# THE SIGNIFICANCE OF FUNGAL DECAY OF *EUCALYPTUS REGNANS* F. MUELL. HEARTWOOD FOR SOME ASPECTS OF THE BIOLOGY OF *COPTOTERMES LACTEUS* (FROGGATT)

A thesis submitted for the degree of Master of Science of the Australian National University.

by
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## Statement of Originality

Except where specific acknowledgement is given this thesis is my original work

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## Table of Contents

## List of Tables

## List of Figures

## List of Plates

## List of Appendices

## Abstract

The biology of the termite *Coptotermes lacteus* (Froggatt) was investigated with the hope of understanding the basis for this species preferential attack of decaying rather than sound wood. Six wood rotting basidiomycetes, three brown rots, *Coniophora olivacea* (Fr.) *Trametes lilacino-gilva* (Berk.) Lloyd and *Gloeophyllum trabeum* (Pers. ex Fr.) Murr.and three white rots- *Perenniporia tephropora* (Mont.) Ryv., *Pycnoporus coccineus* (Fr. ) Bond & Singer and *Tinctoporellus epimiltinus* (Berk. & Br.) Ryv., were used to inoculate *Eucalyptus regnans* F. Muell. heartwood. Interspecific differences in fungal decomposition rate and wood consumption by termites were examined. Fungal species was a significant factor in determining wood consumption and termite survival. Wood consumption was inversely related to termite survival. Termites maintained on wood inoculated with *T. lilacino-gilva* had the highest consumption rate and the lowest mortality level. Brown rots in general were found to enhance termite colony development. The amount of nitrogen in the wood could not be related to colony development as it did not vary significantly between the wood treatments used to investigate that aspect of termite biology. Nonetheless, there was a trend towards greater wood consumption at higher nitrogen levels.

The preference of *C. lacteus* for wood inoculated with *T. lilacino-gilva* has the potential to be used in the development of integrated termite control based on using wood treated in such a fashion as a biological bait. This would allow a reduction in the amount of toxic chemicals currently being applied which would have both ecological and economic advantages.

# CHAPTER 1

## General introduction

## Introduction

Termites play an important role in recycling of nutrients locked up in wood. Some termite species attack living wood, others feed on sound dead wood and yet others use decayed wood as a principal food source (Lee and Wood 1971). However, when termites attack and destroy commercially valuable trees and timber in use, they antagonise human interests and come to be regarded as pests. Exotic trees are particularly vulnerable to attack when grown in the tropics and sub-tropics where they are subject to drought stress and have no physiological strategies to withstand the difficult dry months (Harris 1971) . Studies of the relationship between termite hazard and amount of rainfall received in parts of Uganda showed that rainfall distribution contributed to the gravity of the problem. Rainfall only needed to fall below a minimum requirement for a given time period and trees growing in areas with extreme rainfall distribution become more prone to termite damage.

Exotic tree species have advantages over local tropical species in some, but not all, areas. In Kenya, for example, some species of the exotic genus *Eucalyptus* are suitable for fuelwood production in the arid and semi-arid areas because, among other things, the trees grow more rapidly than the local species. They are, however, subject to drought stress which makes them vulnerable to termite attack (Evans 1990; Wood 1991; Harris 1971). Juvenile stages of exotic tree species are often more susceptible to termite attack is often higher native tree species which have evolved as part of the local ecosystem. *Macrotermes* were reported to be serious pests of eucalypt and wattle saplings (from planting time up to 9 months) when these were grown on previously unforested land in South Africa (Atkinson 1989). Bowler and Forti (1990) reported serious termite problems in eucalypt plantations which are grown for pulp and paper production in Brazil. Termite control is therefore essential for the success of forestry projects in which exotic tree species are grown.

The long-persistence organochlorine-based termiticides which were commonly used in the past are now banned from use in many developed countries after they were linked with the contamination of the food chain and human dwellings. Developing countries are also under great external and local pressure to minimize the use of such compounds especially because the required standards of pesticide management are rarely achieved (Wood 1991). Nevertheless, most successful methods of termite control still involve the application of repellent chemicals around the structure to be protected. This has been adopted in the protection of tree seedlings by growing them in treated soil. In the S. African situation referred to earlier, for example, contact and systemic chemicals such as controlled -release granules provided effective protection against the termite pest.

Difficulties associated with the application of contact insecticides often reduce the effectiveness of chemical control.

A biological control method directed at the source of termites would be a more practical, cost-effective and environmentally safe measure of protection. Also, as more than one termite species is often present in a given locality, it is important to understand the biology of the particular problem termite in order to formulate control strategies which target it. Measures founded on such a basis would be ecologically sound, causing the least ecological disruption while keeping the termite problem under control.

The evolution of safe termite control methods has focussed on limiting the amount of chemical introduced in the environment. Much research is therefore being undertaken to formulate suitable alternatives which use small chemical doses of short-lived and specific chemicals (Jones, 1988). Slow acting poisons which allow the workers time to reach their nests and pass the poison to the dependent castes are recommended. In addition, a suitable dispensation mechanism for the chemical is required (LaFage 1988) and it is with regard to this that the wooden bait system has received a great deal of attention.

Many of the studies on the interaction between termites and wood decay fungi are centred on the evaluation of wood suitability as baits, especially after Beal and Esenther (1980) demonstrated that *Reticulitermes* species were drawn to decayed wood in which non-repellent Dechlorane (mirex) was impregnated. Termites consumed it and took the poison back to their colonies where the population declined and this suppressed termite activity in the area for 3.5 years. The desirable characteristics in a bait system were outlined by Mauldin *et al.* (1985). One characteristic of a successful bait would be that termites would choose to consume it more than all other available food.

Termites are especially well-suited for control with a bait-system as they have a specialised caste system in their social organisation which assigns the duty of food collection to the workers. In subterranean termites, workers forage for food and take it back to feed the dependent castes in the colony. The food that termites take is governed by their preference for the decayed or sound wood. Termite responses to wood in its sound and decayed state have therefore become the subject of much research.

Termite feeding responses where they are provided with a single choice of food have dominated termite feeding response studies. The situation where termites have a choice of wood must therefore receive more attention now more than ever before. More work on termite feeding responses has also been carried out in the laboratory than in the field. Ruyooka and Groves (1980) found that it was complex interactions between several factors that governed the choice of wood. These people are among the few who have

carried out choice-feeding studies both in the laboratory and field. They found that the radial variability in *E. regnans*, for example, was only portrayed in the field but not in the laboratory.

Broad comparisons of consumption of wood by termites have been made between termite species, wood species, the type and species of fungus responsible for wood decay as well as the decay level in the wood. Amburgey and Smythe (1977a, 1977b), Amburgey (1979), Becker (1965) , French *et al.* (1981) and Ruyooka 1978 reported that the various species of termites in their experiments consumed more decayed than undecayed wood. Benefits that could attract termites to feed on decayed wood include its decreased mechanical strength and increased nitrogen content (Hendee 1935; ; Waller and LaFage 1987). The nitrogen content of wood could also be an important factor of wood consumption (Collins 1983). Whether the wood was sterilized or not after it was decayed also had an impact on its consumption (Amburgey and Smythe 1977a; French *et al.* 1981; Lenz *et al.* 1980; Smythe *et al.* 1971; Tyangi *et al.* (1982,1984)). It would be expected that termites would show preference in the the long-term for those types of wood which enhance their survival. The findings of various people suggest that on decayed wood, survival can be affected significantly by the species of fungus responsible for decay in the wood (Amburgey 1979; Becker 1965).

It is also not well-understood how the termite colony development proceeds under different diets. Becker (1965) and Lenz and Becker (1975) found decayed pine wood increased neotenic development rate of *Heterotermes indicola.* Much more work remains to be done in this area . We have especially no understanding about the extent to which the quality of wood governs the choice of nutrition for a colony founding reproductive pair.

It follows that the study of wood consumption, termite survival and incipient colony development should be of value. I chose to work with *C. lacteus* which prefers feeding on decayed to sound wood (Lenz *et al.* 1987), to find out more about the significance of decayed wood for its biology. Unsterilized wood has been used only in a few studies even though there is awareness that changes which occur in decayed wood during the sterilization process could affect the manner in which termites respond to the wood (Ruyooka 1978). I therefore decided to use air -dried wood as it approximated the state in which wood-eating subterranean termites were likely to encounter dry wood when foraging.

## Objectives

The main objectives of the study were to quantify the effects of decayed heartwood *Eucalyptus regnans* F. Muell. on the following aspects of *Coptotermes lacteus* (Froggatt) biology:

i) wood consumption where a single wood treatment was offered

ii) wood consumption where a choice of decayed wood blocks was offered

iii) factors governing survival of the termite species

iv) incipient colony development in the laboratory

A further aim was to assess the suitability of *E. regnans* heartwood after exposure to selected basidiomycete fungi as a bait for *C. lacteus* based on laboratory-collected data.

The work is presented in sections each of which deals with a different aspect of the study. It is intended to submit Chapters 4 - 6 as papers for publication in scientific journals in future. Thus, results, discussion and literature cited are presented for every chapter.

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# CHAPTER 2

## General materials and methods

## Introduction

Several experiments investigated the significance of decayed *Eucalyptus regnans* F. Muell. heartwood for the biology of *Coptotermes lacteus* (Froggatt). Termite bioassays used wood which was exposed to basidiomycete fungi in a preceding fungal bioassay. An analysis of the ammonium nitrogen content of this wood was also done to assess whether there was a correlation between the responses reported in the termite bioassays and the nitrogen content of the wood presented to the termites. This chapter deals with the general materials and methods used for the termite and fungus experiments leaving out the fine details which are covered in the relevant chapters.

## Materials

### Timber

*Eucalyptus regnans* is classified as a non-durable commercial timber (durability class 4 - a few years' or less service) in the 'tentative durability rating' of Australian timbers by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) (Thornton *et al.* (1983). The basis of the classification is the performance of untreated heartwood of reasonable dimensions (> 40mm thick) when placed in ground contact. The conditions under which the timber specimens are tested include exposure to varying degrees of decay and termite hazards. The low rating of *E. regnans* in this respect made it an attractive choice for studying the relationship between termites and decayed wood. For this reason, this timber species has been used for decades in various experiments with Australian termites.

### Fungi

Only basidiomycete fungi were tested in this study. The brown and white rots on which much of the previous work was based both in Australia and abroad (Amburgey 1979; French 1978; Lenz *et al.* 1980; Ruyooka 1978) were reviewed. Three fungi capable of causing each rot type were selected on the basis of

i) their growth rates on malt-agar and
ii) their ability to decay *E. regnans* wood in service

One of the advantages of working with fast- growing fungi is that the chances of contamination of the decay chambers are greatly reduced. The fungi which were finally selected (Table 2.1) were cultured from the paraffin oil collection of the CSIRO Division of Forest Products, Melbourne.

The full descriptions of the fungi are given in various texts (Cunningham 1965; Gilbertson and Ryvarden 1986; Gilbertson and Ryvarden 1987; Ginns 1982; Stalpers 1978; Walters 1973). Summaries compiled from these texts are presented in Appendix 2.

The observations made during the fungal bioassay have also been provided as supplementary data in Appendix 2.

Table 2.1 Fungi which were selected for the study of termite-fungus-wood relationships.

| Type of rot | Fungus species | CSIRO DFP Strain No. |
|---|---|---|
| Brown cubical rot | *Coniophora olivacea* (Fr.) Karst. | 1779 |
| | *Trametes lilacino-gilva* (Berk.) Lloyd | 1109 |
| | *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. | |
| | (Syn. *Lenzites trabea* (Pers. ex Fr.) ( Fr.) | 7520 |
| White stringy rot | *Perenniporia tephropora* (Mont.) Ryv. | |
| | (Syn. *Fomes lividus* (Kalch.) (Sacc) | 7904 |
| | *Pycnoporus coccineus (Fr.)* Bond et. Singer | 1095 |
| | *Tinctoporellus epimiltinus* (Berk. & Br.) Ryv. | |
| | (Syn. *Poria barbonica* Pat.) | |
| | *Polyporus epimiltinus* Berk. & Br.) | 14579A |

**Termites**

Rhinotermitidae are described as "small wood-eating termites, subterranean in habit" by Harris (1971). The subfamily Coptotermitinae, to which the *Coptotermes* genus belongs, has alates with circular heads, small clypeus and relatively broad pronotum. Soldiers have more or less pear-shaped heads, narrower towards the front, with large fontanelle and pointed labrum. Mandibles are slender and sharp-pointed without marginal teeth.

Members of the genus *Coptotermes* are present throughout the tropics and are especially well-represented in the Australian and Malayan regions. Some species in this genus build mounds in all parts where they occur in Australia. It has been proposed that this unusual mound-building behaviour fills the ecological niche that is occupied by fungus-growing termites in Africa and Asia (Harris 1971). There are six species in the genus *Coptotermes*, one of which, *Coptotermes lacteus*, was used for this investigation. According to (Gay *et al.*(1955), *C. lacteus* occurs as an obligatory mound-builder in the Canberra District.

The genus *Coptotermes* is of outstanding importance in in ecological terms in most parts of Australia. The genus is largely dependent on eucalypts for food with the exception of one species (*C. dreghorni* Hill) which occurs in a limited area of tropical forest. *C. acinaciformis* (Froggatt) and *C. frenchi* Hill attack growing trees to the extent of being considered forest pests (Gay and Calaby 1970).

A full description of *C. lacteus* was given by Gay and Calaby (1970). The species occurs along the south-eastern coast of Australia, from Victoria to southern Queensland. It is found in dense or open forest country or in pastoral country at elevations up to 1000m (3500ft) above sea level. *C. lacteus* builds mounds over most of this range except in northern New South Wales and southern Queensland where colonies occupy rotting logs. The food of *C. lacteus* consists mainly of wood litter on the ground, as well as stumps and logs. *C. lacteus* is also recorded to attack fence posts, poles and occasionally, the timber in buildings in rural areas, but is not of any significant economic importance. It rarely attacks living trees (Gay and Calaby 1970; Watson 1988).

*C. lacteus* mounds attain a height of 2.5m (8ft) and a basal diameter of 2.1m (7ft). The inner nest comprises many galleries and chambers which termites occupy. It is surrounded by a thick clay casing (up to 30cm and more) which has very few galleries. Food sources away from the mound are reached by a series of underground tunnels which are built at a depth of 10-40cm (4-16in) below ground level. The galleries extend up to 18m (60yds) away from the centre of the mound.

The species is taken to be representative of the *Coptotermes* genus in the Canberra region where *C. acinaciformis*, the main pest species, occurs only in a "few, widely-scattered localities" (Watson 1988). *C. lacteus* was therefore chosen for this study on the basis of its local availability, its mound-building characteristics which make it easy to collect in large numbers (Gay *et al.* 1955) and its preference for decayed wood as reported by Lenz *et al.* (1980) and Lenz *et al.* (1991).

## Methods

### Fungal bioassay: Preparation of decayed wood specimens

A plank of air -dried *E. regnans* heartwood from two different trees was randomly selected from the supplies available at the CSIRO Division of Entomology. Each plank was sawn into blocks whose final sanded dimensions were approximately 20 x 20 x 10mm. This size was small enough for substantial mass-losses to occur in the short duration of the bioassay and for the blocks to remain firm enough to handle after the bioassay. The plank from tree 1 had fewer defects (resin veins, knots) than the one from tree 2. Consequently, tree 1 yielded more defect-free (clear) blocks than tree 2. The clear blocks were randomly allocated into groups, consisting of 25-30 blocks, from each tree.

The groups of wood blocks were laid out on wire racks and kept in a temperature- and relative humidity (RH)-controlled room in the Division of Forestry and Forest Products, Highett, Melbourne where the fungal bioassay was carried out. The room conditions approximated 22°C and 43% RH. The equilibrated mass of the blocks, before and after the bioassay, was recorded under these conditions. As a final step in their preparation for the fungal bioassay, the blocks which were to go into decay chambers were placed into plastic containers and sterilized by exposure to 2.5 kGy of Gamma radiation.

The untreated/moisture controls consisted of wood blocks that remained on wire racks in the incubation room throughout the period of the bioassay.They were sterilized in the same manner as described above at the conclusion of the bioassay.The sterile control blocks were treated like those placed in decay chambers except that the control chambers were not inoculated with decay fungi.

Wood blocks were exposed to basidiomycete fungi for 5, 7 or 9 weeks with the aim of producing a range of mass-loss levels in the wood. At the conclusion of the fungal bioassay, the wood blocks were air-dried and not sterilized. Others have used mycelia that are killed using various sterilization methods and very few studies have been carried out with live mycelia (Ruyooka 1978). The studies currently reported therefore involved mycelia in a state as close as possible to what occurs naturally in dry wood.

More details of the fungal bioassay are given in Chapter 3.

**Termite bioassays**

*C. lacteus* was obtained from mounds at "Birkenburn", (149° 37' E, 35° 15'S), a grazing property in the Bungendore area, 50km east-north-east of Canberra. The termite bioassays were carried out in the CSIRO Division of Entomology laboratories, Canberra, Australia.

**Alate collection for colony development studies**

Gay *et al.* (1955) reported that *C. lacteus* alates are released from September to January in the Canberra region. Colonizing flights usually take place late in the afternoon, especially on calm days. Alates leave the colony in a single flight, but occasionally, second and sometimes third flights follow (Gay and Calaby 1970).

In late September, 1990, a total of six noticeably active mounds (from evidence of recent construction on their exterior) were monitored for alate flight. Details of all the six mounds are given in Appendix 3. The alates for colony development studies would be collected from the first two mounds which released them the earliest and termites for the other aspects of termite studies would subsequently be obtained from the same two

colonies. Details about the trapping method for alates and the culturing of incipient termite colonies are given in Chapter 6.

**Collection methods for studies of the feeding responses of *C. lacteus***

The method of termite collection followed was one described by Gay *et al.* (1955). It is a standard handling procedure for *C. lacteus* in laboratory tests in Canberra. The caste composition of termites in the experiments remained as determined by this method of collection.

**Methods used for studies of the termite feeding response**

Details of the methods used for studying the feeding response and survival of termites when put where only one type of wood was available are presented in Chapter 4. The methods of assessing the termite feeding responses where termites were offered a choice of wood blocks are detailed in Chapter 5.

**Termite maintenance conditions in the laboratory**

*C. lacteus* is most successfully maintained on a matrix of inner carton material of its own nests . This material has a good water-holding capacity and contains a high percentage of nutritious organic material (Lee and Wood 1971). A slight modification of the method described by Lenz *et al.* (1987) for *C. lacteus* maintenance in the laboratory was followed. Inner carton material (14% moisture content (MC) in its laboratory equilibrated state) was ground to a fine powder. To provide the correct environment for termites in all experiments, the moisture content of the matrix was raised to 100%.

Plastic vials (120ml) were used for incipient colony development and the termite feeding response experiments. After introducing termites into the vials, a filter paper strip was placed on the rim of the vial to absorb any moisture condensing at that point. This ensured that air continued to flow freely in and out of the vial even after the screw type lid was loosely fitted.

For the choice-feeding experiments, the carton material was placed in 1.5l plastic bowls (Decor super-see-through storage bowls) such that when the carton material was moistened to 100% MC with distilled water, it swelled to fill the containers up to an approximate diameter of 16.3 cm. The lids of the containers were each fitted with a small section of fine wire gauze to permit air circulation.

Experiments were conducted in a constant temperature room at 29°C and 83% RH for varying lengths of time details of which are provided in the appropriate chapters. The wood which remained at the end of the termite experiments was conditioned in a room at 23°C and 65% RH and the equilibrated final mass of the wood recorded.

**Analysis of wood for its ammonium nitrogen content**

Each wood block was comminuted in a grinding mill fitted with a # 30 mesh sieve. The resultant sawdust was dried at 70°C for 48 hours. Samples (0.5g) of the sawdust were analysed for ammonium nitrogen. The method followed is described in greater detail in Chapter 7.

**Statistical analysis of results**

The analysis was based on statistical modelling and predictions in the range of values considered most appropriate for each set of experiments. Studies of termite colony development and the analysis of nitrogen in the wood exposed to the different fungi were based on balanced designs in which all factors were at equal levels. In the termite colony development experiment, this happened because the alates with which colonies were founded were released before the analysis of the fungal bioassay was completed. The assumption at that stage was that the mass-loss in wood exposed to the fungi was proportional to the duration of wood block exposure to the fungi. All other termite bioassay experimental designs were greatly influenced by the results of the analysis of the fungal bioassay. The levels of factors in these experiments differed one from the other and the designs were consequently unbalanced.

Since the range of mass-loss in wood varied with the fungus to which wood was exposed and and with the tree from which wood was exposed (for details see Chapter 3) the statistical analysis was done in two parts. To begin with, all fungi were compared by restricting the mass-loss level in the analysis to equivalent or less than 3%. The restriction of mass-loss to levels greater than 3% was the basis of the other part of the analysis.

The significance of results was based on the F- and t-tests at the 1% or 5% probability levels. Tables of means were provided for the significant factors and factor interactions. In the balanced experiments, ANOVA tables provided adequate summaries of the results. The standard error of the differences of means (SED) was doubled to give the least significant differences (LSD) with which levels of factors could be compared.

In the unbalanced experiments, a standard error of the mean (SEM) was given for each value in the table. The largest of these standard errors was chosen to calculate the LSD with which levels of the factors were compared. The LSD values were therefore approximate.

For those figures in the results sections which depict interaction effects, the LSD values provided facilitate comparisons within levels of the independent variate. Line graphs have also been used to enhance the differences between qualitative variables and caution is advised in interpreting the results. Only the labelled points have real meaning.

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# CHAPTER 3

## Fungal bioassay

## Introduction

The mass production of decayed wood blocks is an essential part of the decayed wood-block bait method of termite control. One of the methods of exposing wood to fungi is the soil-block technique (ASTM 1975) which was used by Amburgey and Smythe (1977a, 1977b). Another technique later developed by Amburgey *et al.* (1981) for decaying large numbers of blocks by *Gloeophyllum trabeum* was described as simple and inexpensive. The method developed by Smith (1982) was an improvement of (ASTM 1976) for the large-scale production of baits. The work of French *et al.* (1981) and French *et al.* (1987) was based on a different laboratory decay technique devised by Thornton (1979).

The technique used in this bioassay has been used by Johnson (pers. comm, 1991) in the bioassay of preservative-treated particle-board and plywood. It aimed at causing the least possible likelihood of contamination of the decay chambers by other microorganisms. It did not conform to any previously published method. Double the estimated number of wood blocks required was prepared in case of contamination(see Appendix 1). While ensuring adequate rates of decay for most fungi, the exposure of wood blocks to the fungi for five, seven or nine weeks was expected to give a wide range of mass-loss.

## Methods

### Timber

Details about the timber and the preparation of wood blocks for the fungal bioassay are given in Chapter 2.

### Fungi

Only basidiomycete fungi were involved in this study. Details about them are given in Chapter 2 and Appendix 2.

### Subculturing of decay fungi

All fungi were initially subcultured from storage vials onto media in in Petri dishes to provide inoculant for the decay chambers of the fungal bioassay. These Petri dishes were placed in a 25°C room to stimulate fungal growth.

**Preparation of decay chambers**

Each decay chamber was prepared by boiling 1 litre of nutrient medium ( 2% malt extract (Oxoid), 3% agar (Davis), 1l tap water) and pouring it into a 373 x 228 x 100mm stainless steel tray. The trays with the medium in them were wrapped in plastic bags and autoclaved at 121°C for one hour. The trays were then transferred to a laminar flow unit where the medium was allowed to cool and set. Inoculation and inspection of decay chambers took place in this unit. The stainless steel tray containing the medium and a sterile piece of Trical (see full details below) placed over the surface of the medium and with the autoclavable bag wrapped around it is hereafter referred to as a decay chamber.

**Inoculation of decay chambers with wood decay fungi**

To inoculate a decay chamber with its designated fungus, the agar in the Petri dish on which the inoculum was growing was cut into 0.5 $cm^3$ squares. The vigorously growing area around the edge of the fungal colony was the preferred inoculum. Six of these inoculant squares were introduced to the decay chamber and placed equidistantly over the surface of the nutrient medium. A 345 x 210mm piece of sterilized sheet of poly(vinyl chloride) mesh (Trical ®) was then placed on top of the inoculated nutrient medium in each chamber. This precaution was taken to prevent the blocks of wood, which would later be introduced to the chamber, from coming into direct contact with the surface of the medium. Such contact might have led to water-logging of the blocks, a condition which could have led to failure of colonisation and decay by the fungus due to inadequate aeration.

Decay chambers were each wrapped in a second plastic bag as a precaution against contamination during transit to the incubation room and while the colonisation of the culture medium by the fungi was in progress. The chambers remained in the incubation room approximately at 22°C and 43% RH until the culture medium in each chamber became fully colonised by fungal mycelia. Plate 3.1 shows, as an example, the state of the chambers inoculated with each fungus at the end of 4 weeks.

**Introduction of sterile wood blocks into decay chambers**

The bags wrapping the decay chambers were surface sterilised with 70% alcohol before being carefully opened to the barest extent necessary for the sterilized wood blocks to be shaken out of the sterile plastic containers and into the decay chambers. After rewrapping them, the chambers were shaken about lightly to distribute blocks more evenly on the surface of the mycelium.

Sterile control chambers were also prepared at this stage. Chambers were then transferred back to the 22°C incubation room where the outer wrapping bag was

discarded. Colonisation of blocks was allowed to proceed for 5, 7 or 9 weeks. The appearance of the incubation room during the period when the fungal bioassay was under way is portrayed in Plate 3.2.

**Removal of wood blocks from decay chambers.**

At the expiry of exposure of the wood blocks to the decay fungi, the decay chambers were taken out of the incubation room and opened. All the mycelia that could be peeled off each block were removed.

The blocks of wood were not to be sterilized and as such, rapid drying was considered instrumental in arresting the activities of the fungi. Thus the wood blocks were placed in a fume cupboard to accelerate air movement and hence to accelerate initial moisture loss. Their mass stabilized by the fourth day after which they were transferred to equilibrate in the incubation room ( 22°C, 43% RH). The equilibrated final mass of the blocks was recorded. Mass-loss was the measure of the amount of fungal activity that occurred in the wood blocks.

## Results

Details about the general growth features of the fungi and the condition of the wood blocks at the end of each duration of the fungal bioassay are given in Appendix 2. The state of the decay chambers at the end of 7 weeks is illustrated in Plates 3.3-4.

The tree from which wood was obtained was a significant factor of mass-loss at the 1% probability level. Blocks from tree 1 lost significantly less mass than those obtained from tree 2 (Fig. 3.1).

When data from both trees were combined and examined at the 1% probability level, the species of fungus the duration of wood block exposure to the fungi was found to be a significant factor of mass-loss. It was significantly higher at 9 weeks than at 5 or 7 weeks (Fig. 3.2(a)). There was no significant difference in mass-loss between 5 and 7 weeks of wood block exposure to fungi.

When data for all incubation periods were combined, the species of fungus to which wood was exposed was also a significant factor of mass-loss at (Fig 3.2 (b)). Overall, wood blocks exposed to the white rots lost significantly more mass than those exposed to the brown rots. Furthermore, among the brown rots, exposure to *Trametes lilacino-gilva* caused significantly higher mass-loss in wood than exposure to the other fungi. Among the white rots, the wood exposed to *Perenniporia tephropora* lost significantly more mass than that which was exposed to the other fungi.

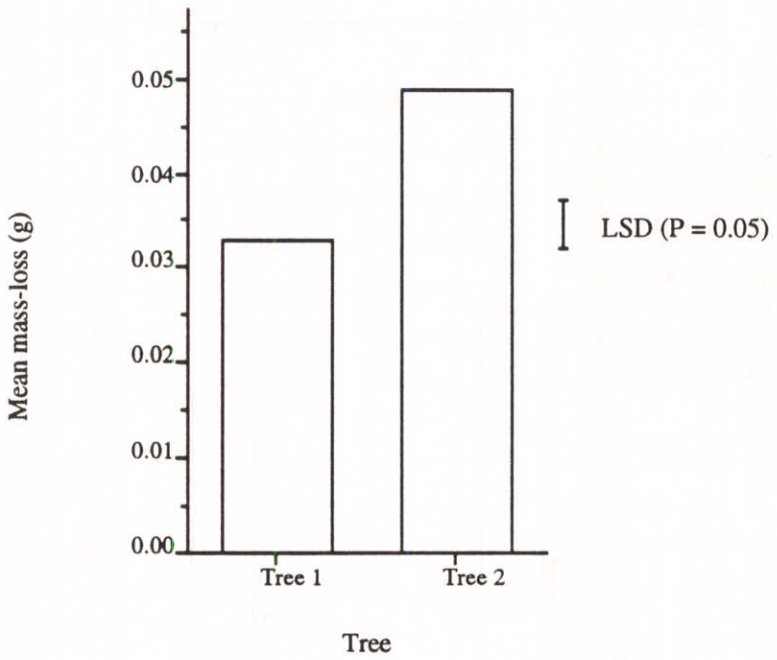



Figure 3.1: Mean mass-loss caused by all six fungi in wood, according to tree from which wood was obtained. LSD are approximate and based on minimum replication i.e. they are conservative.

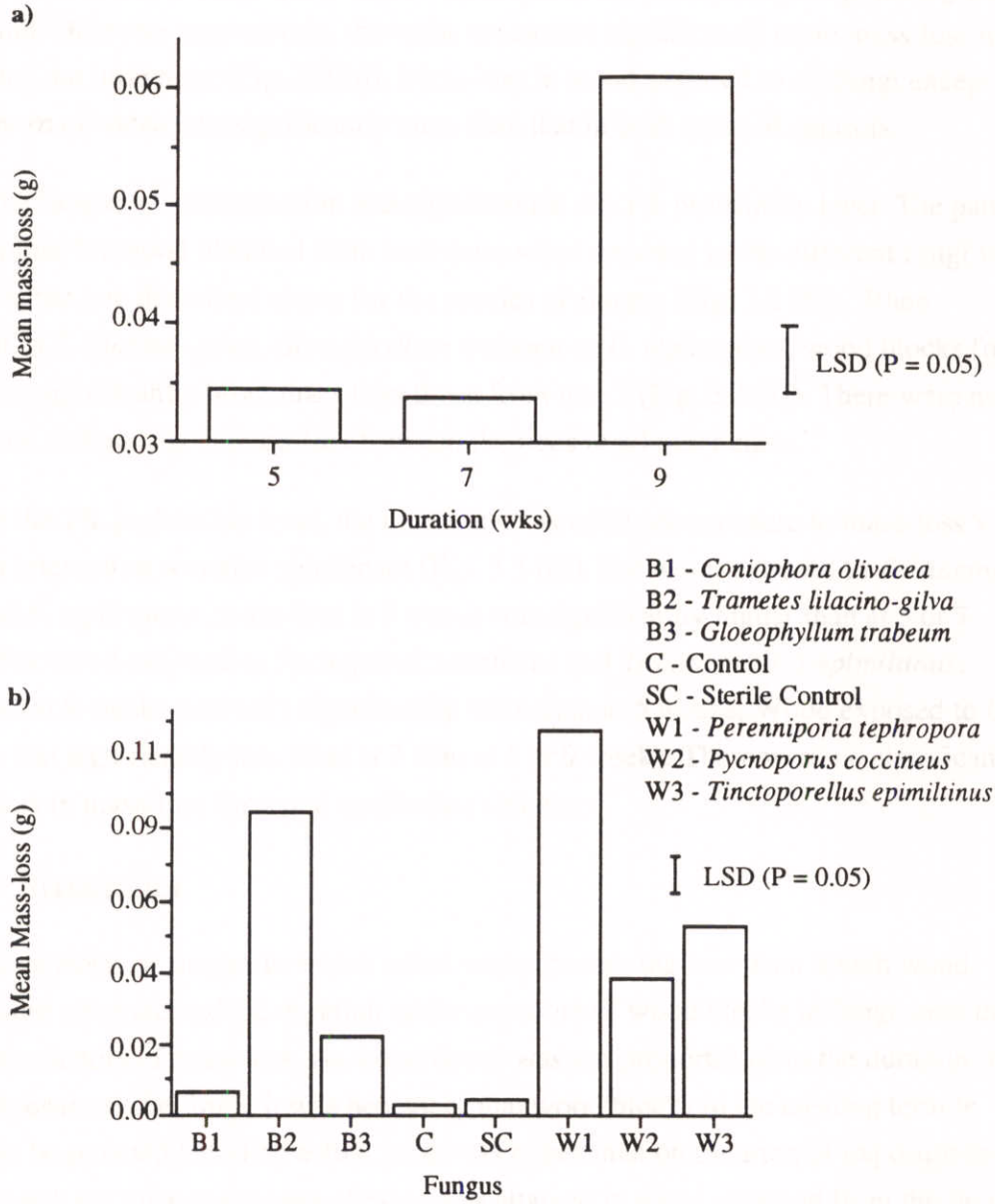



Figure 3.2: Other main factors of the fungal bioassay
a) duration of wood exposure to decay fungi
b) fungus species to which wood was exposed.
LSD are approximate and based on minimum replication i.e. they are conservative.
Fungi coded B produced brown rot while those coded W caused white rot. The untreated control is coded C and the Sterile control , SC.

Between *T. lilacino-gilva* and *P. tephropora,* the outstanding fungi among the brown and white rots respectively, the white rot caused significantly more mass-loss in wood than the brown rot (Fig. 3.2(b)). Mass-loss in wood exposed to all fungi except *Coniophora olivacea* was significantly more than that in both types of controls.

The fungus x tree interaction was significant at the 1% probability level The pattern of mass-loss for wood obtained from both trees when exposed to the different fungi was similar to the one described above for the species of fungus (Fig. 3.2 (b)). When exposed to *T. lilacino-gilva*, *Gloeophyllum trabeum* or *P. tephropora*, wood blocks from tree 2 lost significantly more mass than those from tree 1 (Fig. 3.3 (a)). There were no significant differences in mass-loss between the trees in all other cases.

At the 1% probability level, the duration of wood block exposure to mass-loss x duration interaction was also significant (Fig. 3.3 (b)). For wood exposed to *T. lilacino-gilva* and *P. tephropora*, mass-loss at 9 weeks was significantly higher than at 5 or 7 weeks. For wood exposed to *Pycnoporus coccineus* and *Tinctoporellus epimiltinus*, mass-loss at 9 weeks was only significantly more than at 5 weeks. Wood exposed to *G. trabeum* lost significantly less mass at 7 than at 5 or 9 weeks. There were no significant differences in mass-loss for wood in all other situations.

## Discussion

The species of fungus to which wood was exposed, the tree from which wood blocks were obtained and the duration of the exposure of wood blocks to fungi were the significant factors of mass-loss. As wood decay was not proportional to the duration of wood exposure to the fungi, it was necessary that wood blocks in the ensuing termite bioassays be selected based on actual mass-loss rather than on duration of exposure to fungi. Due to the different ranges of mass-loss attained in wood obtained from the two trees, some aspects of the termite bioassay could be pursued on different levels of mass-loss with wood blocks from the different trees. However, the termite colony development experiments were set up before the analysis of the fungal bioassay data was completed and these experiments were based on an assumption that mass-loss in wood was proportional to duration of exposure to fungi.

Ruyooka (1978) and Lenz *et al.* (1980) reported relatively low mass-loss in *E. regnans* exposed to *G. trabeum* compared to *Fomes lividus* (= *P.tephropora*) when specimens were incubated at 25°C for six weeks. In a second study with the two fungal species, Ruyooka (1978) allowed 9 weeks for appreciable decay to occur in wood incubated at 29°C. The conditions of incubation therefore play a part in the amount of

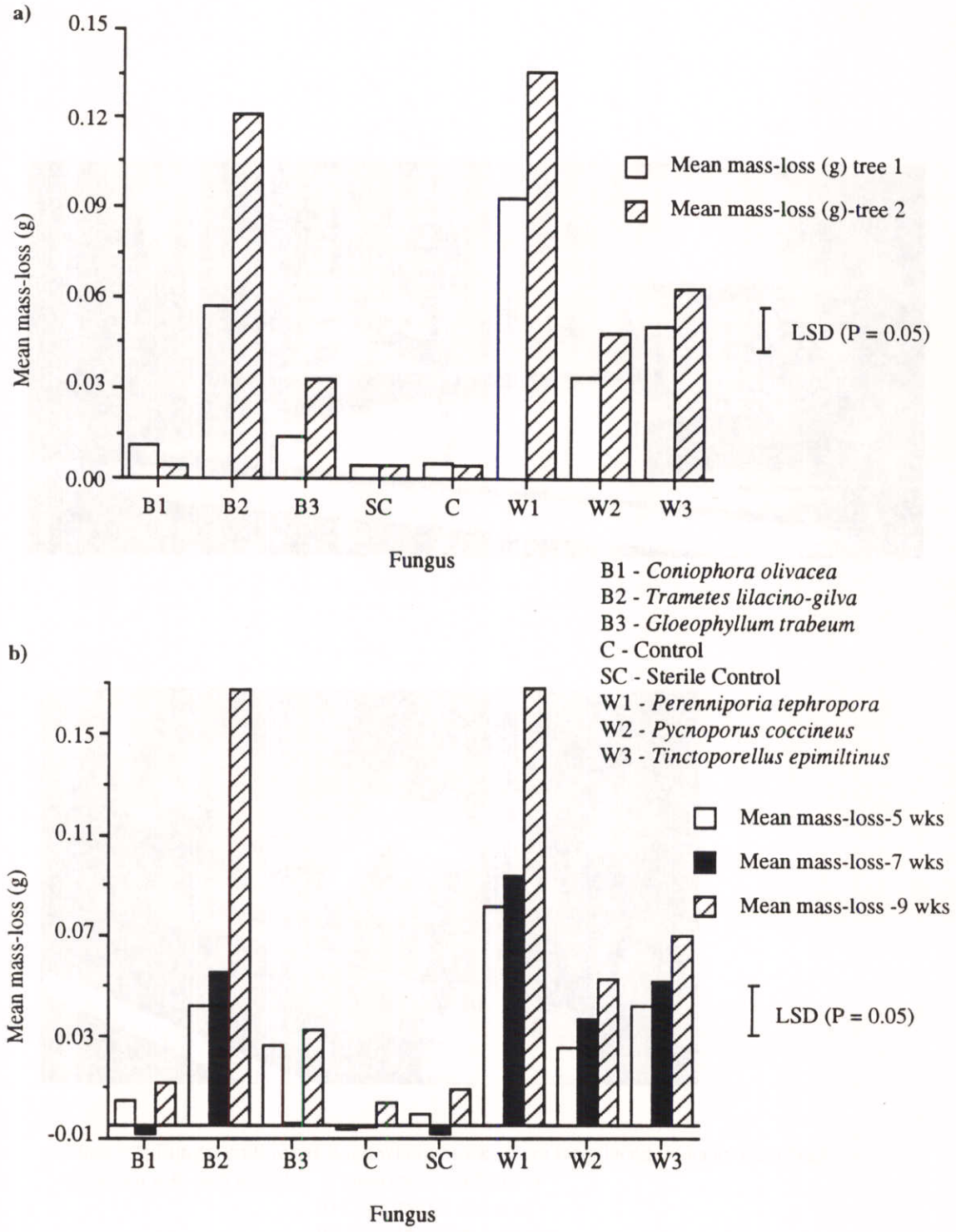
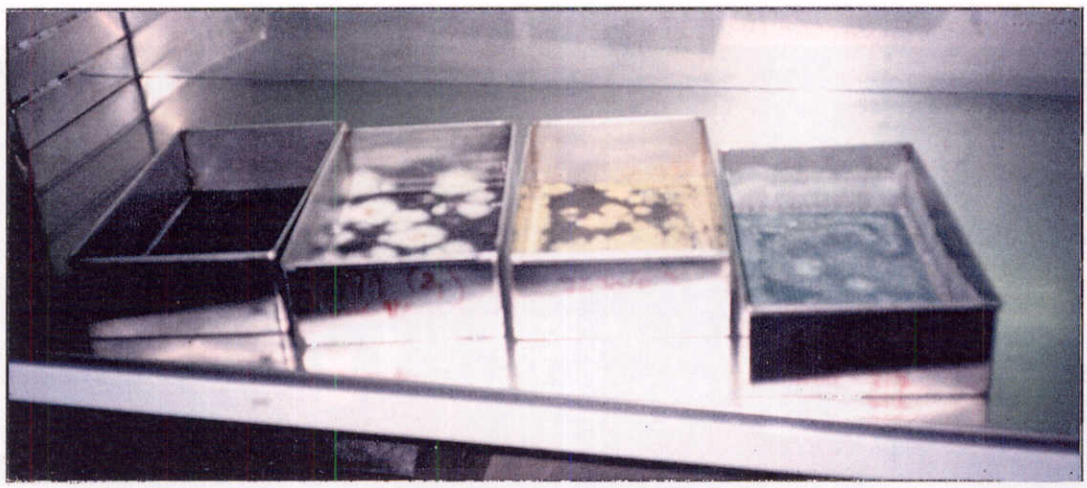



Figure 3.3: Interaction of factors of fungal bioassay
a) fungus x tree interaction
b) fungus x duration interaction
LSD are approximate and based on minimum replication i.e. they are conservative

a)

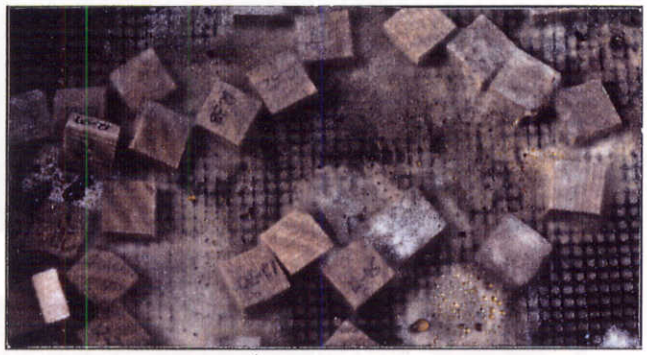


b)

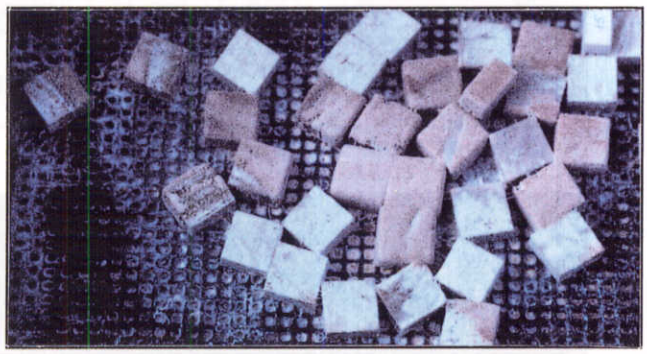

Plate 3.1: Fungal cultures at stage where blocks were introduced in decay chambers
a) brown rots- left to right- i) uninoculated chamber
ii) *Coniophora olivacea*
iii) *Gloeophyllum trabeum*
iv) *Trametes lilacino-gilva*
b) white rots- left to right- i) *Perenniporia tephropora*
ii) *Pycnoporus coccineus*
iii) *Tinctoporellus epimiltinus*
iv) uninoculated chamber


Plate 3.2: The appearance of the wood block conditioning/incubation room during the course of the fungal bioassay.

a)



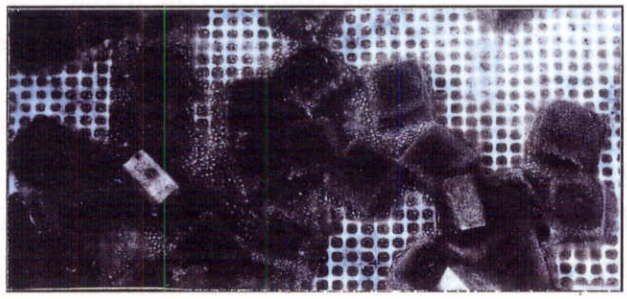
b)



c)

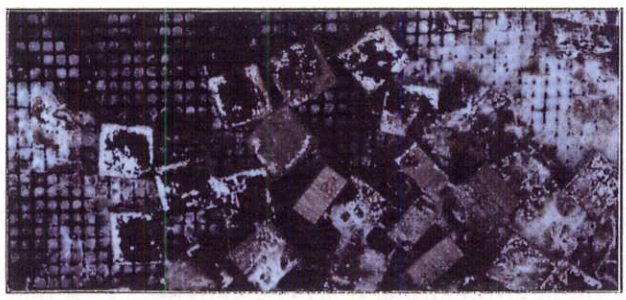
Plate 3.3: The colonisation of wood blocks by the brown rot fungi at 7 weeks
a) *Coniophora olivacea*
b) *Trametes lilacino-gilva*
c) *Gloeophyllum trabeum*

a)



b)

c)



d)

Plate 3.4: The colonisation of wood blocks by the white rot fungi at 7 weeks
a) *Perenniporia tephropora*
b) *Pycnoporus coccineus*
c) *Tinctoporellus epimiltinus*
d) Sterile control blocks. Note set up of chamber was like the rest but not inoculated with a fungus.

decay caused by different fungi. In the current study, it has been shown that the mass of wood did not change significantly with time when wood was exposed to several of the fungi. The conditions might therefore have been more limiting for some of the fungi involved in the bioassay than for others.

