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Proceedings of the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use

September 13, 1989, Bend, Oregon



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In 1989 the Western International Forest Disease Work Conference and the Western Forest Insect Work Conference met jointly in Bend, Oregon, during the week of September 11-15, 1989. One of the 90-minute, concurrent workshops scheduled during this period was a discussion of the biology and present and future control strategies of wood-destroying organisms. This subject area has not traditionally been of concern to these two organizations, either as it might relate to protection of wood in use or to the degradation of wood in a forest environment. It was felt that many of the professionals attending the meeting would be interested in a discussion of current research in the general area of wood-destroying organisms and the future for protection of wood in service. To ensure sufficient participation by knowledgeable pathologists and entomologists, the technical coordinators organized a day-long technical session on wood-destroying organisms, which emphasized state-of-the-art and future research needs and control practices. The subjects presented included: research on wood decay by the USDA Forest Service, new developments in wood-deterioration research from the International Research Group on Wood Preservation, methods for nondestructive evaluation of infestations and infections of wood-destroying insects and decay in structures, role of termites in forest management in Australia, chemotaxonomy of termites, agonistic behavior of termites, tunneling behavior of subterranean termites, bait/toxicant strategies for control of subterranean termites, and training of the pest control industry to utilize the new technologies.

Retrieval Terms; wood biodeterioration, decay mechanisms, fungi, biosystematics, nondestructive evaluation, acoustic emission, termites, Australian forests, cuticular hydrocarbons, agonistic behavior, semiochemicals, tunneling behavior, foraging behavior, termiticides, bait toxicants.

Authors assumed full responsibility for the submission of camera-ready manuscripts.

Views expressed in each paper are those of the authors and not necessarily those of the sponsoring organizations.

Trade names and commercial enterprises mentioned are solely for information and do not imply endorsement by the sponsoring organizations.

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# Proceedings of the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use

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Michael I. Haverty and W. Wayne Wilcox, technical coordinators

### **CONTENTS**

| In Brief   | iii |
|--|-----|
| Introduction   | v   |
| Current Research on Wood Decay in the USDA Forest Service                        | 1   |
| Harold H. Burdsall, Jr.  |     |
| New Developments in Wood-Destroying Organisms from the International Research    |     |
| Group on Wood Preservation   | 4   |
| Elmer L. Schmidt   |     |
| Need for Nondestructive Evaluation (NDE) in the Detection of Decay in Structures | 7   |
| W. Wayne Wilcox  |     |
| Fermites and Forest Management in Australia                                      | 10  |
| Don McG. Ewart   |     |
| Cuticular Hydrocarbons: Species and Population-Level Discrimination in Termites  | 15  |
| Michael I. Haverty, Marion Page, Barbara L. Thorne, and Pierre Escoubas          |     |
| An Assessment of the Potential Uses of Agonistic Behaviors in Termite Control    | 24  |
| Barbara L. Thorne and Michael I. Haverty   |     |
| Factors Affecting the Tunneling Behavior of the Western Subterranean Termite,    |     |
| Reticulitermes hesperus Banks  | 28  |
| James L. Smith and Michael K. Rust   |     |
| The Potential of Using Acoustical Emission to Detect Termites Within Wood        | 34  |
| Vernard R. Lewis and Richard L. Lemaster   |     |
| Direct Colony Baiting of Termite Colonies: A Tool for Ecological Studies         | 38  |
| Don McG. Ewart   |     |
| Behavioral Ecology of Subterranean Termites and Implications for Control         | 43  |
| J. Kenneth Grace   |     |
|  |     |

### **CONTENTS**

| Baiting Techniques for Control of Coptotermes Species Within Existing      |    |
|--|----|
| Buildings in Australia   | 46 |
| J.R.J. French  |    |
| Population Suppression of Subterranean Termites by Slow-Acting Toxicants   | 51 |
| Nan-Yao Su and Rudolf H. Scheffrahn  |    |
| How Do We Advise the Pest Control Industry in the Post-Organochlorine Era? | 58 |
| J.R.J. French  |    |
| Discussion   | 63 |

### IN BRIEF..

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Retrieval Terms: wood biodeterioration, decay mechanisms, fungi, biosystematics, nondestructive evaluation, acoustic emission, termites, Australian forests, cuticular hydrocarbons, agonistic behavior, semiochemicals, tunneling behavior, foraging behavior, termiticides, bait toxicants.

The USDA Forest Service's research on decay fungi and decay caused by fungi is done mainly in two research work units at the Forest Products Laboratory in Madison, Wisconsin. One unit, the Center for Forest Mycology Research, performs biosystematic research on root-rot and products-rot fungi in the genera *Armillaria, Phellinus*, and *Phlebia*. This group also maintains the culture collection supporting research on decay fungi. A second unit, Biodeterioration Research, concentrates on studies of the basic mechanisms of wood decay.

New developments in wood-destroying organisms and in wood protection from the 20th annual meeting (May 1989 at Lappeenranta, Finland) of the International Research Group on Wood Preservation are highlighted in the areas of biological control of fungi, dry rot, decay mechanisms and product problems, new techniques, insect problems and control, and developments in wood preservatives.

Examples of the need for nondestructive evaluation and remote sensing technologies for evaluating early stages of decay are presented. The need is critical to develop methods of analyzing internal decay at the center of large wood members, and decay behind concealing coverings. Current technologies are reviewed and their inadequacies are discussed. Acoustic emission (AE) and acousto-ultrasonics appear to hold the greatest promise among existing technologies.

Acoustic emission equipment was used to detect drywood termites, *Incisitermes minor*, in ponderosa pine blocks under laboratory conditions. Using a 60 kHz transducer, AE levels were recorded for 0, 5, 10, 15, and 20 termites per block. The association of AE and varying numbers of drywood termites best fit an exponential curve. These data suggest that the detection capabilities of AE increase with increasing numbers of termites. The implications of this finding to the potential use of AE in detecting termites under field conditions are discussed.

Termites have long been regarded as major pests of Australian forests. Drawing together research on termite ecology and mammal conservation, the argument is presented that this perception is wrong: termites are a vital component of native forests and are quite necessary for the survival of many wildlife species. Hydrocarbons in the cuticle of insects are essential in protecting them from desiccation. The vast variety of hydrocarbons synthesized by insects and the apparent species-specificity of cuticular hydrocarbon mixtures make them excellent taxonomic characters for separating species within termite genera. Preliminary data suggest that cuticular hydrocarbons might be used to sort specimens and to search for morphological characters within the subterranean termite genus *Reticulitermes*. Cuticular hydrocarbon profiles enabled population-level discrimination of *Coptotermes formosanus* from different geographic locations.

