into the laboratory.

## CONCLUSIONS

The ability of *N. exitiosus* to degrade lignin was found to decrease after laboratory maintenance periods of four or more months. For example, a population maintained for six months degraded 78% less lignin than freshly collected termites from the field. Therefore, bioassays using *N. exitiosus* that were maintained for these periods may also become less relatable to field results.

## REFERENCES

1. Becker, G. 1969. Rearing of termites and testing methods used in the laboratory. In "Biology of Termites". Vol. 1 (Eds K. Krishna and F.M. Weesner). New York, Academic Press, pp. 351-385.
2. Breznak, J.A. and Pankratz, H.S. 1977. In situ morphology of the gut microbiota of wood-eating termites [*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki]. Appl. Environ. Microbiol. 33: 406-426.
3. Butler, J.H.A. and Buckerfield, J.C. 1979. Digestion of lignin by termites. Soil Biol. Biochem. 11: 507-511.
4. Cohen, W.E. 1934. The chemistry of Australian timbers. Part 4 - A study of the lignin determination II. CSIR Pamphlet No. 51.
5. Cookson, L.J. 1983. The role of lignin in the nutrition of several Australian termites. Internat. Res. Group on Wood. Pres. Document No. IRG/WP/1191.
6. Crawford, D.L. 1978. Lignocellulose decomposition by selected *Streptomyces* strains. Appl. Environ. Microbiol. 35: 1041-1045.
7. Crawford, D.L. and Crawford, R.L. 1976. Microbial degradation of lignocellulose: the lignin component. Appl. Environ. Microbiol. 31: 714-717.
8. Crawford, R.L. 1981. Lignin biodegradation and transformation. New York, John Wiley & Sons.
9. Crawford, R.L. and Crawford, D.L. 1978. Radioisotopic methods for the study of lignin biodegradation. Dev. Ind. Microbiol. 19: 35-49.
10. Effland, M.J. 1977. Modified procedure to determine acid-insoluble lignin in wood and pulp. Tappi 60: 143-144.
11. El-Basyouni, S.Z., Neish, A.C. and Towers G.H.N. 1964. The phenolic acids in wheat III. Insoluble derivatives of phenolic cinnamic acids as natural intermediates in lignin biosynthesis. Phytochem. 3: 627-639.

12. French, J.R.J. and Bland, D.E. 1975. Lignin degradation in the termites *Coptotermes lacteus* and *Nasutitermes exitiosus*. *Material u Organismen* 10: 281-288.
13. Gay, F.J., Greaves, T., Holdaway, F.G. and Wetherly, A.H. 1955. Standard laboratory colonies of termites for evaluating the resistance of timber, timber preservatives, and other materials to termite attack. *Bull. CSIRO No.* 277.
14. Kirk, T.K. 1971. Effects of microorganisms on lignin. *Ann. Rev. Phytopathol.* 9: 185-210.
15. Kirk, T.K., Connors, W.J., Bleam, R.D., Hackett, W.F. and Zeikus, J.C. 1975. Preparation and microbial decomposition of synthetic [<sup>14</sup>C] lignins. *Proc. Natl. Acad. Sci. USA* 72: 2515-2519.
16. Krasil'nikov, N.A. and Satdykov, S.I. 1970. Bacteria of termites' intestines. *Microbiologiya* 39: 562-564.
17. Moore, W.E. and Johnson, D.B. 1967. Procedures for the chemical analysis of wood and wood products. USDA Forest Service, Forest Products Laboratory, Madison.
18. Prestwich, G.D., Bentley, B.L. and Carpenter, E.J. (1980). Nitrogen sources for neotropical nasute termites: fixation and selective foraging. *Oecologia (Berl.)* 46: 397-401.
19. Zimmerman, P.R., Greenberg, J.P., Wandiga, S.O. and Crutzen, P.J. 1982. Termites: a potentially large source of atmospheric methane, carbon dioxide, and molecular hydrogen. *Science* 218: 563-565.

# 21st FOREST PRODUCTS RESEARCH CONFERENCE

19-23 NOVEMBER, 1964

HELD AT

**CSIRO**

DIVISION OF CHEMICAL AND WOOD TECHNOLOGY  
CLAYTON, VICTORIA,  
Australia

VOLUME 1

- BIODETERIORATION AND CHEMICAL TREATMENT OF WOOD
- FIBRE AND CHEMICALS FROM WOOD