From their work based on species in the northern hemisphere, Fengel and Wegener (1989) suggested that brown rot preference for softwoods, and the preference of white rots for hardwoods, was probably a world-wide situation. Thornton *et al.* (in press) presented data indicating that white rot is more significant than brown rot in affecting the condition of eucalypts exposed in-ground in Australia. While in the current study the low mass-loss in wood blocks exposed to *C. olivacea* and *G. trabeum* conformed to this concept, *T. lilacino-gilva* defied it. Indeed, there were wide variations in the patterns of decay among the white and brown rots. Such variations were acknowledged by Rayner and Boddy (1988). This fungal bioassay also demonstrated that the performance of a fungus does not necessarily represent in all aspects the response of the type (white or brown) of fungi to which it belongs. The bioassay used imposed the same conditions on all fungi regardless of the optimum condition ( °C, moisture content of blocks etc.) for them to cause decay.

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# CHAPTER 4

## Feeding and survival response of *Coptotermes lacteus* when offered a single treatment of blocks of *Eucalyptus regnans* heartwood which were exposed either to brown or white rots.

## Introduction

Different termite species respond to wood exposed to decay fungi in different ways. Some find it attractive while others find it repellent (Amburgey 1979). The responses elicited by attractive wood include feeding, shelter-tube construction and orientation. It is on these positive responses that research has concentrated in the hope of producing a suitable bait for termite control.

For the species of termite which preferentially feed on decayed wood, the type of rot responsible for the wood decay plays a part in determining how much wood they consume (Amburgey and Smythe (1977a, 1977b); Becker and Lenz 1975; French *et al.* 1981) and their survival (Becker 1965; Lenz *et al.* 1980; Lenz *et al.* 1991; Ruyooka 1978; Smythe *et al.* 1971). Among basidiomycete fungi, for instance, there is general agreement that wood decayed by brown rots is more preferred by *Reticulitermes* species to that which is decayed by white rots (Amburgey 1979). Within a fungus species, different isolates can produce further variation in termite response. The level of decay in the wood and the state of the mycelia also influence feeding responses in termites (Becker 1965; Lenz *et al.* 1980; Ruyooka 1978).

The initial attractiveness of wood to termites does not necessarily culminate in feeding (Becker 1965; French 1978; Smythe *et al.* 1971) and neither does chewing of wood always result in ingestion of the wood (Amburgey and Smythe 1977a). Nonetheless, preferences of wood decayed by some types of fungi over those decayed by others are evident even in short-term studies lasting only hours (French 1978; Tyangi *et al.* 1982; Tyangi *et al.* 1984). Brown rots reportedly made wood more attractive to the different termite species involved than the other fungi.

This study aimed to establish the possible long-term benefits of termite association with wood exposed to basidiomycete fungi. The blocks were not sterilized in order to keep the situation as close as possible to what occurs naturally. Feeding and survival responses of *Coptotermes lacteus* when termites were offered *Eucalyptus regnans* heartwood decayed by a particular fungus to a certain mass-loss level were assessed. The most preferred type of rot and whether there was a level of decay at which the feeding and survival responses were most enhanced were of particular interest.

## Methods

### Timber

Details about the timber are given in Chapter 2. Two blocks of wood from the same tree, exposed to the same fungus and of similar mass-loss level were placed in each vial. Wood blocks were briefly dipped in distilled water before being pushed in, side by

side, just beneath the surface of the matrix. A detailed description of the preparation of the matrix is found below.

**Fungi**

Details about the fungi are given in Chapter 2 while the fungal bioassay is covered in detail in Chapter 3. Three brown and three white rots were used in the experiments and the mass-loss levels investigated per fungus depended on the tree from which the wood was obtained. The percentage mass-loss levels of wood in the experiments are listed in Table 4.1.

Table 4.1 Percentage mass-loss levels under investigation where termites were offered a single wood treatment

| Tree | Fungus type & species | % mass-loss levels in the blocks | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | Brown rots | | | | | | | |
| | *Coniophora olivacea* | 0 | 1 | 2 | | | | |
| | *Trametes lilacino-gilva* | 2 | 3 | 4 | 5 | 10-12 | | |
| | *Gloeophyllum trabeum* | 0 | 1 | 2 | 3 | | | |
| | White rots | | | | | | | |
| | *Perenniporia tephropora* | 2 | 3 | 5 | 7 | 9 | 10-12 | 13 |
| | *Pycnoporus coccineus* | 0 | 1 | 2 | 3 | | | |
| | *Tinctoporellus epimiltinus* | 2 | 3 | 5 | 6 | 7 | 10-12 | |
| | Control | 0 | | | | | | |
| | Sterile Control | 0 | 1 | 2 | | | | |
| 2 | Brown rots | | | | | | | |
| | *Coniophora olivacea* | 0 | 1 | 2 | | | | |
| | *Trametes lilacino-gilva* | 2 | 3 | 5 | 10-12 | 15-17 | 22-24 | |
| | *Gloeophyllum trabeum* | 0 | 1 | 2 | 3 | | | |
| | White rots | | | | | | | |
| | *Perenniporia tephropora* | 2 | 5 | 7 | 13 | 15-17 | 19-21 | 22-24 |
| | *Pycnoporus coccineus* | 1 | 2 | 3 | 6 | 11,13 | | |
| | *Tinctoporellus epimiltinus* | 2 | 3 | 4 | 6 | 11,13 | | |
| | Control | 0 | | | | | | |
| | Sterile Control | 0 | 1 | 2 | | | | |

**Termites**

Details about the field collection method and termite maintenance in the laboratory are provided in Chapter 2. One gram of freshly collected termites, which on average consisted of 280 individuals, was introduced into each vial in the experiments.

**Preparation of matrix**

The amount of distilled water required to raise the moisture content of the finely ground carton material to 100% was calculated as follows:

| | |
|---|---|
| 30ml of inner carton material weighed | 16.16g at 14% MC |
| Amount of moisture in the material (14% of 16.16g) | 2.26g |
| Amount of dry matter | 13.90g |
| Amount of distilled water required to bring dry matter to 100% saturation level (13.90g - 2.26g) | 11.64g |

1ml water approximates 1g hence 11.64g = 12ml (to nearest ml)

Mound material and water were thoroughly mixed by stirring; thereafter the matrix occupied nearly all of the vial's volume.

Two wood blocks were placed in the vial, just beneath the surface of the matrix. Termites were then placed on the surface of the matrix in each vial after which the lid was loosely fitted. The experiment lasted for eight weeks. When this period expired, the live mass of surviving termites and their number as well as the equilibrated final mass of the remnant wood were recorded.

Although the decayed wood in this experiment was not sterilized, it would appear that termites were drawn rapidly to it as it was the only choice of wood they had. As such, termite activity prevented the excessive growth of mycelia in the blocks. It was consequently considered that mass-loss was a realistic reflection of termite attack and was the sole measure of wood consumption.

## Results

**Wood consumption**

For mass-loss levels under 3% in wood, the fungus species to which wood was exposed and the source of the termites to which it was presented were significant factors of consumption at the 1% probability level (Fig. 4.1). Termites from colony 1 consumed significantly more wood than those from colony 2 (Fig. 4.1(a)) yet had lower survival and proportion of workers than colony 2. Wood exposed to *T. lilacino-gilva* was consumed significantly more than wood exposed to all other fungi as well as both types of controls (Fig. 4.1 (b)). Wood exposed to *P. tephropora* and *T. epimiltinus* was

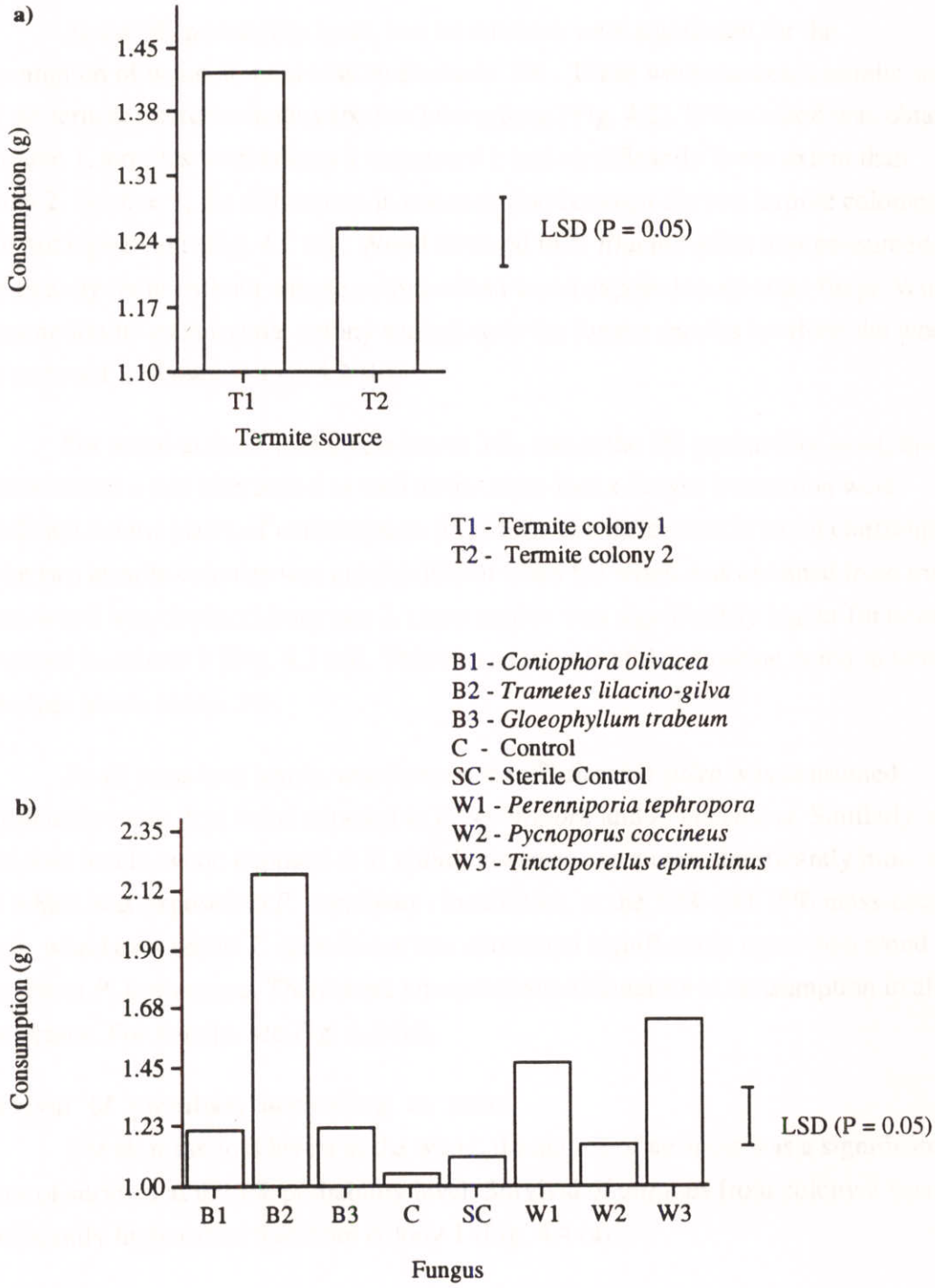



Figure 4.1: Wood consumption at mass-loss levels under 3%: main factors. LSD are approximate and based on minimum replication i.e. they are conservative.
a) termite source
b) species of fungus.

consumed significantly more than both types of controls. The remaining fungal treatments did not differ significantly between themselves or from both types of controls.

At the 5% probability level, two interactions were significant for the consumption of wood at mass-loss levels under 3% . These were the tree x termite source and the termite source x fungus species interactions (Fig. 4.2). When wood was obtained from tree 1, termites from colony 1 consumed it to a significantly lower extent than colony 2. For tree 2, the differences in consumption between the two termite colonies were not significant (Fig. 4.2 (a)). Wood exposed to *T. lilacino-gilva* was consumed significantly more by both termite colonies than wood exposed to all other fungi. Wood consumption by each termite colony according to the fungus species to which the wood was exposed is shown in Fig. 4.2 (b).

For wood at mass-loss levels above 3%, and at the 1% probability level, the termite source x tree interaction as well as the mass-loss x fungus interaction were significant determinants of consumption (Fig. 4.3). The difference in wood consumption by the two termite colonies was not significant when the wood was obtained from tree 1. When wood was obtained from tree 2, consumption was significantly higher for colony 2 compared to colony 1 (Fig. 4.3 (a)). This was a reversal of the situation noted in wood at mass-loss levels below 3%.

At all mass-loss levels, wood exposed to *T. lilacino-gilva* was consumed significantly more than wood exposed to *P. tephropora* and *P. coccineus*. Similarly, at all mass-loss levels, wood exposed to *T. epimiltinus* was consumed significantly more than that which was exposed to *P. coccineus* . In addition, at the 10% and 15% mass-loss levels, wood exposed to *T. epimiltinus* was consumed significantly more than wood exposed to *P. tephropora*. There were no significant differences in consumption in all other cases. For details, see Fig. 4.3 (b).

**Survival of termites, according to mass.**

For all mass-loss levels in the wood, the source of termites was a significant factor of survival at the 1% probability level. Survival of termites from colony 2 was significantly higher than that from colony 1 (Fig. 4.4 (a)).

Termite survival on wood exposed to all the fungi could be compared at mass-loss levels under 3%. The species of fungus was a significant factor of survival at the 1% probability level (Fig. 4.4 (b)). Survival on wood exposed to *T. lilacino-gilva* was significantly higher than on wood exposed to all other fungi and both types of controls. Survival on the wood exposed to *T. epimiltinus* was also significantly higher than on wood exposed to *P. tephropora*. Survival on wood exposed to the rest of the fungi was

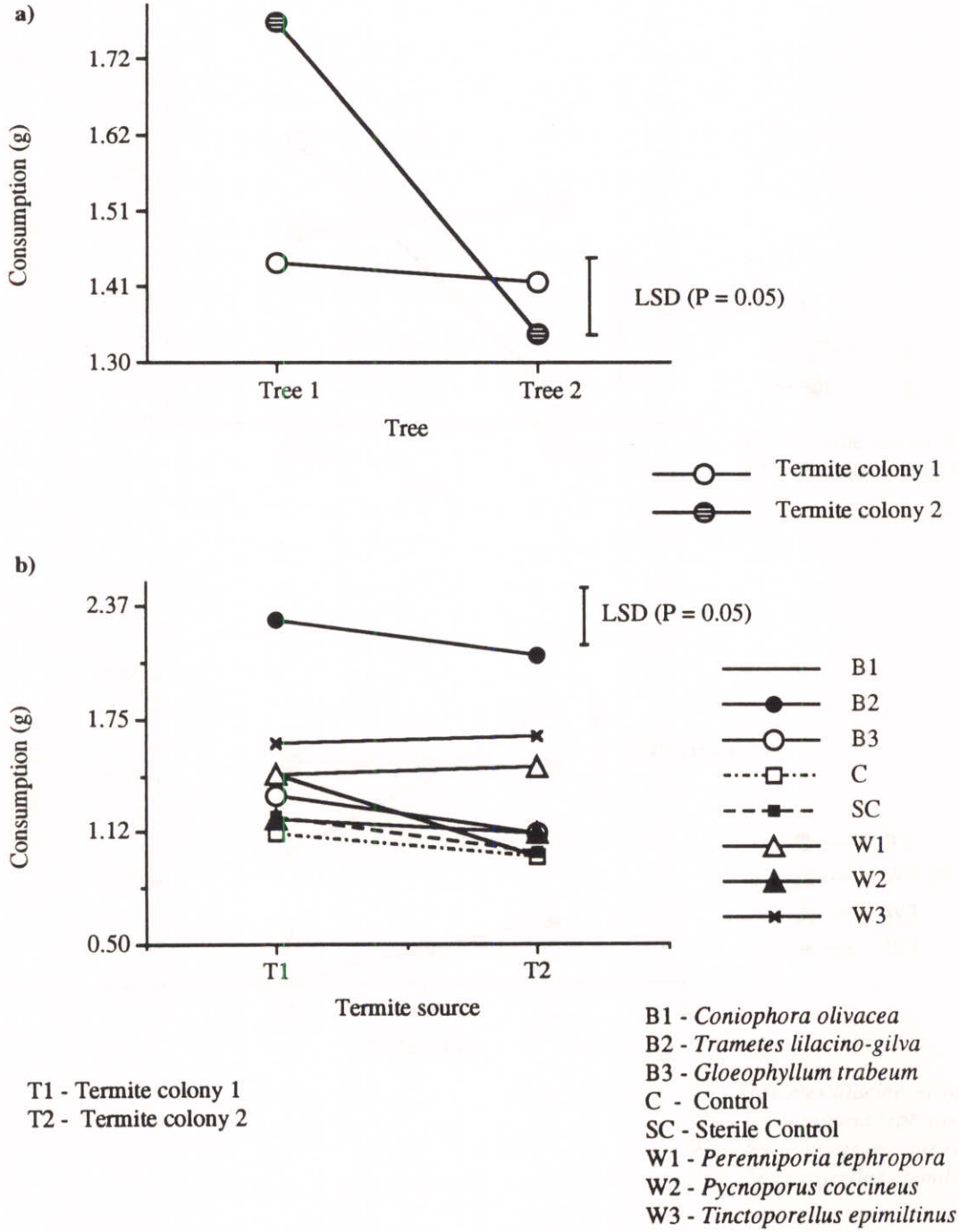



Figure 4.2: Effect of interaction of factors on wood consumption at mass-loss levels under 3%. LSD are approximate and based on minimum replication i.e. they are conservative.
a) termite source x tree interaction
b) termite source x fungus interaction.

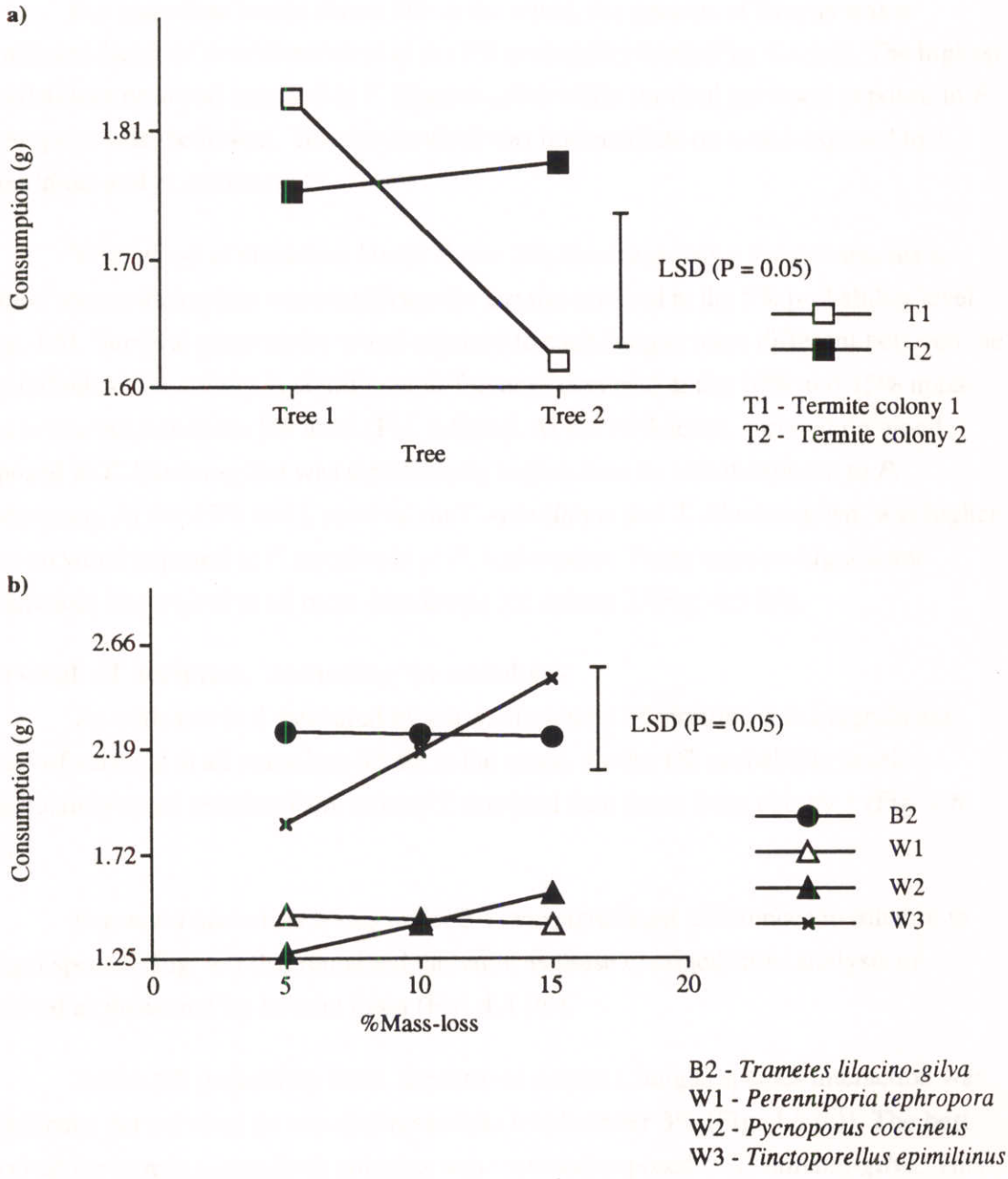



Figure 4.3: Effect of interaction of factors on wood consumption at mass-loss levels above 3%. LSD are approximate and based on minimum replication i.e. they are conservative.
a) termite source x tree interaction
b) mass-loss x fungus interaction

not significantly different when one was compared to the other or with both types of controls.

For mass-loss levels above 3% in the wood, the species of fungus was a significant factor of termite survival at the 1% probability level (Fig. 4.4 (c)). The highest survival was on wood exposed to *T. lilacino-gilva* while survival on wood exposed to *P. tephropora* was the lowest. Termite survival was intermediate on wood exposed to *T. epimiltinus* and *P. coccineus*.

With wood at mass-loss levels above 3%, the mass-loss x fungus species x termite source interaction was significant for termite survival at the 5% probability level (Fig. 4.5). Survival patterns for wood exposed to each fungus were different between the two colonies. For colony 1, significant differences occurred at the 10% and 15% mass-loss levels but not at the 5% level (Fig. 4.5 (a)). At the 10% level, survival on wood exposed to *T. lilacino-gilva* was significantly higher than on wood exposed to *P. tephropora.* At the 15% level, survival on *T. epimiltinus* and *T. lilacino-gilva* was higher than on wood exposed to *P. coccineus* or *P. tephropora.* There were no significant differences in survival at all mass-loss levels for colony 2 (Fig. 4.5 (b)).

**Survival of termites, according to numbers**

As with survival measured by mass, the source of termites was a significant factor of survival at all mass-loss levels in the wood. At the 1% probability level, significantly more termites from colony 2 survived than those from colony 1 (Fig. 4.6 (a)).

For wood mass-loss levels under 3%, the significant differences in relation to fungal species (Fig. 4.6 (b)) remained the same as those obtained from analysis of survival as measured by termite mass (Fig. 4.4 (b)).

At the 5% probability level, the termite source x fungus species interaction was significant for survival on wood at mass-loss levels under 3% (Fig. 4.6 (c)). The best survival for termites from both colonies was on wood exposed to *T. lilacino-gilva*. This was significantly more than survival on wood exposed to all other fungi and both types of controls.

For wood at mass-loss levels above 3% and at the 1% probability level, the fungus species was a significant factor of termite survival as measured by numbers (Fig. 4.6 (d)). The significant differences in termite survival according to numbers were the same as those obtained for termite survival as measured by mass.

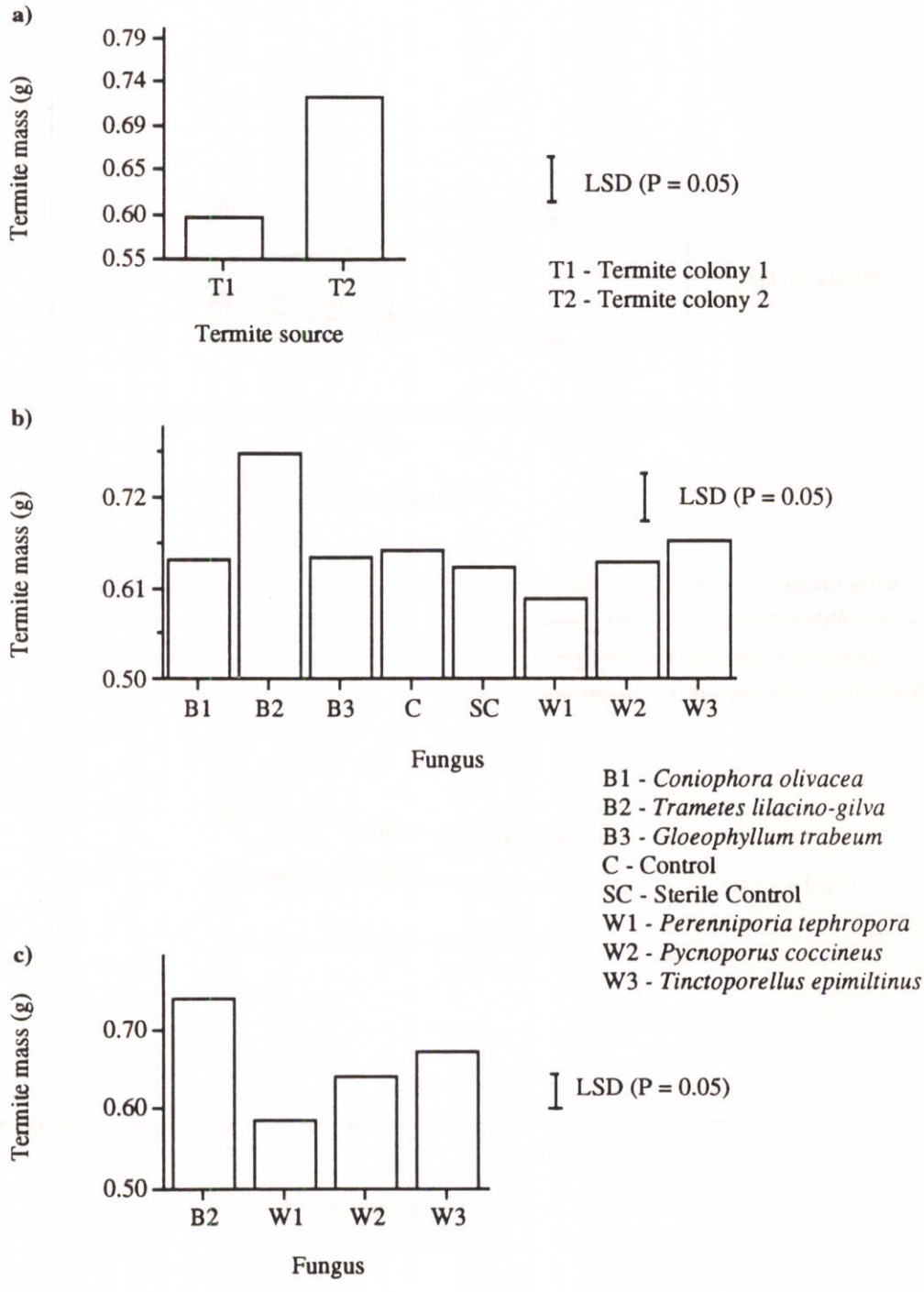



Figure 4.4: Survival of 1g termite groups by mass (g). LSD are approximate and based on minimum replication i.e. they are conservative.
a) effect of termite source on survival at all mass-loss levels
b) effect of species of fungus to which wood was exposed at mass-loss levels under 3%
c) effect of species of fungus to which wood was exposed at mass-loss levels above 3%.

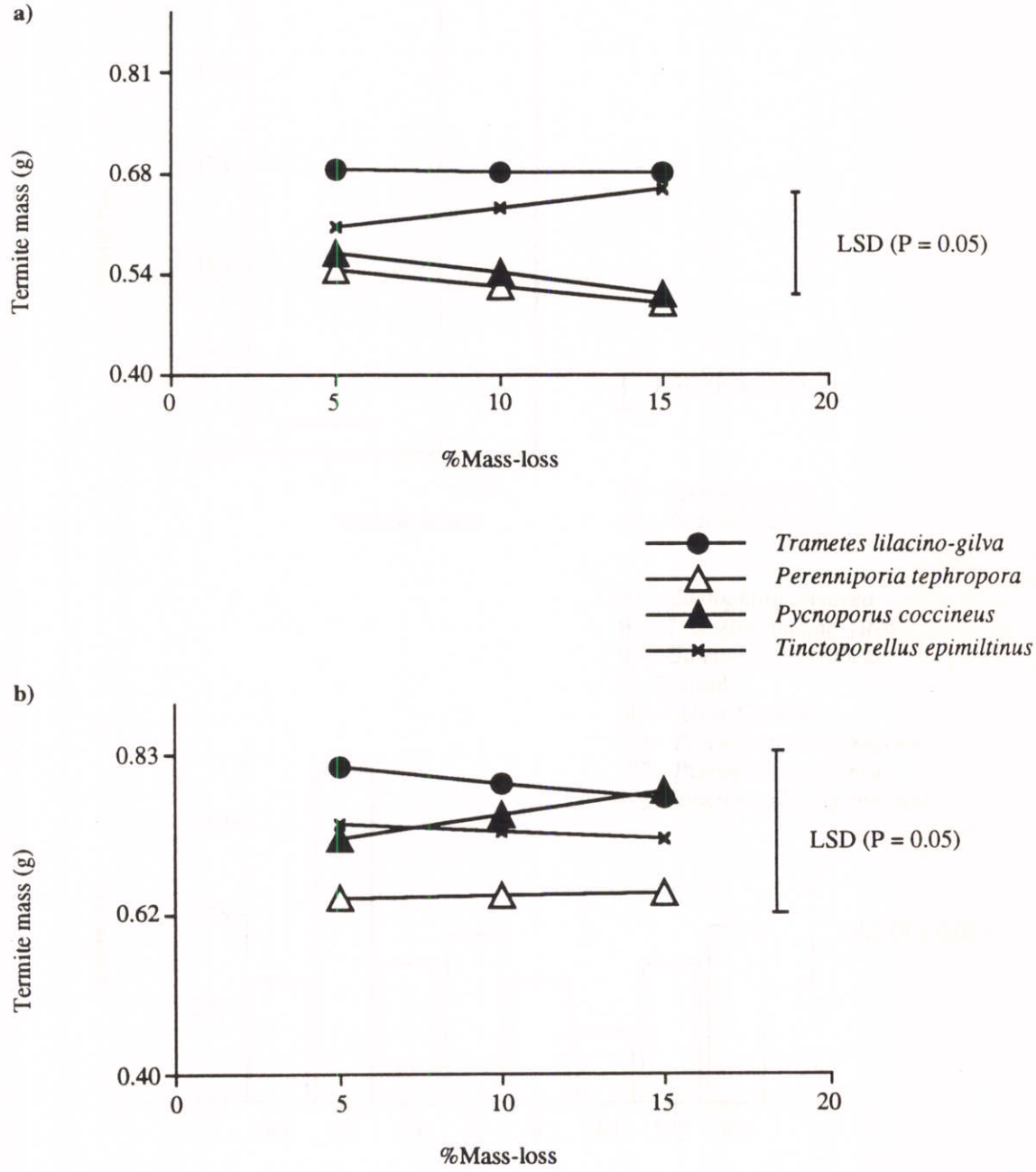



Figure 4.5: Effect of the mass-loss x fungus species x termite source interaction on survival of 1g termite groups, by mass (g), on wood at mass-loss levels above 3%. LSD are approximate and based on minimum replication i.e. they are conservative.
a) mass-loss x fungus species x termite colony 1
b) mass-loss x fungus species x termite colony 2.

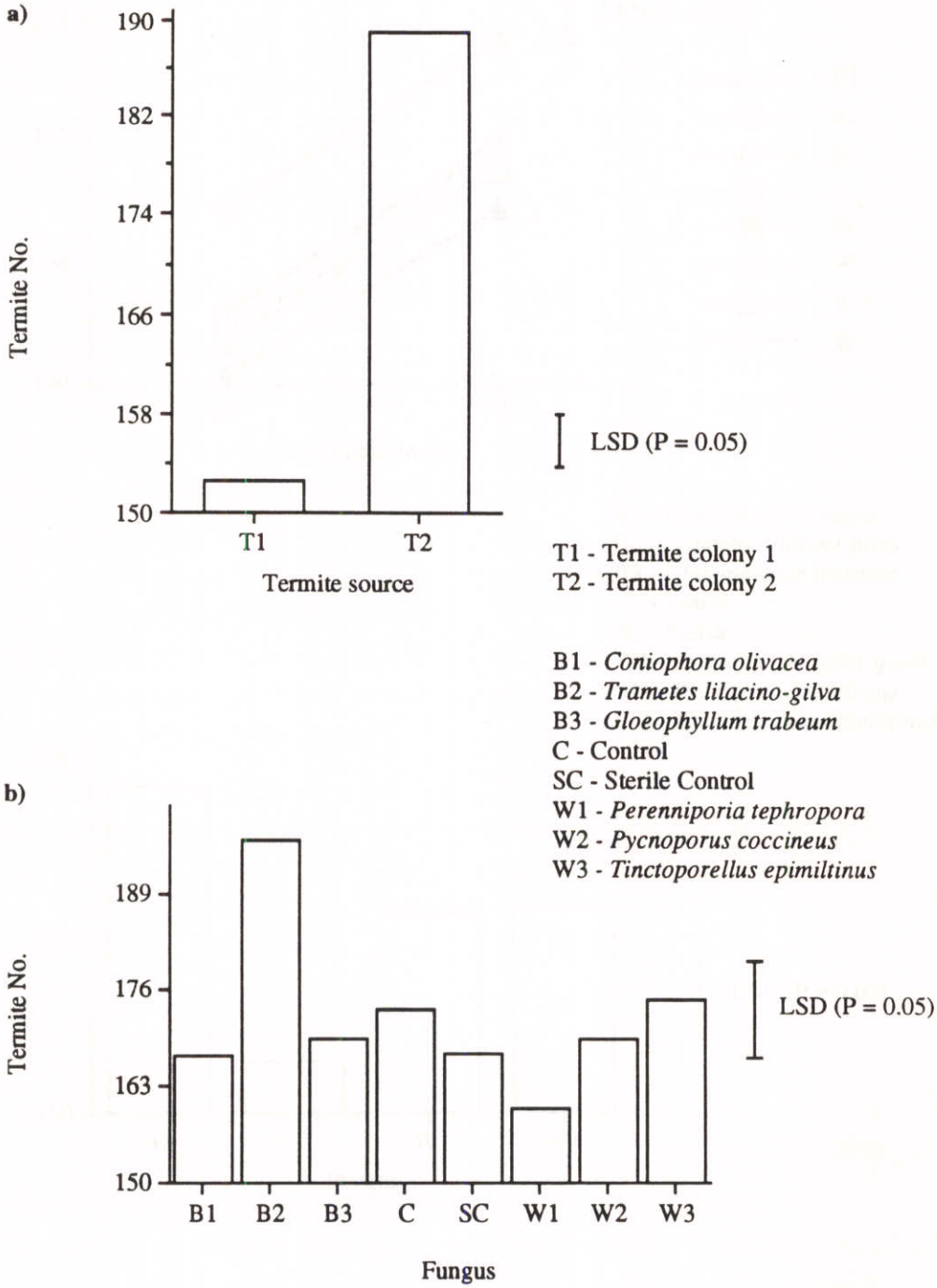



Figure 4.6(a, b): Termite survival, by numbers, at all mass-loss levels in the wood. LSD are approximate and based on minimum replication i.e. they are conservative.
a) effect of termite source at all mass-loss levels
b) effect of fungus species on wood at mass-loss levels under 3%.

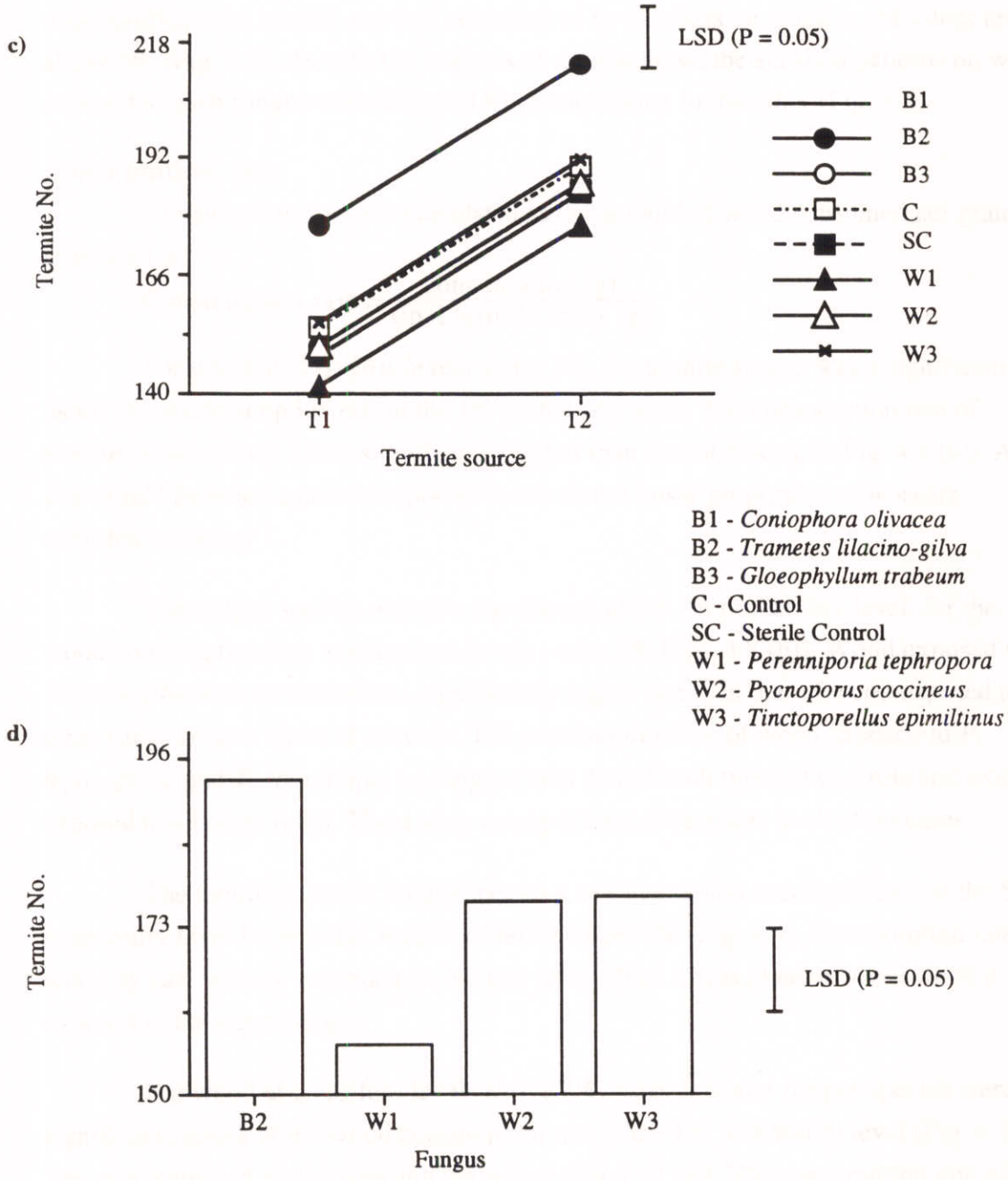



Figure 4.6 (c, d): Survival of termites, by numbers, on wood at all mass-loss levels. LSD are approximate and based on minimum replication i.e. they are conservative.
c) effect of the termite source x fungus species interaction on wood at mass-loss levels under 3%
d) effect of species of fungus on wood at mass-loss levels above 3%.

At the 1% probability level, the mass-loss x fungus species x termite source interaction was significant for termite survival as measured by numbers on wood at mass-loss levels above 3% (Fig. 4.7). As with the analysis of termite mass, the survival patterns on wood exposed to each fungus were different from one colony to the other (Fig. 4.7).

**Consumption rate**

Consumption rate was calculated as the amount of wood consumed per gram of termites i.e.

$$\text{Consumption rate} = \frac{\text{consumption (g)}}{\text{final termite mass (g)}}$$

For wood at mass-loss levels under 3%, the termite source was a significant factor of the consumption rate at the 1% probability level. The consumption rate of termites from colony 1 was significantly higher than that of colony 2 (Fig. 4.8 (a)). Again this should be noted against the poorer survival and lower proportion of workers recorded in colony 1.

The fungus species was also significant, at the 1% probability level, for the wood consumption rate at mass-loss levels under 3% (Fig. 4.8 (b)). Wood exposed to *T. lilacino-gilva* was consumed at a significantly higher rate than that of wood exposed to all other fungi or both types of controls. The consumption rate of wood exposed to *P. tephropora* and *T. epimiltinus* was higher than that of both types of controls and wood exposed to all other fungi. There were no significant differences in all other cases

The termite source x fungus species x tree interaction was significant at the 5% probability level for wood at mass-loss levels under 3% (Fig. 4.9). Consumption rate of wood by each colony depended on the tree from which it was obtained even when it was exposed to the same fungus.

For wood at mass-loss levels above 3%, mass-loss and fungus species were significant factors of the wood consumption rate at the 1% probability level (Fig. 4.10). The rate increased with increasing mass-loss (Fig 4.10 (a)). The consumption rate of wood exposed to *P. coccineus* was the lowest. Wood exposed to *T. lilacino-gilva* was consumed at a significantly higher rate than wood exposed to *P. tephropora* and *P. coccineus* but not *T. epimiltinus* (Fig. 4.10 (b)).

Also significant at the 1% probability level for wood consumption rate at mass-loss levels above 3% were the tree x termite source and mass-loss x fungus species interactions (Fig. 4.11). The consumption rate of wood by termite colony 1 was significantly higher on wood obtained from either tree than that of colony 2 (Fig. 4.11(a)).