The potential use of termite-termite agonism in pest control is explored and evaluated. Intra- and interspecific encounters among termites from different colonies are known to result in aggressive or avoidance behaviors in a variety of species. Recent studies suggest, however, that intraspecific confrontations only rarely evoke aggressive responses in several economically important termite genera in the United States (*Coptotermes, Reticulitermes,* and *Zootermopsis*). Thus the most promising applied use of natural agonistic behaviors among termites appears to be in research on individual- and colony-recognition systems. Artificial manipulation of recognition and response cues might facilitate novel control methodologies based on masking or interfering with natural stimuli. Directions for future research are discussed.

Laboratory studies were conducted to determine factors that affect the tunneling behavior of *Reticulitermes hesperus*. Soil particles between 0.84 and 2.36 mm in size prevented tunneling. Exposure to solid layers of calcium, magnesium, or zinc borate did not repel workers, but produced greater than 87 percent mortality within 10 days. No tunneling occurred in soils treated with registered termiticides tested at concentrations above 50 ppm. Exposure to bifenthrin and cypermethrin deposits as low as 1 ppm immobilized termite workers and prevented them from tunneling.

Termites failed to penetrate a layer of dead termites killed by earlier exposure to chlorpyrifos. Contact with termites killed with chlorpyrifos resulted in 96 percent mortality of the tunneling workers. Workers readily penetrated layers of termites freshly killed by freezing.

At 15°C, the distance tunneled by acclimated termites was significantly greater than those of unacclimated termites. At 21-32°C, the acclimation period did not affect the distance tunneled. Tunneling rates were significantly lower at 15°C than at 21, 27, or 32°C and were significantly greater at 27°C than at 21 or 32°C.

The benefits of direct colony baiting of *Coptotermes lacteus* are described. Bait substrates enclosed in polyvinyl chloride tubes were applied in direct contact with the galleries of a termite mound. Attention of researchers is drawn to the potential of this method for studying and controlling species other than the mound-building *Coptotermes lacteus*.

Studies of Reticulitermes flavipes colonies in Ontario,

Canada, indicate that these colonies contain greater foraging populations and forage over larger territories than was previously thought. Results are consistent with those obtained elsewherewith *Coptotermes formosanus* and *Heterotermes aureus*. Implications for the development of baiting techniques for termite control are discussed, and several laboratory investigations of potential bait toxicants and insecticidal dusts are reviewed. Behavioral chemicals are also potentially useful in termite control, and bioassays with tree extractives indicate that semiochemicals affecting termite orientation offer an explanation for the observed pattern of *R. flavipes* infestations in street trees in Toronto.

Baiting techniques have been developed over the years to control *Coptotermes* species, the most economically important termite group in Australia. Given the restrictions on organochlorines as termiticides in North America, research has focussed on baiting programs against *Coptotermes* as alternatives to the current control measures of drenching soil with pesticides or fumigation of entire structures. Details of the baiting proce-

dures, toxins used, and their suitability for use in eradicating *Coptotermes* infestations in existing buildings are described. Suggestions are offered for the direction in which future baiting techniques may prove productive.

Historic background and the concept of slow-acting toxicants for population suppression of subterranean termites are reviewed. Information needed for development of bait-toxicants and studies needed to generate such information are summarized.

Given the loss of organochlorines as termiticides in North America, and with the possibility of such restrictions occurring in the future in Australia and elsewhere, how are entomologists to advise the pest control industry in termite control? A brief historical review of pesticide bans and the relevance of these bans to the present termite control situation are discussed. Suggestions are offered for entomologists to pursue an integrated pest management approach to termite control based on sound ecological parameters and social priorities.

### **PREFACE**

Early in 1989, one of us (MIH) suggested to the organizing committee of the joint meeting of the Western International Forest Disease Work Conference (WIFDWC) and the Western Forest Insect Work Conference (WFIWC) that they include wood-destroying organisms on the program. They accepted the idea and wanted the workshop to be interdisciplinary and comoderated by an entomologist and a pathologist. We then began to formulate an agenda and to develop a list of likely participants. This subject area was not traditionally of concern to these two organizations, either as it might relate to protection of wood in use or to the degradation of wood in a forest environment. In addition, the likely participants on the list do not often attend either of these annual meetings.

We feared very few of the attendees would have much to contribute to this workshop because there are so few entomologists or pathologists in these organizations who conduct research on wood-destroying organisms. We did think, however, that many of the professionals attending this meeting would be interested in a discussion of current research in the general area of wood-destroying organisms and the future for protection of wood in service.

To attract scientists currently involved in research on wood-destroying organisms, we convened (by invitation) a technical session held during the joint meeting of the WIFDWC and WFIWC. We arranged an agenda of 15- to 20-minute presentations, divided equally between entomology and pathology. Unfortunately, the American Wood-Preservers' Association held their annual meeting concurrent with the joint meeting of the WIFDWC and WFIWC. Thus many of the wood pathologists who might have otherwise attended and made presentations were already committed to another meeting. As a result, the number of presentations by entomologists was much greater than those by pathologists. On the afternoon of September 14, we held a 90-minute workshop to summarize the presentations and discuss future direction of research on wood-destroying organisms.

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## Current Research on Wood Decay in the USDA Forest Service<sup>1</sup>

### Harold H. Burdsall, Jr.<sup>2</sup>

Abstract: The Forest Service's research on decay fungi and decay caused by fungi is done mainly in two research work units at the Forest Products Laboratory. One unit, the Center for Forest Mycology Research, performs biosystematic research on root-rot and products-rot fungi in the genera <u>Armillaria</u>, <u>Phellinus</u>, and <u>Phlebia</u> and maintains the culture collection supporting this research and other research on decay fungi. A second unit, Biodeterioration Research, concentrates on studying the basic mechanism of wood decay.

Most of the Forest Service's work on wood decay is being done in two work units in Forest Insect and Disease Research (FIDR) housed at the Forest Products Laboratory (FPL) in Madison, Wis. Work on wood preservation and biotechnological applications of wood decay fungi are also in progress at the FPL in Forest Products and Harvesting Research units, but the research on the decay fungi and biodeterioration is located in the FIDR units, the Center for Forest Mycology Research (CFMR) and Biodeterioration Research.