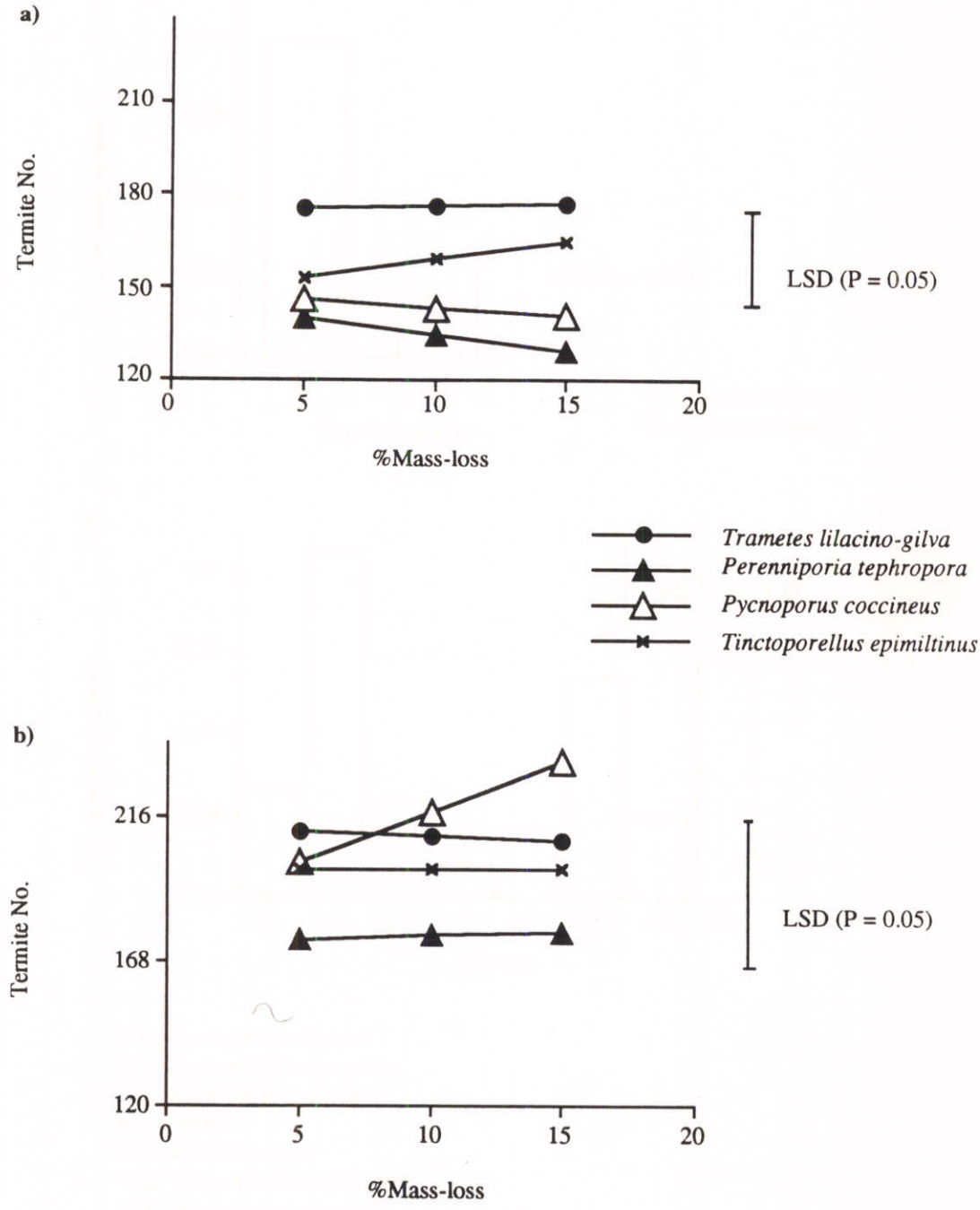
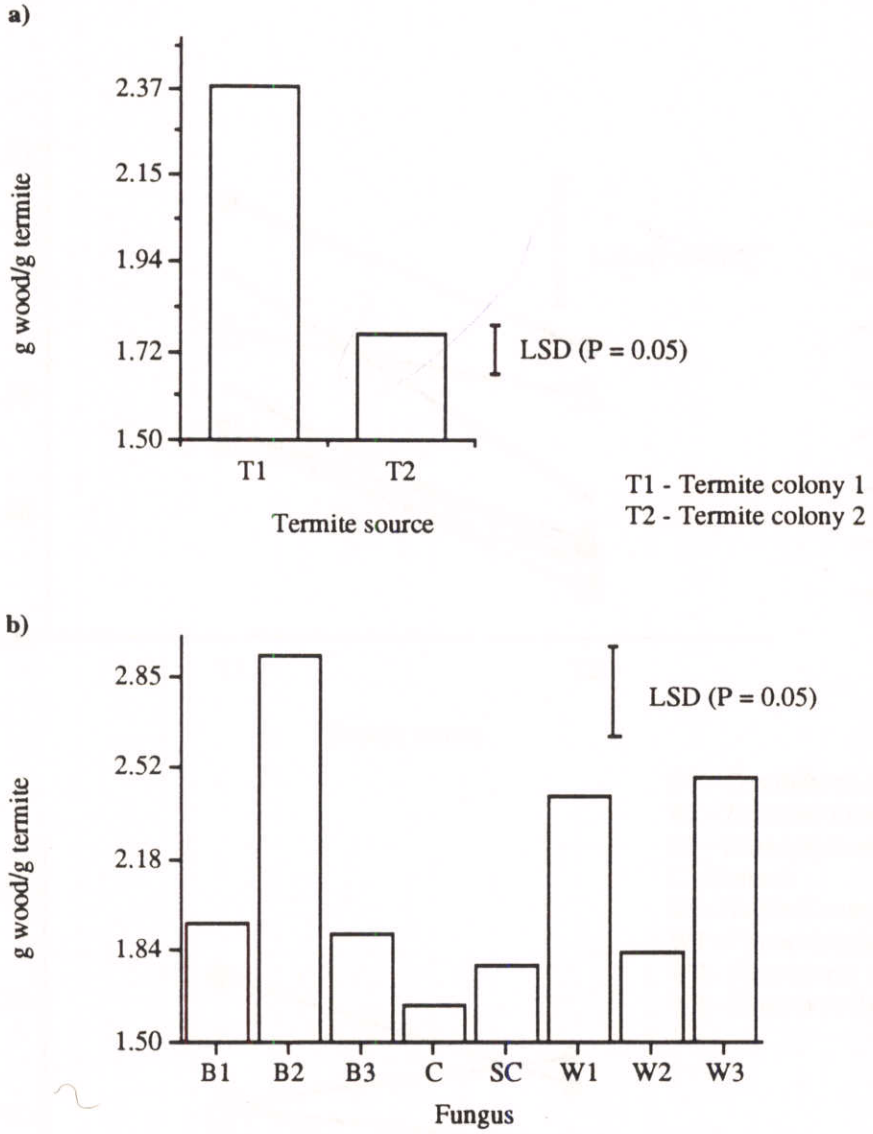



Figure 4.7: Effect of mass-loss x fungus species x termite source interaction on termite survival , by numbers, on wood at mass-loss levels under 3%. LSD are approximate and based on minimum replication i.e. they are conservative.
a) mass-loss x fungus species x termite colony 1
b) mass-loss x fungus species x termite colony 2.




B1 - *Coniophora olivacea*
B2 - *Trametes lilacino-gilva*
B3 - *Gloeophyllum trabeum*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus coccineus*
W3 - *Tinctoporellus epimiltinus*

Figure 4.8: Consumption rate of wood at mass-loss levels under 3%: main factors. LSD are approximate and based on minimum replication i.e. they are conservative.
a) termite source
b) fungus species.

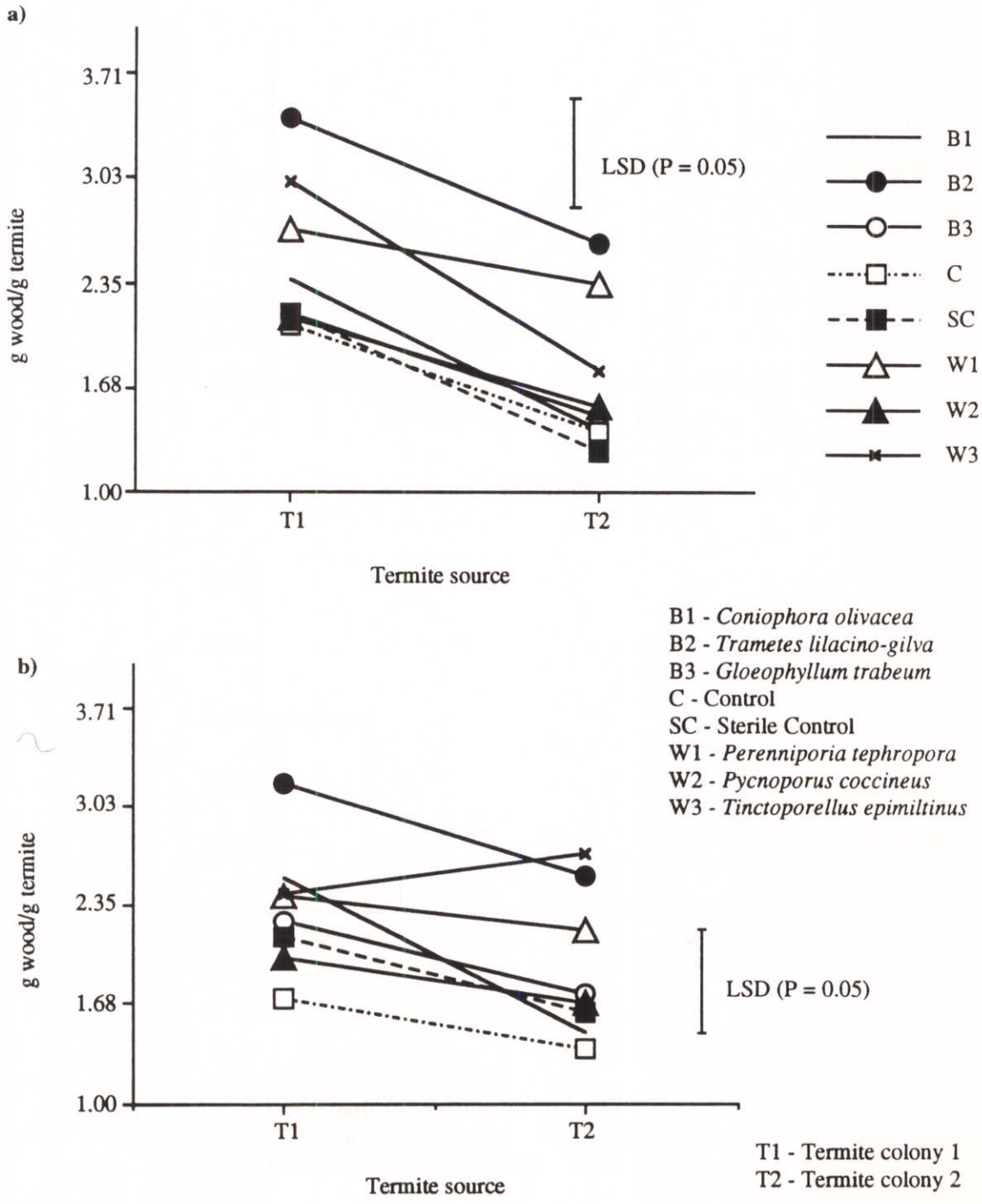
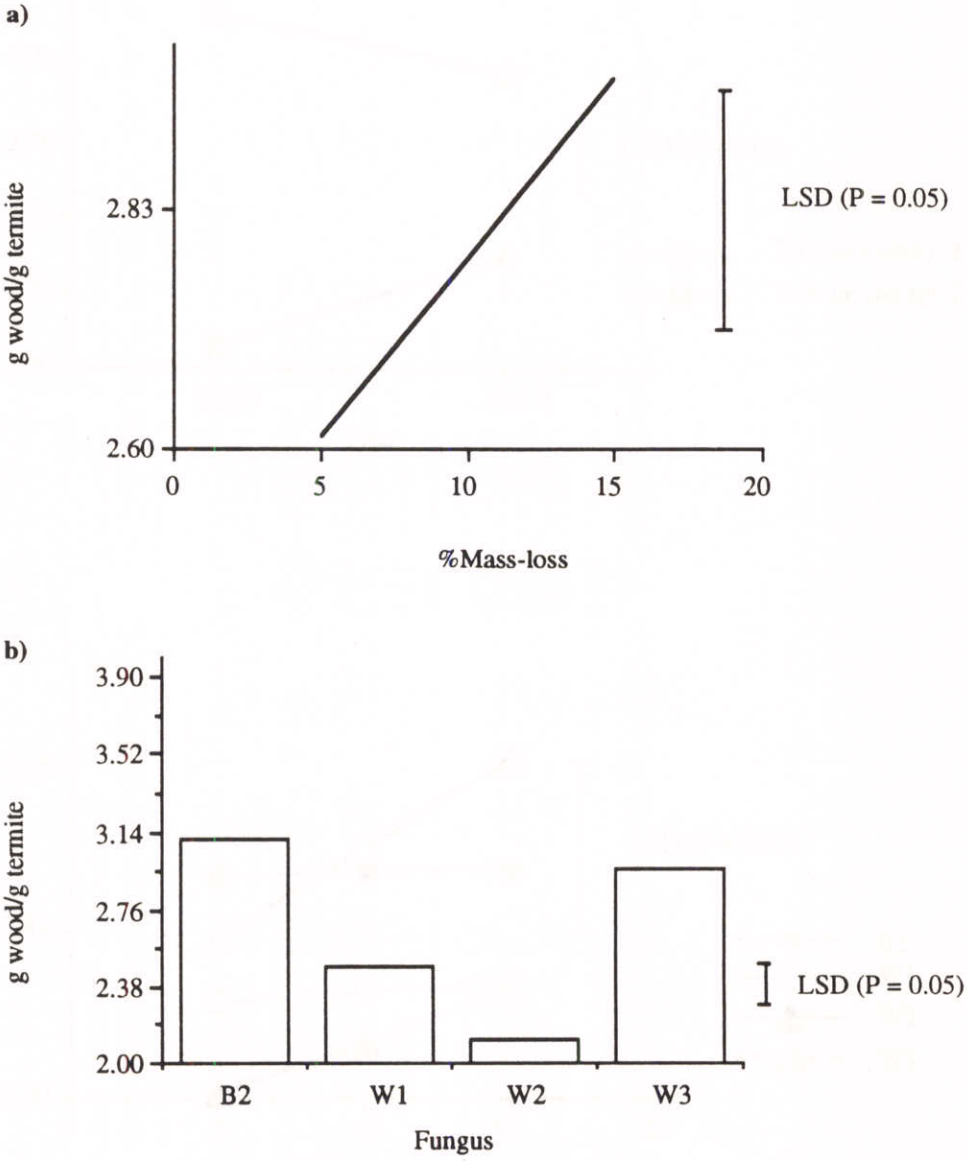



Figure 4.9: Consumption rate of wood at mass-loss levels under 3%. The termite source x tree x fungus species interaction. LSD are approximate and based on minimum replication i.e. they are conservative.

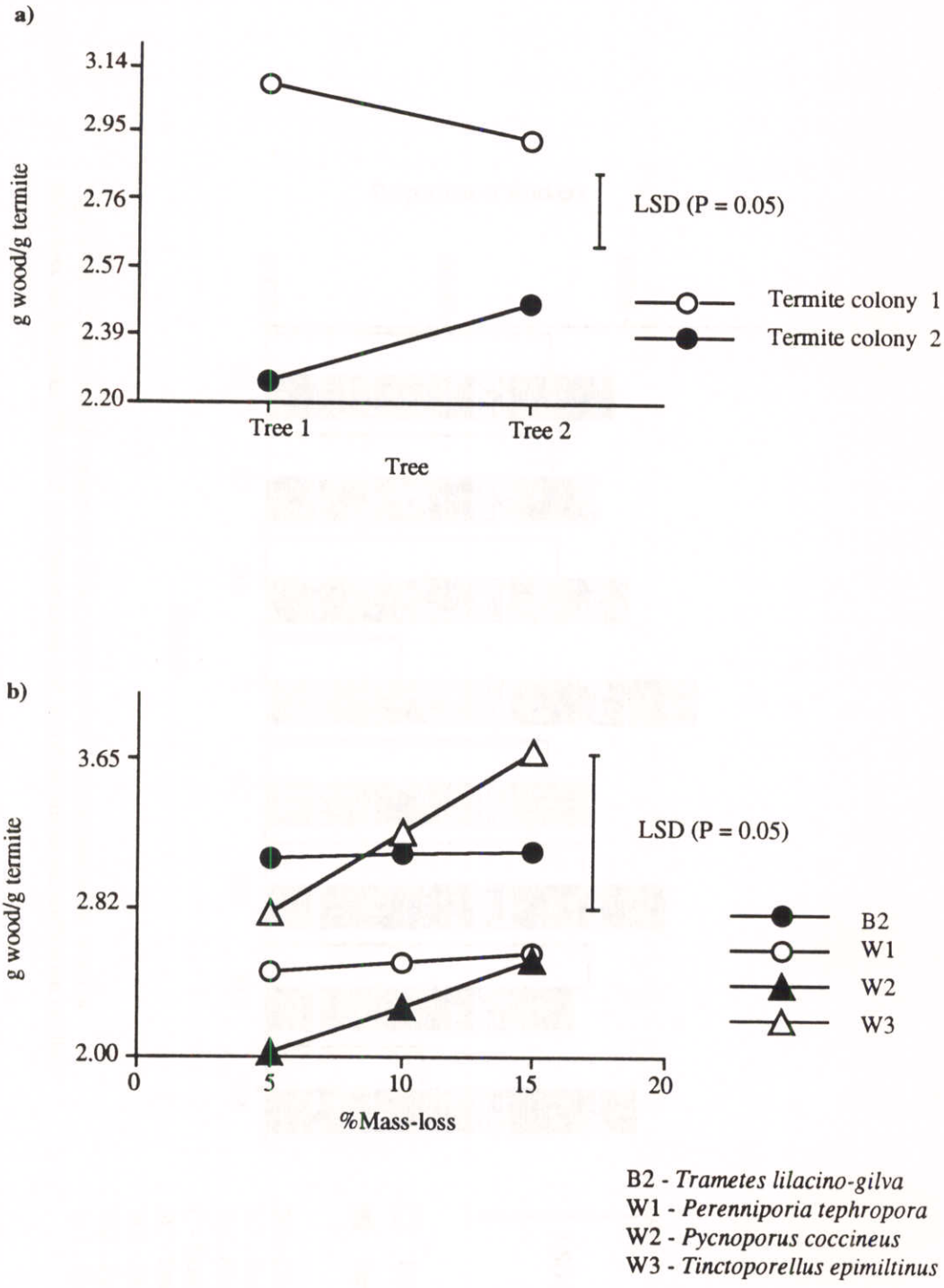
a) termite source x fungus x tree 1

b) termite source x fungus x tree 2.




B2 - *Trametes lilacino-gilva*
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus coccineus*
W3 - *Tinctoporellus epimiltinus*

Figure 4.10: Consumption rate of wood at mass-loss levels above 3%: main factors. LSD are approximate and based on minimum replication i.e. they are conservative.
a) mass-loss
b) fungus species to which wood was exposed.




B2 - *Trametes lilacino-gilva*
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus coccineus*
W3 - *Tinctoporellus epimiltinus*

Figure 4.11: Consumption rate of wood at mass-loss levels above 3%: interaction of factors. LSD are approximate and based on minimum replication i.e. they are conservative.
a) tree x termite source interaction
b) mass-loss x fungus species interaction.

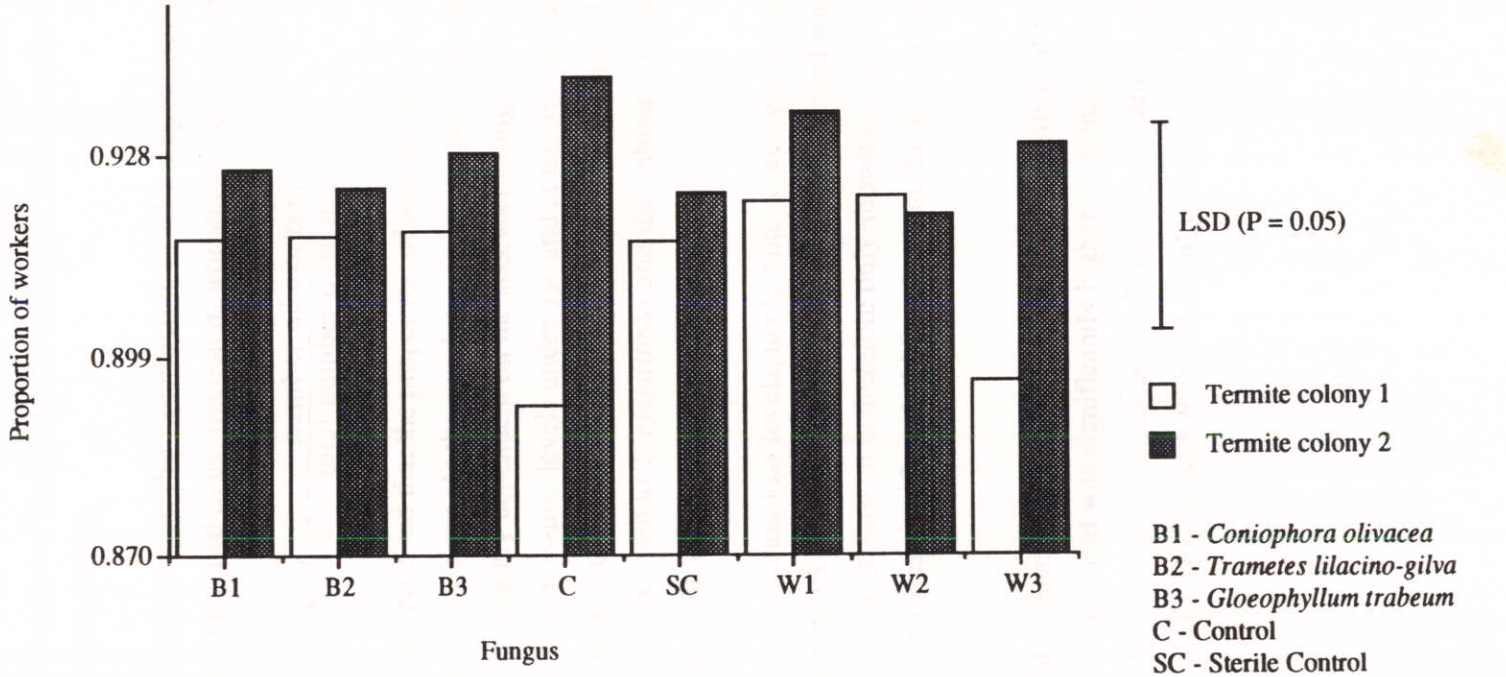



Figure 4.12: Proportion of workers surviving at the end of the experiments on wood at mass-loss levels under 3%. LSD are approximate and based on minimum replication i.e. they are conservative.

Wood exposed to *T. lilacino-gilva* was consumed at a significantly higher rate than that of wood exposed to *P. coccineus* at the 5% mass-loss level. At the 10% level, the consumption rate of wood exposed to *T. epimiltinus* was significantly higher than that of wood exposed to *P. coccineus*. At the 15% mass-loss level, the consumption rate of wood exposed to *T. epimiltinus* was significantly higher than that of wood exposed to *P. tephropora* or *P. coccineus*. There were no significant differences in consumption rates in all other cases (Fig. 4.11 (b)).

**Proportion of workers at the conclusion of the experiment**

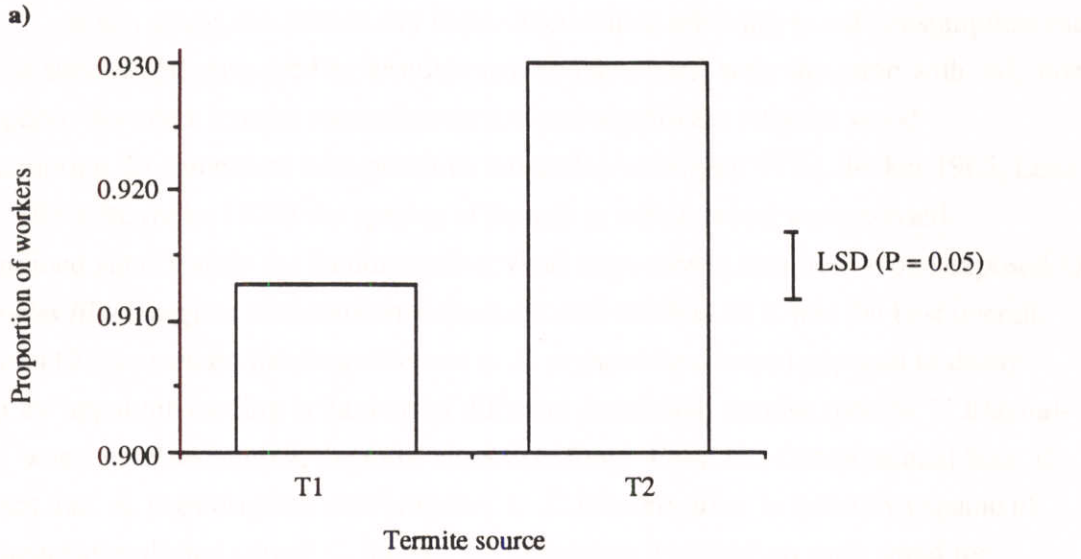
This was calculated as the fraction of workers in the total number number of termites i.e.

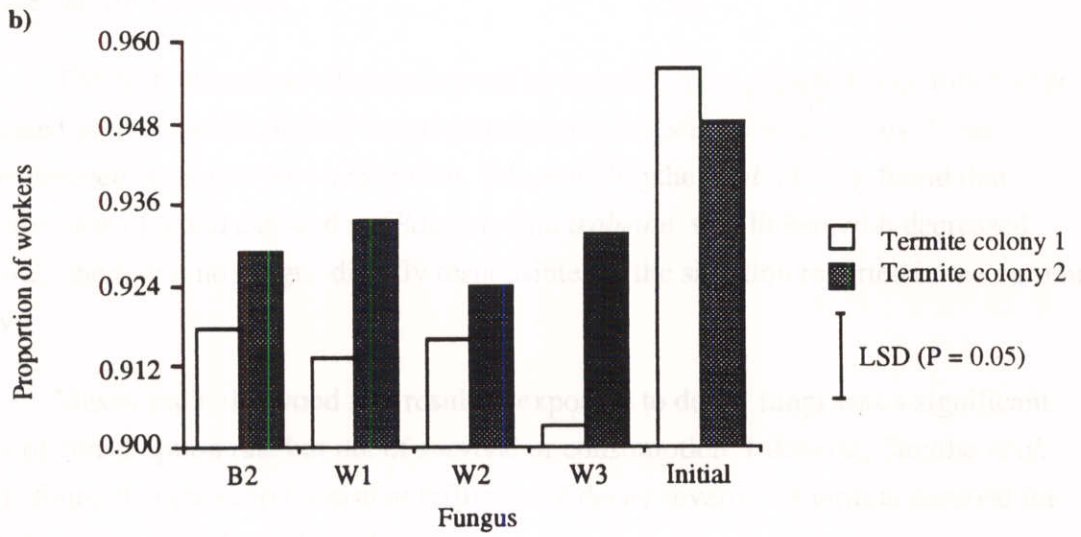
$$\text{Proportion of workers} = \frac{\text{number of workers}}{\text{total number of termites}}$$

The initial caste data indicated that the proportion of workers in the two termite colonies was not significantly different. At the conclusion of the experiment, however, this proportion was significantly dependent on the species of fungus to which the wood was exposed. For wood at mass-loss levels under 3% and at the 5% probability level, the proportion of workers in colony 2 was significantly higher than in colony 1 when presented with wood exposed to *T. epimiltinus* and the untreated control. There were no significant differences in other situations (Fig. 4.12).

For wood at all mass-loss levels, the termite source was a significant factor of the proportion of workers at the 1% probability level. As the trend was the same both below and above 3% mass-loss, it is therefore only shown for mass-loss above 3%. The proportion of workers was significantly higher for termites from colony 2 than colony 1 (Fig 4.13 (a)).

For wood at mass-loss levels above 3%, the proportion of workers at the conclusion of the experiment was significantly higher for termites from colony 2 compared to colony 1 when wood exposed to *P. tephropora* and *T. epimiltinus* was presented (Fig 4.13 (b). Differences were not significant for wood exposed to the other fungi.




B2 - *Trametes lilacino-gilva*
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus coccineus*
W3 - *Tinctoporellus epimiltinus*

Figure 4.13: Proportion of workers surviving at the end of the experiments on wood at mass-loss levels above 3%. LSD are approximate and based on minimum replication i.e. they are conservative.
a) effect of termite source on final proportion of workers
b) effect of termite source x fungus species interaction on final proportion of workers compared with the initial proportion.

## Discussion

In this study, the factors and factor interactions affecting wood consumption and termite survival (as measured by termite mass and numbers) were the same with only one exception: the tree x termite source interaction was significant only for wood consumption. In agreement with previous research (Amburgey 1979, Becker 1965; Lenz *et al.* 1980; Ruyooka 1978) the species of fungus to which wood was exposed determined significantly the feeding and survival responses in termites. Wood exposed to *Trametes lilacino-gilva* was consumed the most and survival on it was the best overall. French (1978) assessed the attractiveness of *E. regnans* heartwood exposed to decay fungi by 'apparent' feeding behaviour of different Australian termite species. *T. lilacino-gilva* was mentioned among the most attractive fungi. From the data presented here, it appears that *E. regnans* heartwood exposed to *T. lilacino-gilva* is not only capable of producing stimuli that attract *C. lacteus* but also cause it to feed on such wood for prolonged periods, at the same time enhancing the survival of the termites. It is a combination of such qualities that would make *E. regnans* decayed by the fungus a suitable bait for *C. lacteus*.

The high overall wood consumption by termites from colony 1 was linked with decreased survival while the low wood consumption associated with colony 2 was complemented by higher termite survival. Whereas Smythe *et al.* (1971) found that attractiveness of wood exposed to *Gloeophyllum trabeum* was linked with decreased survival, there was no fungus directly responsible for the situation reported in the current study.

Mass-loss in the wood as a result of exposure to decay fungi was a significant factor of consumption rate but not of survival or consumption. Likewise, Smythe *et al.* (1971) found there was no consistent influence of decay severity on termite survival for the different fungi and woods in their study.

Whereas at mass-loss levels below 3% in the wood it was the main factors and two-way factor interactions which influenced the results, beyond the 3% mass-loss level, interactions with as many as three factors were nearly always significant. In such a case, it was impossible to determine which of the of the particular components in a given situation had the major influence on termite response. Two-way interactions were easier to interprete. The termite source x species of fungus interaction, for example, once again stressed that best survival was on wood exposed to *T. lilacino-gilva* . In addition, the interaction of tree x the source of termites highlighted the influence of intraspecific tree differences on wood consumption.

Another striking source of variation in wood consumption and termite survival

was the source of termites. Termites from the two colonies gave opposite results for wood consumption and survival. The survival by mass and numbers followed similar trends for each termite source and so the possible differences in condition of the termites were unlikely to have contributed to the contradictory behaviour between termites from both colonies. The survival of workers (by proportion) was also not only dependent on the fungus to which the wood presented to termites was exposed but also on the source of termites. Termites from colony 2 when compared to colony 1 had a higher proportion of workers surviving at the end of the experiments when they were presented with wood exposed to *T. epimiltinus*. This happened at mass-loss levels in wood both below and above 3%. It also happened with wood exposed to *P. tephropora* at mass-loss levels above 3%.

Intraspecific differences between colonies have been reported for other species of *Coptotermes(*Lenz 1985). The differences in the response of the two colonies of *C. lacteus* in the study reported here are of an order one would normally associate with different species of termites. This indicates that intercolony variability has to be taken into account not only for the measurement of the resistance of materials to termites (Howick and Creffield 1983, Su and LaFage 1984) but also in studies of aspects of termite biology.

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# CHAPTER 5

## Feeding response of *Coptotermes lacteus* to a choice of blocks of *Eucalyptus regnans* heartwood which were exposed to brown and white-rots.

## Introduction

Under natural conditions, subterranean termites have to choose what to feed on out of a vast range of materials which they encounter. Wood-eating termites are guided in so doing by their familiarity with different types of food (Lenz *et al.* 1991; Ruyooka 1978). Waller and Fage (1987) reported that termites remained faithful to food to which they were pre-conditioned even when they were given the opportunity to feed on wood they would normally have consumed under natural conditions. It is therefore not easy to determine termite food preferences. Nonetheless, three groups of dietary habits emerged from the study by Lenz *et al.* (1991). There were those termites which fed almost exclusively on sound wood, others to which the state of the wood was less important and yet others which thrived on wood decayed by particular fungi. *Coptotermes lacteus* (Froggatt) is in the last category.

Hendee (1935) found that rotten Douglas fir provided a better diet for *Zootermopsis angusticollis* than sound wood. She suggested that the superior nutritive qualities associated with decayed wood were only part of the reason why termites might have preferred to feed on it compared to sound wood. She proposed that extractives which repelled termites from attacking certain woods might be detoxified by fungi, turning the wood into suitable food.

In this study, *C. lacteus* was presented with varying numbers of blocks of *Eucalyptus regnans* F. Muell. which had previously been exposed to brown or white rots and had attained various mass-loss levels. Wood blocks were not sterilized after exposure to the fungi and there was a high probability that they contained live mycelia. The study was designed to measure the response of termites to blocks of wood that:

1) had attained the same mass-loss level (1%) after exposure to each of the fungi,
2) had attained the same mass-loss level following exposure to each of the different white or the brown rots,
3) had attained different mass-loss levels after exposure to the same fungus.

## Methods

### Timber

Details about the timber are given in Chapter 2.

### Fungi

Details about the fungi are given in Chapter 2 while the fungal bioassay is covered in detail in Chapter 3.

**Termites**

The details of collection and maintenance of termites in the laboratory are given Chapter 2. The two-gram termite groups used in these choice-feeding experiments were obtained from termites which had been maintained for 16 weeks in the laboratory.

**Experiments**

In all, five experiments were conducted.

**Experiment 1** was designed to compare termite feeding response towards wood exposed to all fungal treatments at the 1% mass-loss level. A total of 8 wood blocks, one each from each of the six fungal treatments and the two types of controls, were presented to the termites. Both types of controls provided contrasts to the fungus-treated wood not only in this experiment but in all the others as well.

**Experiment 2** compared the feeding response of termites towards wood exposed to brown rots. Termite response to two levels of mass-loss- 0% and 3%- in wood exposed to each of the three brown rot fungi was investigated. With only one block from each fungal treatment and each mass-loss level represented per container, 8 blocks were available for the termites to choose on what to feed.

Since there was a wider mass-loss range attained by wood blocks exposed to white rots, in experiment 3, 4 and 5, the response of termites to wood exposed to white rots was investigated.

**Experiment 3** assessed the feeding response of termites when presented with wood blocks exposed to all white rots in two groups of mass-loss levels, that is, (2, 4)% and (3, 9)%. Only one block from each fungal treatment and each mass-loss level was represented per container and therefore 8 blocks in total were available for the termites to choose from.

**Experiment 4** investigated the feeding response of termites towards wood exposed to *Perenniporia tephropora* which had attained 5, 6, 8, 10, 12 and 14% mass-loss levels. Again, with only one block from each fungal treatment and each mass-loss level represented per container, there were 8 blocks for the termites to choose from.

In **experiment 5**, the wood blocks presented to termites were those which had been exposed to *Tinctoporellus epimiltinus* and had attained 0, 5, or 8% mass-loss. Only one wood block per level of mass-loss was made available in each container, bringing the total number to 5.

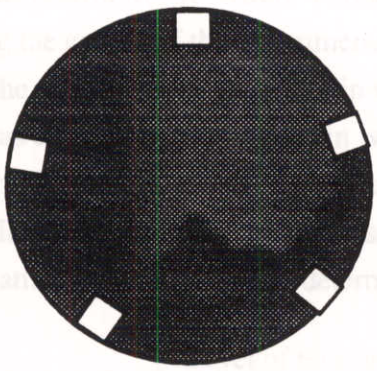
A summary of the experimental details is provided in Table 5.1. Blocks of wood were sorted according to the fungi to which they had been exposed and the tree from which they had been obtained as well as levels of mass-loss associated with each experiment.

Table 5.1 The fungi and mass-loss levels in wood in the choice feeding experiments

| in Experiment No. | Fungal treatment of wood | Percentage mass -loss levels wood under investigation |
|---|---|---|
| 1 | *Coniophora olivacea* | 1 |
| | *Trametes lilacino-gilva* | 1 |
| | *Gloeophyllum trabeum* | 1 |
| | *Perenniporia tephropora* | 1 |
| | *Pycnoporus coccineus* | 1 |
| | *Tinctoporellus epimiltinus* | 1 |
| | Sterile Control | 1 |
| | Control | 1 |
| 2 | *Coniophora olivacea* | 0, 3 |
| | *Trametes lilacino-gilva* | 0, 3 |
| | *Gloeophyllum trabeum* | |
| | Sterile Control | 0 |
| | Control | 0 |
| 3 | *Perenniporia tephropora* | (2, 4) (3, 9) |
| | *Pyynoporus coccineus* | (2, 4) (3, 9) |
| | *Tinctoporellus epimiltinus* | (2, 4) (3, 9) |
| | Sterile Control | 0 |
| | Control | 0 |
| 4 | *Perenniporia tephropora* | 5, 6, 8, 10, 12 14 |
| | Sterile Control | 0 |
| | Control | 0 |
| 5 | *Tinctoporellus epimiltinus* | 0, 5, 8 |
| | Sterile Control | 0 |
| | Control | 0 |

Wood blocks were dipped momentarily in distilled water before placing them in the containers for the experiments so as to minimise the amount of water that they absorbed once placed in the matrix. An approximately equal distance was maintained between all blocks as they were placed around the periphery of each container. The sequence of the blocks was randomised for each container. Figure 5.1 shows the arrangement of blocks in the containers.

a)



b)

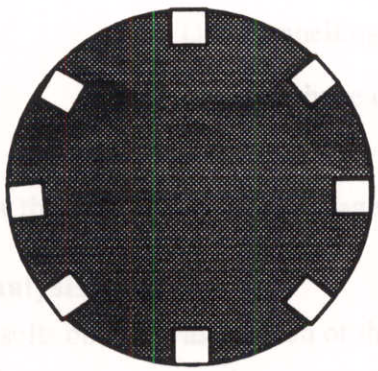


Figure 5.1: A sketch of the arrangement of wood blocks in the containers in choice-feeding experiments
a) container with 5 wood blocks
b) container with 8 wood blocks.

Two grams of *C. lacteus* were placed at the centre of the surface of the matrix in containers in which the blocks had previously been laid out. On average, the total number of individual termites in these groups was 590. The experiments lasted for 3 weeks.

**Methods of assessment of wood consumption**

During the course of the experiments, the mycelia on some of the blocks were reactivated by the conducive environment in which the blocks were maintained. Mass-loss in wood was consequently assessed in two ways. One method attributed mass-loss to combined termite and fungal attack. The other assigned a visual score of termite attack on each block. It took account of physical loss of the woody material and attributed it only to termite attack. The scores were determined as follows:

| Score | Level of termite attack on wood blocks |
|---|---|
| 0 | No visible attack |
| 1 | Light etchings on surface |
| 2 | Tunnelling or deep etchings at one or two spots |
| 3 | Tunnelling right through the block at several places |
| 4 | Deep tunnelling and severe etchings all over block |
| 5 | Original shape of block severely distorted by termite attack |

Plate 5.1-2 show the extent of termite damage associated each score

**Statistical analysis**

The results obtained using each of the methods are considered in turn. All statistical significance tests were done using a t- or F-test at the 1% or 5% probability level. The LSD values provided in the figures are to facilitate comparison within levels of the independent variable. They are not to be used for comparisons between levels of factors.

**Results**

To confirm whether the fungi which colonised the blocks in the course of the experiments were the original cultures with which they were inoculated, samples of blocks from the experiments were sent back to the CSIRO Division of Forestry and Forest Products. With the exception of *C. olivacea* and *G. trabeum*, the rest of the fungi were recovered from the blocks and their identity confirmed.

### i) Assessment of termite feeding preferences according to visual scores

#### Experiment 1: Consumption of wood exposed to one each of all the fungi and at 1% mass-loss level

At the 1% probability level, the species of fungus was the only significant factor of wood consumption. Consumption of wood exposed to *T. lilacino-gilva* was significantly higher than of both types of controls and wood exposed to all the other fungi. The differences in consumption were not significant in all other cases (Fig. 5.2).

#### Experiment 2: Consumption of wood exposed to brown rots at the 0% and 3% mass-loss levels.

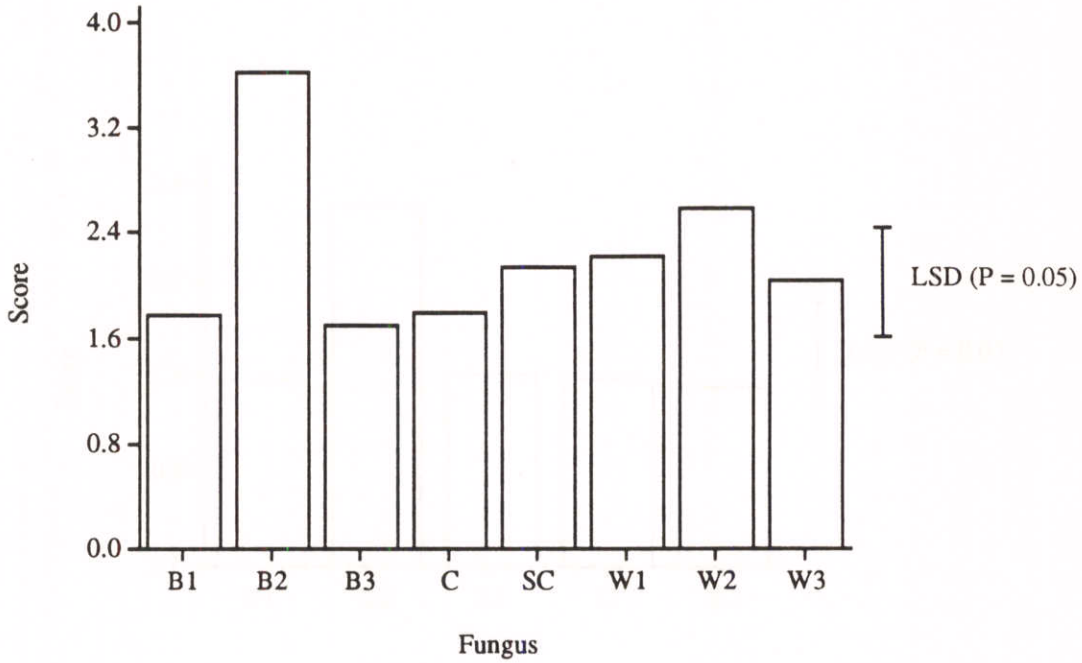
The fungal species to which wood was exposed was a significant factor of wood consumption at the 1% probability level (Fig.5.3). Wood exposed to *T. lilacino-gilva* was consumed significantly more than both types of controls and wood which was exposed to *C. olivacea* or *G. trabeum*. The differences in consumption were not significant in all other cases.

The mass-loss x fungus x tree interaction was also significant at the 1% probability level. Consumption patterns were just the reverse when wood obtained from one tree was compared to the other. See Fig. 5.4 for more details.

#### Experiment 3: Consumption of wood exposed to white rots and at various mass-loss levels

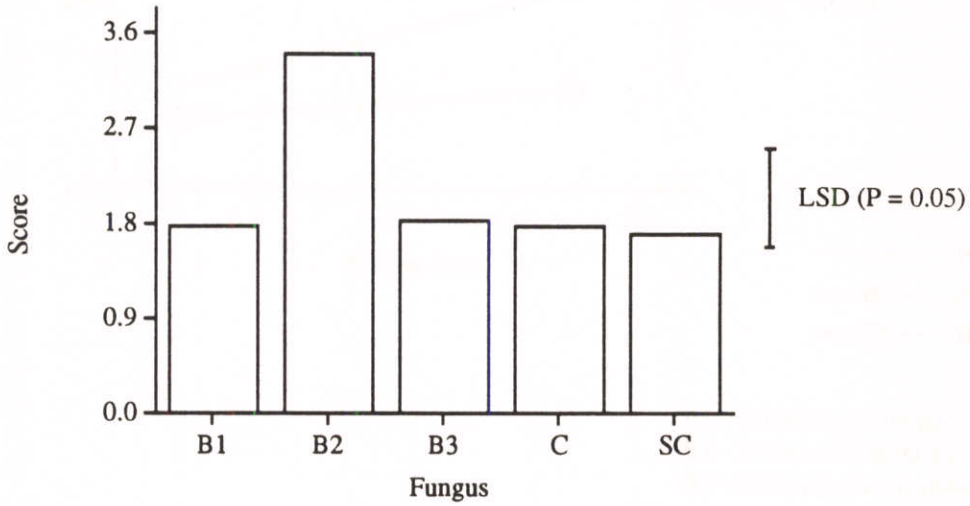
The tree x termite source x mass-loss level interaction was significant at the 1% probability level (Fig. 5.5). Wood consumption patterns were different for the two termite colonies. For colony 1, no differences were found between the wood obtained from both trees at all mass-loss levels. For termite colony 2, the consumption of wood obtained from tree 1 was higher at the 9% mass-loss levels than that obtained from tree 2. At all other levels, consumption of wood obtained from the two trees was not significantly different.