### CENTER FOR FOREST MYCOLOGY RESEARCH

The investigations of the Center for Forest Mycology Research (CFMR) (Research Work Unit FPL 4501) mainly address the biosystematics of wood decay fungi and the maintenance of the Forest Service's culture collection of wood-rotting fungi. The problems under investigation by the scientists include the biosystematics and species delimitation in the genus Armillaria (the root- and butt-rot fungus), the taxonomy of the genus Phellinus (which causes substantial root and heart rots of forest trees), and the biosystematics of the genus Phlebia, (which, although it occurs mainly as a slash-rotting organism, is also found in products). In addition, several members of the genus Phlebia are of possible use in biopulping or other biotechnological endeavors. The other problem area under investigation in the CFMR involves the resolution of species complexes that are found within the culture collection.

### Biosystematics and Species Delimitation in Armillaria

The work on the genus <u>Armillaria</u> has involved several approaches. One of the main approaches is the use of a rather

<sup>1</sup> Presented at the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1989, Bend, Oregon.

unique serological technique to distinguish species of Armillaria. Instead of being inoculated into mice, goats or rabbits, the antigen (Almillaria fungal tissue) is inoculated into both sides of the breast and both legs of laying hens. After a second inoculation a week later, and a wait of 2 weeks for incubation within the chicken, the antibodies are at sufficient titer and deposited in the yolk of the eggs. The eggs are collected, the antibodies isolated, and the ELISA technique used to distinguish species on the basis of optical density of the color reaction. Our results to date indicate that we can distinguish several of the species readily. We will be putting more species into the system to see how widely applicable this technique might be, because now the system shows a great deal of promise. Using laying hens for antibody development is advantageous because of the ease of inoculation, the ease of obtaining the antibody material through the collection of eggs, the rapid increase of titer, and the apparent greater specificity (that is, less cross reactivity) of the antibodies developed in the egg yolk vs. the blood of the other animals.

Other investigations on <u>Armillaria</u> are also in progress, including attempts to elucidate the nuclear cycle within the life cycle of the fungus. Attempts are also being made to develop a method for consistent fruiting of the various <u>Armillaria</u> species so that genetic material will be regularly available for research on the <u>Armillaria</u> species. Refinement of the mating compatibility system and development of methods for cultural identifications are both under study.

### Taxonomy of Phellinus

Research on the genus <u>Phellinus</u> has resulted in a published synopsis of the species of the genus <u>Phellinus</u> in the world. It is complete with keys and descriptions—many taken from the literature, but otherwise not available. Work is also progressing on the delimitation of <u>Phellinus</u> species especially as it concerns the variability within species. <u>Phellinus tremulae</u> (Bond.) Bond. <u>et</u> Boriss. has been collected and isolates made from all across North America, and the variability within this species is being studied. The genetic compatibility within the species and other variations, both morphological and biological, are being examined. Other species complexes in this group are being examined as well.

### Biosystematics of Phlebia

Research on the genus <u>Phlebia</u> is taking a number of different routes: morphological, genetic, and molecular-biological. The genus fruits well in culture so that single-spore isolates for incompatibility studies are easily available. Morphological studies are combined with these incompatibility studies

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and cultural studies to aid in distinguishing the species. In addition, the utility of restriction fragment length polymorphisms and gene sequencing is being appraised. This should give an idea about the reliability of this methodology/technology to the wood-rotting basidiomycetes and give support to the other types of taxonomic investigations that are proceeding.

To date, the <u>Phlebia rufa</u> complex has been examined and found to consist of two different species. This information was supported by morphological and genetic data as well. Other species of this group are now under investigation. The results will not only give us a better idea of the taxonomy and identity of the various <u>Phlebia</u> species, but will also indicate the relationships among genera which are closely related to <u>Phlebia</u>.

### Resolution of Species Complexes

We also have a program that is aimed at resolving species complexes in the culture collection. In cases in which we have numerous isolates of the same species and it is obvious that there are some misidentifications, these misidentifications are being resolved by finding the isolates that are similar in culture and then going to the fruiting body and finding the differences there as well. Two of the genera under investigation are <u>Bjerkandera</u> and <u>Phellinus</u>. A number of species that were previously put into genus <u>Poria</u> are also under investigation.

### BIODETERIORATION AND PRESERVATION OF WOOD

Research Work Unit FPL 4551, Biodeterioration of Wood, is determining basic mechanisms involved in the decay process. The purpose of this work is to find the "Achilles heel" in the decay process that could guide the development of new or improved treatment. One approach is to use serological techniques including monoclonal antibody methodology to determine how the enzymes get into the wood. These studies use monoclonal antibodies to locate and characterize the enzymes responsible for wood decay. The presence of the enzymes in the sheath surrounding the hyphae and the delivery system transporting the enzymes into the wood are being studied. In addition, a technique for visualizing the delivery system of the enzymes into the wood substrates has been developed using the Scanning Electron Microscope. Results of this work indicate that the fungus does develop a sheath as has been suggested in other work. This sheath forms microfibrils and microfibrillar materials that penetrate the woody tissues and deliver the enzymes deep into the wood. The concept of the enzymes merely being distributed at random by diffusion is now regarded as improbable. This work is still in progress but may well provide information that will explain exactly how the enzymes are delivered to the woody substrate.

Considerable progress has been made in understanding the nature of the cellulose-degrading agent produced by brown-rot fungi. The physical and chemical properties of brown-rotted cellulose have been elucidated, and electron spin resonance (ESR) has been used to identify formation of hydroxyl radical and paramagnetic metals. These results demonstrate that brown-

rot fungi depolymerize cellulose by oxidative cleavage. Because of the rapidity of the degradation, oxidative cleavage most likely takes place by formation of cellulose-metal ion complexes and reaction with oxygen radicals. Understanding the biochemistry of this system should provide tremendous opportunities to thwart decomposition of wood.

Other work is aimed at the use of innocuous fungi as biocontrol agents against mold, stain and decay fungi. Species of <u>Trichoderma</u> and <u>Scytalidium</u> are the most promising. Methods of applying the control agent and the most effective species and strains to use are still being evaluated. Field tests to date have provided mixed results.

Determining in a nondestructive manner that decay is present at an early stage involves the use of electron spin resonance. This technique is based on the idea that as decay progresses the element magnesium changes states. By using ESR the change in valence of the magnesium can be sensed, and the amount of decay can be determined at early stages. This work is still in progress.

From the standpoint of control, the regulation of key enzymes in digestion and growth is of interest. Important digestive enzymes are those decomposing cellulose, hemicellulose, and lignin. For growth, enzymes of importance are those involved in nitrogen metabolism, such as glutamine synthetase and polyamine enzymes, and those involved in cell wall formation, such as chitin synthetase.