Wood exposed to white rots was consumed significantly more than both types of controls at the 1% probability level (Fig. 5.6).




B1 - *Coniophora olivacea*
B2 - *Trametes lilacino-gilva*
B3 - *Gloeophyllum trabeum*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus epimiltinus*
W3 - *Tinctoporellus epimiltinus*

Figure 5.2: Experiment 1. Visual scores of termite attack on wood at the 1% mass-loss level. LSD are approximate and based on minimum replication i.e they are conservative.




B1 - *Coniophora olivacea*
B2 - *Trametes lilacino-gilva*
B3 - *Gloeophyllum trabeum*
C - Control
SC - Sterile Control

Figure 5.3: Experiment 2. Visual score of termite attack on the wood exposed to brown rots: effect of species of fungus. LSD are approximate and based on minimum replication i.e they are conservative.

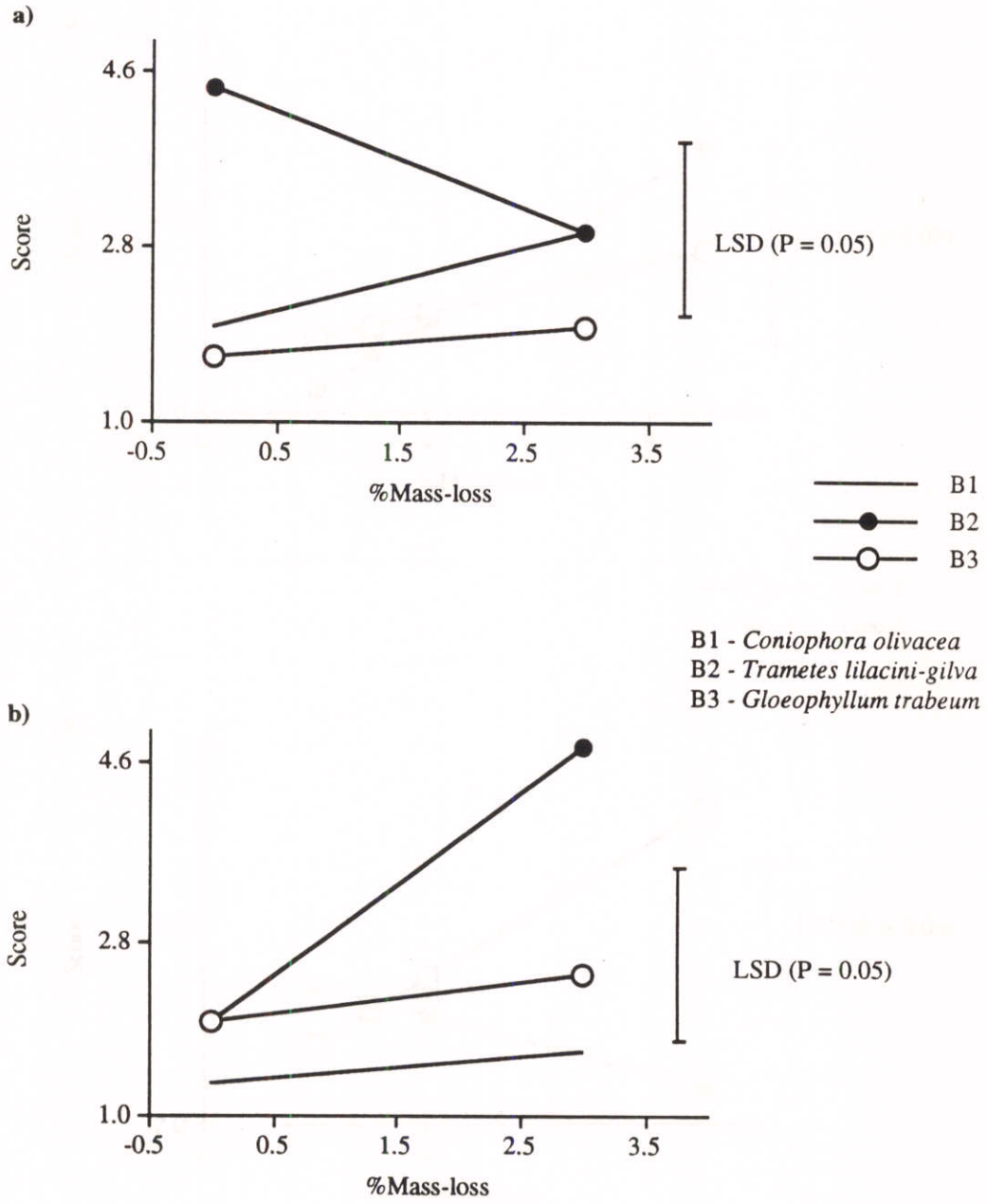



Figure 5.4: Experiment 2. Visual scores of termite attack on the wood exposed to brown rots: effect of the mass-loss x fungus x tree interaction. LSD are approximate and based on minimum replication i.e they are conservative
a) mass-loss x fungus x tree 1
b) mass-loss x fungus x tree 2.

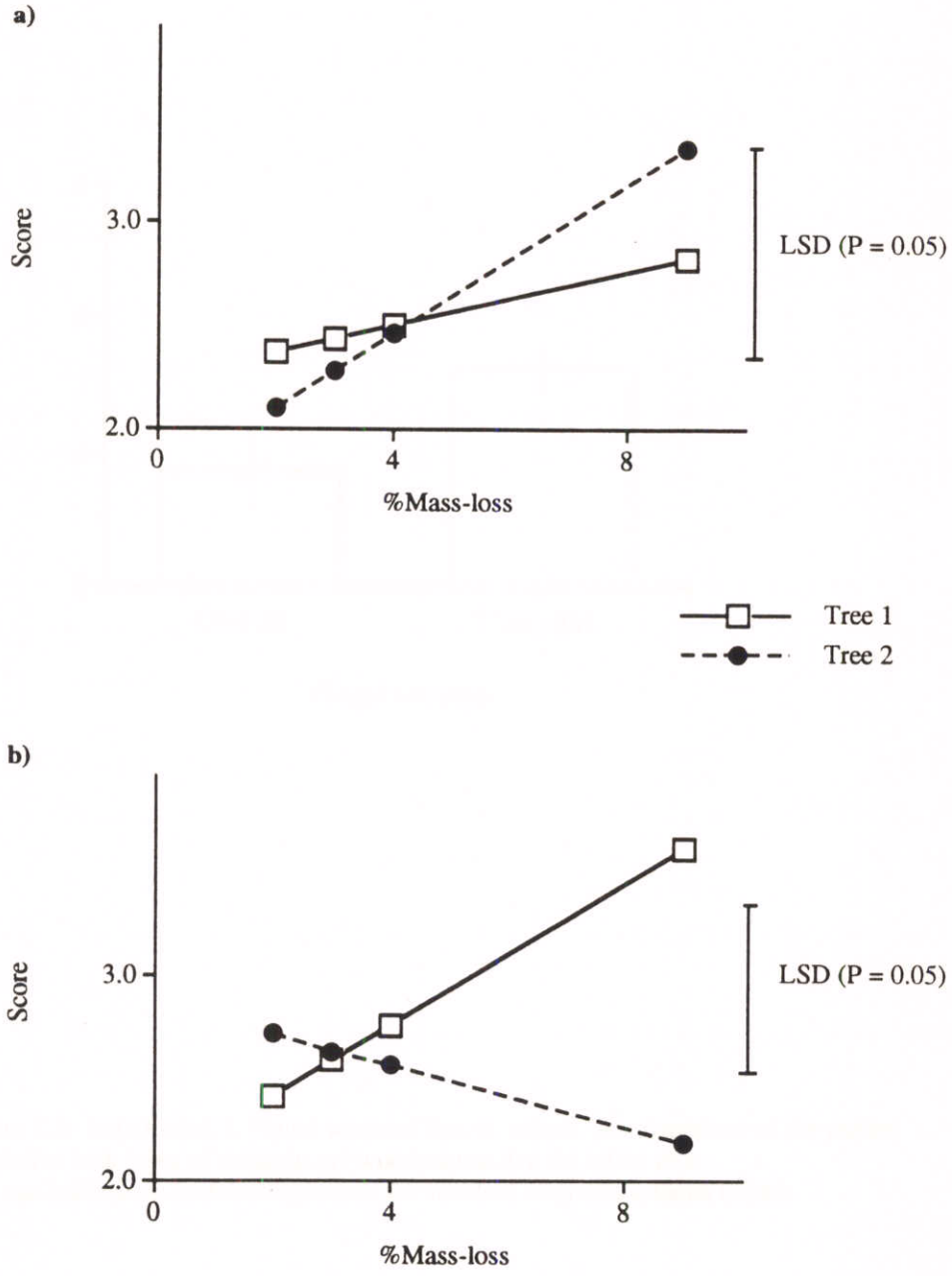



Figure 5.5: Experiment 3. Visual scores of termite attack on wood exposed to white rots: the effect of tree x termite source x mass-loss interaction. LSD are approximate and based on minimum replication i.e they are conservative.
a) tree x mass-loss x termite colony 1
b) tree x mass-loss x termite colony 2.

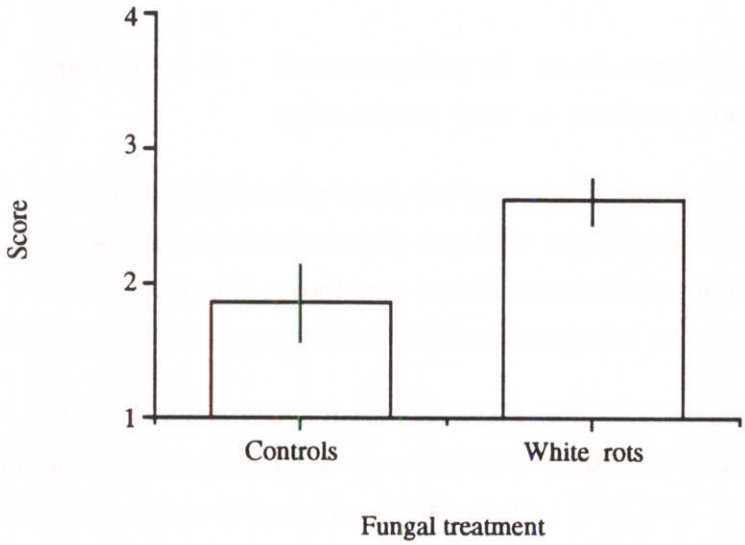



Figure 5.6: Experiment 3. Visual scores of termite attack. A comparison of the pooled results for both types of controls and wood exposed to the white rots.
The vertical line on each bar represents the standard error of the mean (SEM).

**Experiment 4: Consumption of wood exposed to *P. tephropora*. and at various mass-loss**

At the 5% probability level, there were no significant differences in wood consumption due to different mass-loss levels or the species of fungi to wood was exposed.

**Experiment 5: Consumption of wood exposed to *T. epimiltinus* and at various mass-loss**

At the 5% probability level, the fungus x tree interaction was significant (Fig. 5.7). For wood obtained from tree 2, the sterile control was consumed significantly more than wood exposed to *T. epimiltinus* or the untreated control. Consumption was not significantly different between the wood treatments for wood obtained from tree 1.

The termite source x mass-loss interaction was also significant at the 5% probability level. Significantly more wood was consumed at the 8% mass-loss level by termites from colony 2 than those from colony 1 (Fig. 5.8).

At the 1% probability level, the consumption of wood exposed to *T. epimiltinus* was significantly higher than that of the pooled results for both types of controls (Fig. 5.9).

**ii) Mass-loss according to combined action of termites and fungi in the wood blocks**

These results are to be interpreted with caution since they reflect combined action of termites and fungi on mass-loss rather than the customary mass-loss due to termite attack.

**Experiment 1: Mass-loss in wood exposed to one each of all the fungi and at the 1% mass-loss level**

The species of fungus to which wood was exposed was a significant factor of mass-loss at the 1% probability level. Wood exposed to *Trametes lilacino-gilva, P. coccineus* and *Perenniporia tephropora* lost significantly more mass than the controls and wood exposed to the rest of the fungi (Fig. 5.10). There was no significant difference in mass-loss in wood in all other cases.

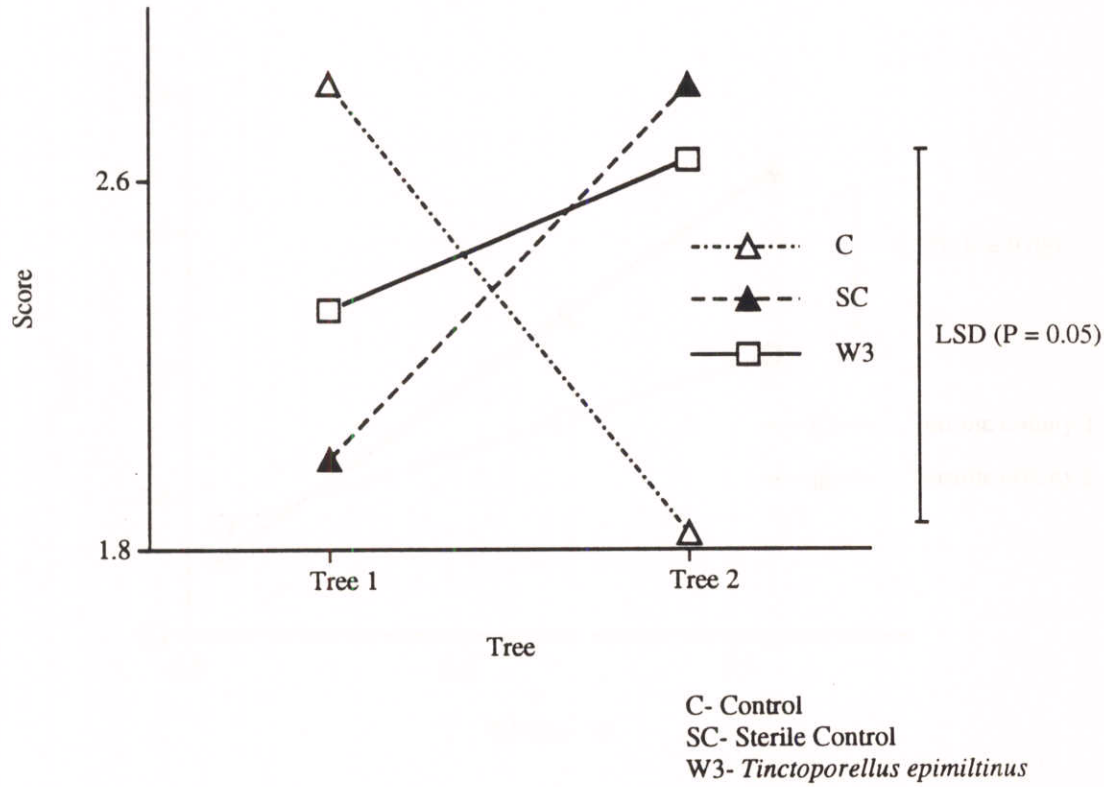



Figure 5.7: Experiment 5. Visual scores of termite attack on wood exposed to *Tinctoporellus epimiltinus:* the effect of the species of fungus x tree interaction. LSD are approximate and based on minimum replication i.e they are conservative.

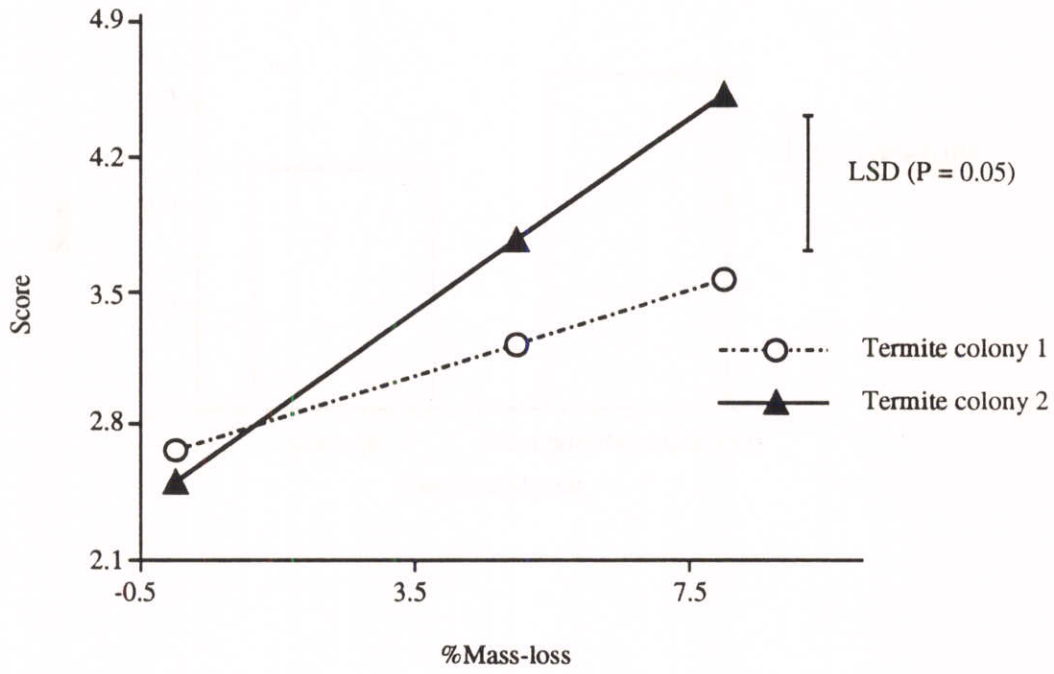



Figure 5.8: Experiment 5. Visual scores of termite attack on wood exposed to *T. epimiltinus*: the effect of mass-loss x termite source interaction. LSD are approximate and based on minimum replication i.e they are conservative.

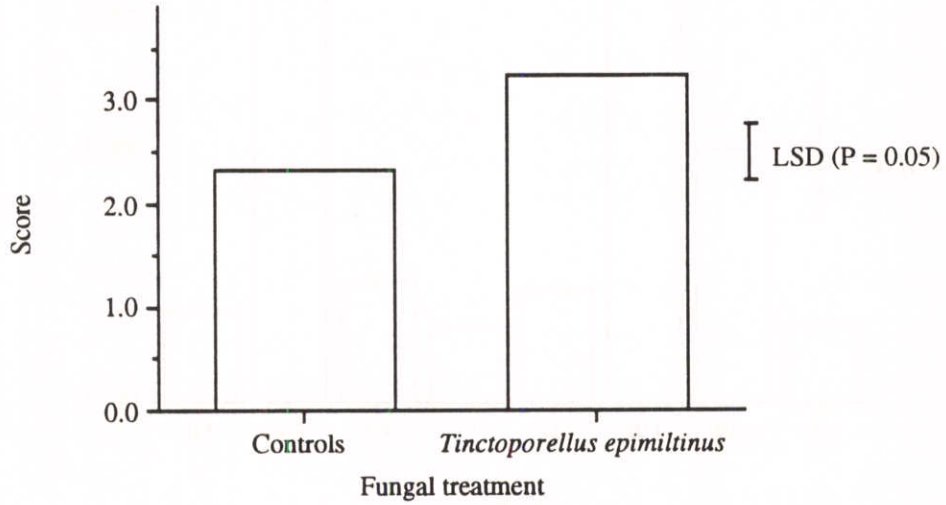
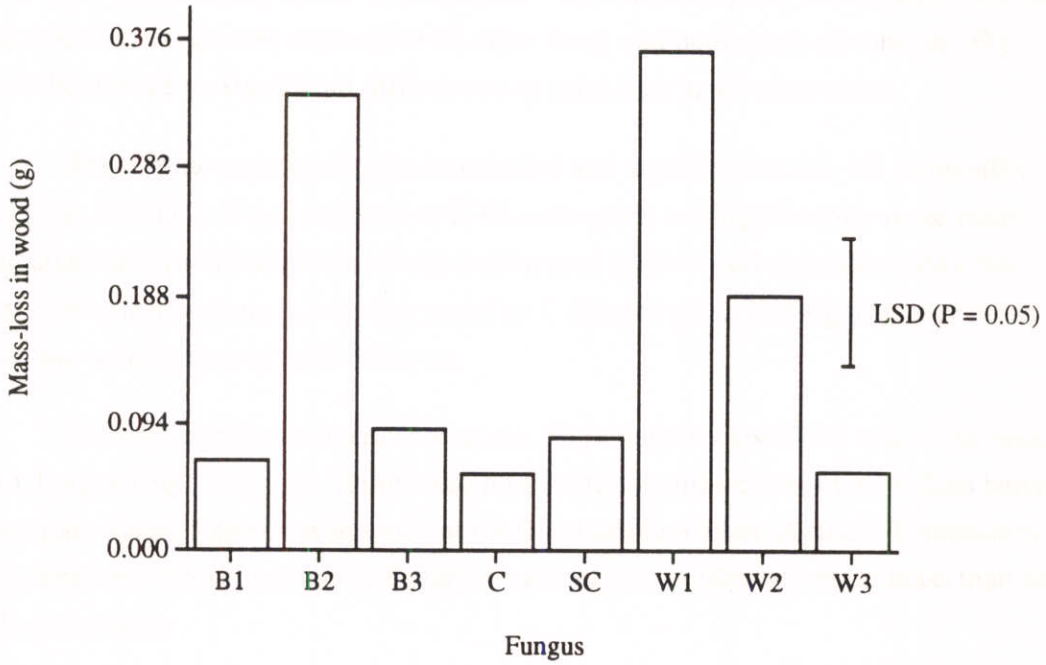



Figure 5.9: Experiment 5. Visual scores of termite attack. A comparison of the pooled results for both types of controls and wood exposed to *TTinctoporellus epimiltinus.* LSD are approximate and based on minimum replication i.e they are conservative.




B1- *Coniophora olivacea*
B2- *Trametes lilacino-gilva*
B3- *Gloephyllum trabeum*
C- Control
SC- Sterile Control
W1- *Perenniporia tephropora*
W2- *Pycnoporus coccineus*
W3- *Tinctoporellus epimiltinus*

Figure 5.10: Experiment 1. The combined effect of termite and fungal attack on mass-loss (g) in all wood treatments at the 1% mass-loss level. LSD are approximate and based on minimum replication i.e they are conservative.

### Experiment 2: Mass-loss in wood exposed to brown rots and at the 0% and 3% mass-loss levels.

The fungal species to which wood was exposed was a significant factor of mass-loss at the 1% probability level. Wood exposed to *T. lilacino-gilva* lost significantly more mass than that which was exposed to all other fungi and both types of controls (Fig. 5.11).There were no significant differences in mass-loss in all other cases.

The termite source x fungus interaction was significant at the 1% probability level (Fig. 5.12 (a)). Wood exposed to *T. lilacino-gilva* lost significantly more mass when attacked by termites from colony 2 compared to the wood exposed to the other fungi. For termite colony 1, wood exposed to *T. lilacino-gilva* lost significantly more mass than wood exposed to *C. olivacea.*

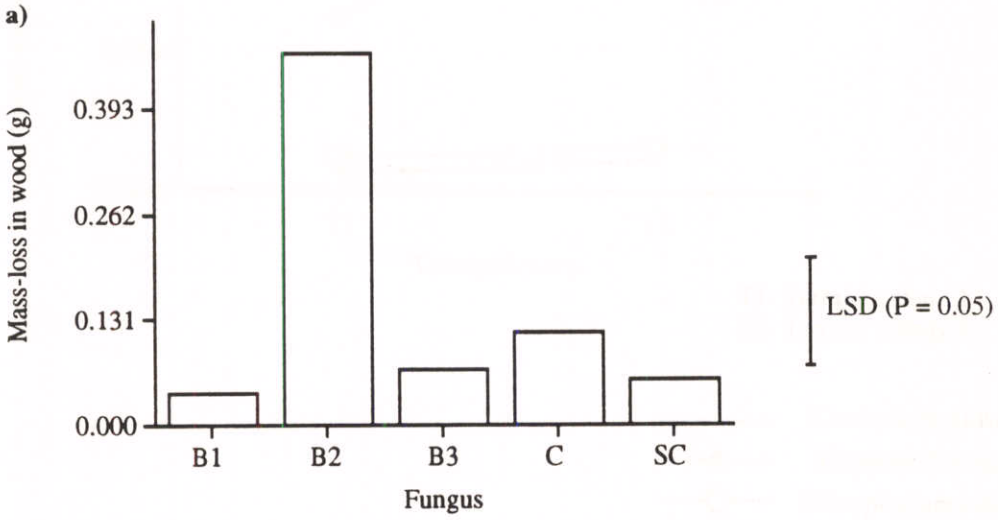
Another significant interaction at the 1% probability level was that of the mass-loss x fungus (Fig. 5.12 (b)). There were no significant differences in mass-loss between the various fungal treatments in wood at the 0% mass-loss level. At the 3% mass-loss level, however, wood exposed to *T. lilacino-gilva* lost significantly more mass than wood in all other cases.

Also significant at the 1% probability level was the termite source x fungus x tree interaction (Fig 5.13). The patterns of mass-loss varied from one tree to the other, especially for termite colony 2.

### Experiment 3: Mass-loss in wood exposed to white rots and at various mass-loss levels

At the 1% probability level, the fungal species and mass-loss were significant factors affecting mass-loss in wood. Wood exposed to *P. tephropora* lost significantly more mass than wood exposed to the other white rots (Fig. 5.14 (a)). Wood exposed to *T. epimiltinus* lost the least mass while mass-loss in wood exposed to *P. coccineus* was intermediate. Mass-loss increased significantly with increasing level of mass-loss in the wood (Fig. 5.14 (b)).

In addition, at the 1% probability level, mass-loss in wood exposed to white rots lost significantly more mass than both types of controls. The analysis of results was conducted by contrasting pooled results of mass-loss in wood exposed to white rots with the pooled results for the sterile and untreated controls (Fig. 5.15).




B1 - *Coniophora olivacea*
B2 - *Trametes lilacino-gilva*
B3 - *Gloeophyllum trabeum*
C - Control
SC - Sterile Control

Figure 5.11: Experiment 2. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to the brown rots according to species of fungus. LSD are approximate and based on minimum replication i.e they are conservative.

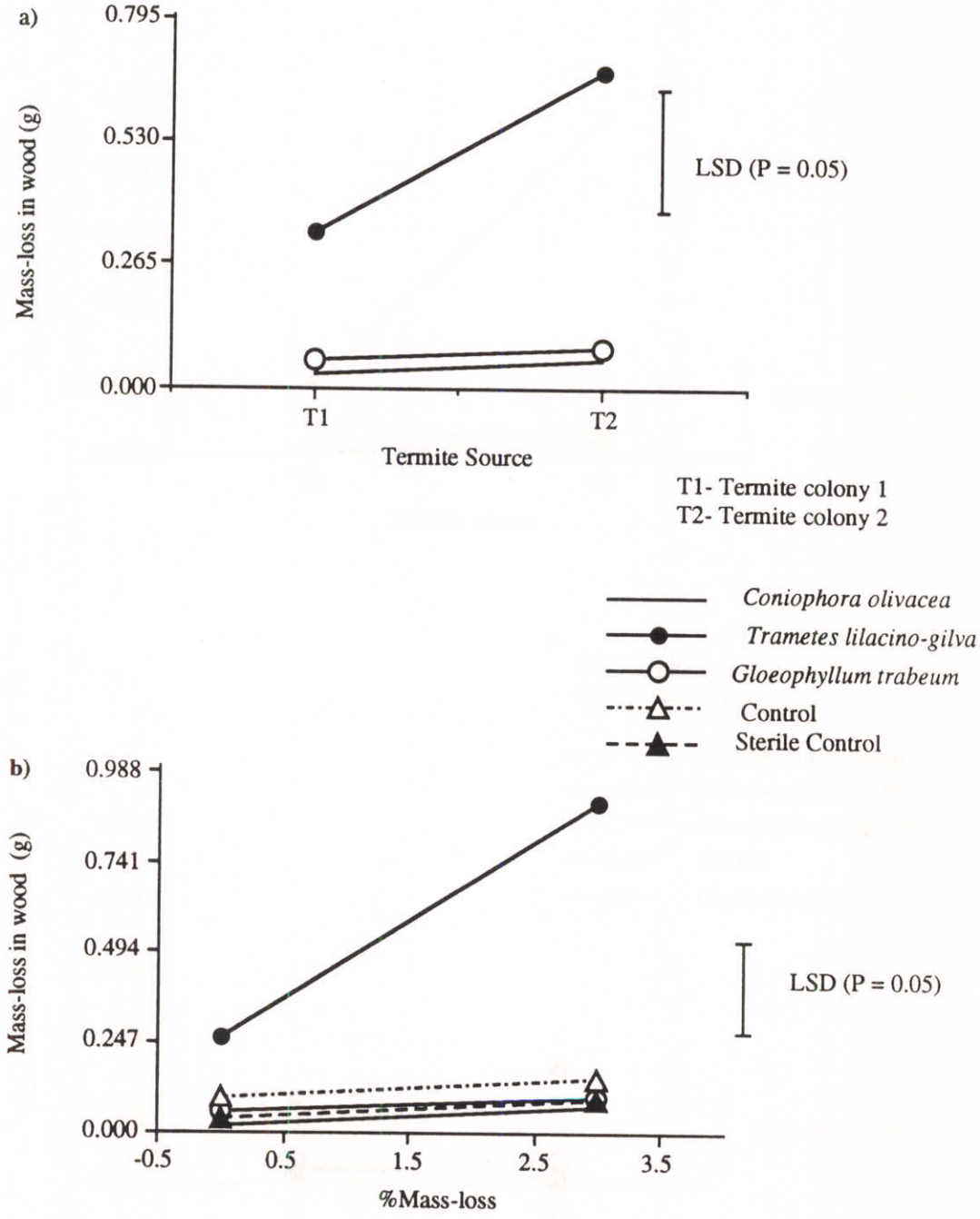



Figure 5.12: Experiment 2. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to brown rots: two-way factor interactions. LSD are approximate and based on minimum replication i.e they are conservative.
a) termite source x fungus interaction
b) mass-loss x fungus interaction.

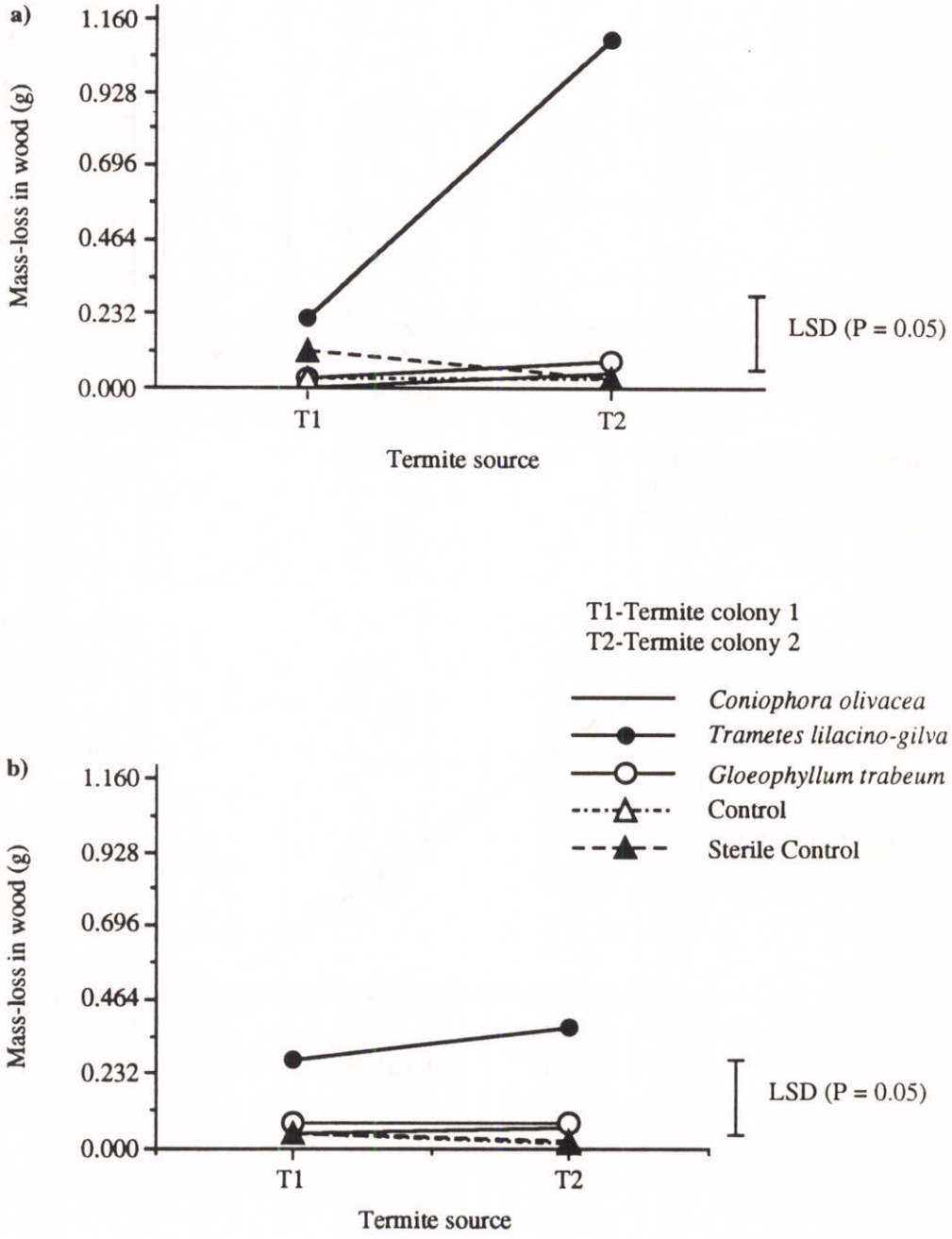



Figure 5.13: Experiment 2. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to brown rots: three-way factor interactions. LSD are approximate and based on minimum replication i.e they are conservative.
a) termite source x fungus x tree 1
b) termite source x fungus x tree 2.

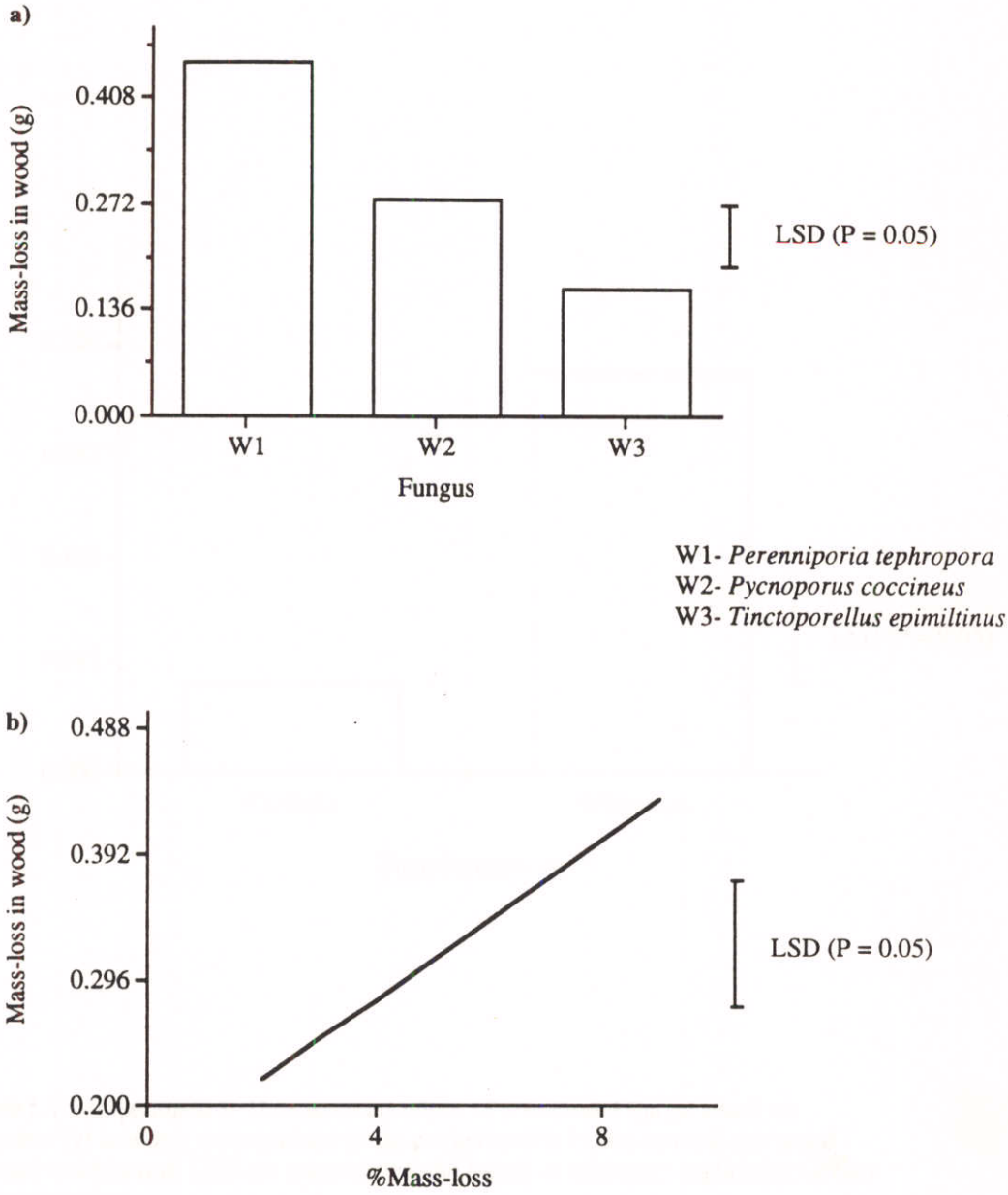



Figure 5.14: Experiment 3. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to the white rots: main factors of the experiment. LSD are approximate and based on minimum replication i.e they are conservative.
a) fungal species to which wood was exposed
b) mass-loss level in the wood.

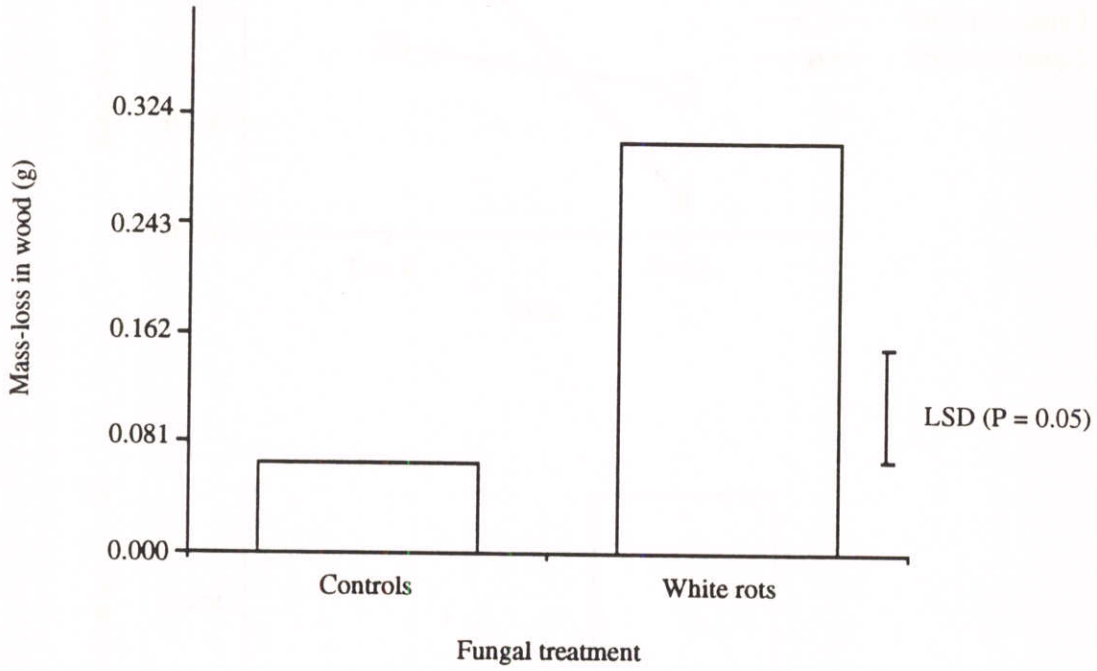



Figure 5.15: Experiment 3. The combined effect of termite and fungal attack on mass-loss (g) in wood: a comparison of the pooled results for the controls and wood exposed to white rots. LSD are approximate and based on minimum replication i.e they are conservative.

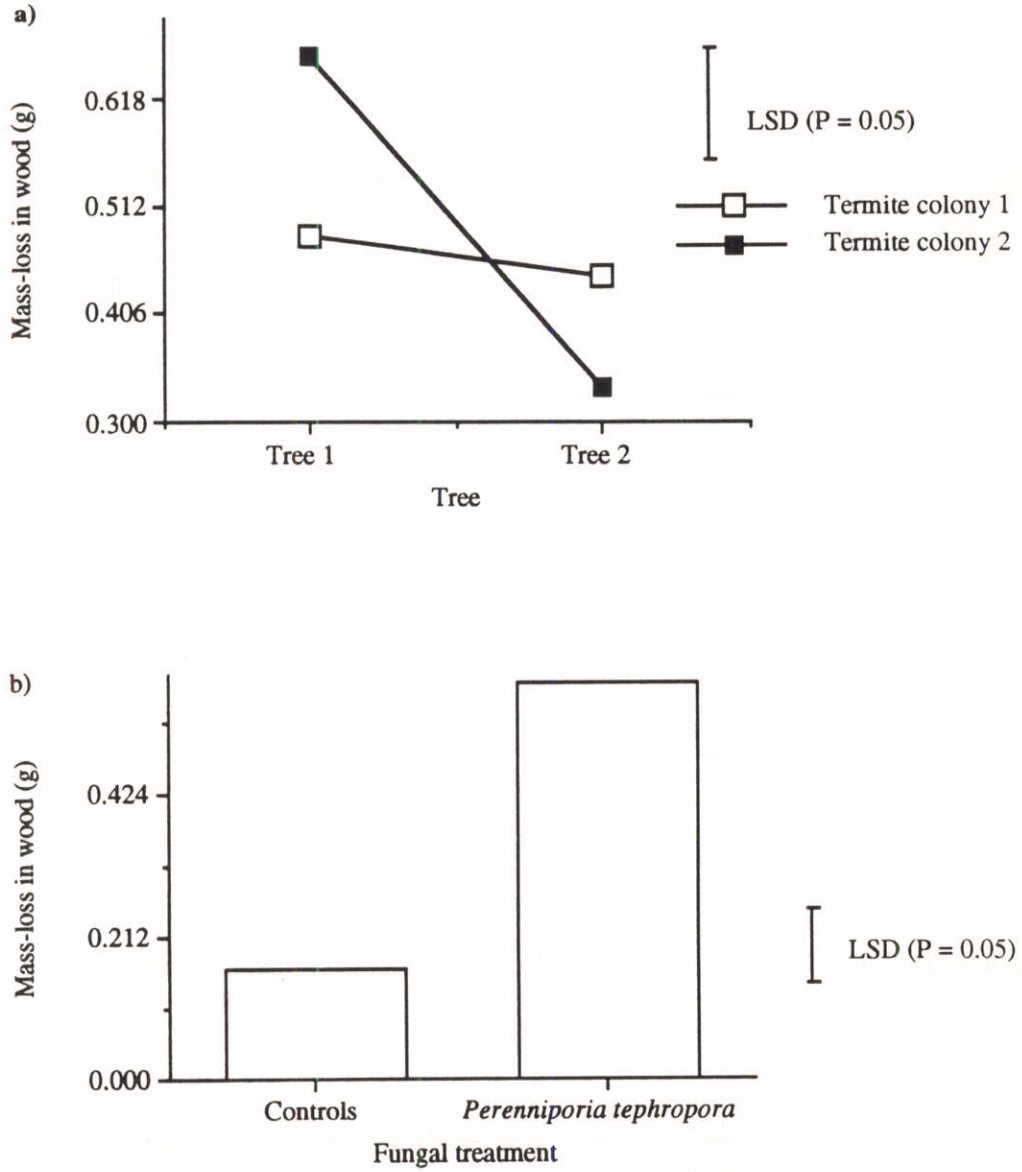



Figure 5.16: Experiment 4. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to *Perenniporia tephropora*. LSD are approximate and based on minimum replication i.e they are conservative.
a) effect of termite source x tree interaction
b) a comparison of wood exposed to the fungus and the pooled results for both types of controls.