Almost nothing is known about nitrogen metabolism in wood decay fungi, but it plays the most important role of the nonstructural nutrients in wood. The very low amounts of nitrogen in wood indicate that wood decay fungi have a very efficient mechanism for metabolizing and reusing nitrogen.

Polyamines are nitrogen compounds found in fungi that are essential for growth and development. The biosynthesis of polyamines in most organisms can proceed through one of two metabolic pathways. However, only one pathway has been reported to be the route of synthesis in fungi. This feature makes the pathway an ideal target for specific regulation of polyamine production in wood decay fungi. The result would be the specific control of decay by affecting only fungal growth and development. We are using "suicide" inhibitors such as difluoromethylomithine (DFMO) in the study of polyamine transport systems and determining reaction steps in metabolic pathways. The inhibitors are enzyme specific and irreversible, acting at the catalytic site of the enzyme, which results in "suicide." These inhibitors are ideal candidates for studies to target the "Achilles heel" of the decay fungi.

Another approach that might be utilized for specific inhibition of fungal growth and development is the use of chemicals that prevent synthesis of chitin, which is an essential component of fungal cell walls. Vertebrates do not possess chitinous tissue. This basic biological difference has stimulated interest in the development of pesticides that specifically inhibit chitin synthesis and have little or no effect on non-target organisms. We are presently studying inhibition of chitin synthetase from various fungi with chitin synthetase inhibitors.

To digest the cell wall constituents of wood, enzymes must be released external to the fungus. Thus, one method of preventing fungal attack of wood would be to inactivate the extracellular degrading enzymes in situ or prevent their release from fungal hyphae. Extracellular enzymes, however, appear quite stable—higher concentrations of chemical are often required to inactivate the enzyme than to prevent growth. A more promising approach, therefore, would be to find means to interfere with processes involved in enzyme secretion.

Tunicamyacin and calmodulin antagonists are compounds that we found to inhibit secretion of carbohydrate-degrading enzymes by wood decay fungi. Tunicamyacin interferes with glycosylation or sugar attachment to enzymes, and calmodulin antagonists interfere with calcium transport, which appears to be a part of the secretory process. We do not propose to use these materials as wood preservatives, but they may serve as model systems in the continuing search for more environmentally compatible fungicides.

Monitoring of the decay tests in both the deep South and other areas of the country is continuing, to learn about the effectiveness of various preservative treatments in preventing decay and stopping internal decay.

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# New Developments in Wood-Destroying Organisms from the International Research Group on Wood Preservation (IRG)<sup>1</sup>

### Elmer L. Schmidt<sup>2</sup>

Abstract: New developments in wood-destroying organisms and in wood protection from the 20th annual meeting (May 1989 at Lappeenranta, Finland) of the International Research Group on Wood Preservation (IRG) are highlighted in the areas of biological control of fungi, dry rot, decay mechanisms and product problems, new techniques, insect problems and control, and developments in wood preservatives.

The aim of the International Research Group on Wood Preservation (IRG) is "to promote research throughout the world on the subject of wood preservation" (Anonymous 1988). With members in 55 nations around the world, its annual meetings and the documents presented provide a stimulating forum for exchange and discussion of new ideas in research and problem-solving in the areas of wood-destroying organisms and methods of wood protection.

The IRG Secretariat is currently housed in Sweden (Drottning Kristinas vag 47C, S-114 28, Stockholm). The presentations at the annual meeting were organized into four main Working Groups with a number of Subgroups in each:

### Working Groups IA & IB--Flora and Fauna

Microbial breakdown mechanisms
The problem of pretreatment decay
The problem of dry rot
ermite behavior associated with new methods
of termite control
Insects in dry wood (excluding termites)

### Working Group II -- Fundamentals of Testing

Soft rot tests
Field tests out of ground
contact Accelerated aging
tests
Non-destructive testing for defects in wood
structures

### <u>Working Group III--Preservatives and Methods</u> of Treatment

Collaborative field experiment Health and safety aspects Remedial treatments Refractory timbers

### Working Group IV--Marine Wood Preservation

Leaching of some copper-based preservatives The use of wood in boats Sheathing materials

Given the great number of research documents (>125) discussed at the meeting, it was necessary to only skim the bulk of information for topics of interest to those attending the joint Western International Forest Disease Work Conference and Western Forest Insect Work Conference meeting. For more details and data on, the topics briefly discussed, please contact the IRG Secretariat for copies of specific documents.

### BIOLOGICAL CONTROL DEVELOPMENTS

Control of blue stain on <u>Araucaria</u> <u>cunninghamii</u> Aiton in Australia with the bacterium <u>Pseudomonas cepacia</u> (ex Burkholder) Palleroni and Holmes was excellent in the laboratory, but only 50-65 percent effective in 2-month field trials (Benko 1989). Wood from a Scots pine pole treated 7 years earlier with <u>Trichoderma</u> spp. still resisted decay by <u>Lentinus lepideus</u> Fries in soil-block tests (Bruce and others 1989).

<sup>&</sup>lt;sup>1</sup>Presented at the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1989, Bend, Oregon.

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NEW INFORMATION ON THE DRY ROT FUNGUS <u>Serpula</u> <u>lacrymans</u> (Wulfen ex Fries) Schroeter

Dry rot fungus is a major destroyer of buildings in Europe and in certain areas of Japan and Australia. Mortar or some source of calcium seems necessary for growth to neutralize the copious oxalic acid produced by this fungus by the formation of calcium oxal ate (Bech-Andersen 1989, Trong 1989). A heat treatment has been developed to eradicate the fungus from structures (40°C for 24 h), and fungus cell viability can be checked by Janus Green B stain (a method more sensitive than culture attempts) (Koch and others 1989). This fungus can remove nitrogen from the soil to enhance growth in wood (Doi and Togashi 1989). Temperature apparently restricts its distribution in Australia and its growth rate is not related to decay capacity (Thornton 1989a, b).