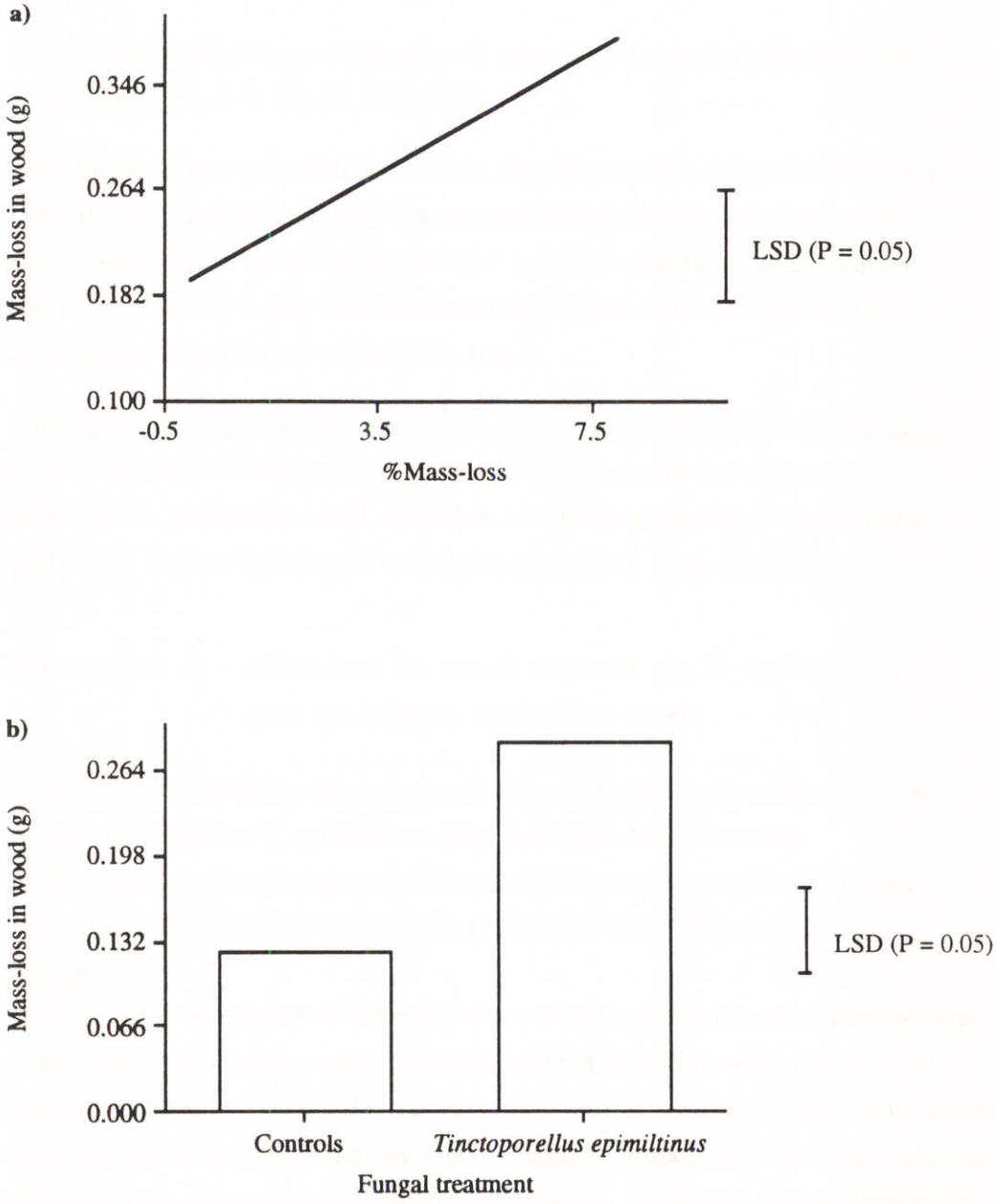



Figure 5.17: Experiment 5. The combined effect of termite and fungal attack on mass-loss (g) in wood exposed to *Tinctoporellus epimiltinus*. LSD are approximate and based on minimum replication i.e they are conservative.
a) effect of mass-loss
b) a comparison of the wood exposed to the fungus and the pooled results for both types of controls.

### Experiment 4: Mass-loss in wood exposed to *P. tephropora*.and at various mass-loss levels

There were no significant differences in mass-loss in wood at all mass-loss levels investigated (5, 6, 8, 10, 12 and 14%).

The termite source x tree interaction was significant at the 1% probability level (Fig. 5.16 (a)). Termites from colony 2 were associated with significantly more mass-loss in wood when compared to colony 1 and when the wood was obtained from tree 1. There were no significant differences in mass-loss between the two colonies when the wood was obtained from tree 2.

When the analysis of results was conducted by contrasting mass-loss in wood exposed to *P. tephropora* with the pooled results for the sterile and untreated controls, at the 1% probability level, mass-loss in wood exposed to *P. tephropora* was significantly higher than that of both types of controls (Fig. 5.16 (b)).

### Experiment 5: Mass-loss in wood exposed to *T. epimiltinus* and at various mass-loss levels

At the 1% probability level, mass-loss increased significantly with mass-loss level for wood exposed to *T. epimiltinus* (Fig. 5.17 (a)). Mass-loss was significantly higher for wood exposed to *T. epimiltinus* when compared to pooled results of both types of controls at the same probability level (Fig. 5.17 (b)).

**Discussion**

Since termites encounter unsterilized wood in the field, one would expect that wood containing live mycelia would contribute most information with regard to the significance of decayed wood for the biology of the termite species. In this study, all the wood blocks exposed to decay fungi were considered to contain live mycelia. Failure to recover *Coniophora olivacea* and *Gloeophyllum trabeum* at the end of the experiments did not necessarily mean that the fungi were not present or alive in the wood blocks. Rather, it could be that there were other microorganisms in the wood which competed better on the isolation medium (G. C. Johnson, CSIRO Division of Forestry, pers. comm., 1991).

Smythe *et al.* (1971) did not attempt to separate fungus from termite effects when they presented wood with live mycelia to termites. No conclusions were reached from those experiments. Faced with similar difficulties and having failed to distinguish between the causes of mass-loss by using damage ratings, French *et al.* (1981) recommended that termite attack on wood blocks containing live mycelia would be

assessed best by visual ranks in addition to assigning damage ratings. Ruyooka (1978) took the combined action of termites and fungi to be responsible for the mass-loss in such wood and this approach enabled him to reach some conclusions.

In the current study, mass-loss in the wood blocks due to the combined action of termites and fungi was assessed by mass (g). However, because the choice of wood blocks provided was wide and decay could have progressed unaffected in the blocks on which termites did not spend much time, visual scores of termite attack were assigned to each block to distinguish the mass-loss which was solely the result of termite attack form that caused by both agents. This discussion is based on the findings obtained with assessment of wood consumption by visual scores.

The species of the fungus to which wood was exposed was an important factor of wood consumption when all fungi were compared and also for the wood exposed to brown rots. Wood exposed to *Trametes lilacino-gilva* was consumed the most in both cases. This agreed with the earlier report (Chapter 4) whereby termites had only one type of wood to feed on

When the white rots were compared one against the other, the consumption of wood was not influenced by fungus species. Instead, it was influenced by the interaction of the tree, the mass-loss in wood and the termite source. The influence of mass-loss could not be isolated from the effects of these other factors in the determination of the amount of wood consumed.

Matsuo and Nishimoto (1973) who found that *Coptotermes formosanus* consumed more pine wood with increasing decay in the wood.In the current study, wood exposed to *P. tephropora* was not eaten whatever the mass-loss level it had attained. Perhaps the wood exposed to *P.tephropora* acquired substances from the fungus which were repellent to termites. Amburgey (1979) reported that wood exposed to some, but not all, white rots portrayed such a characteristic.

In the case of a choice between wood exposed to white rots and both types of controls, sound wood was consumed less than decayed wood. This applied to the wood exposed to the white rots in general. With a choice of brown rots, the palatability of wood exposed to *T. lilacino-gilva* seems to have biased the termites towards consuming it far more than wood exposed to the other brown rots or the controls.

This preferential consumption of wood exposed to *T. lilacino-gilva*, with a correspondingly low consumption of other wood treatments on offer, also happened when all other fungi, including white rots, were included in the choice. Wood exposed to

*T. lilacino-gilva* twas therefore the most preferred food for *C. lacteus* amongst all the wood treatments in this study.

French *et al.* (1981) found that upon presenting decayed and undecayed *Pinus radiata* sapwood to *Coptotermes acinaciformis* , the state of the mycelia was an important determinant of consumption. The most attractive wood when sterilized was the least palatable when it contained live mycelia. The state of wood may thus determine the suitability of decayed wood as a bait. In this study where the wood was not sterilized, wood exposed to *T.lilacino-gilva* was the most attractve to *C. lacteus.*

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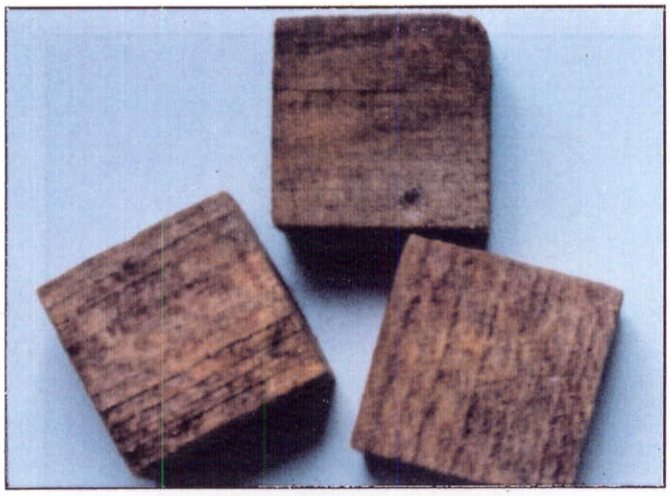

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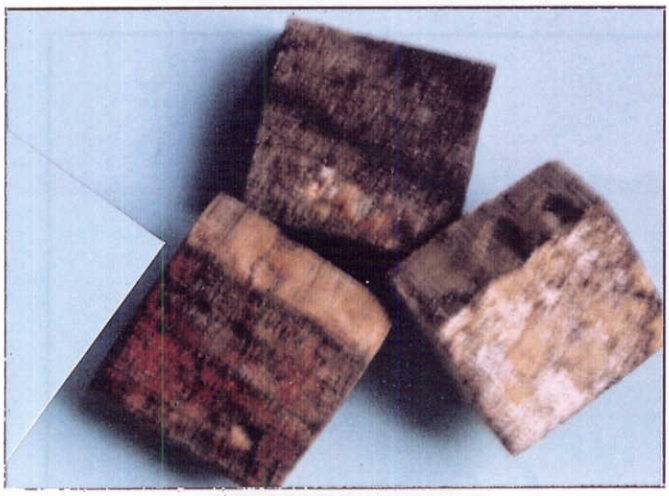

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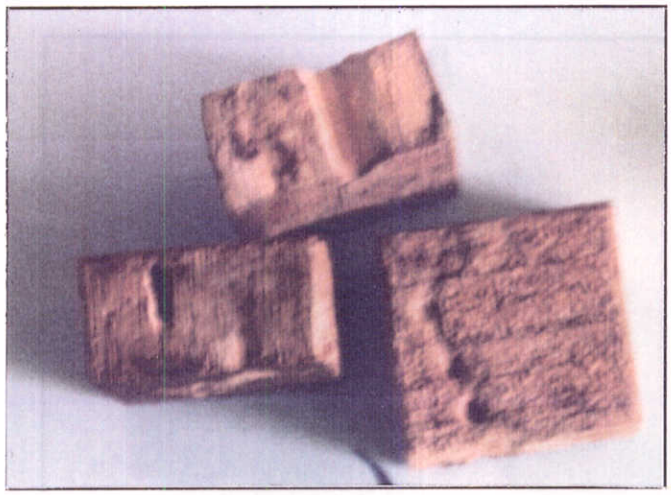

Plate 5.1: The extent of termite attack associated with the visual scores method of assessment of wood consumption
0 - No visible sign of attack
1 - Light etchings on surface
2 - Tunnelling or deep etchings at one or two spots

3

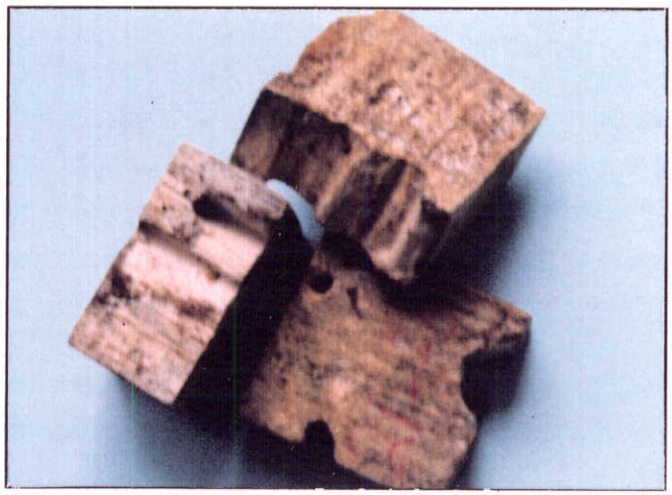

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Plate 5.2: The extent of termite attack associated with the visual scores method of assessment of wood consumption
3 - Tunnelling right through blocks at several places
4 - Deep tunnelling and severe etchings all over block
5 - Original shape of block severely distorted by termite attack

# CHAPTER 6

## Termite colony development.

## Introduction

Termites are cryptobiotic in nature and this severely hinders colony development studies. For this reason, only about 2% of the 2500 termite species have been studied in this respect. Studies have focussed on such aspects of colony development as the relationship between temperature and colony growth rate as well as the timing of the appearance of different castes (Becker 1961; Garcia and Becker 1975; Han and Noirot 1983).

The growth dynamics of termites were linked with food quality by Becker (1965). He reported that the nutritive value of wood exposed to favourable basidiomycetes was approximately double that of sound wood and that it furthered development in the three species of termites he investigated. Such superior nutritive value was attributed to vitamins and nitrogen-rich tissues in decayed wood which sound wood does not possess, a suggestion with which Hendee (1935), Ruyooka (1978), Waller and Fage (1987) and Wood (1978) agreed. For *Coptotermes lacteus*(Froggatt), a feeding preference for decayed over sound wood has already been documented by Lenz *et al.* (1980). The impact of microbially-modified wood on colony development has not been previously investigated.

In this study, *C. lacteus* primary reproductives (dealated pairs) were presented with wood that had been exposed either to a brown- or a white-rot fungus. The species of fungi selected from the six basidiomycetes available were some of those previously involved in the study of other aspects of termite-fungi relationships in Australia (French 1978; French *et al.* 1981; Lenz *et al.* 1980). The level of decay in the wood was shown to be an important determinant of wood consumption (Becker and Lenz 1975) and its impact on colony development was assessed in this study so as to make it as comprehensive as possible.

The time available for the study reported here was limited and consequently, development of colonies of *C. lacteus* in the laboratory was studied for only six and a half months.

## Methods

Colony development experiments were set up before the statistical analysis of the fungal bioassay was done. The knowledge gained from the bioassay (Chapter 3) and from the feeding response and survival experiments reported in Chapters 4 and 5 could not therefore have been utilized in the setting up of the colony development experiment. The assumption at the stage when the experiment was set up was that mass-loss was proportional to the duration of exposure of wood blocks to fungi .

**Timber**

Details about the timber are given in Chapter 2 while the fungal bioassay is covered in detail in Chapter 3. Mass-loss in the wood was selected according to the duration of time to which wood blocks were exposed to the decay fungi. This might have led to the overlap of mass-loss levels in the specified low, medium and high categories as listed below.

For the brown rot, wood blocks were available only at the low mass loss levels (0-3%). With the white rot, it was possible to allocate wood blocks for the experiment at three levels of mass loss-low, medium and high details of which depended on the tree from which wood was obtained (Table 6.1).

Table 6.1 Mass-loss levels at which wood blocks exposed to *Perenniporia tephropora*, a white rot, were provided as food for incipient colonies.

| Source of wood blocks | Termite source | % Mass-loss category | % mass-loss level |
|---|---|---|---|
| Tree 1 | Colony 1 | Low | 3-8 |
| | | Medium | 9-15 |
| | | High | 16-25 |
| | 2 | Low | 3-8 |
| | | Medium | 9-21 |
| | | High | 14-30 |
| Tree 2 | Colony 1 | Low | 2-13 |
| | | Medium | 9-21 |
| | | High | 16-26 |
| | 2 | Low | 3-8 |
| | | Medium | 9-21 |
| | | High | 15-30 |

Each primary reproductive pair was presented with a group of three wood blocks which were randomly selected from one of the trees and at one of the three mass-loss categories.

**Fungi**

Details about the fungi are given in Chapter 2. The representative white and brown rot fungi for this study were chosen at random out of the six available for the experiments.

**Termites**

*Coptotermes lacteus* alates were collected from two field colonies in partly cleared pastureland in the Bungendore area, NSW, Australia. The first colony inhabited a mound which was 1m high and had a basal circumference of 6.25m. It was located at the edge of a patch of dry sclerophyll forest (See Plate 6.1 (a)). The second colony occupied a mound which was 0.6m high and had a basal circumference of 3.18m. It was established against a tree stump in an open area. Both colonies released alates in mid-late October, the usual time for this occurrence in the area (Gay and Calaby 1970).

**Alate collection**

To collect alates, sack-like traps which were slightly larger in size than the mound in question were made-to-measure out of terylene material. These were placed open-side-down over mounds. The base of each of the traps was firmly attached to the mound with a sisal cord that could be drawn tightly around the mound. Soil was heaped around the base of traps wherever irregularity of the shape of mound made it necessary to enhance this seal. The sides of the traps were tied with ribbons to supporting steel fencing pickets to give alates room to fly, behaviour which stimulates shedding of wings (Plate.6.1 (b)).

Emerged alates were placed in large plastic boxes which were padded with moistened paper and fitted loosely with lids (See Plate 6.2). Some of the alates shed their wings during transportation back to the laboratory but the wings of others had to be taken off manually.

The sex of the individuals was determined. Male and female individuals were placed in separate containers. By picking an individual at random from each container, male/female pairs were formed.

Details about the preparation of the matrix in which the colonies were established are provided in Chapter 2. Plastic vials (120ml) were filled with 14g of powdered inner carton material from *C. lacteus* mounds. This was moistened to 100% moisture content so as to make it a suitable substrate for the primary reproductives and their brood. Three wood blocks were buried in the centre of the vial. These blocks were in the same mass-loss category following exposure to the same fungus.

The vials in which the reproductive pairs were placed were arranged according to replicates in rectangular plastic containers. A small amount of water was maintained in the base of these containers to provide a high relative humidity in the environment of the colonies. The alate pairs were left to breed for 6.5 months in a constant temperature room (29° C, 83% RH). The parameters of assessment of colony growth were amount of wood

consumed, the mass of the offspring, the total number of offspring reared and the proportion of each caste relative to total number of offspring reared.

**Statistical methods**

The significance of results was tested with the F- and t-tests at the 1% or 5% probability levels. In the results section, the LSD values provided are to facilitate comparison of results within a given level of the independent variable in figures which depict factor interactions. They are not to be used for making comparisons between levels of the factors.

**Wood consumption by incipient colonies**

Because unsterilized blocks were used, mass-loss due to the continued action of the fungus could not be isolated from the termite component. The extent of termite attack on wood blocks was assessed by assigning them a visual score according to severity of attack as follows:

0 - No attack on wood blocks

1 - Wood blocks barely attacked

2 - Wood blocks moderately tunnelled through

3 - Wood blocks extensively tunnelled through

4 - Wood blocks completely disintegrated as a result of termite attack.

## Results

The behaviour of different alate pairs varied right from the start of the experiment. Some burrowed their way through the matrix to establish a network of galleries at the base of the vial. Others made their nest adjacent to the wood blocks in the middle section of the vial while yet others occupied the top of the matrix. Mortality rate was 25%, with the majority of the primary reproductives which never got established dying within a few weeks of the start of the experiment. There was a number of lone survivors which lasted through the duration of the experiment. The activities of the primary reproductive pairs which established themselves against the sides of the vials were visible for the initial part of the experiment but as the colonies grew, the clear sides of the vials were plastered with wet deposits which finally hid the colonies from sight. The observations reported here were those made where circumstances permitted.

Primary reproductive pairs moved actively around the galleries and were in constant physical contact; stopping to acknowledge each other with their antennae whenever they went past each other. Up to 14 eggs were counted on the 15$^{th}$ day after incipient colonies were set up with the alate pairs from the first field colony. In some of those colonies initiated with primary reproductive pairs from the second field colony, up

to 5 eggs were found on the sixth day. Larvae hatched 30 days after the colonies were initiated. The reproductive pairs took care of their broods and were assisted in this task at a later stage by the young workers they had reared. By the second month when colonies sealed themselves out of sight, the egg, larvae and worker stages were all represented in some cases. The white mycelia of *P. tephropora* grew actively in all tubes where blocks exposed to the fungus were presented to the incipient colonies.

In the most successful colonies, caste development at the end of 6.5 months was as follows: - i) eggs in different embryonic development stages appeared in clusters. These separated easily into individual eggs when disturbed, ii) 2 stages of fragile opaque-white larvae, iii) 2 stages of workers distinguishable from larvae by their more developed head features and deeper pigmentation on body and iv) presoldiers (with white mandibles) and soldiers (with dark brown mandibles). The relative success of colonies was centred around which of these castes was represented, in what number and their mass. The full range of data collected is given in Appendix 4. See also Plates 6.3 - 6.5.

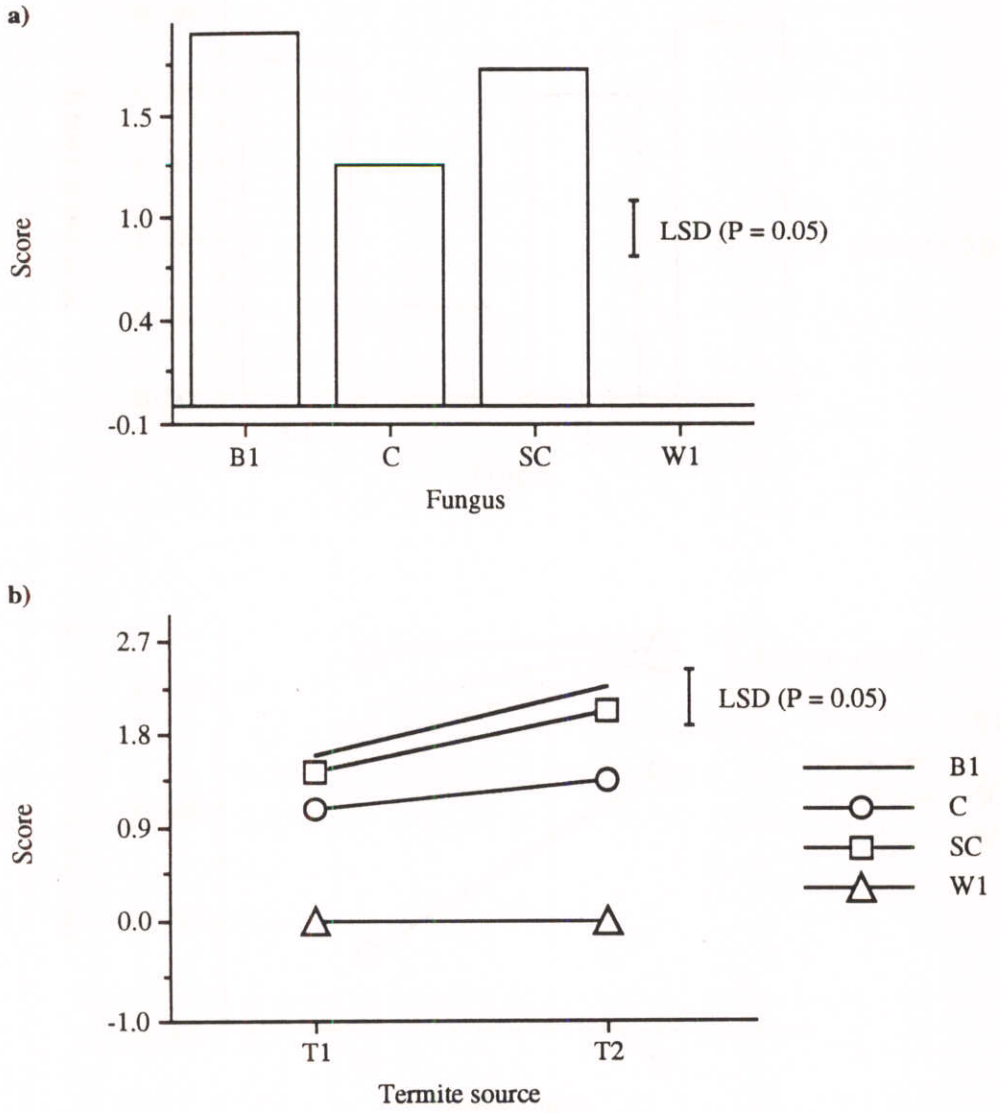
**Wood consumption: Visual scores.**

The fungus species to which wood was exposed was a significant factor of wood consumption at the 1% probability level (Fig. 6.1 (a)). There was no significant difference in consumption of wood exposed to *C. olivacea* and the sterile control. Wood exposed to *P. tephropora* was not noticeably eaten. The untreated control was consumed significantly less than wood exposed to *C. olivacea* or the sterile control.

The termite source x fungus species interaction was significant at the 1% probability level (Fig. 6.1 (b)). For both termite colonies, wood exposed to *P. tephropora* was consumed significantly less than all other wood. With termites from colony 1, there were no significant differences in the consumption of wood exposed to *C. olivacea* and both types of controls. Termites from colony 2 consumed significantly more wood when it was exposed to *C. olivacea* compared to the control. See Fig. 6.1 (b) for more details.

**Mass-loss in the wood due to combined action of termite and fungal attack**

At the 1% probability level, the fungus species to which wood was exposed was a significant factor of mass-loss (Fig. 6.2 (a)). Wood exposed to *Coniophora olivacea* lost significantly more mass than the untreated control but not more than the sterile control. Wood exposed to *Perenniporia tephropora* lost significantly more mass than either type of control. There was no significant difference in mass-loss in wood exposed to *P. tephropora* and *C. olivacea.*




T1 - Termite colony 1
T2 - Termite colony 2

B1 - *Coniophora olivacea*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*

Figure 6.1: Termite colony development. Visual scores of wood consumption by incipient colonies
a) fungus species to which wood was exposed
b) termite source x fungus species interaction
LSD are approximate and based on minimum replication i.e. they are conservative.

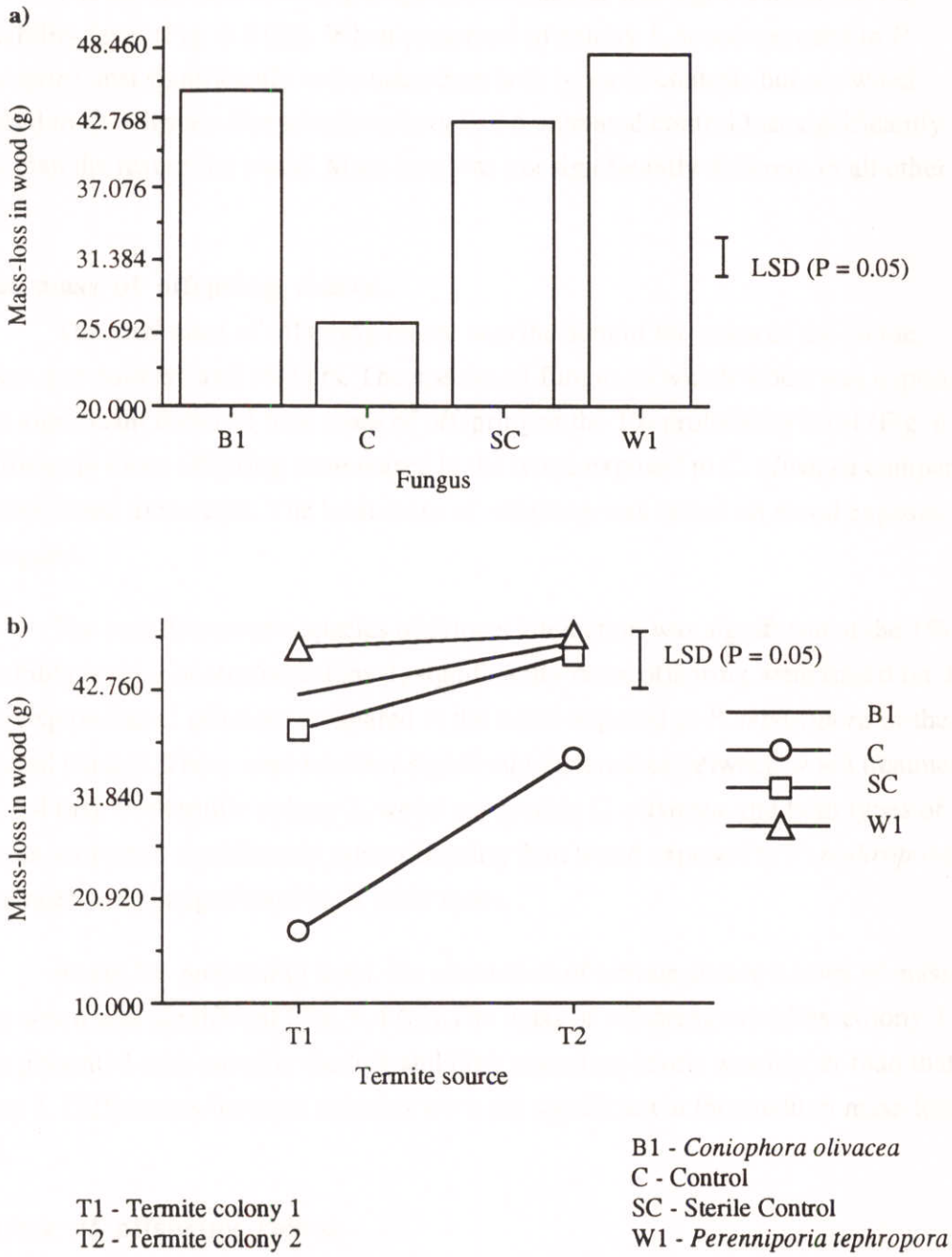



Figure 6.2: Termite colony development. The combined effect of termite and fungal attack on mass-loss (g) in wood
a) fungus species to which wood was exposed
b) termite source x fungus species interaction
LSD are approximate and based on minimum replication i.e. they are conservative.

The termite source x fungus species interaction was significant at the 1% probability level (Fig. 6.2 (b)). When presented to colony 1, wood exposed to *P. tephropora* lost significantly more mass than both types of controls but not wood exposed to *C. olivacea.* For termite colony 2, the untreated control lost significantly less mass than the rest of the wood. Mass-loss was not significantly different in all other cases.

**Total mass of offspring reared**

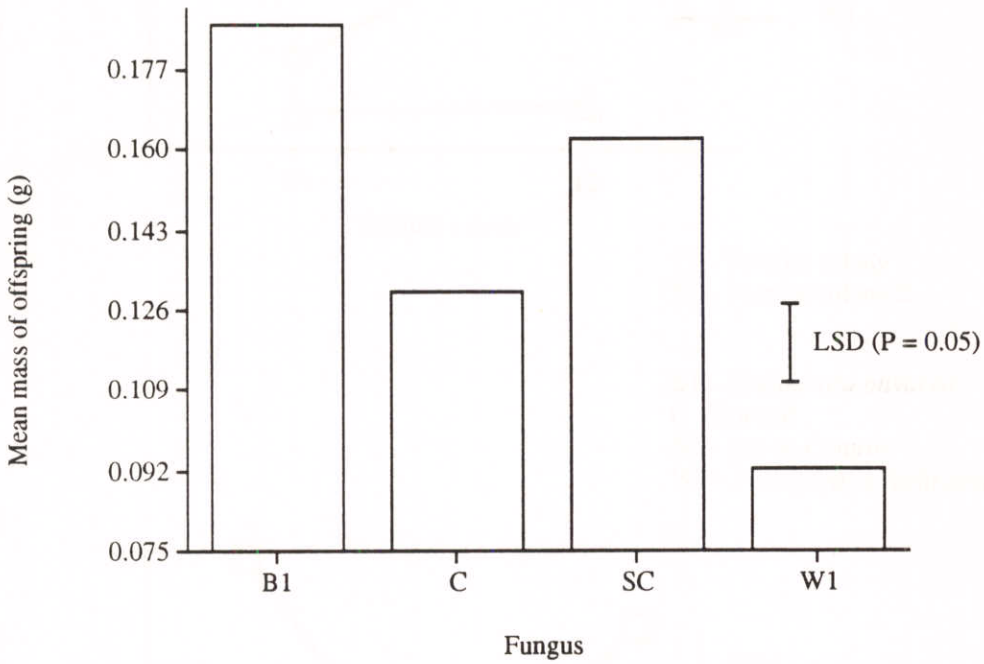
The total mass of offspring reared was the sum of the mass of the larvae, worker, pre-soldiers and soldiers. The species of fungus to which wood was exposed was a significant factor of total mass of offspring at the 1% probability level (Fig. 6.3). Significantly more offspring were reared in the wood exposed to *C. olivacea* compared to all other wood treatments. The least mass of offspring was raised on wood exposed to *P. tephropora.*

The termite source x species of fungus interaction was significant at the 1% probability level. For termite colony 1, significantly more offspring were raised on the wood exposed to *C. olivacea* compared to the wood exposed to *P. tephropora* or the untreated control. There were no other significant differences between wood treatments (Fig. 6.4 (a)). For termite colony 2, wood exposed to *C. olivacea* and both types of controls supported significantly more offspring than wood exposed to *P. tephropora.* The differences were insignificant in all other cases.

At the 5% probability level, the interaction of termite source x level of mass-loss in the wood was significant (Fig. 6.4 (b)).The mass of offspring raised by colony 2 when presented with wood at the low and high mass-loss levels was higher than that by colony 1. Differences between colonies were not significant at the medium mass-loss level.

**Number of offspring raised**

The total number of offspring raised was the sum of the number of larvae, workers, pre-soldiers and soldiers. At the 1% probability level, the species of fungus to which wood was exposed was a significant factor of the number of offspring raised (Fig. 6.5). The least number of offspring was raised on wood exposed to *P. tephropora.* The number of offspring raised on wood exposed to *C.olivacea* was significantly more than the number raised on the untreated control but not significantly more than the number raised on the sterile control. See Fig. 6.5 for more details.




B1 - *Coniophora olivacea*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*

Figure 6.3: Termite colony development. The effect of fungus species to which wood was exposed on the mean mass (g) of offspring raised. LSD are approximate and based on minimum replication i.e. they are conservative.

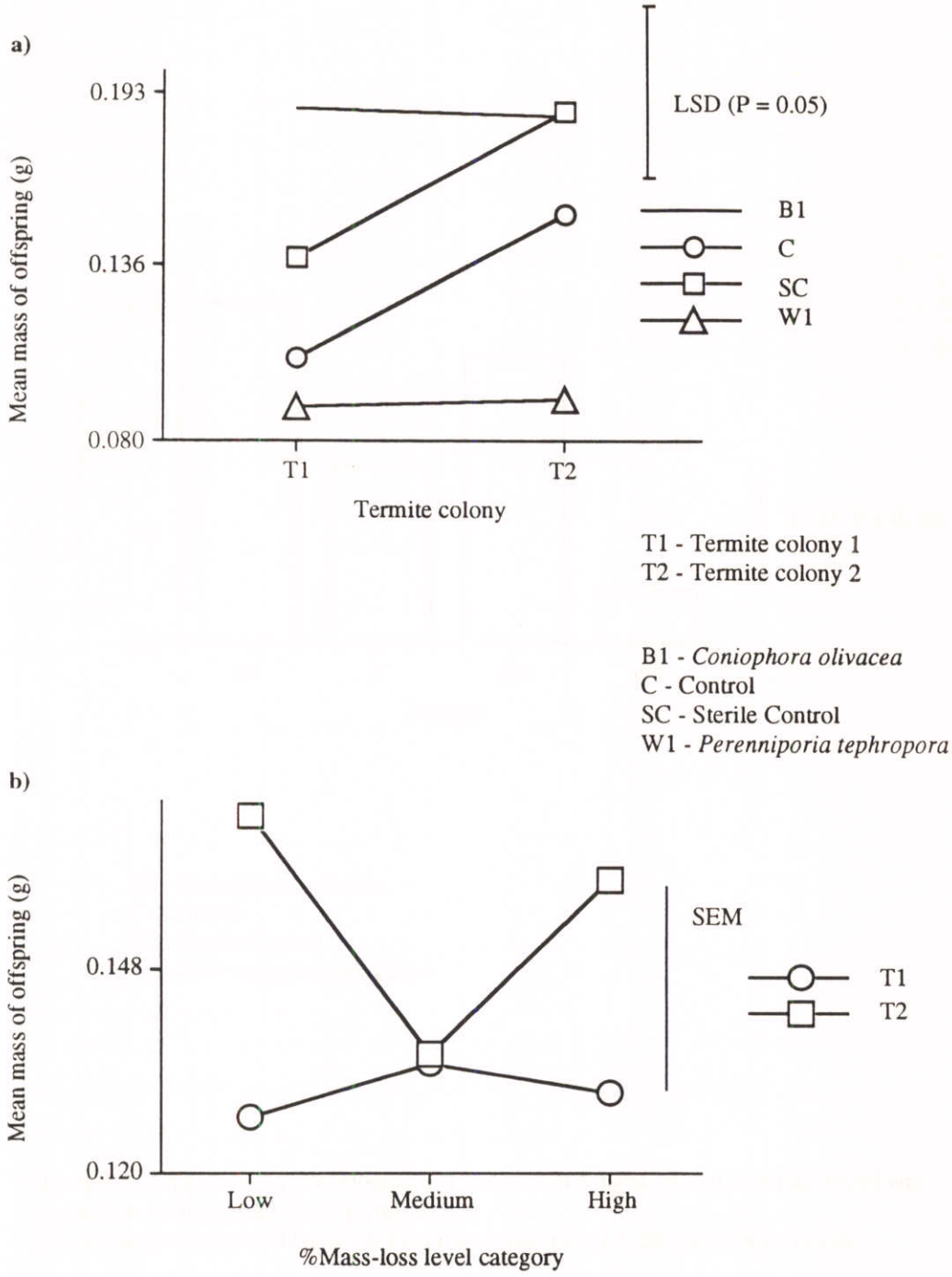
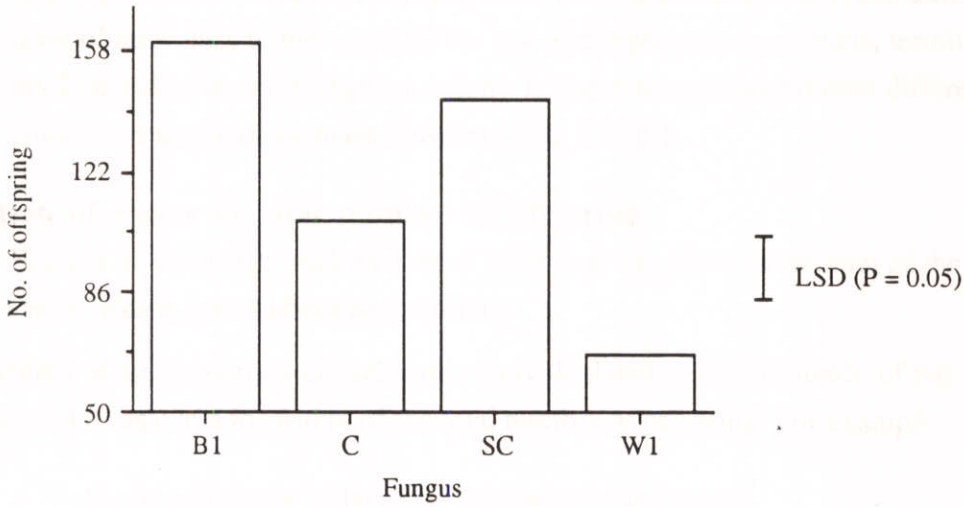



Figure 6.4: Termite colony development. Effects of two-way factor interactions in the experiment on the mean mass (g) of offspring raised.
a) termite source x species of fungus. LSD are approximate and based on minimum replication i.e. they are conservative.
b) termite source x level of mass-loss in the wood. SEM value is provided to facilitate comparison within levels of the factors.




B1 - *Coniophora olivacea*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*

Figure 6.5: Termite colony development. The effect of fungus species to which wood was exposed on total number of offspring raised.
LSD are approximate and based on minimum replication i.e. they are conservative.

The termite x fungus species interaction was significant at the 1% probability level (Fig. 6.6 (a)). Termites from colony 1 raised significantly more offspring on wood exposed to *C. olivacea* than on the untreated control or wood exposed to *P. tephropora*. With termites from colony 2, significantly more offspring were raised on both types of controls and wood exposed to *C. olivacea* than on wood exposed to *P. tephropora*. There were no other significant differences between treatments.

Also significant at the 5% probability level was the interaction between termite source x level of mass-loss in the wood.At the low and high mass-loss levels, termites from colony 2 raised more offspring than colony 1. There were no significant differences between colonies at the medium mass-loss level (Fig 6.6 (b)).

**Proportion of castes to total number of offspring**

For this analysis, the total number of offspring was taken as the sum of the eggs, larvae, workers, pre-soldiers and soldiers.

The logarithm of the proportion of each caste was calculated as the difference of the logarithm of the caste and logarithm of the total number of offspring. For example:

log of proportion of larvae = log (larvae) - log (total).

A point to note is that the proportion of eggs and the soldier caste was not quantifiable on the same basis as workers and larvae. Consequently, only the results of last two castes are presented.

**Proportion of larvae relative to the total number of offspring in incipient colonies**

At the 1% probability level, the interaction of colony x fungus species x mass-loss level in the wood was significant (Fig. 6.7). The proportion of larvae varied significantly when one colony was compared to the other. For colony 1, there were no differences between the proportion of larvae raised at all mass-loss levels on the wood exposed to the different treatments. For colony 2, however, the differences between the proportion of larvae raised on the different wood treatments were significant at the low and high mass-loss levels in the wood. See Fig. 6.7 for more details.

**Proportion of workers relative to the total number of offspring in incipient colonies**

The significant factors at the 1% probability level were the tree from which wood was obtained and the fungus species to which wood was exposed. Particular attention should be paid when interpreting Figure 6.8 as it portrays negative logarithms and the visual impression gives the reverse mathematical meaning. Wood obtained from tree 1 supported a higher proportion of workers workers than tree 1 (Fig. 6.8 (a)).