### MISCELLANEOUS POINTS ON WOOD DECAY

<u>Peni ophora gi gantea</u> (Fri es) Massee was found to be the most common decay fungus in Scots pine logs during air seasoning in the United Kingdom, and may well be so because of its use as a biological control agent against <u>Heterobasidion</u> <u>annosum</u> (Fries) Brefeld in the region of tree harvest (Zahora and Dickinson 1989). Soft rot (not detectable by hammer sounding) appears not to be a problem in CCAtreated pine poles in Sweden where earlier treatments lacking copper failed in service (Friis-Hansen and Ludstrom 1989). Interesting work on the role of extracellular matrix in decay was presented by workers from the USDA Forest Products Laboratory in Madison, Wisconsin (Green and others 1989; Micales and others 1989), and work from Sweden has shown localization of lignin degrading enzymes in wood (Daniel and others 1989). Work from South Africa has demonstrated that strictly anerobic fungi (from ruminates) can colonize and may degrade wood (Wiederhold and others 1989). New methods for detection of organisms include use of fluorescein diacetate (FDA) hydrolysis to measure mold growth on wood indirectly (Bjurman 1989), and ion mobility spectrometry (IMS) to detect bacterially infested red oak in the field (Lawrence and others 1989).

### **INSECTS**

A review of toxicants used around the world to control termites was presented (French and LaFage 1989), and an excellent review on nonchemical termite barriers noted promise for small particle applications (French 1989). Chlordane residues in soil around homes in Louisiana reflect the fact that 14 percent of the treatment sites may be potentially susceptible to the Formosan

termite (LaFage and Delaplane 1989). Early promise was demonstrated in the use of acoustic emissions to detect termites feeding in wood (Fujii and others 1989). Problems with weevils (Curculionidae) and death watch beetles (Anobiidae) in historical/heritage buildings were noted in Spain and Finland (Trobajo 1989, Viitanen and Pulkkinen 1989).

### WOOD PRESERVATIVE DEVELOPMENTS

New generation organic formulations for mold and stain control were compared in a study from the United Kingdom (Williams and Lewis 1989). Acetylated wood has now found commercial application in Japan (Takahashi and others 1989). Proprionic acid is used to protect wood chips for export from South Africa (Ismail and others 1989). Other problems discussed included mention of the surface hardness resulting from CCA treatment of pine poles (Jonnsson and others 1989), increasing interest in the use of borates for decay protection and remedial treatment (Barnes and others 1989; Henningsson and others 1989), surface enhancement of wood with metal tannates (Plackett and Cronshaw 1989), and performance of pressure-treated spruce in field trials (Hosli and Doyle 1989).

Again, many other topics of interest were presented at the IRG meeting in Finland in 1989, but were not discussed at this meeting due to time constraints of the presentation. Interested persons should examine the Annual Proceedings of the American Wood Preservers' Association (AWPA) which lists titles of IRG papers, or contact the IRG Secretariat.

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# Need for Nondestructive Evaluation (NDE) in the Detection of Decay in Structures<sup>1</sup>

### W. Wayne Wilcox<sup>2</sup>

Abstract: Examples of the need for nondestructive and remote sensing technologies for evaluating early stages of decay are presented. The need is critical to develop methods of analyzing internal decay, at the center of large wood members, and decay behind concealing coverings. Current technologies are reviewed and their inadequacies discussed. Acoustic emission and acoustoultrasonics appear to hold the greatest promise among existing technologies.

Sometimes, nondestructive evaluation (NDE) is not needed to determine that a potentially serious decay problem exists in a building. If the plywood siding, a hand railing, or a major glulam beam sprouts mushrooms (fig. 1), for example, you can be pretty sure you have a decay problem on your hands. Unfortunately, those are rare occurrences. White mycelium growing on the surface of wooden structural members in moist areas (fig. 2) is also a pretty good clue that you should be concerned about the structural integrity of the member involved, but that, too, occurs relatively infrequently in structures. Once the structure is opened and advanced

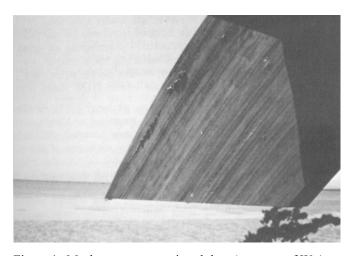


Figure 1--Mushrooms on exterior glulam (courtesy of W.A. Dost).

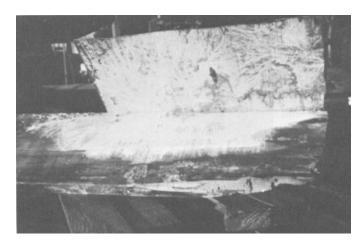


Figure 2--Mycelial mat on joist and underside of plywood subfloor.

decay is revealed, little additional information is necessary to assess the structural impact of the decay in many cases. But, in the majority of cases of wooden structures with water problems, visual indicators are not present and some form of internal assessment must be incorporated.

Two broad categories of internal decay are encountered in buildings. One is where wood is exposed to the weather and is sufficiently open that the surfaces of the wood members remain dry enough that any decay occurs inside the member without telegraphing to the surface (i.e., internal decay in exposed members). The second circumstance is where wooden structural components are contained behind surface finishes which effectively conceal any evidence of the decay process.

Here are some examples of both such circumstances.

An A-frame structure supported on glulam beams which extend beyond the perimeter walls and roof-line allowed the glulams to be subjected to internal decay (fig. 3). No external evidence of decay was apparent, but probing revealed sufficient softness to warrant concern for the structural integrity of the members. A combination of increment boring and ultrasonic pulse timing provided sufficient evidence of advanced decay to convince the engineer that reinforcement was required (fig. 4).

Elsewhere, the entire Indian Valley College campus was temporarily condemned while exposed glulam beams were investigated and a structural solution was

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Figure 3--Glulam and connections exposed to the weather.

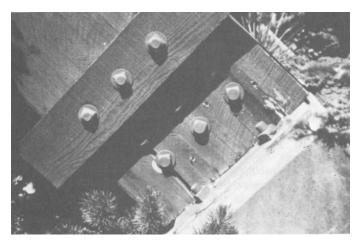


Figure 4--Internal decay was found by boring and ultrasonic pulse timing.

developed. This design proved particularly difficult, since glulams played such an important role in all structural aspects of the building, from lateral and vertical support to floor and roof support, blocking and decorative trim. However, even glulams used as nonstructural elements for their esthetic appeal can constitute life hazard if they fail. Decay initiated in exposed parts of large members may extend past the perimeter wall, through load-bearing areas and into the interior of the structure. Although evaluation of internal decay in exposed members currently presents significant challenges, at least it is possible; evaluation of decay which extends into the structure is often impossible without major demolition. These are two different challenges for NDE.

For some time, there was a significant tendency on the part of designers to expose glulams to the weather on the outside of the building for esthetic reasons. We now know that this is not a good idea. Once the major check system develops in the top of a glulam in exterior exposure, it appears to be doomed. Even solid timbers exposed to the weather, with numerous contact points and bolt holes (fig. 5), present a significant hazard, because water is trapped long enough to be absorbed.

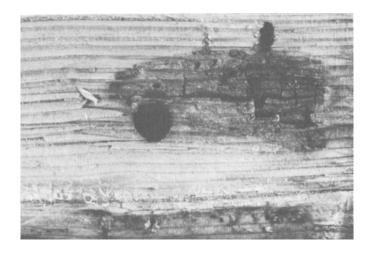


Figure 5--Solid timber with internal decay around a bolt hole.

In both of these cases (exposed glulams, and timbers with exposed joints and fastenings) it might be justifiable to just call for removal in any structure over six years old, and you would not be wrong too much of the time. However, because you would end up removing some perfectly sound wood if you operated that way, and also because the people paying for such treatment usually demand fairly firm evidence of a problem before being willing to expend the large sums of money involved, some sort of internal property evaluation is necessary. As far as destructive sampling goes, if it turns out to be decayed, it does not matter that you have put a rather large hole in it. However, if it turns out to be sound, then it would have been better to have had a nondestructive method of analyzing the interior of the member. Sometimes the life hazard involved in the failure of an individual wood structural member is so great that a means of continually monitoring its strength, as part of preventive maintenance, would be an extremely valuable tool. This is another role which NDE could play.

In all the previous examples, at least portions of the wood structural members were exposed to view. However, often, structural members are covered with a material which effectively conceals any evidence of the existence of a decay problem. Stucco probably is the most troublesome, and common, example of such covering. In one case, glulam beams and solid wood columns were encased in stucco and, in the corners, were affixed with planter boxes containing their own irrigation system. Unfortunately, this system irrigated both the soil and the supporting wood structure, causing decay (fig. 6) which was not at all evident behind the stucco (fig. 7). Although the prior example was inside a structure, stucco is most commonly used as an exterior finish. Both inside and out, stucco is just as concealing and, at the moment, requires actual demolition in order to assess the condition of wood beneath it. Remote, nondestructive assessment is truly needed where stucco covers structural wood.

An elastomeric compound, which mimics stucco, failed miserably when applied directly to plywood siding. Because it acted as a membrane, the cracks which developed let water in but the uncracked portions prevented it from getting back out. Therefore, it was both part of the decay problem and the concealment of the problem.

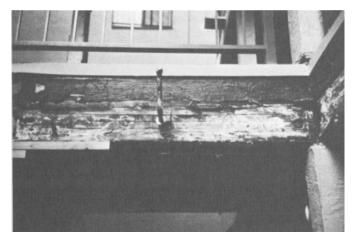


Figure 6--Decayed beam which was behind stucco as in Fig. 7.

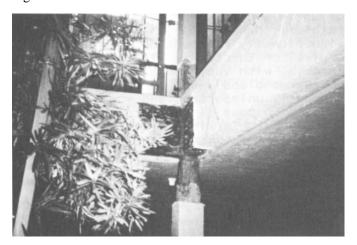


Figure 7--Stucco wall showing no external evidence of decay behind it.

At the time of this writing, the most reliable field test available for diagnosing decay is the "pick test." Unfortunately, it does not begin to become reliable until the equivalent of approximately 10-15 percent weight loss, at which point a significant amount of wood strength has already been lost. Also, it is effective only when decay is present on the surface, which is frequently not the case with major wood structural members in exposed service. An effective way of examining the inside of a large member is to use an increment borer. This instrument produces a core of actual wood from the interior of the member, which can be either visually examined or microscopically evaluated. Microscopical examination can effectively diagnose decay in its earliest stages, before significant loss in strength. However, it is an arduous, time-consuming and highly specialized technique which cannot be applied to a large number of wood members, and still leaves behind a hole.

Probably the most commonly used device for detecting decay in the field is the screwdriver, or some other type of penetrating probe. Probing allows evaluation of both the hardness of the wood (a strength property not particularly sensitive to early stages of decay), and its toughness (which is very sensitive to early stages of decay) through application of the "pick test." When a building is undergoing evaluation for the presence of decay in structural members the screwdriver is likely to

be present. However, information gained from probing may be misleading.

Properly evaluated and maintained, wooden buildings are capable of providing long service. Around 250 years is about the best that we can show in this country. But elsewhere, over 800 years can be demonstrated.

The big problem is that most of the strength of wood is lost in such an early stage of decay that we have no current field-applicable technology capable of evaluating it with reliability. Professional judgment and experience may be our most effective current means of evaluating decay in structures. Actually, the intuitive expertise we've developed, out of necessity, may be an impediment to the development of electronic technology. A fairly recent survey by Barry Goodell and Oregon State University (Goodell and Graham, 1983) found that a majority of utility pole inspectors are confident that they can detect early stages of decay by hammer soundings!

Moisture meters are used in the field, and can indicate whether wood is in a moisture condition capable of supporting decay. Units with long insulated pins are capable of giving such information, even from the inside of a member. This technique, however, does not allow evaluation of strength loss. A sonic device has been in use, by utilities, on poles for many years and appears to be effective in locating advanced decay and voids. However, there is no information to suggest that it can detect early stages of decay. Experiments have shown that ultrasonic pulse transit time measurement is capable of detecting early stages of decay in some circumstances, but not others (Wilcox, 1988). Acoustic emission under compression appears to be extremely sensitive to early stages of decay, but is not nondestructive (Beall and Wilcox, 1987). Acousto-ultrasonics appears to be able to detect early stages of decay under nondestructive application, but has not yet been adequately researched to know for sure. (The primary difference between acoustic emission and acousto-ultrasonics is that acoustic emission testing involves analysis of sound being generated within a material being stressed, whereas acousto-ultrasonics involves analysis of sound which has traveled through the material but is induced by a source external to the material being tested.) Resonant vibration and electrical resistance methods may bear promise for nondestructive evaluation of wood structural members, but are just beginning to be researched for this application.

There is a need for more sensitive tools for inspecting wood structural members for damage zones and wood decay. Significant efforts are in progress to apply emerging technologies to the evaluation and inspection of wood members. These tools are being developed considering the need to correlate NDE measurements with member strength and stiffness. Whether such technology can be developed for non-destructive application in structures remains to be seen.

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### Termites and Forest Management in Australia<sup>1</sup>

### Don MG. Ewart<sup>2</sup>

Abstract: Termites have long been regarded as major pests of Australian forests. Drawing together research on termite ecology and mammal conservation, this paper argues that this perception is wrong; termites are a vital component of native forests.