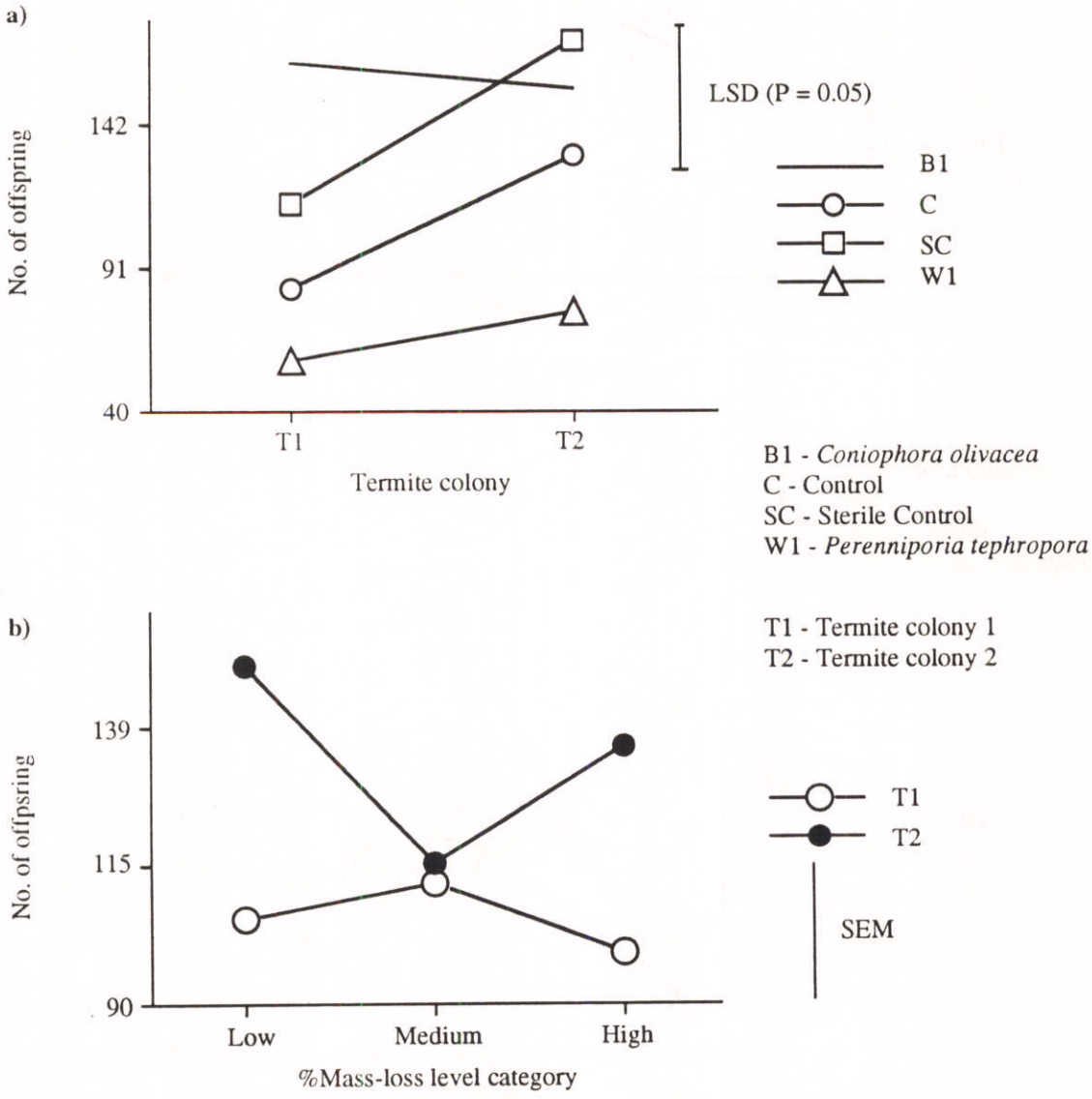



Figure 6.6: Termite colony development. Effects of two-way factor interactions in the experiment on the total number of offspring raised
a) termite source x fungus species interaction. LSD are approximate and based on minimum replication i.e. they are conservative.
b) termite source x level of mass-loss in the wood. SEM value is provided to facilitate comparison within levels of the factors.

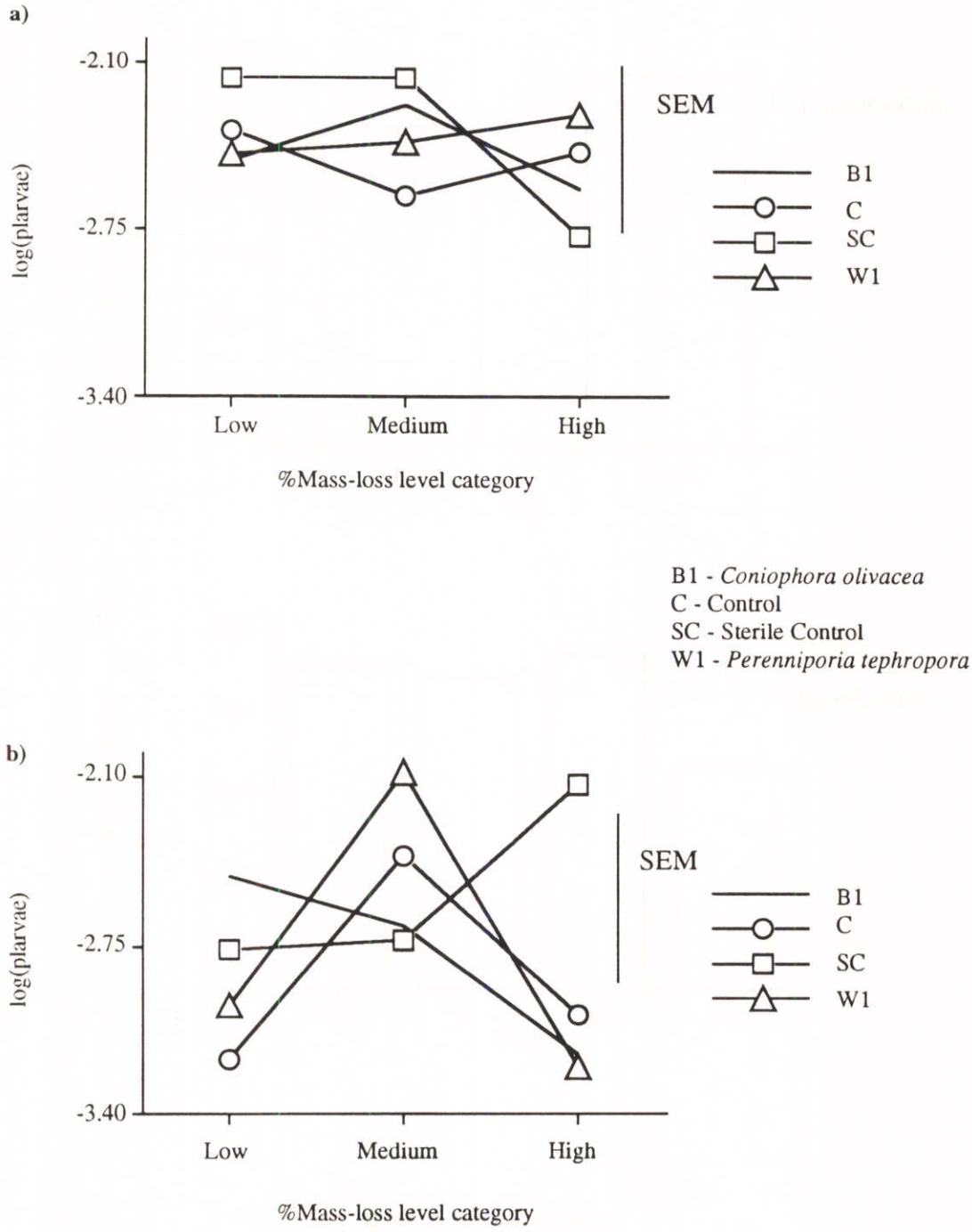
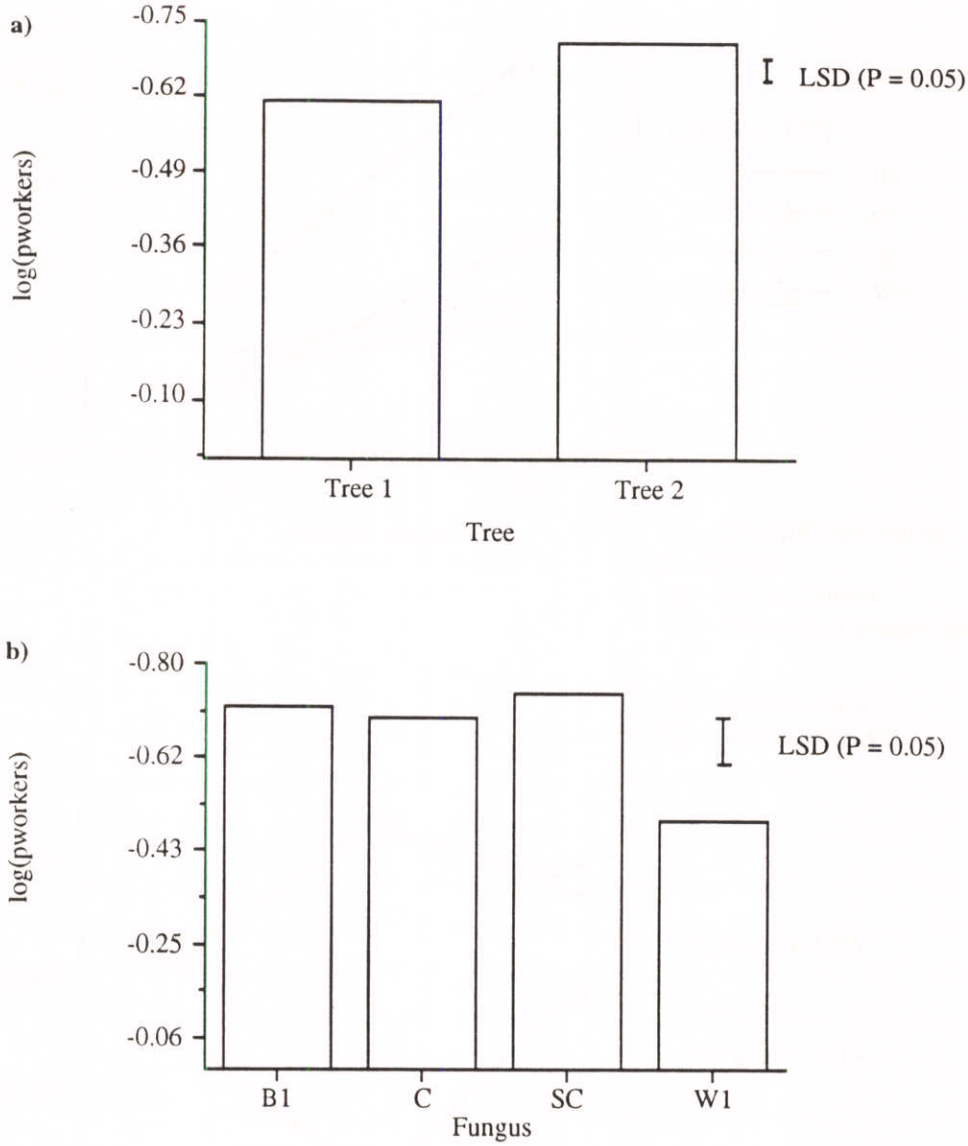



Figure 6.7: Termite colony development. Effect of fungus species x mass-loss x termite source interaction on the proportion of larvae relative to the total number of offspring raised. SEM values are provided to facilitate comparison within levels of the factors.
a) fungus species x mass-loss level x termite colony 1
b) fungus species x mass-loss level x termite colony 2




B1 - *Coniophora olivacea*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*

Figure 6.8: Termite colony development. Main factors of the proportion of workers relative to the total number of offspring raised. LSD are approximate and based on minimum replication i.e. they are conservative.
a) effect of tree from which wood was obtained
b) effect of fungus species.

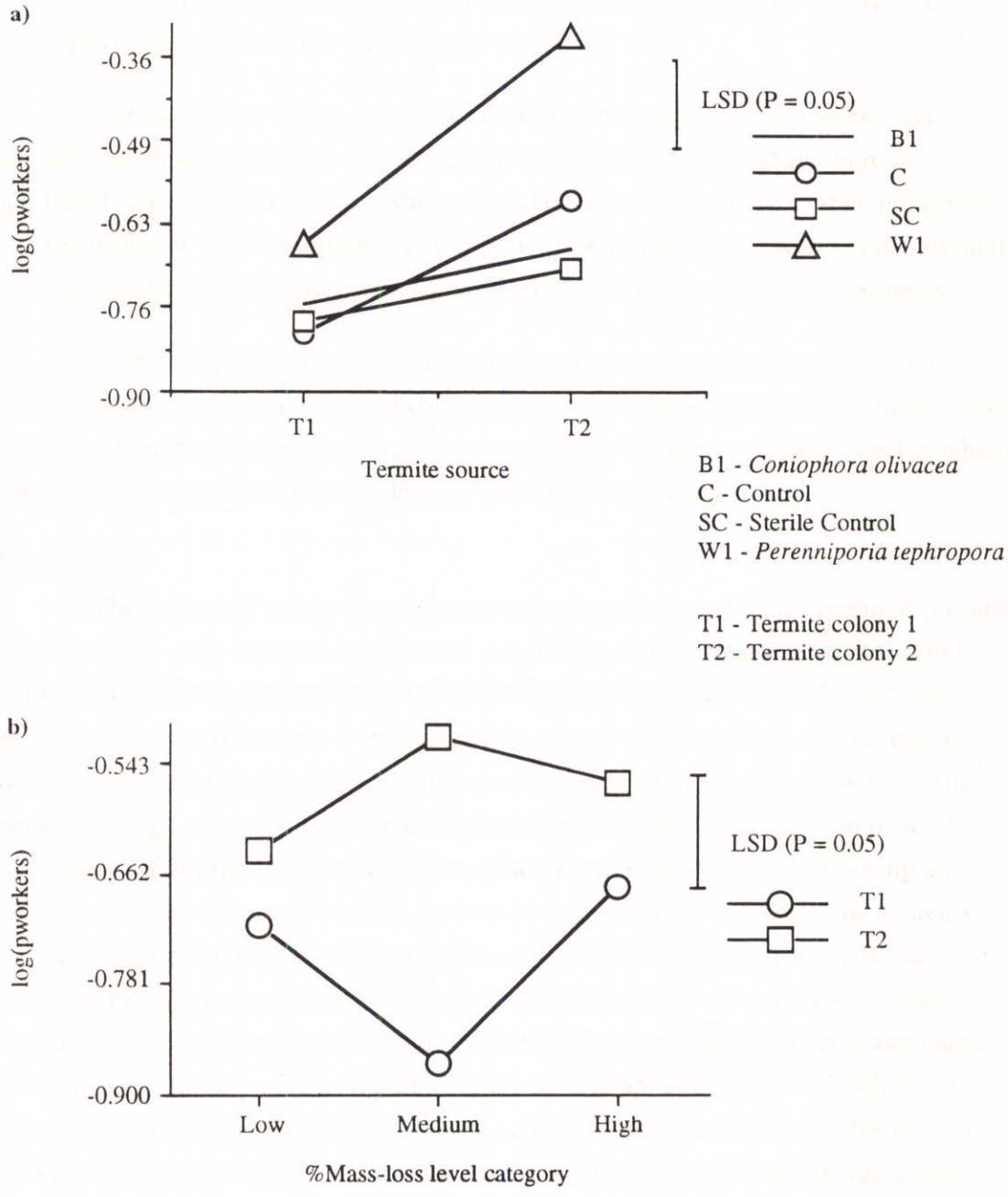



Figure 6.9: Termite colony development. Effects of two-way factor interactions on the proportion of workers relative to the total number of offspring raised.
LSD are approximate and based on minimum replication i.e. they are conservative.
a) fungus species x termite source
b) mass-loss level x termite source

Wood exposed to *P. tephropora* supported a significantly higher proportion of workers compared to both types of controls and wood exposed to *C. olivacea* (Fig. 6.8 (b)). The proportion of workers raised on wood exposed to *C. olivacea* and both types of controls was not significantly different.

The interaction of termite source x species of fungus to which wood was exposed was significant at the 5% probability level (Fig. 6.9 (a)). There were no significant differences in the proportion of workers raised by colony 1 when presented with the different wood treatments. For colony 2, the proportion of workers reared on the wood exposed to *P. tephropora* was significantly lower than in all other treatments.

At the 1% probability level, the termite source x mass-loss level in the wood interaction was significant (Fig. 6.9 (b)). Differences in the proportion of workers raised by the two termite colonies were significant only at the medium mass-loss level in which case the proportion was higher for termite colony 2 than colony 1.

## Discussion

The pattern of colony development was comparable to the one reported for other colonies which were initiated from paired, virgin first-form reproductives (King and Spink 1974; Han and Noirot 1983; Becker 1961; Garcia and Becker 1975). All aspects of incipient colony development were universally governed by the species of fungus to which wood was exposed and the termite source x species of fungus interaction. The species of fungus x mass-loss interaction was significant for the proportion of workers and mass of offspring. For the proportion of workers, total number of offspring and their mass, the termite source x mass-loss interaction was significant. The termite source x fungus species x mass-loss level was significant only for the proportion of larvae. The overlap in mass-loss categories in the current study might have obscured the effect of mass-loss level on colony development. Hence there was no particular level associated with enhanced development as reported by Becker (1965) and Lenz and Becker (1975). In particular, the continued growth of the fungus in some cases accounted for the most of the mass-loss, as was clearly indicated by the visual scores of termite damage to the wood. Thus no consumption was recorded for blocks exposed to P. tephropora, yet as this showed the highest mass-loss this compounding effect of variable feeding by termites and variable mass-loss caused by subsequent fungal growth complicated the analysis.

The proportion of workers is an especially important parameter of growth given that the successful development of a colony depends on a numerically strong worker caste (Garcia and Becker 1975). The results obtained must, however, be interpreted with caution because the large proportion of workers was not always associated with large termite colonies. A case in point is the large proportion of workers which was raised on

wood exposed to *P. tephropora*. Even though this wood supported colonies with the highest proportion of workers, colony development when assessed by the number of offspring raised showed that wood exposed to the fungus supported among the lowest populations. In actual fact, the performance of termites on wood exposed to the fungus was one of the poorest in terms of colony size and caste differentiation, and also amount of wood eaten.

Incipient colonies which were presented with wood exposed to *P. tephropora* were faced with unfavourable food as shown by the wood consumption scores. Indeed, the most common score for the termite attack on wood blocks was 0, signifying that the wood was untouched. Such a restrictive diet may have encouraged cannibalistic tendencies which Hendee (1935) reported under stressful conditions. In their studies, Becker (1961) and Garcia and Becker (1975) reported that eggs and larvae were the stages targetted for cannibalism. Such tendencies could explain why in the study reported here, only late-stage workers were present in the nests with the parents when wood exposed to *P. tephropora* was presented to the termite colonies.

According to Becker (1965), wood exposed to certain brown brown rots had higher nutritional value than sound wood and furthered termite development for the *Kalotermes flavicollis* (Fabr.), *Heterotermes indicola* (Wasmann) and *Reticulitermes lucifugus* (Rossi). When mass and number of offspring in the current study were put into consideration, wood exposed to the brown rot *C. olivacea* supported more offspring than wood exposed to *P. tephropora* or sound wood. The slow growth of *C. lacteus* colonies on wood exposed to *P. tephropora* could therefore also have been due to poor nutrition.

In the colonies with low populations, a prolonged pause in egg-laying might also have occurred after the first larvae hatched. The findings of Becker (1961) were that such a pause coincided with the rearing of larvae and that oviposition resumed with the appearance of the first workers. Under the circumstances of the study reported here, it appears that oviposition did not recur in some cases such as on wood exposed to *P. tephropora*. Small populations could therefore have been the result of low egg production where termites found themselves faced with unfavourable food as suggested by Waller and Fage (1987).

Termites respond to the odour produced by fungal mycelia by increasing their food intake where they find it favourable (Becker and Lenz 1975). On the other hand, the white-rotted wood could have termite-repellent compounds which, for instance, cause *Reticulitermes* species to naturally avoid it (Amburgey 1979). Wood exposed to *P. tephropora*, a white rot, might therefore have repelled *C. lacteus* because of an odour

produced by its mycelia or because, like most but not all white rots, it produced negative feeding stimuli in termites.

The size of colonies in this study was also affected by of the colony from which the queen was obtained. This effect was, however, only significant in interaction with other factors such as species of fungus to which the wood was exposed and the level of decay it consequently attained.

In this study, the development of the soldier caste was not directly related to the total number of offspring.The observations, however, were that the caste was present in most colonies but was noticeably low in number or missing altogether in the colonies presented with wood exposed to *P. tephropora*. The highest numbers of soldiers recorded was 24 (ca 8% of the particular colony) and was raised on wood exposed to *C. olivacea*. Such a performance in 6.5 months was much better than the one reported by Han and Noirot (1983) for *Cubitermes fungifaber*. They reported only 1 soldier, at most , after the 7$^{th}$ month. After 2.5 years, *Coptotermes formasanus* colonies had ca. 10% soldiers (King and Spink 1974). Oshima (1919) cited in King and Spink (1974) recorded a 1: 9 soldier: worker ratio in the first 942 days.

The study reported here evaluated the effect of food quality on the caste development in *C. lacteus*. There were clear differences in colony development on wood exposed the brown rot and the white rot, overall performance being much brown-rotted wood. There was great similarity of performance between wood exposed to *C. olivacea* and the sterile control. This suggested that at low mass-loss levels in wood, the growth of the colonies was influenced by changes in the wood as a result of its contact with the nutrient medium supplied for the propagation of the fungus.The benefits were outweighed by adverse effects related to the presence of the fungus in the case of wood exposed to *P. tephropora*.

More detailed studies of the effect of environmental factors on colony growth remain to be done to bring the state of knowledge about colony development in *C. lacteus* to the same level as that of termites studied in other instances. Since these laboratory experiments were done under controlled circumstances, unlike the highly variable field conditions, field studies should be undertaken to validate the importance of the various factors studied here for the biology of *C. lacteus*.

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a)

b)

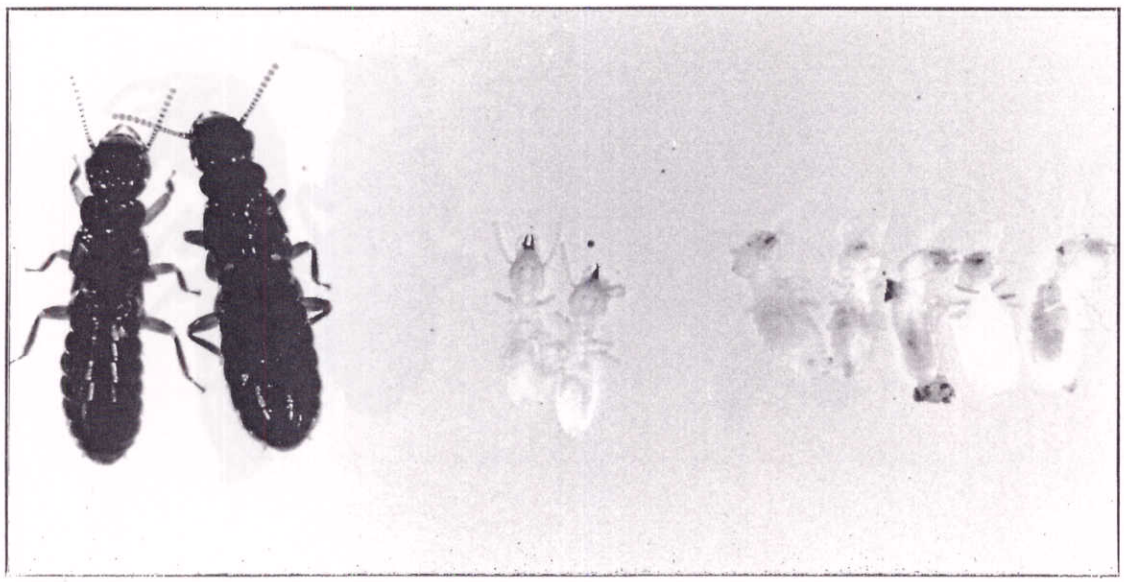
Plate 6.1: Termite collection
a) Typical *Coptotermes lacteus* mound, in this case, the mound from which termite colony 1 was collected
b) Alate trap

a)

b)

Plate 6.2: Alate collection
a) the dark mass near the base of the picket is a large group of alates. Dark spots on the terylene cloth are alates caught inside trap
b) note large boxes padded with wet tissue paper in which alates were transported back to the laboratory.

a)



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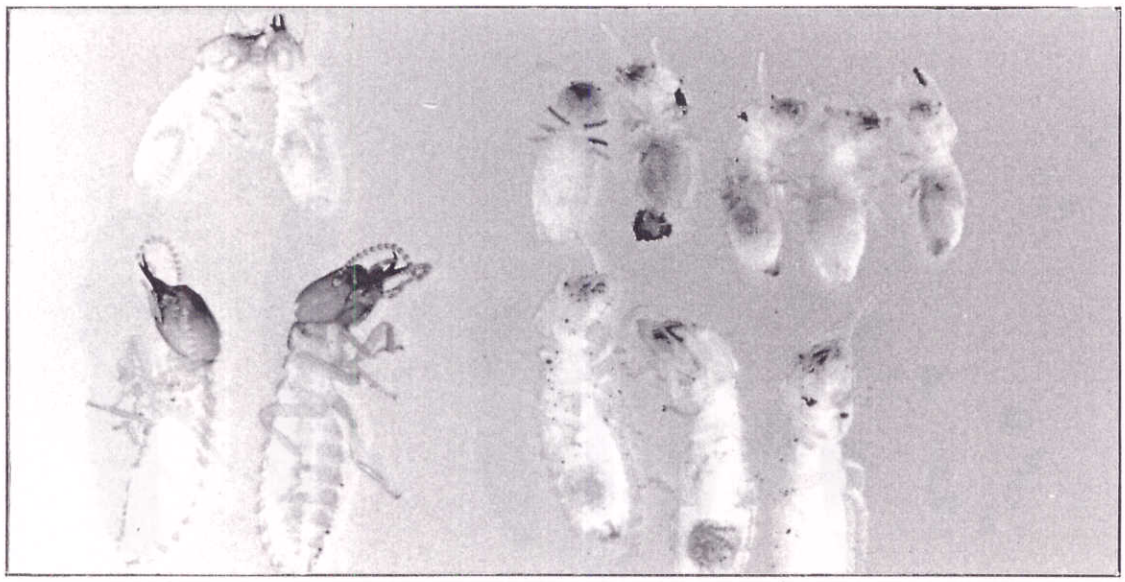
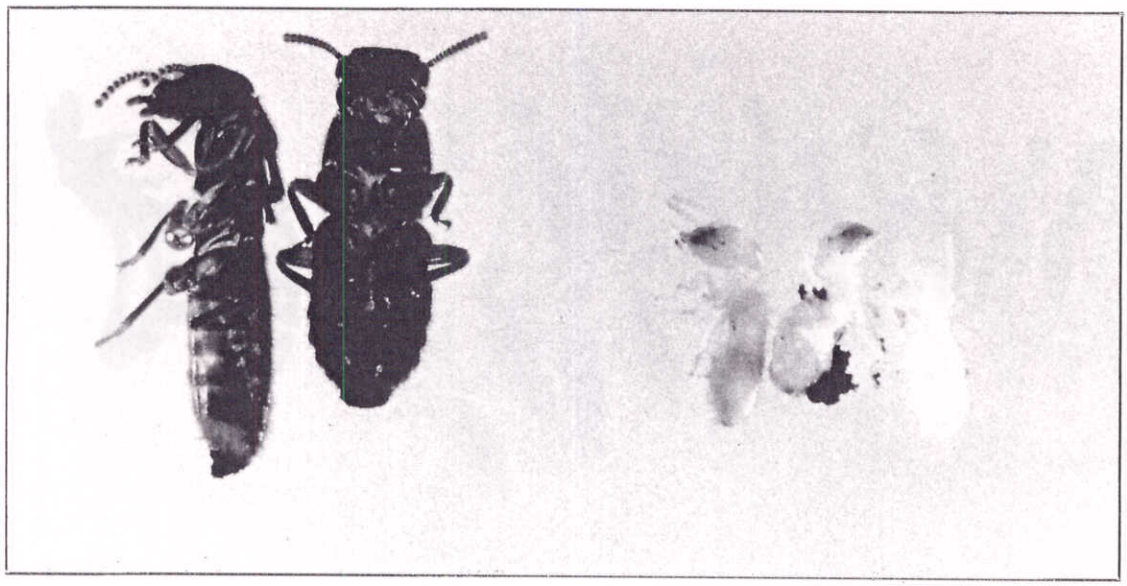


Plate 6.3: Incipient colony development on wood exposed to *Coniophora olivacea*
a) wood obtained from tree 1 and at high mass-loss level (14-30%)- alate pair (left) from colony 2 and the two castes of offspring reared. Total number of offspring reared was 2 soldiers (middle), 5 workers (right).
b) Comparison of sizes of offspring for laboratory-reared (top) and field-collected termites (bottom)- soldiers (left), workers (right).

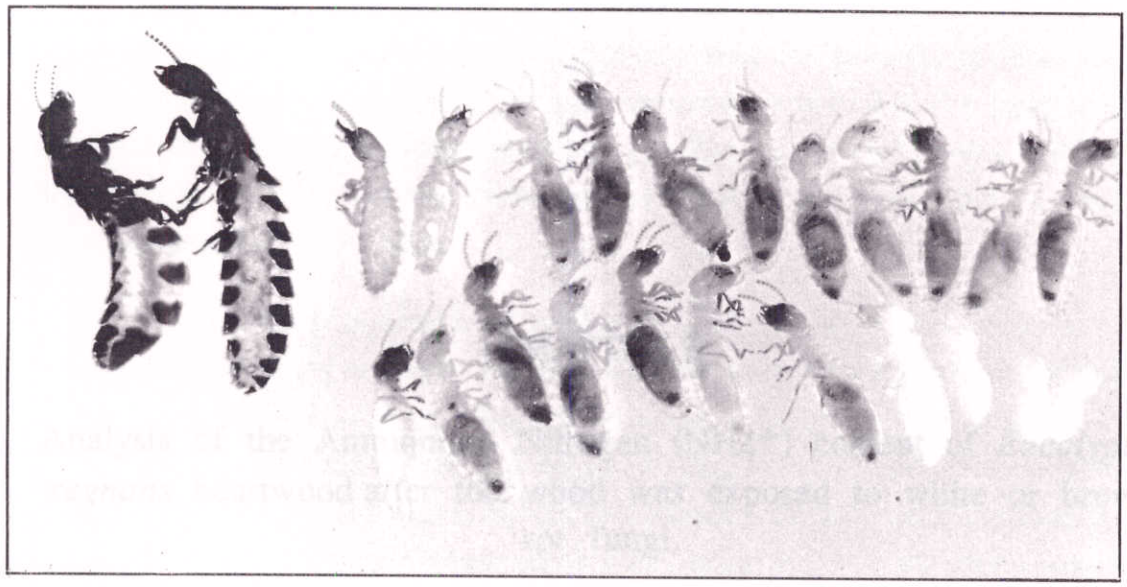
a)



b)

Plate 6.4: Incipient colony development on wood exposed to *Perenniporia tephropor*a and at medium mass-loss level (9-21%)
a) wood obtained from tree 2- alate pair (left) from colony 2 and the only caste of offspring reared i.e 3 workers
b) Wood obtained from tree 2- alate pair (left) from colony 2 and the two castes of offspring reared i.e. 6 workers, 1 pre-soldier (4th individual from left).

a)



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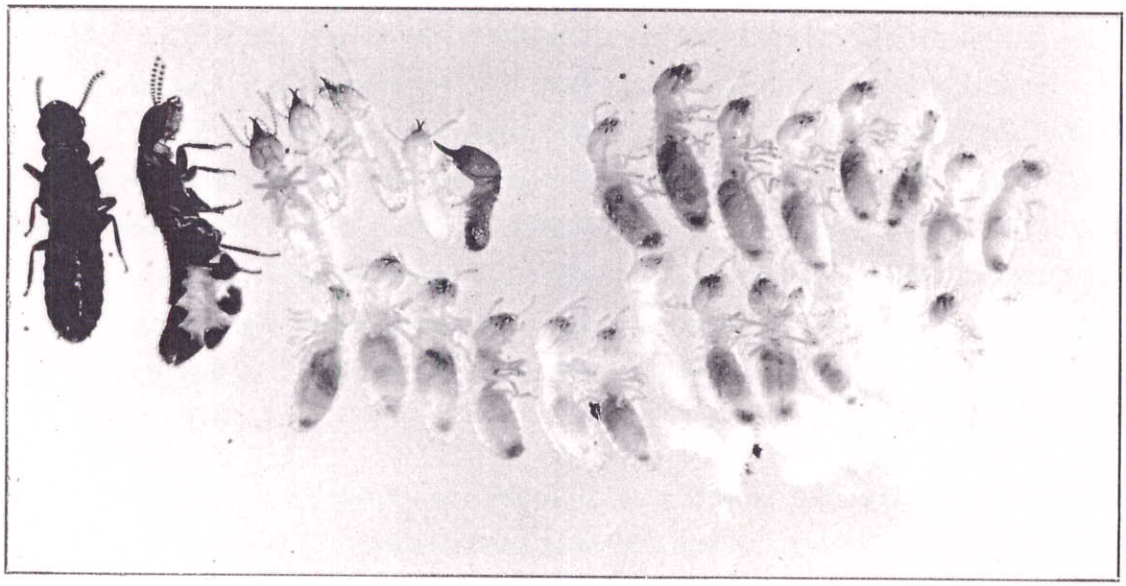


Plate 6.5: Incipient colony development on both types of controls
a) untreated control for medium mass-loss level (9-15%) and wood obtained from tree 1- alate pair (left) from colony 1. The total number of offspring reared was 3 larvae, 16 workers, 1 pre-soldier and 2 soldiers
b) sterile control for low mass-loss level (3-8%) and wood obtained from tree 1- alate pair (left) from colony 2. The total offspring in each caste were 11 larvae, 31 workers and 5 soldiers.

# CHAPTER 7:

## Analysis of the Ammonium Nitrogen ($NH_4^+$) content of *Eucalyptus regnans* heartwood after the wood was exposed to white or brown rot fungi.

## Introduction

Wood has less nitrogen (N) for unit of available carbon (C) than most other plant tissues, especially of the herbaceous type Levi *et al.* (1968); Levi and Cowling (1969). Sound wood is reported to have a nitrogen content of 0.03-0.10% in dry weight. It is rarely more than 0.3% LaFage and Nutting (1978); Merrill and Cowling (1966b). Nitrogen content varies with tree species and position in the tree from which the sample originated MacKay *et al.* (1985).

The variability reported in nitrogen levels is not fully accounted for by the heterogeneity of woody materials. The basic Kjeldahl method for nitrogen analysis which is used in various modifications influences the results of analyses according to the particular method that is followed (LaFage and Nutting 1978). The process itself does not fully recover the nitrogen bound in N-N or N-O linkages, the latter being common in several wood tissues. Of the nitrogen which is recovered, 50% is in the ammonium form (Collins 1983). Lignin-bound proteins which form the residues of the hydrolysis account for 15% of the nitrogen.

Resistance of much of the nitrogen in the wood to extraction by neutral solvents (water, ethanol) and a proteolytic enzyme (Pronase, CalBioChem) led Merrill and Cowling (1966b) to suggest that enzyme systems that modify cellulose, hemicellulose or lignin may be essential before the nitrogen becomes available to microbial proteases or other constituents that act on the cell walls of the wood. Such characteristics have far-reaching effects on the organisms that utilize wood as a source of nutrients and energy. Wood-inhabiting *Anobium* larvae, for example, increased in weight in a manner directly correlated with the N-content of the wood in which they were growing in (Merrill and Cowling 1966a).

Natural variability in the nitrogen content of wood influences its susceptibility to decay (Collins 1983). A definite relationship between the nitrogen content of wood and its susceptibility to fungal attack has been demonstrated (Merrill and Cowling 1966a). Despite this, wood-decay fungi have developed mechanisms of nitrogen conservation that enable them to exploit wood as a medium of growth irrespective of its limited nitrogen content. Such mechanisms come into play when the nitrogen levels become very limiting. White rots, for example, are capable of using all forms of nitrogen available when N is in short supply. Strategies for withdrawing nitrogen from older hyphae by autolysis or transfer from adjacent litter have been reported under some circumstances (Levi and Cowling 1969). Immobilization of nitrogen by fungi was shown when mass losses in wood of up to 30% were found not to alter the amount of nitrogen per unit volume present (Collins 1983).

There is evidence that the N-content of litter is improved by fungal attack (Collins 1983). A highly significant correlation was found when the relationship between wood decay and nitrogen content was examined for wood in soil contact, irrespective of soil-type and wood species (Waite and King 1979). Inorganic nitrogen taken up with the absorption of water by wood buried in soil formed a negligible proportion of the total increase in nitrogen and the possibility that the nitrogen-content of the wood increased due to uptake of inorganic nitrogen ($NO_3^-$) was eliminated under the conditions of the experiment. Increase in N-content was instead equated to a measure of the amount of colonisation by fungi occurring when wood is in contact with soil. The increase in nitrogen in wood during microbial decay was therefore attributed to invading organisms which immobilize nitrogen as its organic form.

For this study, analysis was to find out whether the amount of ammonium nitrogen in *Eucalyptus regnans* F. Muell. heartwood was related to the fungus species to which it was exposed and the level of decay that it had attained after exposure to one each of six wood decay fungi.

## Methods

### Timber

Details about the timber and the preparation of samples (0.5g) of sawdust are covered in detail in Chapter 2. The nitrogen ($NH_4^+$) content of the samples was evaluated colorimetrically against known standards in an auto analyser and chart peak heights fed into a custom written computer program to give nitrogen in parts per million (ppm).

### Fungi

Details about the fungi are given in Chapter 2. The fungal bioassay is covered in detail in Chapter 3. Blocks were categorised according to the mass-loss levels they had attained: low (0-2%), medium (6-10%) and high (≥10%) - ( see Table 7.1). For each fungus and at each category, a representative block of wood was randomly chosen from the available pool of blocks that fell into that category. .

Table 7.1 Percentage mass-loss categories out of which wood blocks were selected for the analysis of nitrogen

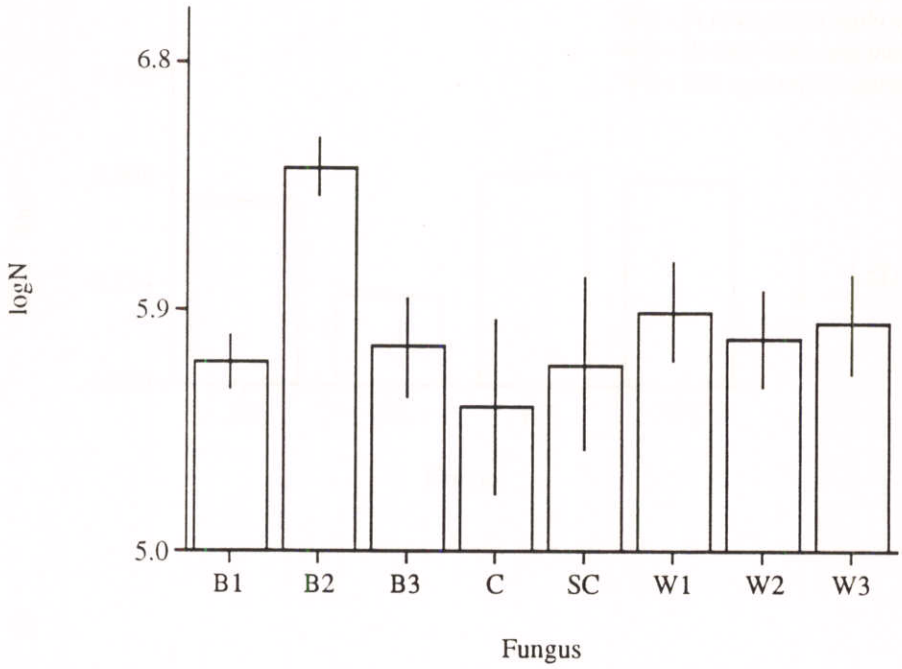
| Tree | Fungal treatment | Percentage mass loss category of the wood blocks | | |
|---|---|---|---|---|
| | | Low | Medium | High |
| 1 | *Coniophora olivacea* | 2 | | |
| | *Trametes lilacino-gilva* | 2 | 8 | 14 |
| | *Gloeophyllum trabeum* | 2 | | |
| | *Perenniporia tephropora* | 2 | 8 | 15 |
| | *pycnoporus coccineus* | 2 | 6 | 10 |
| | *Tinctoperillus epimiltinus* | 2 | 8 | 16 |
| | Sterile control | 0 | | |
| | Control | 0 | | |
| 2 | *Coniophora olivacea* | 1 | | |
| | *Trametes lilacino-gilva* | 1 | 10 | 20 |
| | *Gloeophyllum trabeum* | 2 | | |
| | *Perenniporia tephropora* | 4 | 8 | 14 |
| | *pycnoporus coccineus* | 2 | 6 | 10 |
| | *Tinctoperillus epimiltinus* | 2 | 6 | 10 |
| | Sterile control | 0 | | |
| | Control | 0 | | |

## Results

For mass-loss levels under 3% in the wood, and at the 1% probability level, the species of fungus to which wood was exposed was a significant factor in the determination of amount of nitrogen present in the wood. Wood exposed to *T. lilacino-gilva* yielded significantly more nitrogen than that exposed to all other fungi (Fig. 7.1). The differences between other treatments and the controls were not significant .

The significant factors of the nitrogen content of wood at the 1% probability level and at mass-loss levels above 3% in the wood were the species of fungus and mass-loss level in the wood.

For mass-loss levels above 3% in the wood, the species of fungus to which wood was exposed was a significant factor at the 1% probability level (Fig. 7.2 (a)). The amount was significantly lower in the wood exposed to *P. tephropora* when compared with that in the wood exposed to *T. lilacino-gilva*, *P. coccineus* or *T. epimiltinus*. The amount of nitrogen present in the wood in other fungal treatments was not significantly different, one from the other, or from the controls.




B1 - *Coniophora olivacea*
B2 - *Trametes lilacino-gilva*
B3 - *Gloeophyllum trabeum*
C - Control
SC - Sterile Control
W1 - *Perenniporia tephropora*
W2 - *Pycnoporus coccineus*
W3 - *Tinctoporellus epimiltinus*

Figure 7.1: Amount of nitrogen in the wood at mass-loss levels under 3% following its exposure to different fungal treatments.
The vertical line on each of the bars represents the standard error of the mean (SEM).