Australian forests are dominated by eucalypts. More so than on any comparable landmass, Australian forests are characterized by members of this one genus comprising more than 450 species<sup>3</sup>. Eucalypts first appeared 35 million years ago, and are believed to have evolved on the margins of the then extensive rainforests. With climatic change and the retreat of rainforests in the late Tertiary, distinct seasons arose and the great radiation of the eucalypts began as adaptation to varying water supply became favored (White 1986).

The second major genus of Australian forest trees is <u>Acacia</u>, the wattles. Although Australia has more than 600 species of <u>Acacia</u>, they rarely dominate forests, and usually form an understory below a eucalypt canopy. Wattles are relatively resistant to termites, and standing wattles are mostly degraded by beetles. The third major genus, <u>Callitris</u>, is the only mainland

<sup>1</sup>Presented at the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1989, Bend, Oregon.

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<sup>3</sup>Al though <u>Eucalyptus</u> is still a single genus, revision of the genus was first suggested by Carr and Carr (1959). Pryor and Johnson (1971) recognized four subgenera which in a later (1981) paper they regarded as being equivalent to genera, but did not promote at that time. It is probably only a matter of time before the group is revised into at least four genera (Florence 1981).

gymnosperm to form extensive forest and occurs widely in semi-arid regions of eastern Australia. <u>Callitris</u> is particularly resistant to degradation by termites<sup>4</sup>.

Australia has very little forest. In a land mass of 760 million hectares, only 42.5 million hectares, less than six percent, are forested. These forests occur in a coastal belt, limited by rainfall (Senate 1977), and have been significantly fragmented since the arrival of Europeans. Inland Australia is arid, with vast areas of extremely sparse woodland where herbivory is generally unimportant and much of plant production goes directly to the detrivores. Termites are thus very important and these inland regions may be thought of as lightly wooded deserts with a fauna dominated by termites, ants and lizards. Recent studies (Tongway and others 1989) point to termites as promoters of small-scale fertile patches which are refuges for many desert plant speci es.

LOSS OF TIMBER TO TERMITES--HISTORIC PERSPECTIVES

The earliest records of termite damage to timber in Australia date from the late nineteenth century and report the impact of indigenous termites on introduced horticultural tree crops and vines (French 1893). At this time most of the timber produced by forest clearing was simply burnt, and it was not until the native forests were commercially harvested on a large scale that the activities of termites received attention.

Thirty years ago--even five years ago-- I would have reported that termites are major pests in Australian forests, that they consume large quantities of merchantable timber and that the intensity of their attack is often much greater than in other bioregions (Carne

<sup>&</sup>lt;sup>4</sup>For tabulations of the durability of Australian timbers, see Johnson and others (1986).

and Taylor 1978, Greaves and others 1967, Greaves 1962b, Harris 1966, Perry and others 1985, Ratcliffe and others 1952).

The first researchers calculated the "cost" of termites in terms of the volume of timber lost" by surveying the extent of degraded timber that contained evidence of termite activity. Studies showed that in both highland and lowland forests, termites "reduced" timber yield by 25 percent or more (Greaves and others 1965, 1967). This indicated loss in potential profitability lead to novel control proposals, including the treating of individual trees with arsenic trioxide or organochlorines applied by injection, the removal of all mature trees that might potentially harbour termites and the replacement of susceptible native forest with plantations of Pinus spp. (Greaves 1959, Greaves and others 1967). However, with the exception of conversion to pines, none of these termite control measures have ever been economically feasi bl e.

In the main, only five termite species are responsible for almost all of the attack in commercial Australian forests. The primitive Giant Northern Termite (Mastotermes darwiniensis)
Froggatt) (Mastotermitidae) is a major impediment to tropical forestry, the dampwood termite (Porotermes adamsoni [Froggatt]) (Porotermitidae) occurs in cool or mountainous forests along the eastern seaboard, but by far the most important are of the genus Coptotermes Wasmann (Rhinotermitidae):
C. acinaciformis Froggatt (C.a. raffrayi in Western Australia), C. frenchi Hill and C. lacteus (Froggatt).

Species of <u>Coptotermes</u> are very adaptable; they may nest in the ground, in mounds of their own construction, or in trees. Their distribution in Australia is almost as wide as the eucalypts they eat: desert, woodland, cool temperate forest and rainforest (Calaby and Gay 1959).

### ATTACK ON TREES

Termites prefer to consume decayed wood (Wood 1978, French and others 1981, 1987) and there is evidence that they are healthier if they do (Hendee 1935, Lenz and others 1980). Attack on trees is more likely to occur when the tree is wounded (Greaves and others 1965). Colony formation by founding-pairs is through scar tissue, typically reported to be fire scars at the base of trees

(Greaves and others 1965, Perry and others 1985), but entry through less easily observed routes such as branch stubs, damaged roots and the exit holes of beetles and moths is also probable. Attack is also initiated when termites extend galleries in the soil and enter other trees, typically from galleries running on the underside of roots and entering the heartwood at the root crown, a habit common in Coptotermes. This method of attack may occur over distances of 50 meters with a single colony feeding on more than 15 trees (Greaves 1962a) and is eased by the high incidence of natural root grafting among eucalypts (Ashton 1975).

Coptotermes build a central nest, at the base of the tree, with the nursery being below ground level in cool climates and elevated in warmer areas. The termites consume the heartwood, as its natural anti-feedant chemicals degrade through decay organisms and natural ageing. <u>Porotermes</u> consume the timber in a similar manner, but do not maintain a central nest, rather, they work through the tree as a travelling population. Thus the main "pest" species are consuming dead and decayed heartwood and decayed areas of timber which are of negligible commercial value. Rather than being primary agents of timber degradation, their presence is secondary: an effect rather than a cause. Timber is not a limiting resource for the termites, but appropriately degraded timber is; termites select their food for the presence of appropriate decay.

Shigo's model of compartmentalization of decay in trees (CODIT) (Shigo 1984) is widely ignored by Australians (see, for example, Perry and others 1985), but the patterns of termite activity in eucalypt timber closely follow Shigo's predicted areas of response. His model is particularly useful in understanding the coevolutionary balance between termites and trees: although termites feed within the tree, the tree is able to limit their activity and protect its structural integrity, by compartmentalizing scar tissue. It does a tree no harm to be hollowed and may even provide some structural advantage (Dickinson and Tanner 1978, Janzen 1976). The Longevity of termite infested eucalypts show that this balance between growth and consumption may be held for more than two centuries, with the process of hollowing extending into all the main branches.