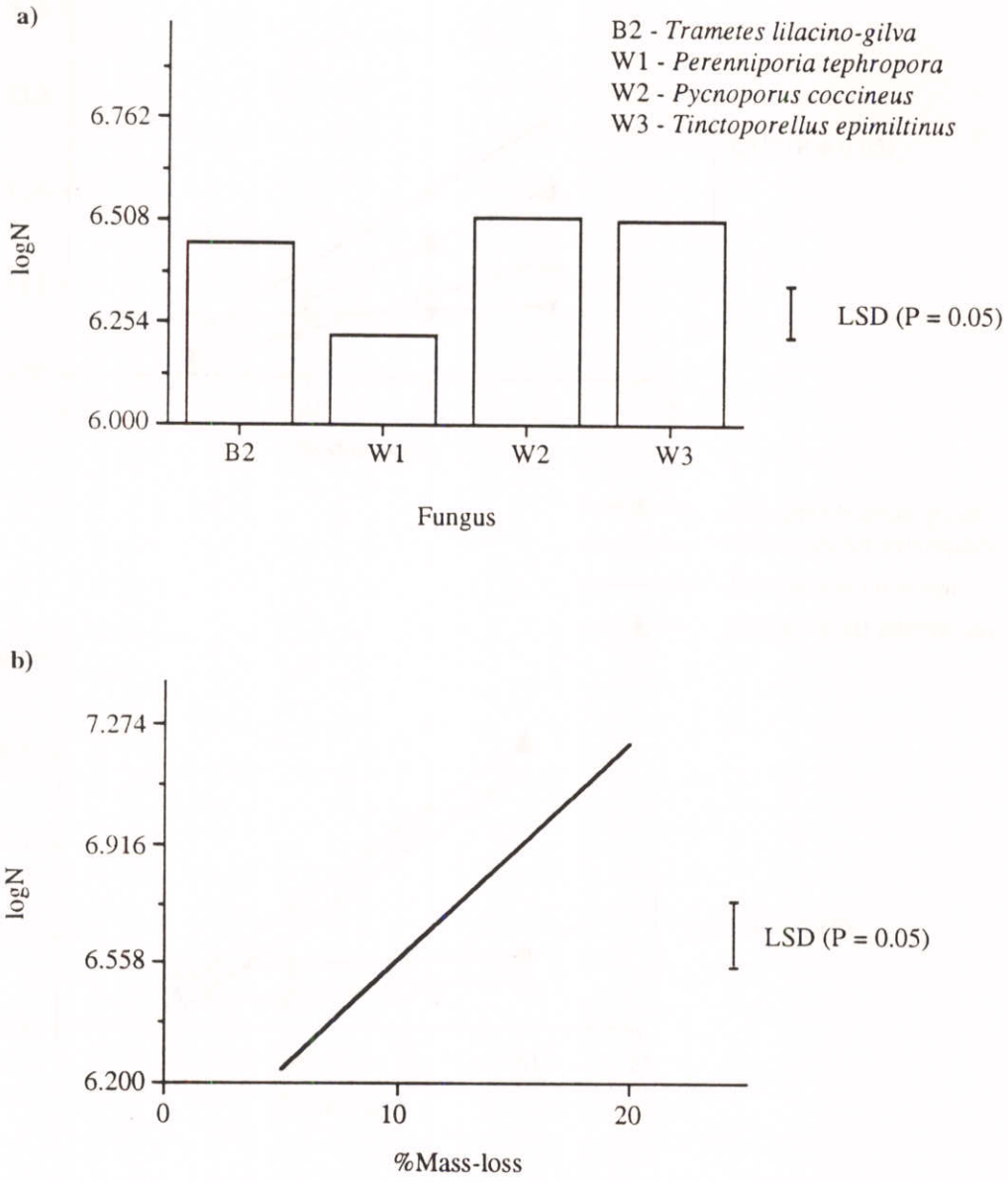



Figure 7.2: Amount of nitrogen in the wood at mass-loss levels under 3% following its exposure to wood decay fungi: main factors of the experiment. LSD are approximate and based on minimum replication i.e they are conservative.
a) fungus species to which wood was exposed
b) mass-loss level in the wood.

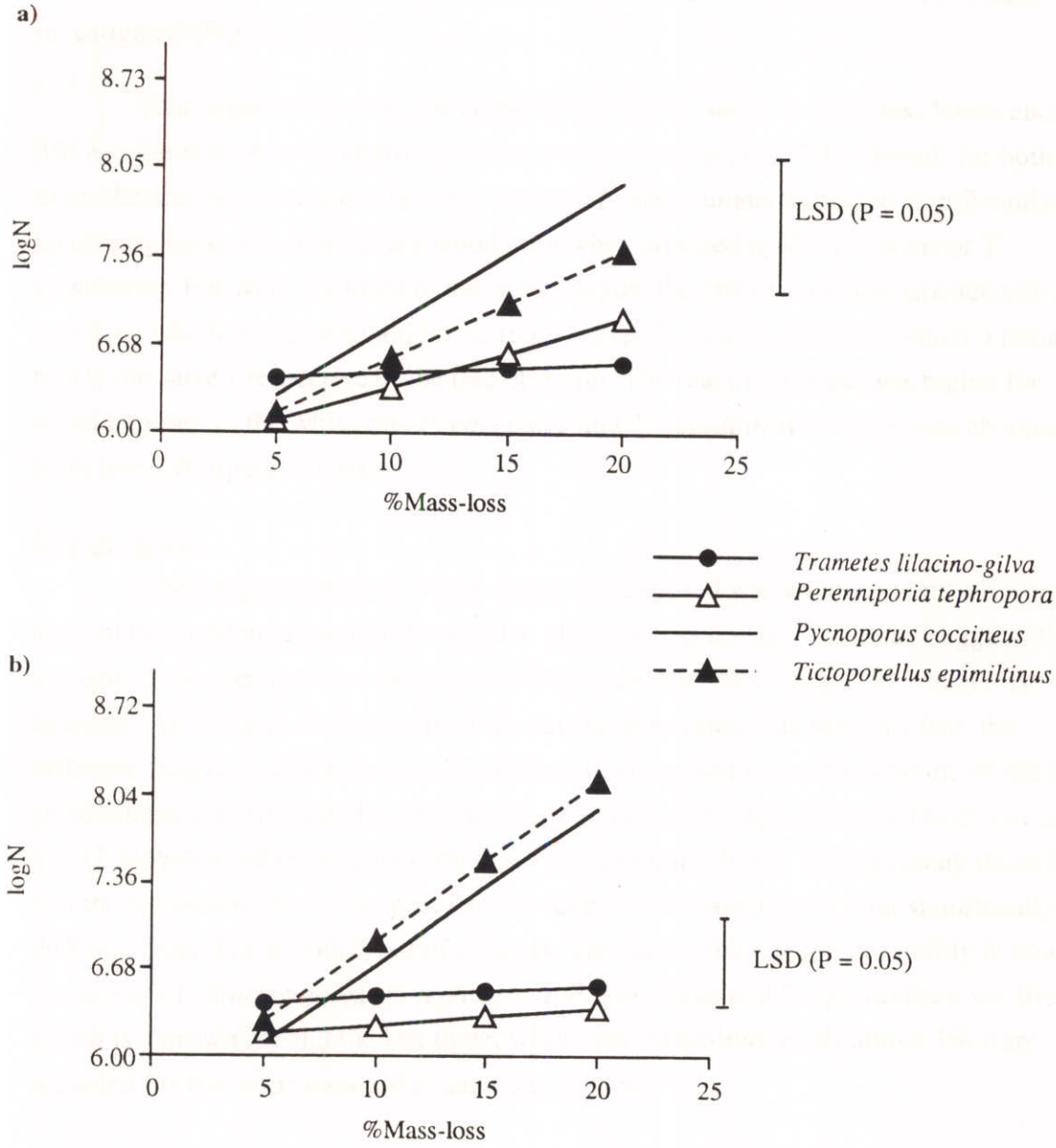



Figure 7.3: Amount of nitrogen in the wood at mass-loss levels above 3% following its exposure to decay fungi: effect of mass-loss x fungus species x tree interaction. LSD are approximate and based on minimum replication i.e they are conservative.
a) mass-loss x fungus x tree 1
b) mass-loss x fungus x tree 2

The level of mass-loss was another significant factor in the determination of the amount of nitrogen in the wood at the 1% probability level. The amount of nitrogen in wood increased significantly with increasing mass-loss level for the 0% - 20% range investigated (Fig. 7.2 (b)).

Also significant at the 1% probability level for wood at mass-loss levels above 3% was the mass-loss x fungus species x tree interaction (Fig. 7.3). Overall for both trees, the amount of nitrogen in wood exposed to each fungus increased significantly with increasing mass-loss level in the wood only when attacked by *P. coccineus* or *T. epimiltinus*. For wood exposed to the same fungus, the rate of increase depended on the tree from which it was obtained for all fungi except *T. lilacino-gilva* for which it remained nearly the same irrespective of the tree of origin. The rate of increase was higher for wood exposed to the white rots *P. coccineus* and *T. epimiltinus* when it was obtained from tree 2 compared to tree 1.

**Discussion.**

The fungus species to which wood was exposed was an important factor of the amount of nitrogen present in the wood at all mass-loss levels. This study suggests the amount of nitrogen in wood varies according to the species of fungus to which it is exposed. As all fungi were grown on the similar substrates, this suggests that the different fungi colonised the wood to different extents and hence the amount of nitrogen accumulated was correlated to the amount of colonisation. Wood exposed to *C. olivacea* and *G. trabeum* fell in the under 3% mass-loss category. It was not surprising therefore that the N-content of wood exposed to the fungi in this category was not significantly different from that of both types of controls. The fungi with the greatest ability to colonise wood were *T. lilacino-gilva, P. tephropora, P. coccineus* and *T. epilmiltinus*, the first of which is a brown rot and the last three, white rots. Mass-loss levels above 3% were recorded for the wood exposed to each one of them.

Wood exposed to *T. lilacino-gilva* yielded significantly more nitrogen than wood exposed to all other fungi only at mass-loss levels under 3% in the wood. At mass-loss levels above 3%, the amount of nitrogen in wood exposed to the brown rot and two of the white rots was not significantly different . The amount of nitrogen in wood exposed to these white rots therefore increased significantly while the amount in the brown rot only increased slightly as the mass-loss level in the wood increased. This larger increase in amount of nitrogen in wood exposed to the white rots with increasing mass-loss may have been what caused the mass-loss level to be a significant factor of amount of nitrogen in wood

Given that white rots can utilize all forms of nitrogen available in wood (Collins

1983), it was unexpected that wood exposed to *P. tephropora,* a white rot, was overtaken by *T. lilacino-gilva*, a brown rot, in its N-content at the higher mass-loss levels. The unusual performance of *T. lilacino-gilva* at all mass-loss levels was more closely related to white rots than the brown rots and shows that generalisations about the characteristics of wood-destroying fungi do not apply to all specific cases.

Waite and King (1979) attributed the increase in nitrogen in wood to invading organisms and took it as a measure of amount of colonisation. Increase in nitrogen content in wood with decay was also reported by Haack and Slansky (1987). These findings also concurred with the views of Collins (1983) who reported that rotten wood accumulates nitrogen and those of the current study.

The environment in which a tree grows can influence the nitrogen content of its timber (Merrill and Cowling 1966a; Waller and Fage 1987). Thus differences in N-content can be expected in wood from trees growing in different environments. However, natural variability appeared to have had the least influence on the nitrogen content in wood in this study since the tree factor was significant only in interaction with other factors. In this case, the factors were mass-loss level in the wood and species of fungus to which wood was exposed.

As already discussed in preceding chapters, the feeding and survival responses of termites were determined to a great extent by the species of fungus responsible for the decay. Consumption of wood exposed to *T. lilacino-gilva* was the significantly higher under all circumstances and this suggests that *C. lacteus* might therefore been to taking advantage of the enhanced nitrogen present in the wood decayed by a favourable fungus as proposed by Waller and Fage (1987). With respect to termite colony development, the amount of nitrogen in wood exposed to *C. olivacea* and that exposed to *P. tephropora* did not vary significantly. The influence of amount of nitrogen in wood on colony development was not clear since colony growth was equally good on the sterile control as on the wood exposed to the brown rot. It would be worthwhile to investigate colony development on wood exposed to *T. lilacinogilva* now that nitrogen in the wood is known to have been among the highest so as to find out if colonies would perform different under circumstances where there are real differences in amount of nitrogen.

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# CHAPTER 8

## General discussion

Basidiomycetes are essential for the fungal decomposition of wood. This group can be subdivided into white or brown rots. In this study the effects of three brown rots (*Trametes lilacino-gilva*, *Coniophora olivacea*, *Gloeophyllum trabeum*), and three white rots (*Perenniporia tephropora*, *Pycnoporus coccineus* and *Tinctoporellus epimiltinus*), on the structure and content of *Eucalyptus regnans* heartwood were investigated. The interaction of fungal decomposition of wood on aspects of the biology of the *Coptotermes lacteus* was examined. The aspects of biology considered included wood consumption and preference, termite survival, caste composition and incipient colony development.

As a food source sound dead wood is characterised by being hard, sometimes harbouring potential toxins and having a low nitrogen content (Waller and LaFage 1987). Wood decay, on the other hand, may favourably or unfavourably alter food quality depending on the microorganisms present in the wood. Dix (1985) and Waller and LaFage (1987) reported that changes in wood associated with decay enhance the wood's moisture holding capacity. Decayed wood might also be advantageous because of enhanced nitrogen and access to nitrogen-rich sporophores and may contain pre-digested wood substances (Waller and LaFage 1987). Various microorganisms, including wood decay fungi, can also detoxify plant extractives present in heartwood and thus create a non-toxic substrate for termites to live on. Decay in wood often results in changes in wood strength -a loss in toughness- (Highley 1987, Wilcox 1978). This might make it mechanically easier for termites to feed on wood.

*C. lacteus* is among those termites that prefer to feed on decayed rather than on sound wood (French *et al.*1981; Lee and Wood 1971, Lenz *et al* .(1980, 1987, 1991). The current study has illustrated that the species of fungus to which *E. regnans* was exposed and the level of decay in the wood were significant factors of wood consumption by this termite. At low decay levels in the wood (mass-loss $\leq$ 3%) and regardless of whether a single treatment or a choice of wood treatments was offered to *C. lacteus*, wood exposed to *T. lilacino-gilva* was consumed significantly more than wood exposed to all other fungi, that is, *C. olivacea, G. trabeum*, *P. tephropora, P. coccineus* and *T. epimiltinus*. It was also consumed more than the untreated and the sterile controls.

At the higher decay levels in wood (mass-loss>3%) where the only brown rot represented was *T. lilacino-gilva* but all white rots were represented, wood exposed to the brown rot remained the most preferred wood treatment to feed on. Termite survival and wood consumption rate was significantly higher on wood exposed to the brown rot fungus. It therefore contributed positively towards the biological aspects under consideration and *C. lacteus* preferred to feed on it above all other wood presented.

The proportion of workers surviving at the end of the experiment , as well as the termite survival, were significantly influenced by the source of termites. Termite survival was lowest in the colony where wood consumption and the proportion of workers were highest. Intercolony variability was so strong that termite colonies behaved as if obtained from different species rather than from different colonies of the same species. Intercolony variability is not often as pronounced as in the current study but as discussed in Chapter 4, it forms an important characteristic of the biology of *C lacteus* as is characteristic of *Coptotermes* genus (French 1981; Howick and Creffield 1983; Lenz 1985; Lenz and Zi-Rong 1985). For this reason, future termite studies must take account of such potential variability in the design of the experiments. Inevitably, this will increase the size and complexity of studies of termite biology, including those in termite /wood decay fungi associations.

Interactions between the tree as a source of wood blocks, fungus species, level of decay in the wood and termite source were significant in different combinations for all the aspects of termite biology studied. These interactions indicate complex relationships between all factors studied which agrees with the general view that many factors are involved in the termites-fungi relationship (Amburgey 1979; Becker 1965; Lenz 1980; Ruyooka 1978). Only by specifying them can the significance of decayed wood for termite biology can be fully appreciated (Lenz 1991).

For the study of termite biology, air-dried unsterilized wood was used since it is decayed wood in such a state that termites encounter in the field. Under such circumstances, mass-loss in wood could have been misleading if taken to represent the amount of wood consumed by termites.as wood in the experiments continued to lose mass both to termites and fungi. This was demonstrated clearly in the choice feeding and termite colony development experiments whereby wood exposed to *P. tephropora* was a favourable wood when assessed by mass-loss but judged on the visual scores of termite attack, it was not eaten at all. Why termites might have consumed so little, if any, of the wood exposed to *P. tephropora* was not investigated but it has been reported that termites find wood exposed to some white rots repellent. It was reported by Hendee (1935) that excessive growth of fungi on decayed wood caused the deaths of many termites in her experiments with *Zootermopsis angusticollis*. Excessive mycelial growth was also observed with wood exposed to *P. tephropora* in the study reported here which made it likely that the wood acquired substances which were toxic to *C. lacteus*. This an area that should be invesigated further in future experiments.

In the colony development experiments, *C. olivacea* was randomly selected to represent the brown rot fungi and *P. tephropora* to represent white rots. Asssessed by wood consumption based on visual scores, total offspring number and mass colony

development proceeded at an equal pace on wood exposed to the brown rot as on the sterile control. This could have been because the brown rot did not cause heavy decay in the wood to which it was exposed (0-3%) and the state of the wood was influenced more by having spent time in contact with a rich nutrient medium from which it might have picked up some nutrients. Likewise, the sterile controls might have taken up nutrients from the medium such that the two wood treatments had much in common and this caused termites to react equally towards them. Such a change in the wood in contact with the nutrient medium might have bestowed favourable characteristics to the wood to which termites were attracted. This was perhaps the cause of higher wood consumption of these wood blocks compared to the untreated control.

With a much wider range of decay levels in the wood exposed to *P. tephropora*, the fungus possibly caused changes in the wood profound enough to be detected by termites. But it appears the changes were unfavourable. Termites consumed untreated controls more than wood exposed to the fungus. It was generally true that wherever species of fungus was a significant factor of the parameters of assesssment of termite colony development, performance was poorest on the wood exposed to this fungus.

An analysis of nitrogen in the wood after it was exposed to one each of the six fungi was carried out. At the lower decay level, there was significantly more nitrogen in wood exposed to *T.lilacino-gilva* than in all other wood. At the higher decay level, there was no significant difference in the amount of nitrogen in wood exposed to this fungus compared to the white rots which were the only other fungi to have caused high decay levels. The relationship between the various aspects of termite biology and the amount of nitrogen present in wood was therefore not clear.

It would have been expected that if it was the amount of nitrogen in wood that termites found most important factor in their choice of food, then wood exposed to *T. lilacino-gilva* at the higher decay levels, due to the overall good performance of this wood treatment, would have had to have significantly more nitrogen than wood exposed to the white rots. This was not the case. Also, the amount of nitrogen in wood increased directly with increasing decay level in the wood but this did not correspond to an increase of wood consumption, for example, in wood exposed to *P. tephropora*. Moreover, the amount of nitrogen in wood exposed to *P. tephropora* was significantly lower than in wood exposed to the other white rots. The amount of N in wood was a function of the fungal species used to inoculate the wood but termites appeared not to have based their behaviour solely on the amount of nitrogen available in the wood. Termite response to the amount of nitrogen in wood was therefore possibly influenced by external factors beyond the scope of this atudy. It has reported, for example, that termites have developed behavourial and physiological adaptations which enable them to live succesfully on such a

substrates low in nitrogen (Collins 1983; Lovelock *et al.*1985; MacKay *et al* 1985;Prestwich *et al.* 1980; Shellman-Reeve 1990; Waller and LaFage 1987). This aspect of *C. lacteus* biology therefore deserves further investigation to establish how the species copes with the limiting amount of nitrogen in wood as it appears not to be directly related to the dietary intake..

This study showed *C. lacteus* has a sound biological basis on which it bases its preferences for decayed wood. With respect to termite control, such a preferred wood could be used as a poisoned bait when impregnated with a non-repellent, slow-acting, short-persistence poison and placed in an area where termite workers would encounter it as they forage for food. Such a system of integrated control should enhance the effect of the chemical by itself . It would therefore be necessary to carry out more work in the field which took into account competition for food with other termites and to determine whether wood exposed to *T. lilacino-gilva* would be a species-specific bait for *C. lacteus* or not. It is important that poisoned baits should target specific termite species as this would cause minimum disruption to the beneficial termites in an area.

Research is under way into the possible control agents that can replace the now-banned organo-chlorine compounds.Wooden baits are also under evaluation as viable options of dispensing the successful ones to termite pests in the forest (Jones 1988, 1989). Such a system of integrated control using small doses of chemicals would be especially welcomed by developing countries. These countries urgently need a cheap, safe method of termite control to protect the fast -growing exotic tree species which are prone to termite attack, but which otherwise have a good potential for meeting the fuelwood requirements and other tree-related daily needs of the local people. Further, such a method would reduce the level of toxic chemicals in the biosphere, an important consideration in light of current ecological and political concerns regarding the safety of toxic chemicals .

The study established that study *C. lacteus* preferred feeding on *E. regnans* heartwood exposed to *T. lilacino-gilva* above wood exposed to other basidiomycete fungi under investigation. This preference shows that *E. regnans* heartwood exposed to *T. lilacino-gilva* has the characteristics of a good bait (Mauldin *et al.* 1985) under laboratory conditions. However, the laboratory conditions in which the work was carried out were not as rigourous or as comprehensive as those in the field and more work remains to be done to find out how *C. lacteus* would behave towards *E. regnans* heartwood exposed to *T. lilacino-gilva* in the field. This is the ultimate test of a good bait.

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## Appendix 1.1: Allocation of wood blocks from tree 1 to various aspects of termite bioassay

| Proposed aspect of investigation in wood x fungus x termite interaction | Source of wood blocks | Test fungi | Termite (mound) sources | Wood blocks per termite bioassay chamber | Replicates | Wood blocks per duration of exposure to wood-decay fungi |
|---|---|---|---|---|---|---|
| Attractiveness of wood blocks to termites after exposure of blocks to fungi | Tree 1 | 8 | 3 | 1 | 3 | 72 |
| Feeding and survival experiments with termites | | 8 | 3 | 3 | 3 | 216 |
| Termite colony development experiments | | 4 | 1 | 10 | 5 | 200 |
| | | | | | | 488 |

## Appendix 1.2: Allocation of wood blocks from tree 2 to various aspects of termite bioassay

| Proposed aspect of investigation in wood x fungus x termite interaction | Source of wood blocks | Test fungi | Termite (mound) sources | Wood blocks per termite bioassay chamber | Replicates | Wood blocks per duration of exposure to wood-decay fungi |
|---|---|---|---|---|---|---|
| Attractiveness of wood blocks to termites after exposure of blocks to fungi | Tree 2 | 8 | 3 | 1 | 2 | 48 |
| Feeding and survival experiments with termites | | 8 | 3 | 3 | 2 | 144 |
| Termite colony development experiments | | 4 | 1 | 10 | 5 | 200 |
| | | | | | | 392 |

**Appendix 1.3: Number of wood blocks in the fungal bioassay and their allocation to different durations of exposure to the fungi.**

| Tree | Duration of wood block exposure to fungi | Number of wood blocks required for the hypothetical fungal bioassay design | Number of wood blocks that were bioassayed |
|---|---|---|---|
| 1 | 5 | 488 | 895 |
| | 7 | 488 | 795 |
| | 9 | 488 | 885 |
| 2 | 5 | 392 | 894 |
| | 7 | 392 | 704 |
| | 9 | 392 | 885 |
| | Total | 2640 | 5058 |

## Appendix 2: Characteristics of the fungi selected for the fungal bioassay

### Brown rots

***Coniophora olivacea*** (Fr.) Karst. -DFP 1779.

Ginns (1982) reported that this fungus is associated with a brown cubical rot of live *Picea abies* in Sweden and live *P. engelmannii* in Colorado, USA, logs and occasionally boards and timbers. It is a fungus typically associated with coniferous wood. In Australian the coniophora spp. attack all timbers up too durability class 2 regardless of whether they are softwood or hardwood (Walters 1973)

In pure culture, the growth rate of *C. olivacea* is variable, with some cultures growing more slowly than others. At two weeks, its mats on plates are white to cream in colour with a few cultures showing yellow patches. Most cultures by this time would have developed raised aerial mycelium extending to the lid and a texture varying from cottony fluctose to woolly fluctose. The mat is concentrically zonate.

At four weeks, some mats are yellow brown, distinctly brown or mottled with brown patches. Hyphal strands are typically brown and the corrosive action of the mycelium turns the agar soft and greasy. A sweet or weakly sweet odour is produced even though occasionally there is no odour given out or if so, it is spicy sweet.

From the fungal bioassay:

At 7 weeks, the growth of mycelia in decay chambers was restricted, for the most part, to the bases. Hyphal strands were found growing against the sides of the trays at some points. Where coverage of the wood blocks was sparse, the mycelia were present in the form of hyphal strands. Where coverage was dense, the mycelia were soft and fluffy and did not form a 'skin' on wood blocks. Colour of mycelia was dark to light golden brown. The colour of exudate was light brown. Wood blocks were firm and were commonly bound by cobweb-like structures of intertwining mycelia. Attempts to clean the blocks led to smearing of surfaces with an unsightly dark brown colour which obscured the identity of blocks. Mycelia were therefore left untouched to dry on the blocks.

At nine weeks, the majority of wood blocks were sparsely to moderately covered by light-golden to brown-coloured mycelia. The hyphal strands of mycelia on sparsely covered units were thin and brown in colour. Growth of fluffy mycelia was restricted mostly to base of trays but was also found on densely covered units. Clear moisture and brown exudate droplets were present as were the cobweb-like mycelial links between neighbouring blocks.

***Trametes lilacino-gilva*** (Berk.) Lloyd -DFP 1109

This fungus is almost confined to Australia where it occurs under conditions ranging from those with an assured water supply to semi-arid regions. It is also almost confined to the genus *Eucalyptus*. It commonly attacks non-durable species but not durable ones. Associated with a brown cubical rot, the fungus is especially destructive should it attack the external timbers of a dwelling (Walters 1973). Worldwide, its distribution is mostly in the southern hemisphere and it has been reported in S. Africa, Australia and New Zealand (Cunningham 1965).

No better description of the fungus could be given than that of Stalpers (1978). The advancing zone is raised and even; the hyphae distant. Its mat is thin cottony to dense cotton-woolly becoming felty. Aerial hyphae contain oil drops. The upper surface of *T. lilacino-gilva* (12-10cm radius) is reddish brown with dark radiating streaks and ridges; becoming darker with age. The lower surface consists of pore mouths, quite visible to the naked eye, reddish lilac while actively growing, easily bruised to deep crimson. When mature, it becomes pale and loses the reddish colour to become mauve or lilac. When dry, it is pale lilac.

From fungal bioassay:

At five weeks, fungal mycelia grew in strands along sides of trays but growth was very sparse on thebase of the tray. Mycelia were off-white in colour and they were fluffy in texture. Colonisation occurred in patches on some of the blocks. Where growth on wood blocks was dense, the colour darkened to dusty pink .The mycelia formed a brittle and tough 'skin' on wood blocks which was difficult to peel off. Moisture droplets were found on wood blocks and on the sides of the tray but the malt agar had shrunk away from the sides of the trays.

At seven weeks, the majority of wood blocks was moderately to densely covered by fungal mycelia. Where coverage was dense, the mycelia formed a tough, leathery dusty pink skin The skin was thin in some cases and would tear to pieces when cleaning was attempted. On some wood blocks, a spongy skin was present which yielded moisture on squeezing. Thick skins peeled off as a whole when wood blocks were cleaned. Such wood blocks had a somewhat streaky apperance. On moderately covered wood blocks, cuboidal chips of wood broke off the edges and in such a delicate situation, cleaning was only partially achieved. The mycelia growing on the base of the tray was uniformly light in colour with dark patches of mycelia only present in a couple of trays. The sides of the trays were covered to about half-way by thick strands of white mycelia.

At nine weeks, most units were covered by leathery, tough, dusty pink skins of mycelia. The bases of the trays were covered by sparse, fluffy white mycelia interspersed with dense

dusty pink patches. White mycelial strands had grown half-way up the sides of trays where moisture droplets were abundant. Such droplets were also common on the plastic mesh situated between the wood blocks and the culture medium. A few droplets were also present on wood blocks. Due to the dense growth of mycelia on the wood blocks, a few of them were firmly 'rooted' to the plastic mesh. The edges of the sparsely-covered wood blocks tended to disintegrate during cleaning. The culture medium had shrunk away from the sides of the trays.

***Gloeophyllum trabeum*** (Fr.ex Pers.) Murr.- DFP 7520

This fungus causes a destructive brown cubical rot and has a preference for softwoods even though it is also found attacking species of eucalypts. Exposed woodwork is preferred. It also likes an assured water supply but in spite of this, it will withstand long periods of dessication. It is one of the most important causes of decay in homes and other structures in North America (Gilbertson and Ryvarden 1986).

Conducting strands are unusual but when formed are substantial and tend to grow downwards to form a sclerotium; an underground organ for food storage and for surviving unfavourable seasons. Surface growths of thick, loose cottony mycelium are common. The mat is cottony to woolly, whitish to cream buff or yellow honey, sometimes with whitish farinacous zones. Odour is sweet (Stalpers 1978).

From fungal bioassay:

At five weeks, growth of mycelia was restricted to the base of the tray where they formed a golden-coloured, fluffy bed. Where wood blocks were moderately-covered, the mycelia were fluffy in texture and yellow in colour. Such blocks were delicate to clean as bits of wood were lost in the areas where the mycelia were most concentrated. Less densely covered units were easier to clean. Moisture droplets were present on small patches of the plastic mesh and along the sides of the tray but not on wood blocks. A characteristic 'sweet' aroma was given off. The culture medium had shrunk away from the sides of the tray.

At seven weeks, most wood blocks had only a sparse coverage of mycelia. The base of the tray was covered by a honey-coloured fluffy bed of mycelia. This apperance of the bed was that of a moonscape. Mycelia were only found on the base of the tray. A few of the wood blocks were covered with yellow, fluffy mycelia which grew in clumps like cactus. There were no moisture or exudate droplets present. Most wood blocks were still firm and loosely attached to the bed of mycelia.

At nine weeks, the characteristics of the fungus were similar to those at five and seven weeks. Most units were sparsely covered by fungal mycelia. The bases of the trays were densely covered by fluffy, off-white or honey-coloured mycelia. During cleaning, the wood

blocks which had a dense cover of mycelia lost bits of wood in the areas where the mycelia were most dense. Moisture droplets were present on small patches of the plastic mesh and along sides of tray but not on wood blocks. The characteristic 'sweet' aroma was given off by the mycelia.

**White rots**

*Perenniporia tephropora* (Mont.) Ryv.(=*Fomes lividus* (Kalch)(Sacc.) - DFP 7904

In Australia, this fungus lives on bark of fallen dead logs or erect stumps. it has a hyphal system with abundant skeletal hyphae and it causes a cheesy white rot of dead hardwoods of numerous genera in North America. It requires an assured water supply. It is characterised by its colouring, at first whitish then colour ranges from ochraceous to pale brown (Gilbertson and Ryvarden 1987).

In Australia, the fungus favours external wood-work and is common on bush timbers and in open air structures in the rain forests of the warmer and wetter regions where it is very destructive. It also vigorously attacks both sapwood and heartwood of durable and less durable species. It occasionally penetrates floors and stumps (Walters 1973).

From fungal bioassay:

At five weeks, the wood blocks were sparsely to densely covered by mycelia. The brown skin of mycelia which was present on wood blocks with dense coverage was easy to peel off as it came off as a whole. Such blocks were firmly attached to the base of the tray by the mycelia. Those blocks with a sparse skin were harder to clean. There were moisture droplets all over the tray while a brown coloured exudate was present in patches along the sides of the tray.

At seven weeks, all woodblocks were covered by a mycelial skin of varying thickness. The colour of the mycelia at the base of the tray was off-white while on units, it was mostly brown but occasionally off-white. The mycelia grew to about the half-way level up the sides of the tray. Hyphal strands grew from that point on to the top of the tray and sometimes over and down the outer sides of the tray. Moisture droplets were abundant and oily exudates were present but had started to dry up in some areas, leaving behind tar-like brown smears.The skin of fungal mycelia on the wood blocks peeled off as a whole where it was thick but with greater difficulty where it was thin. In a such a situation as the latter, thin, white patches of mycelia remained behind.

At nine weeks, the densely covered units had thick, brown skins of mycelia. Moisture was abundant but exudate was scarce or had thickened.The wood blocks were still firm enough to handle with ease and they had a streaky appearance after cleaning.

***Pycnoporus coccineus*** (Fr.) Bond. & Sing. - DFP 1095

This is a fungus that is mainly found in the southern temperate zone of the globe and occurs throughout Australia. *Pycnoporus* is frequent where the water supply is high and in buildings it is confined to external woodwork. It has a wide host range, including all sapwoods and heartwoods up to durability class 3 (moderately durable, seldom more than 15 years' service), and hence its worldwide distribution (Walters 1973).

There is usually no surface mycelium other than an occasional thin white skin and it produces no conducting strands. The rot is a pure white cheesy to stringy rot, tinged scarlet, with occasional sheets of white fungal tissue (Walters 1973).

Colour is light salmon orange to apricot orange, carnelian red or flame scarlet, rarely cinnamon rufous (Stalpers 1978).

From fungal bioassay:

At five weeks, the colour of this fungus ranged from pale to deep apricot (orange/red). It produced a pale yellow exudate which was restricted to the base of the trays. Tiny moisture droplets were present. Wood block coverage was sparse to dense and the skin which was formed was more leathery than that of *Perenniporia tephropora*. The culture medium had shrunk away from the sides of the tray

At seven weeks, the characteristics of mycelial growth were like those at five weeks with the exception that exudate was light orange. The mycelia on wood blocks were difficult to peel off except in cases where the growth was thick.

At nine weeks, the coverage of wood blocks by fungal mycelia ranged from sparse to dense. The colour of mycelia was apricot and the exudate was clear or honey-coloured. The densely covered woodblocks had skins of mycelia which peeled off easily. The wood underneath was tinted orange/red where the skin got crushed during peeling. A sweet, damp, mushroomy smell was given out. The base of the trays were covered by a thick, spongy.mat of mycelia. Patches of the mycelia grew along the sides of the trays where moisture droplets were also found.

***Tinctoporellus epimiltinus*** (Berk. & Br.) Ryv. - DFP 14579A

This is yet another fungus which causes a white rot in dead hardwoods, whether standing or fallen. It has a pan-tropical distribution extending from the North American gulf coast to northern Argentina. It is easily identified as it reddens its substrate (Gilbertson and Ryvarden 1987).

Not much is known about its growth habits in Australia and it was the one fungus incorporated in this project for which the least information was available.

From fungal bioassay:

The fungal mycelial colonising wood blocks formed brittle, papery skins on them. The skins varied in colour from yellw to brown.The coverage ranged from sparse to modereate. The exudate produced was clear in colour . Moisture droplets had condensed on the sides of the tray. Underneath the skins, the mycelia were fluffy and white. White strands of mycelia grew up the sides of the trays and over the brown skins on the wood blocks.

At seven weeks, most of the wood blocks were densely covered by fungal mycelia. These had formed brittle, papery skins on them. The colour ranged from ochre to cream, with fluffy white patches. Brown hyphal strands were also present on the sparsely covered wood blocks. Exudate was found in pools along edges of trays. Where coverage was thick, wood blocks were firmly rooted to the mycelia at the base of the trays.

At nine weeks, the decay chambers were very dry. Moisture covered some patches of the plastic mesh but moisture droplets on the sides of the trays were scanty. The growth of fungal mycelia was restricted mostly to the base of the trays or on wood blocks. A few strands of mycelia were found on the sides of the tray. On the bases of the trays, the mycelial colour ranged from brown/yellow/ochre/white to orange. The sparsely covered units had white/yellow strands traversing their surfaces while on the densely covered wood blocks, ochre or white papery skins were found.

**Appendix 3: A list of the characteristics of termite mounds monitored for alate flight preparedness in the colony development experiment:**

| Mound No. | Height (m) | Basal circumference (m) | Location |
|---|---|---|---|
| 1 | 0.66 | 3.18 | Open pasture. Established on old tree stump |
| 2 | 0.9 | 3.9 | Near base of eucalypt tree at edge of dry sclerophyll forest |
| 3 | 1.6 | 4.83 | On open patch of the above forest type |
| 4 | 1.1 | 4.8 | Next to an isolated casuarina tree in open pasture |
| 5 | 0.65 | 3.4 | Open pasture |
| 6 | 1 | 6.25 | Edge of patch of dry sclerophyll forest |

Mounds 1 and 6 were eventually chosen for termite collection because they prepared for and released their alates the earliest. Mound 6 released alates first and became known thereafter as colony 1 while Mound 1 released alates later and henceforth was identified as colony 2.

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Termite | | | | % mass-loss | Lab. colony | Initial mass | Wet weight | Final mass (g) | % wood | Visual score of | Weight of | Weight of | Termite caste development | | | | |
| 2 | source (field) | Replicate | Tree No. | Fungus | category | No. | of wood (g) | of wood (g) | (equilibrated) | consumption | termite attack | alate pair | offspring | Eggs | Larvae | workers | Pre-soldiers | Soldiers |
| 3 | Colony 1 | 1 | 1 | B1 | Low | 64 | 7.04 | 7.89 | 5.25 | 25 | 0 | 0.0138 | 0.0903 | 76 | 11 | 57 | 0 | 4 |
| 4 | Colony 1 | 1 | 1 | B1 | Medium | 71 | 6.45 | 6.49 | 4.02 | 38 | 2 | 0.015 | 0.1013 | 76 | 20 | 61 | 4 | 5 |
| 5 | Colony 1 | 1 | 1 | B1 | High | 81 | 6.43 | 5.98 | 3.6 | 44 | 0 | * | * | * | * | * | * | * |
| 6 | Colony 1 | 1 | 1 | W1 | Low | 93 | 7.23 | 8.04 | 4.17 | 42 | 0 | 0.0154 | 0.0708 | 63 | 4 | 33 | 0 | 3 |
| 7 | Colony 1 | 1 | 1 | W1 | Medium | 102 | 6.17 | 6.58 | 3.16 | 49 | 0 | * | * | 0 | * | * | * | * |
| 8 | Colony 1 | 1 | 1 | W1 | High | 112 | 5.69 | 5.61 | 2.84 | 50 | 0 | 0.0161 | 0.0284 | 0 | 0 | 13 | 0 | 1 |
| 9 | Colony 1 | 1 | 1 | SC | Low | 34 | 6.77 | 7.48 | 4.51 | 33 | 2 | 0.0146 | 0.0788 | 44 | 3 | 38 | 0 | 6 |
| 10 | Colony 1 | 1 | 1 | SC | Medium | 41 | 6.83 | 6.92 | 4 | 41 | 2 | 0.0135 | 0.0736 | 38 | 5 | 46 | 2 | 4 |
| 11 | Colony 1 | 1 | 1 | SC | High | 55 | 7.18 | 7.01 | 4.05 | 44 | 2 | 0.017 | 0.043 | 18 | 0 | 17 | 1 | 3 |
| 12 | Colony 1 | 1 | 1 | C | Low | 5 | 7 | 9.31 | 6.49 | 7 | 0 | * | * | * | * | * | * | * |
| 13 | Colony 1 | 1 | 1 | C | Medium | 15 | 6.98 | 8.53 | 6.25 | 10 | 1 | 0.0144 | 0.0736 | 37 | 15 | 46 | 1 | 5 |
| 14 | Colony 1 | 1 | 1 | C | High | 22 | 6.5 | 7.38 | 5.3 | 18 | 1 | 0.0145 | 0.0583 | 35 | 6 | 26 | 0 | 3 |
| 15 | Colony 1 | 1 | 2 | BI | Low | 70 | 6.42 | 7.04 | 4.78 | 26 | 0 | 0.0151 | 0.1118 | 95 | 14 | 67 | 2 | 6 |
| 16 | Colony 1 | 1 | 2 | BI | Medium | 77 | 6.44 | 7.31 | 4.94 | 23 | 2 | 0.0159 | 0.0611 | 77 | 6 | 30 | 1 | 4 |
| 17 | Colony 1 | 1 | 2 | BI | High | 87 | 6.77 | 8.36 | 5.73 | 15 | 2 | 0.015 | 0.0706 | 69 | 5 | 29 | 0 | 4 |
| 18 | Colony 1 | 1 | 2 | W1 | Low | 100 | 6.85 | 7.58 | 3.88 | 43 | 0 | 0.0156 | 0.0692 | 41 | 4 | 34 | 0 | 4 |
| 19 | Colony 1 | 1 | 2 | W1 | Medium | 110 | 5.75 | 6.27 | 2.89 | 50 | 0 | 0.0175 | 0.0432 | 37 | 6 | 16 | 0 | 2 |
| 20 | Colony 1 | 1 | 2 | W1 | High | 116 | 5.75 | 6.27 | 3.05 | 47 | 0 | 0.0061 | 0.0251 | 0 | 0 | 20 | 0 | 1 |
| 21 | Colony 1 | 1 | 2 | SC | Low | 37 | 7.17 | 7.84 | 3.99 | 44 | 0 | * | * | * | * | * | * | * |
| 22 | Colony 1 | 1 | 2 | SC | Medium | 50 | 7.31 | 9.9 | 6.62 | 9 | 0 | * | * | * | * | * | * | * |
| 23 | Colony 1 | 1 | 2 | SC | High | 58 | 6.82 | 6.25 | 3.75 | 45 | 1 | 0.0164 | 0.0623 | 30 | 3 | 32 | 0 | 3 |
| 24 | Colony 1 | 1 | 2 | C | Low | 8 | 6.62 | 8.56 | 5.96 | 10 | 1 | 0.0148 | 0.0448 | 33 | 7 | 22 | 0 | 4 |
| 25 | Colony 1 | 1 | 2 | C | Medium | 19 | 6.47 | 8.17 | 5.89 | 9 | 1 | 0.0167 | 0.045 | 26 | 3 | 16 | 1 | 2 |
| 26 | Colony 1 | 1 | 2 | C | High | 27 | * | 9.65 | 7.05 | * | 1 | 0.0142 | 0.0484 | 27 | 0 | 22 | 0 | 2 |
| 27 | Colony 1 | 2 | 1 | B1 | Low | 62 | 6.92 | 6.26 | 3.8 | 45 | 2 | 0.0147 | 0.01697 | 122 | 30 | 96 | 1 | 12 |
| 28 | Colony 1 | 2 | 1 | B1 | Medium | 73 | 6.49 | 5.13 | 3.26 | 50 | 3 | 0.0152 | 0.347 | 118 | 33 | 288 | 1 | 18 |
| 29 | Colony 1 | 2 | 1 | B1 | High | 83 | 6.79 | 6.71 | 3.97 | 42 | 2 | 0.014 | 0.1882 | 181 | 28 | 152 | 0 | 12 |
| 30 | Colony 1 | 2 | 1 | W1 | Low | 103 | 5.98 | 6.24 | 3.18 | 47 | 0 | 0.0112 | 0.1079 | 5 | 7 | 68 | 1 | 7 |
| 31 | Colony 1 | 2 | 1 | W1 | Medium | 92 | 6.7 | 7.6 | 3.34 | 50 | 0 | 0.0146 | 0.1112 | 63 | 11 | 64 | 0 | 5 |
| 32 | Colony 1 | 2 | 1 | W1 | High | 115 | 5.63 | 6.09 | 2.88 | 49 | 0 | * | * | 0 | 0 | 5 | 0 | 2 |
| 33 | Colony 1 | 2 | 1 | SC | Low | 31 | 6.85 | 6.55 | 3.33 | 51 | 3 | 0.0122 | 0.11 | 65 | 20 | 83 | 1 | 7 |
| 34 | Colony 1 | 2 | 1 | SC | Medium | 43 | 7.13 | 5.81 | 3.46 | 51 | 3 | 0.0148 | 0.1558 | 134 | 20 | 94 | 1 | 9 |
| 35 | Colony 1 | 2 | 1 | SC | High | 51 | 6.48 | 5.26 | 2.8 | 57 | 3 | 0.0149 | 0.1795 | 156 | 12 | 111 | 2 | 12 |
| 36 | Colony 1 | 2 | 1 | C | Low | 13 | 7.17 | 8.56 | 6.52 | 9 | 1 | 0.06 | 0.0504 | 0 | 0 | 21 | 0 | 5 |
| 37 | Colony 1 | 2 | 1 | C | Medium | 23 | 7 | 7.38 | 5.21 | 26 | 1 | 0.0141 | 0.1513 | 64 | 15 | 103 | 2 | 8 |
| 38 | Colony 1 | 2 | 1 | C | High | 2 | 6.94 | 7.39 | 4.27 | 38 | 2 | 0.0138 | 0.0711 | 45 | 5 | 49 | 2 | 7 |
| 39 | Colony 1 | 2 | 2 | B1 | Low | 67 | 7.01 | 5.34 | 2.98 | 57 | 3 | 0.0134 | 0.234 | 128 | 23 | 168 | 2 | 18 |
| 40 | Colony 1 | 2 | 2 | B1 | Medium | 79 | 6.77 | 5.65 | 3.15 | 53 | 3 | 0.0147 | 0.1716 | 65 | 18 | 105 | 2 | 11 |
| 41 | Colony 1 | 2 | 2 | B1 | High | 88 | 7.36 | 7.48 | 4.77 | 35 | 2 | 0.0157 | 0.177 | 41 | 22 | 122 | 1 | 10 |
| 42 | Colony 1 | 2 | 2 | W1 | Low | 98 | 6.51 | 7.6 | 3.81 | 41 | 0 | 0.0141 | 0.0818 | 11 | 10 | 48 | 2 | 4 |
| 43 | Colony 1 | 2 | 2 | W1 | Medium | 109 | 6.34 | 7.01 | 3.44 | 46 | 0 | * | * | * | * | * | * | * |
| 44 | Colony 1 | 2 | 2 | W1 | High | 119 | 5.39 | 5.85 | 2.68 | 50 | 0 | 0.013 | 0.0727 | 39 | 3 | 51 | 0 | 6 |
| 45 | Colony 1 | 2 | 2 | SC | Low | 36 | 7.24 | 8.23 | 4.35 | 40 | 1 | 0.0155 | 0.1164 | 67 | 19 | 57 | 1 | 8 |
| 46 | Colony 1 | 2 | 2 | SC | Medium | 48 | 6.84 | 6.52 | 3.16 | 54 | 3 | 0.0137 | 0.1072 | 65 | 13 | 79 | 1 | 7 |
| 47 | Colony 1 | 2 | 2 | SC | High | 57 | 6.96 | 7.05 | 4.39 | 37 | 2 | 0.0146 | 0.1835 | 92 | 9 | 130 | 3 | 9 |
| 48 | Colony 1 | 2 | 2 | C | Low | 7 | 7.24 | 8.82 | 6.58 | 9 | 1 | 0.0142 | 0.2046 | 134 | 14 | 150 | 2 | 14 |
| 49 | Colony 1 | 2 | 2 | C | Medium | 16 | 7.38 | 9.21 | 6.29 | 15 | 1 | 0.0153 | 0.0808 | 74 | 8 | 39 | 0 | 6 |
| 50 | Colony 1 | 2 | 2 | C | High | 26 | * | 9.33 | * | * | 1 | 0.0142 | 0.1103 | 112 | 17 | 77 | 3 | 7 |
| 51 | Colony 1 | 3 | 1 | B1 | Low | 61 | 8.99 | 6.11 | 3.92 | 56 | 1 | 0.0196 | 0.1487 | 79 | 7 | 104 | 1 | 11 |
| 52 | Colony 1 | 3 | 1 | B1 | Medium | 75 | 7.03 | 6.21 | 3.74 | 47 | 2 | 0.0151 | 0.3243 | 75 | 40 | 232 | 2 | 20 |
| 53 | Colony 1 | 3 | 1 | B1 | High | 84 | 7.08 | 6.21 | 3.85 | 46 | 3 | 0.0153 | 0.3022 | 0 | 26 | 219 | 1 | 14 |
| 54 | Colony 1 | 3 | 1 | W1 | Low | 91 | 7 | 7.59 | 3.77 | 46 | 0 | 0.0132 | 0.149 | 38 | 8 | 86 | 0 | 7 |
| 55 | Colony 1 | 3 | 1 | W1 | Medium | 105 | 5.95 | 6.52 | 3.12 | 48 | 0 | 0.0126 | 0.0912 | 47 | 4 | 67 | 0 | 8 |

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | Colony 1 | 3 | 1 | W1 | Medium | 105 | 5.95 | 6.52 | 3.12 | 48 | 0 | 0.0126 | 0.0912 | 47 | 4 | 67 | 0 | 8 |
| 56 | Colony 1 | 3 | 1 | W1 | High | 113 | 5.51 | 6.50 | 2.95 | 46 | 0 | * | * | * | * | * | * | * |
| 57 | Colony 1 | 3 | 1 | SC | Low | 32 | 6.55 | 7.37 | 5.93 | 9 | 1 | 0.0143 | 0.252 | 89 | 24 | 181 | 3 | 13 |
| 58 | Colony 1 | 3 | 1 | SC | Medium | 44 | 7.00 | 8.07 | 4.20 | 40 | 1 | * | * | * | * | * | * | * |
| 59 | Colony 1 | 3 | 1 | SC | High | 52 | 6.77 | 7.38 | 4.72 | 30 | 1 | 0.0147 | 0.1243 | 77 | 4 | 78 | 0 | 9 |
| 60 | Colony 1 | 3 | 1 | C | Low | 4 | 6.59 | 7.76 | 4.17 | 37 | 0 | * | * | * | * | * | * | * |
| 61 | Colony 1 | 3 | 1 | C | Medium | 14 | 7.31 | 8.29 | 5.44 | 26 | 1 | 0.0137 | 0.1485 | 75 | 9 | 121 | 0 | 12 |
| 62 | Colony 1 | 3 | 1 | C | High | 24 | 6.81 | * | * | * | * | * | * | * | * | * | * | * |
| 63 | Colony 1 | 3 | 2 | B1 | Low | 68 | 7.38 | * | * | * | * | * | * | * | * | * | * | * |
| 64 | Colony 1 | 3 | 2 | B1 | Medium | 78 | 6.60 | * | 2.71 | 59 | 1 | * | * | * | * | * | * | * |
| 65 | Colony 1 | 3 | 2 | B1 | High | 89 | 6.95 | 6.57 | 4.36 | 37 | 1 | 0.0142 | 0.2235 | 69 | 33 | 163 | 3 | 15 |
| 66 | Colony 1 | 3 | 2 | W1 | Low | 97 | 6.19 | 7.20 | 3.50 | 43 | 0 | 0.0134 | 0.1027 | 17 | 5 | 56 | 0 | 7 |
| 67 | Colony 1 | 3 | 2 | W1 | Medium | 108 | 6.01 | 6.54 | 3.15 | 48 | 0 | * | * | * | * | * | * | * |
| 68 | Colony 1 | 3 | 2 | W1 | High | 120 | 5.73 | 6.33 | 3.00 | 48 | 0 | 0.015 | 0.088 | 77 | 7 | 49 | 0 | 6 |
| 69 | Colony 1 | 3 | 2 | SC | Low | 39 | 6.79 | 6.59 | 2.95 | 57 | 2 | 0.141 | 0.174 | 108 | 32 | 62 | 1 | 14 |
| 70 | Colony 1 | 3 | 2 | SC | Medium | 46 | 6.56 | 6.27 | 3.62 | 45 | 0 | 0.0139 | 0.1501 | 13 | 15 | 91 | 0 | 8 |
| 71 | Colony 1 | 3 | 2 | SC | High | 60 | 7.17 | 6.46 | 3.90 | 46 | 1 | 0.014 | 0.2165 | 96 | 30 | 165 | 2 | 19 |
| 72 | Colony 1 | 3 | 2 | C | Low | 6 | 6.51 | 8.01 | 5.70 | 12 | 1 | 0.0151 | 0.091 | 55 | 8 | 50 | 0 | 6 |
| 73 | Colony 1 | 3 | 2 | C | Medium | 18 | 7.05 | 9.21 | 5.92 | 16 | 1 | 0.0164 | 0.0727 | 113 | 12 | 39 | 0 | 3 |
| 74 | Colony 1 | 3 | 2 | C | High | 28 | 7.44 | 8.85 | 6.15 | 17 | 1 | 0.014 | 0.2368 | 101 | 36 | 173 | 1 | 8 |
| 75 | Colony 1 | 4 | 1 | B1 | Low | 65 | 6.79 | 6.43 | 3.76 | 45 | 2 | 0.0138 | 0.1169 | 46 | 15 | 75 | 1 | 5 |
| 76 | Colony 1 | 4 | 1 | B1 | Medium | 72 | 7.01 | * | * | * | * | * | * | * | * | * | * | * |
| 77 | Colony 1 | 4 | 1 | B1 | High | 82 | 6.82 | * | * | * | 1 | * | * | * | * | * | * | * |
| 78 | Colony 1 | 4 | 1 | W1 | Low | 95 | 6.66 | 7.21 | 3.79 | 43 | 0 | 0.015 | 0.1303 | 67 | 24 | 65 | 0 | 6 |
| 79 | Colony 1 | 4 | 1 | W1 | Medium | 104 | 5.74 | 6.25 | 3.03 | 47 | 0 | 0.0075 | 0.0613 | 0 | 0 | 25 | 0 | 4 |
| 80 | Colony 1 | 4 | 1 | W1 | High | 111 | 5.68 | 5.58 | 2.93 | 48 | 0 | 0.0126 | 0.0794 | 33 | 11 | 33 | 1 | 7 |
| 81 | Colony 1 | 4 | 1 | SC | Low | 35 | 7.08 | 6.78 | 4.46 | 37 | 2 | 0.0135 | 0.1904 | 102 | 32 | 135 | 1 | 12 |
| 82 | Colony 1 | 4 | 1 | SC | Medium | 45 | 6.97 | 6.87 | 4.12 | 41 | 0 | 0.014 | 0.1373 | 25 | 31 | 78 | 2 | 7 |
| 83 | Colony 1 | 4 | 1 | SC | High | 54 | 6.93 | 9.27 | 6.01 | 13 | 0 | * | * | * | * | * | * | * |
| 84 | Colony 1 | 4 | 1 | C | Low | 3 | 6.70 | 8.07 | 5.39 | 20 | 2 | 0.0145 | 0.126 | 36 | 27 | 72 | 2 | 6 |
| 85 | Colony 1 | 4 | 1 | C | Medium | 11 | 6.94 | 8.61 | 5.83 | 16 | 1 | 0.0136 | 0.0687 | 19 | 10 | 35 | 1 | 2 |
| 86 | Colony 1 | 4 | 1 | C | High | 25 | 7.23 | 9.22 | 6.25 | 14 | 1 | 0.0148 | 0.0495 | 33 | 10 | 23 | 0 | 4 |
| 87 | Colony 1 | 4 | 2 | B1 | Low | 69 | 7.14 | 6.52 | 3.71 | 48 | 3 | 0.0142 | 0.1846 | 129 | 49 | 114 | 0 | 9 |
| 88 | Colony 1 | 4 | 2 | B1 | Medium | 76 | 6.86 | 6.77 | 4.38 | 36 | 0 | 0.0152 | 0.2557 | 198 | 63 | 143 | 0 | 18 |
| 89 | Colony 1 | 4 | 2 | B1 | High | 86 | 6.91 | 7.15 | 3.99 | 42 | 0 | * | * | * | * | * | * | * |
| 90 | Colony 1 | 4 | 2 | W1 | Low | 96 | 6.76 | 6.78 | 3.44 | 49 | 0 | 0.017 | 0.0712 | 30 | 6 | 29 | 0 | 6 |
| 91 | Colony 1 | 4 | 2 | W1 | Medium | 106 | 6.13 | 6.69 | 3.09 | 50 | 0 | * | * | * | * | * | * | * |
| 92 | Colony 1 | 4 | 2 | W1 | High | 117 | 5.62 | 5.72 | 2.62 | 53 | 0 | * | * | * | * | * | * | * |
| 93 | Colony 1 | 4 | 2 | SC | Low | 38 | 7.38 | 8.61 | 5.89 | 20 | 1 | 0.0145 | 0.1452 | 124 | 33 | 73 | 0 | 6 |
| 94 | Colony 1 | 4 | 2 | SC | Medium | 49 | 6.81 | 8.49 | 5.91 | 13 | 2 | 0.0148 | 0.1232 | 68 | 24 | 72 | 1 | 6 |
| 95 | Colony 1 | 4 | 2 | SC | High | 56 | 6.85 | 7.23 | 5.23 | 24 | 0 | 0.0141 | 0.1793 | 162 | 31 | 124 | 1 | 9 |
| 96 | Colony 1 | 4 | 2 | C | Low | 9 | 6.70 | 8.56 | 5.75 | 14 | 1 | 0.0156 | 0.0746 | 28 | 9 | 44 | 0 | 5 |
| 97 | Colony 1 | 4 | 2 | C | Medium | 17 | 7.09 | 8.77 | 6.17 | 13 | 1 | 0.0151 | 0.109 | 30 | 19 | 51 | 0 | 6 |
| 98 | Colony 1 | 4 | 2 | C | High | 29 | 6.89 | 8.91 | 6.04 | 12 | 1 | 0.014 | 0.0846 | 28 | 9 | 48 | 0 | 5 |
| 99 | Colony 1 | 5 | I | B1 | Low | 63 | 6.88 | 6.30 | 4.18 | 39 | 3 | 0.0153 | 0.21 | 159 | 30 | 143 | 4 | 9 |
| 100 | Colony 1 | 5 | I | B1 | Medium | 74 | 7.14 | 6.69 | 3.71 | 48 | 0 | 0.0149 | 0.1906 | 160 | 34 | 119 | 1 | 12 |
| 101 | Colony 1 | 5 | I | B1 | High | 85 | 6.41 | 5.90 | 3.22 | 50 | 2 | 0.0063 | 0.137 | 0 | 0 | 44 | 0 | 4 |
| 102 | Colony 1 | 5 | I | W1 | Low | 94 | 6.50 | 6.56 | 3.26 | 50 | 0 | 0.0118 | 0.109 | 10 | 5 | 69 | 1 | 6 |
| 103 | Colony 1 | 5 | I | W1 | Medium | 101 | 5.86 | 6.27 | 3.25 | 45 | 0 | 0.0132 | 0.0988 | 44 | 11 | 64 | 0 | 5 |
| 104 | Colony 1 | 5 | I | W1 | High | 114 | 5.23 | 5.78 | 2.72 | 48 | 0 | * | * | * | * | * | * | * |
| 105 | Colony 1 | 5 | I | SC | Low | 33 | 6.25 | 6.35 | 3.28 | 48 | 3 | 0.0132 | 0.1779 | 76 | 38 | 109 | 2 | 8 |
| 106 | Colony 1 | 5 | I | SC | Medium | 42 | 6.88 | 6.30 | 3.93 | 43 | 2 | 0.014 | 0.1402 | 103 | 34 | 77 | 1 | 8 |
| 107 | Colony 1 | 5 | I | SC | High | 53 | 6.83 | 6.50 | 3.43 | 50 | 2 | 0.0141 | 0.1415 | 48 | 27 | 87 | 1 | 5 |
| 108 | Colony 1 | 5 | I | C | Low | 1 | 6.58 | 6.81 | 4.29 | 35 | 2 | 0.0139 | 0.1648 | 33 | 25 | 116 | 2 | 11 |

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 109 | Colony 1 | 5 | 1 | C | Medium | 12 | 7.34 | 8.64 | 6.25 | 15 | 1 | 0.015 | 0.0887 | 129 | 0 | 32 | 0 | 7 |
| 110 | Colony 1 | 5 | 1 | C | High | 21 | 7.04 | 7.85 | 5.64 | 20 | 2 | 0.015 | 0.2239 | 47 | 26 | 106 | 0 | 12 |
| 111 | Colony 1 | 5 | 2 | B1 | Low | 66 | 6.49 | 5.03 | 2.84 | 56 | 3 | 0.0146 | 0.2932 | 105 | 19 | 192 | 2 | 18 |
| 112 | Colony 1 | 5 | 2 | B1 | Medium | 80 | 6.82 | 7.11 | 4.21 | 38 | 0 | * | * | * | * | * | * | * |
| 113 | Colony 1 | 5 | 2 | B1 | High | 90 | 6.90 | 6.60 | 4.47 | 35 | 2 | 0.0136 | 0.272 | 170 | 34 | 177 | 2 | 16 |
| 114 | Colony 1 | 5 | 2 | W1 | Low | 99 | 6.91 | 7.98 | 3.99 | 42 | 0 | 0.015 | 0.1174 | 8 | 11 | 69 | 1 | 5 |
| 115 | Colony 1 | 5 | 2 | W1 | Medium | 107 | 6.14 | 6.71 | 3.16 | 49 | 0 | * | * | * | * | * | * | 8 |
| 116 | Colony 1 | 5 | 2 | W1 | High | 118 | 5.39 | 6.47 | 2.70 | 50 | 0 | * | * | 0 | 0 | 0 | 0 | 1 |
| 117 | Colony 1 | 5 | 2 | SC | Low | 40 | 6.58 | 6.99 | 3.83 | 42 | 0 | 0.0156 | 0.1073 | 0 | 11 | 50 | 0 | 4 |
| 118 | Colony 1 | 5 | 2 | SC | Medium | 47 | 7.07 | 6.39 | 3.59 | 49 | 3 | 0.0142 | 0.1847 | 89 | 53 | 130 | 3 | 7 |
| 119 | Colony 1 | 5 | 2 | SC | High | 59 | 6.53 | * | * | * | * | * | * | * | * | * | * | * |
| 120 | Colony 1 | 5 | 2 | C | Low | 10 | 6.60 | 8.18 | 5.14 | 22 | 1 | 0.0145 | 0.1035 | 38 | 25 | 51 | 0 | 5 |
| 121 | Colony 1 | 5 | 2 | C | Medium | 20 | 7.47 | 8.94 | 6.61 | 12 | 1 | 0.0148 | 0.1921 | 124 | 24 | 115 | 1 | 8 |
| 122 | Colony 1 | 5 | 2 | C | High | 30 | 6.80 | 8.91 | 5.65 | 17 | 2 | 0.0143 | 0.1093 | 64 | 14 | 51 | 1 | 4 |
| 123 | Colony 2 | 1 | 1 | B1 | Low | 183 | 7.29 | * | * | * | * | * | * | * | * | * | * | * |
| 124 | Colony 2 | 1 | 1 | B1 | Medium | 192 | 6.94 | * | * | * | * | * | * | * | * | * | * | * |
| 125 | Colony 2 | 1 | 1 | B1 | High | 202 | 6.72 | 5.95 | 3.35 | 50 | 2 | 0.0138 | 0.1921 | 78 | 23 | 133 | 4 | 15 |
| 126 | Colony 2 | 1 | 1 | W1 | Low | 215 | 6.77 | 6.99 | 3.96 | 42 | * | 0.0113 | 0.1089 | 50 | 14 | 76 | 2 | 9 |
| 127 | Colony 2 | 1 | 1 | W1 | Medium | 221 | 5.70 | 5.85 | 2.81 | 51 | 0 | * | * | * | * | * | * | * |
| 128 | Colony 2 | 1 | 1 | W1 | High | 233 | 5.20 | 4.94 | 2.33 | 55 | 0 | 0.0068 | 0.0794 | 0 | 0 | 63 | 0 | 7 |
| 129 | Colony 2 | 1 | 1 | SC | Low | 152 | 6.95 | 6.83 | 4.01 | 42 | 2 | 0.0128 | 0.159 | 126 | 29 | 143 | 1 | 12 |
| 130 | Colony 2 | 1 | 1 | SC | Medium | 164 | 6.94 | 6.54 | 3.68 | 47 | 2 | 0.0136 | 0.1736 | 206 | 15 | 120 | 1 | 16 |
| 131 | Colony 2 | 1 | 1 | SC | High | 172 | 6.71 | * | * | * | * | * | * | * | * | * | * | * |
| 132 | Colony 2 | 1 | 1 | C | Low | 124 | 6.94 | 7.74 | 4.72 | 32 | 2 | 0.0123 | 0.1059 | 55 | 12 | 65 | 2 | 8 |
| 133 | Colony 2 | 1 | 1 | C | Medium | 135 | 6.92 | 7.11 | 4.16 | 40 | 2 | 0.0132 | 0.16 | 85 | 20 | 118 | 0 | 12 |
| 134 | Colony 2 | 1 | 1 | C | High | 142 | 6.51 | 6.72 | 4.12 | 37 | 2 | 0.012 | 0.1402 | 56 | 2 | 103 | 0 | 10 |
| 135 | Colony 2 | 1 | 2 | B1 | Low | 187 | 7.42 | * | * | * | * | * | * | * | * | * | * | * |
| 136 | Colony 2 | 1 | 2 | B1 | Medium | 196 | 6.58 | 5.82 | 3.27 | 50 | 2 | 0.0075 | 0.155 | 43 | 2 | 85 | 1 | 9 |
| 137 | Colony 2 | 1 | 2 | B1 | High | 207 | 7.15 | 6.95 | 4.03 | 44 | 2 | 0.014 | 0.2148 | 144 | 25 | 100 | 1 | 17 |
| 138 | Colony 2 | 1 | 2 | W1 | Low | 218 | 7.18 | 7.63 | 4.18 | 42 | 0 | 0.012 | 0.1001 | 51 | 3 | 83 | 0 | 8 |
| 139 | Colony 2 | 1 | 2 | W1 | Medium | 229 | 5.48 | 6.12 | 2.94 | 46 | 0 | * | * | * | * | * | * | * |
| 140 | Colony 2 | 1 | 2 | W1 | High | 240 | 5.92 | 5.36 | 2.75 | 54 | 0 | 0.013 | 0.1029 | 27 | 7 | 76 | 0 | 9 |
| 141 | Colony 2 | 1 | 2 | SC | Low | 156 | 7.15 | 6.54 | 3.22 | 55 | 2 | 0.0127 | 0.175 | 66 | 14 | 122 | 2 | 14 |
| 142 | Colony 2 | 1 | 2 | SC | Medium | 168 | 6.62 | * | * | * | * | * | * | * | * | * | * | * |
| 143 | Colony 2 | 1 | 2 | SC | High | 178 | 6.55 | * | 3.34 | 49 | 1 | * | * | * | * | * | * | * |
| 144 | Colony 2 | 1 | 2 | C | Low | 128 | 6.20 | 7.34 | 4.43 | 29 | 1 | 0.0131 | 0.1457 | 47 | 9 | 115 | 1 | 9 |
| 145 | Colony 2 | 1 | 2 | C | Medium | 136 | 6.98 | 7.50 | 4.36 | 38 | 1 | 0.0138 | 0.1693 | 21 | 14 | 107 | 3 | 9 |
| 146 | Colony 2 | 1 | 2 | C | High | 149 | 7.16 | 7.17 | 4.34 | 39 | 2 | 0.013 | 0.1782 | 122 | 30 | 139 | 0 | 12 |
| 147 | Colony 2 | 2 | 1 | B1 | Low | 181 | 6.94 | 6.11 | 3.31 | 52 | 3 | 0.0133 | 0.2136 | 140 | 21 | 189 | 2 | 17 |
| 148 | Colony 2 | 2 | 1 | B1 | Medium | 195 | 6.93 | 6.96 | 3.45 | 50 | 3 | 0.0136 | 0.1276 | 74 | 23 | 100 | 2 | 7 |
| 149 | Colony 2 | 2 | 1 | B1 | High | 201 | 6.56 | 5.63 | 3.31 | 50 | 3 | 0.0136 | 0.2257 | 87 | 18 | 152 | 0 | 18 |
| 150 | Colony 2 | 2 | 1 | W1 | Low | 214 | 6.32 | 6.34 | 3.52 | 44 | 0 | 0.013 | 0.1332 | 47 | 8 | 85 | 1 | 11 |
| 151 | Colony 2 | 2 | 1 | W1 | Medium | 225 | 6.05 | 5.84 | 2.79 | 54 | 0 | 0.0123 | 0.0005 | 0 | 0 | 3 | 0 | 0 |
| 152 | Colony 2 | 2 | 1 | W1 | High | 234 | 5.23 | 6.00 | 2.75 | 47 | 0 | * | * | * | * | * | * | * |
| 153 | Colony 2 | 2 | 1 | SC | Low | 154 | 6.99 | 7.85 | 4.12 | 41 | 0 | * | * | * | * | * | * | * |
| 154 | Colony 2 | 2 | 1 | SC | Medium | 162 | 6.19 | * | * | * | * | * | * | * | * | * | * | * |
| 155 | Colony 2 | 2 | 1 | SC | High | 171 | 7.44 | 7.60 | 4.34 | 42 | 2 | 0.0138 | 0.1494 | 89 | 9 | 111 | 3 | 11 |
| 156 | Colony 2 | 2 | 1 | C | Low | 122 | 6.90 | 6.63 | 3.90 | 43 | 2 | 0.0137 | 0.1824 | 108 | 7 | 135 | 2 | 15 |
| 157 | Colony 2 | 2 | 1 | C | Medium | 133 | 7.17 | 7.48 | 4.38 | 39 | 1 | 0.0129 | 0.1922 | 51 | 11 | 134 | 3 | 13 |
| 158 | Colony 2 | 2 | 1 | C | High | 144 | 6.68 | 7.60 | 3.88 | 42 | 0 | * | * | * | * | * | * | * |
| 159 | Colony 2 | 2 | 2 | B1 | Low | 186 | 6.71 | 6.03 | 3.29 | 51 | 3 | 0.0126 | 0.2021 | 101 | 19 | 141 | 0 | 15 |
| 160 | Colony 2 | 2 | 2 | B1 | Medium | 200 | 6.65 | 6.33 | 3.14 | 53 | 3 | 0.0155 | 0.2114 | 63 | 5 | 86 | 0 | 11 |
| 161 | Colony 2 | 2 | 2 | B1 | High | 210 | 7.21 | * | * | * | * | * | * | * | * | * | * | * |
| 162 | Colony 2 | 2 | 2 | W1 | Low | 219 | 6.42 | 6.72 | 3.43 | 47 | 0 | 0.0134 | 0.1457 | 36 | 4 | 65 | 2 | 7 |

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 163 | Colony 2 | 2 | 2 | W1 | Medium | 230 | 5.64 | 5.88 | 2.55 | 55 | 0 | * | * | * | * | * | * | * |
| 164 | Colony 2 | 2 | 2 | W1 | High | 238 | 5.74 | 5.83 | 2.89 | 50 | 0 | * | * | * | * | * | * | * |
| 165 | Colony 2 | 2 | 2 | SC | Low | 159 | 6.72 | 5.60 | 3.49 | 48 | 3 | 0.0136 | 0.2736 | 103 | 19 | 202 | 1 | 121 |
| 166 | Colony 2 | 2 | 2 | SC | Medium | 170 | 6.57 | 5.88 | 3.08 | 53 | 3 | 0.0125 | 0.1343 | 115 | 18 | 85 | 1 | 13 |
| 167 | Colony 2 | 2 | 2 | SC | High | 179 | 7.04 | * | * | * | * | * | * | * | * | * | * | * |
| 168 | Colony 2 | 2 | 2 | C | Low | 126 | 7.28 | 7.88 | 4.62 | 37 | 1 | 0.0125 | 0.1803 | 95 | 18 | 111 | 4 | 10 |
| 169 | Colony 2 | 2 | 2 | C | Medium | 139 | 7.23 | 8.13 | 5.59 | 23 | 1 | 0.0134 | 0.1457 | 91 | 17 | 97 | 0 | 12 |
| 170 | Colony 2 | 2 | 2 | C | High | 146 | 6.88 | 7.00 | 3.91 | 43 | 2 | 0.0135 | 0.1639 | 100 | 4 | 126 | 1 | 11 |
| 171 | Colony 2 | 3 | 1 | B1 | Low | 185 | 6.59 | 6.84 | 3.80 | 42 | 2 | 0.0121 | 0.0991 | 31 | 23 | 49 | 0 | 7 |
| 172 | Colony 2 | 3 | 1 | B1 | Medium | 191 | 6.65 | 5.06 | 2.66 | 60 | 3 | 0.0144 | 0.2545 | 189 | 21 | 212 | 1 | 23 |
| 173 | Colony 2 | 3 | 1 | B1 | High | 204 | 6.80 | 6.45 | 3.12 | 54 | 2 | 0.0139 | 0.0093 | 0 | 0 | 5 | 0 | 2 |
| 174 | Colony 2 | 3 | 1 | W1 | Low | 212 | 6.46 | 6.16 | 3.21 | 50 | 0 | 0.0124 | 0.137 | 44 | 8 | 101 | 0 | 12 |
| 175 | Colony 2 | 3 | 1 | W1 | Medium | 224 | 5.59 | 6.27 | 2.77 | 50 | 0 | * | * | * | * | * | * | * |
| 176 | Colony 2 | 3 | 1 | W1 | High | 231 | 5.80 | 5.84 | 3.40 | 41 | 0 | 0.0136 | 0.0951 | 12 | 3 | 55 | 0 | 5 |
| 177 | Colony 2 | 3 | 1 | SC | Low | 153 | 6.90 | 6.36 | 4.04 | 41 | 2 | 0.0143 | 0.0531 | 21 | 11 | 31 | 0 | 5 |
| 178 | Colony 2 | 3 | 1 | SC | Medium | 165 | 6.38 | 5.03 | 2.99 | 53 | 3 | 0.0123 | 0.116 | 28 | 15 | 82 | 1 | 6 |
| 179 | Colony 2 | 3 | 1 | SC | High | 174 | 6.78 | 6.77 | 4.21 | 38 | 2 | 0.0128 | 0.1539 | 31 | 42 | 110 | 2 | 7 |
| 180 | Colony 2 | 3 | 1 | C | Low | 125 | 6.96 | 6.70 | 4.04 | 42 | 2 | 0.012 | 0.1083 | 40 | 8 | 91 | 0 | 9 |
| 181 | Colony 2 | 3 | 1 | C | Medium | 132 | 6.95 | 6.83 | 4.03 | 42 | 0 | * | * | * | * | * | * | * |
| 182 | Colony 2 | 3 | 1 | C | High | 141 | 6.78 | 6.85 | 4.66 | 31 | 1 | 0.0124 | 0.1341 | 81 | 12 | 100 | 1 | 8 |
| 183 | Colony 2 | 3 | 2 | B1 | Low | 190 | 7.29 | 8.07 | 5.18 | 29 | 1 | 0.0136 | 0.1438 | 53 | 17 | 71 | 2 | 7 |
| 184 | Colony 2 | 3 | 2 | B1 | Medium | 199 | 6.49 | * | * | * | * | * | * | * | * | * | * | * |
| 185 | Colony 2 | 3 | 2 | B1 | High | 209 | 7.03 | 6.28 | 3.92 | 44 | 2 | 0.0144 | 0.1 | 31 | 3 | 56 | 0 | 7 |
| 186 | Colony 2 | 3 | 2 | W1 | Low | 217 | 6.37 | 6.51 | 3.46 | 46 | 0 | * | * | 24 | 6 | 85 | 2 | 8 |
| 187 | Colony 2 | 3 | 2 | W1 | Medium | 228 | 5.60 | 5.80 | 2.82 | 50 | 0 | 0.0136 | 0.0088 | 0 | 0 | 5 | 1 | 0 |
| 188 | Colony 2 | 3 | 2 | W1 | High | 239 | 5.79 | 6.33 | 3.12 | 46 | * | 0.0128 | 0.097 | 34 | 5 | 67 | 0 | 5 |
| 189 | Colony 2 | 3 | 2 | SC | Low | 160 | 6.94 | 6.56 | 3.35 | 52 | 3 | 0.0147 | 0.093 | 57 | 8 | 40 | 2 | 5 |
| 190 | Colony 2 | 3 | 2 | SC | Medium | 167 | 7.05 | 6.60 | 3.73 | 47 | 2 | 0.0118 | 0.0993 | 10 | 0 | 54 | 0 | 5 |
| 191 | Colony 2 | 3 | 2 | SC | High | 180 | 6.58 | 6.93 | 3.91 | 41 | 0 | * | * | * | * | * | * | * |
| 192 | Colony 2 | 3 | 2 | C | Low | 129 | 7.16 | 7.66 | 4.69 | 34 | 2 | 0.0153 | 0.01184 | 45 | 19 | 72 | 1 | 7 |
| 193 | Colony 2 | 3 | 2 | C | Medium | 137 | 7.13 | 9.30 | 5.85 | 18 | 1 | 0.0137 | 0.0801 | 29 | 9 | 44 | 0 | 4 |
| 194 | Colony 2 | 3 | 2 | C | High | 150 | 6.87 | 8.01 | 5.41 | 21 | 1 | 0.014 | 0.0809 | 19 | 3 | 39 | 0 | 4 |
| 195 | Colony 2 | 4 | 1 | B1 | Low | 184 | 6.76 | 5.72 | 3.82 | 43 | 2 | 0.014 | 0.2851 | 205 | 52 | 250 | 2 | 24 |
| 196 | Colony 2 | 4 | 1 | B1 | Medium | 193 | 6.87 | 6.61 | 3.63 | 47 | 2 | 0.0132 | 0.1675 | 109 | 21 | 96 | 0 | 15 |
| 197 | Colony 2 | 4 | 1 | B1 | High | 205 | 7.13 | 5.69 | 3.16 | 56 | 3 | 0.0142 | 0.2325 | 152 | 15 | 201 | 0 | 20 |
| 198 | Colony 2 | 4 | 1 | W1 | Low | 213 | 6.46 | 3.82 | 3.55 | 45 | 0 | 0.006 | 0.1355 | 0 | 0 | 59 | 0 | 6 |
| 199 | Colony 2 | 4 | 1 | W1 | Medium | 222 | 5.67 | 6.05 | 2.90 | 49 | 0 | * | * | * | * | * | * | * |
| 200 | Colony 2 | 4 | 1 | W1 | High | 235 | 5.64 | 6.18 | 3.14 | 44 | 0 | 0.0139 | 0.1212 | 18 | 5 | 82 | 0 | 6 |
| 201 | Colony 2 | 4 | 1 | SC | Low | 155 | 6.50 | 5.32 | 2.80 | 57 | 3 | 0.0128 | 0.3643 | 137 | 28 | 210 | 3 | 12 |
| 202 | Colony 2 | 4 | 1 | SC | Medium | 161 | 6.90 | * | * | * | * | * | * | * | * | * | * | * |
| 203 | Colony 2 | 4 | 1 | SC | High | 173 | 6.95 | 6.39 | 3.58 | 48 | 2 | 0.0141 | 0.2673 | 233 | 36 | 221 | 3 | 17 |
| 204 | Colony 2 | 4 | 1 | C | Low | 121 | 6.92 | 7.03 | 3.97 | 43 | 2 | 0.0133 | 0.1636 | * | 18 | 115 | 1 | 13 |
| 205 | Colony 2 | 4 | 1 | C | Medium | 134 | 6.85 | 6.73 | 4.24 | 38 | 1 | 0.0128 | 0.1809 | 40 | 28 | 114 | 2 | 15 |
| 206 | Colony 2 | 4 | 1 | C | High | 143 | 6.94 | 8.38 | 5.19 | 25 | 1 | * | * | * | * | * | * | * |
| 207 | Colony 2 | 4 | 2 | B1 | Low | 188 | 7.10 | 5.95 | 3.65 | 49 | 3 | 0.0142 | 0.3112 | 180 | 50 | 258 | 2 | 21 |
| 208 | Colony 2 | 4 | 2 | B1 | Medium | 197 | 6.80 | 6.97 | 3.34 | 51 | 2 | 0.0129 | 0.1799 | 128 | 40 | 129 | 4 | 12 |
| 209 | Colony 2 | 4 | 2 | B1 | High | 206 | 7.49 | 6.00 | * | * | * | * | * | 48 | 2 | 119 | 0 | 10 |
| 210 | Colony 2 | 4 | 2 | W1 | Low | 220 | 6.57 | | 3.54 | 46 | 0 | * | * | * | * | * | * | * |
| 211 | Colony 2 | 4 | 2 | W1 | Medium | 226 | 5.86 | * | 3.00 | 49 | 0 | * | * | * | * | * | * | * |
| 212 | Colony 2 | 4 | 2 | W1 | High | 237 | 5.72 | 5.74 | 3.06 | 47 | 0 | 0.0122 | 0.1383 | * | 2 | 93 | 0 | 8 |
| 213 | Colony 2 | 4 | 2 | SC | Low | 157 | 7.01 | * | * | * | * | * | * | * | * | * | * | * |
| 214 | Colony 2 | 4 | 2 | SC | Medium | 166 | 6.71 | 7.45 | 4.05 | 40 | 2 | 0.0135 | 0.1817 | 79 | 30 | 124 | 0 | 11 |
| 215 | Colony 2 | 4 | 2 | SC | High | 176 | 7.21 | 8.12 | 4.14 | 43 | 0 | * | * | * | * | * | * | * |
| 216 | Colony 2 | 4 | 2 | C | Low | 130 | 6.78 | 7.11 | 4.12 | 39 | 1 | 0.0135 | 0.1939 | * | 17 | 125 | 2 | 14 |

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 217 | Colony 2 | 4 | 2 | C | Medium | 138 | 7.18 | 7.44 | 4.38 | 39 | 2 | 0.0134 | 0.1693 | 35 | 16 | 117 | 1 | 10 |
| 218 | Colony 2 | 4 | 2 | C | High | 147 | 7.36 | 7.79 | 4.09 | 44 | 2 | 0.0131 | 0.2164 | 108 | 29 | 130 | 1 | 13 |
| 219 | Colony 2 | 5 | 1 | B1 | Low | 182 | 6.96 | 6.91 | 4.37 | 37 | 2 | 0.0134 | 0.2021 | 107 | 6 | 121 | 0 | 12 |
| 220 | Colony 2 | 5 | 1 | B1 | Medium | 194 | 6.55 | 6.05 | 3.28 | 50 | 3 | 0.013 | 0.185 | 29 | 13 | 106 | 0 | 9 |
| 221 | Colony 2 | 5 | 1 | B1 | High | 203 | 5.94 | 6.10 | 3.48 | 41 | 2 | 0.0152 | 0.1749 | 132 | 2 | 114 | 0 | 11 |
| 222 | Colony 2 | 5 | 1 | W1 | Low | 211 | 6.71 | 6.68 | 3.77 | 44 | 0 | 0.0138 | 0.1542 | 45 | 12 | 112 | 0 | 11 |
| 223 | Colony 2 | 5 | 1 | W1 | Medium | 223 | 5.63 | 6.49 | 2.68 | 52 | 0 | 0.0125 | 0.0673 | 3 | 2 | 40 | 0 | 6 |
| 224 | Colony 2 | 5 | 1 | W1 | High | 232 | 5.19 | 5.71 | 2.85 | 45 | 0 | 0.014 | 0.094 | 4 | 0 | 46 | 0 | 4 |
| 225 | Colony 2 | 5 | 1 | SC | Low | 151 | 6.94 | 7.01 | 4.35 | 37 | 2 | 0.0138 | 0.1968 | 81 | 4 | 129 | 1 | 11 |
| 226 | Colony 2 | 5 | 1 | SC | Medium | 163 | 7.04 | 6.53 | 3.95 | 44 | 2 | 0.013 | 0.2156 | 66 | 24 | 161 | 1 | 16 |
| 227 | Colony 2 | 5 | 1 | SC | High | 175 | 4.66 | 6.42 | 3.24 | 30 | 3 | 0.0121 | 0.1579 | 27 | 11 | 115 | 0 | 11 |
| 228 | Colony 2 | 5 | 1 | C | Low | 123 | 7.08 | 7.84 | 4.58 | 35 | 2 | 0.0136 | 0.169 | 65 | 0 | 112 | 1 | 12 |
| 229 | Colony 2 | 5 | 1 | C | Medium | 131 | 6.72 | 7.79 | 4.41 | 34 | 0 | * | * | * | * | * | * | * |
| 230 | Colony 2 | 5 | 1 | C | High | 145 | 6.82 | 6.74 | 4.01 | 41 | 2 | 0.0131 | 0.1572 | 21 | 9 | 98 | 1 | 8 |
| 231 | Colony 2 | 5 | 2 | B1 | Low | 189 | 7.41 | 6.23 | 3.50 | 53 | 3 | 0.0139 | 0.2191 | 71 | 17 | 146 | 1 | 14 |
| 232 | Colony 2 | 5 | 2 | B1 | Medium | 198 | 6.74 | 7.30 | 3.34 | 50 | 3 | 0.0136 | 0.1646 | 63 | 15 | 104 | 0 | 13 |
| 233 | Colony 2 | 5 | 2 | B1 | High | 208 | 7.11 | 8.31 | 4.20 | 41 | 0 | * | * | * | * | * | * | * |
| 234 | Colony 2 | 5 | 2 | W1 | Low | 216 | 6.70 | 7.27 | 3.57 | 47 | 0 | 0.0131 | 0.137 | 40 | 13 | 89 | 0 | 8 |
| 235 | Colony 2 | 5 | 2 | W1 | Medium | 227 | 5.60 | 5.91 | 2.56 | 54 | * | * | * | * | * | * | * | * |
| 236 | Colony 2 | 5 | 2 | W1 | High | 236 | 5.46 | 4.46 | 2.20 | 60 | 0 | 0.0124 | 0.077 | 7 | 3 | 62 | 0 | 6 |
| 237 | Colony 2 | 5 | 2 | SC | Low | 158 | 6.79 | | * | * | * | * | * | * | * | * | * | * |
| 238 | Colony 2 | 5 | 2 | SC | Medium | 169 | 7.63 | 8.31 | 4.11 | 46 | 3 | 0.0121 | 0.1926 | 60 | 10 | 125 | 0 | 9 |
| 239 | Colony 2 | 5 | 2 | SC | High | 177 | 7.02 | 5.68 | 3.21 | 54 | 3 | 0.0131 | 0.2048 | 104 | 35 | 148 | 6 | 15 |
| 240 | Colony 2 | 5 | 2 | C | Low | 127 | 7.10 | | 5.15 | 27 | 2 | 0.0138 | 0.1451 | 60 | 1 | 83 | 0 | 11 |
| 241 | Colony 2 | 5 | 2 | C | Medium | 140 | 7.74 | 8.08 | 4.87 | 37 | 1 | 0.0137 | 0.1897 | 105 | 25 | 150 | 0 | 12 |
| 242 | Colony 2 | 5 | 2 | C | High | 148 | 7.49 | * | * | * | * | * | * | * | * | * | * | * |
| 243 | | | | | | | | | | | | | | | | | | |

## Appendix 5: Nitrogen-content ($NH_4^+$) of wood.

a) Method of analysis. The procedure is summarised from the auto analyser technical sheet.

Samples were placed in 75ml Pyrex digestion tubes. Five millilitres of digestion acid (details in appendix part (b)) were added to each sample after which digestion tubes were placed in a block digester. This was set to run at a low temperature (200°C) for 20 minutes. Tubes were removed and after slight cooling, approximately 5 minutes, 2 millilitres of 100% (vol.) Hydrogen peroxide were added to each of them. Tubes were returned to the digester and it was set to run at 370°C for 1 hour. The tubes were removed from the block and covered with alumunium foil and allowed to cool in a fumehood. Taking care to avoid spillage, the contents of the tubes were shaken in a Vortex shaker and simultaneously filled with water to just below the 75ml graduation mark. Corking the tubes and shaking them further ensured total mixing.

Standards were made for the analysis as indicated on index cards and run through the analyser. Only when they gave linear results were samples analysed. The standards were run again after every 40 samples.

b) Reagents of the analysis.

i) digestion acid: dissolve 600g potassium sulphate in 2 l sulphuric acid by heatng in a 5 l conical flask until clear. Cool. Store in a stoppered flask.

ii) working buffer solution: dilute stock sodium potassium tartrate (20%, 250ml), stock buffer (0.5M, 200ml) and sodium hydroxide solution ( 20%, 63ml) to 1l with distilled water. Add 1ml BRIJ 35 wetting agent. Filter before use.

- stock buffer - dissolve 134g sodium phosphate (dibasic, crystal - $Na_2HPO_4.7H_2O$) in about 800ml of distilled water. Add 40g NaOH. Dilute to 1l with distilled water.

- stock sodium potassium tartrate solution (20%) - dissolve sodium potassium tartrate (200g) in 600ml of distilled water. Dilute to 1l.

iii) sodium nitroprusside solution: weigh 150g sodium salicylate and 0.3g sodium nitroprusside. Dilute to 1l with distilled water. Add 1ml BRIJ 35.

iv) saline diluent: sodium chloride (9g) and 1ml BRIJ in 1l of distilled water.

v) sodium hypochlorite solution (made up daily): BRIJ 35 (1ml), 20ml sodium hypochlorite bleach (Marvolyn). Fill up to 300ml with distilled water.

vi) nitrogen wash: BRIJ 35( 1ml) in 1l distilled water.

vii) hydrogen peroxide: A.R. 100 vol. This reagent should be chosen with particular view to freedom from phosphorus content.