Early growth of eucalypts concentrates on the attainment of height.

Crown growth dominates mid-life, with maturity occurring when the crown reaches the limits of dimensional stability. The term "overmature" is used to describe trees where the growth of the crown is balanced by more or less continual branch shedding. Most young trees do not suffer termite infestation until about 70 cm dbhob<sup>5</sup> although the risk is greater where injuries have occurred (Carne and Taylor 1978, Elliott and Bashford 1984, Mackowski 1984).

Fire is considered to be the major cause of such injury (Elliott and Bashford 1984, Perry and others 1985) particularly when prescribed burning is involved. Even where eucalypts are planted as exotics, termite attack is secondary to some other stress-causing factor (Chatterjee and Singh 1967). Any significant breach of the tree's defences will result in the formation attractive scar tissue. Thus today, a tree's chances of early termite infestation are increased by roading and logging machinery, which cause mechanical damage to the cambium at the base of trees and also, by logging and silvicultural thinning due to collisions with falling trees.

However, my observations in the Boola Boola State Forest in eastern Victoria (Ewart 1988) suggest that regrowth is being rotated while it is too small to support large colonies of the major pest species. These termites require large timber for nesting, and attack on small regrowth trees is most commonly initiated from colonies located in remnant old-growth trees or stumps. Thus while management may make more palatable timber available to termites, the populations of termites will diminish drastically, unless sufficient colonysites remain.

### SPECIES DEPENDENT ON TERMITE-CREATED HOLLOWS

The excavations of termites alter the structure of trees and provide spaces which have become a necessary part of the habitat of many vertebrate species. These beneficial activities are increasingly recognized by wildlife researchers (Ambrose 1982, Mackowski 1984, Saunders 1979) but remain largely ignored by termite biologists (e.g. McMahan 1986). Termites do not create these hollows directly; their excavations, apart from the minimum space

<sup>5</sup>Diameter Breast Height Over Bark.

needed for access, are quickly filled with mud and feces ("mudgut"). Mudgut pipes may extend the full height of the tree and into each of the major limbs. Eventually the mudgut column compacts under its own weight or erodes as water enters through openings created by shed branches. This creates cavities which other animals readily utilize.

A wide range of bats, birds and arboreal marsupials are entirely dependent on hollows of particular volume, depth and opening diameter for nest sites. Australian vertebrates, unlike some of their northern hemisphere counterparts, do not excavate their own tree hollows but are limited in their efforts to minor maintenance, relying instead on the actions of termites.

Fallen, hollowed limbs and trunks of overmature trees provide habitat for ground-dwelling reptiles and mammals. A particular example of this is the specialist termite eater, the Numbat (<u>Myrmecobius</u> <u>fasciatus</u> Waterhouse) (Marsupialia: Myrmecobiidae). Numbats feed exclusively at secondary aggregations of termites on the forest floor and require numerous hollow logs for shelter. They were once widespread across southern Australia, but their range has now been reduced to a small area of southwestern Australia due to the loss of habitat (Christensen and others 1984, Friend 1982). Wildfire and logging reduce the supply of suitably hollowed fallen wood and without shelter, numbats quickly fall prey to the introduced European foxes.

#### FUTURE MANAGEMENT CONSIDERATIONS

Typically, a eucalypt does not develop hollows until between 90 to 120 years old, well after the age at which termites begin to feed, but also well beyond the present 40 to 80 year age at rotation. Management prescriptions call for the retention of a few overmature "habitat" trees in each logging coupe. However, although eucalypts may persist for two to three centuries after developing hollows, they will in time die and eventually fall. Thus the prescriptions are inadequate because they make no provision for recruitment to replace these habitat trees as they are lost.

The impending threat to wildlife populations is potentially serious. Populations will decline rapidly as nest sites become limiting. Even if corrective action is taken immediately, many forests will soon face a gap of a

century or more before another generation of suitably hollowed trees can develop (Mackowski 1984).

This problem is compounded by the negative effects of logging on the retained habitat trees: few will be able to live to their full potential as the coupes in which they stand will be logged between three and seven times during this period. The cost of providing artificial hollows during this period will be enormous. Thus in areas where multiple use management includes the maintenance of wildlife, there is a need to retain not just habitat trees but also cohorts of replacement trees and populations of termites to transform them. Managers of native eucalypt forest need to change their perception of termites: termites are not pests, but have a vital role in forest ecology and must be seen as an intrinsic part of the system.

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# Cuticular Hydrocarbons: Species and Population-Level Discrimination in Termites<sup>1</sup>

### Michael I. Haverty Marion Page Barbara L. Thorne Pierre Escoubas<sup>2</sup>

Abstract: Hydrocarbons in the cuticle of insects are essential in protecting them from desiccation. The vast variety of hydrocarbons synthesized by insects and the apparent species-specificity of cuticular hydrocarbon mixtures make them excellent taxonomic characters for separating species within termite genera. Hydrocarbon phenotypes of dampwood termites, Zootermopsis, correspond to species diagnoses. A morphological character, the subsidiary tooth of the right mandible, was found that correlates exactly with the hydrocarbon phenotypes of the three described species of Zootermopsis. Two distinct hydrocarbon phenotypes within Z. nevadensis have been used to identify a new subspecies, Z. n. nuttingi, which is morphologically indistinguishable from its conspecific, Z. n. nevadensis. This subspecies designation was corroborated with studies of agonistic behavior. Preliminary data suggest that cuticular hydrocarbons might be similarly used to sort specimens and to search for morphological characters within the subterranean termite genus Reticulitermes. This may facilitate a revision of this important genus. Cuticular hydrocarbon profiles enabled population-level discrimination of the Formosan subterranean termite, Coptotermes formosanus, from different geographic locations.

The outer layer of the cuticle of all terrestrial insects consists of a thin layer of wax (Hadley 1985). This wax plays a key role in survival of the insect by providing protection from desiccation (Hadley 1980), as well as serving as a barrier to abrasion, microorganisms, and chemicals (Blomquist and Dillwith 1985). Hydrocarbons are ubiquitous components in insect cuticular lipids and can comprise up to 90 percent of the material (Blomquist and Dillwith 1985, Hadley 1985). They have been shown to be important semiochemicals, and have been postulated as species and caste recognition cues in termites (Howard and Blomquist 1982).

Alkanes occur in all insect surface lipids investigated so far. n-Alkanes generally range from 21 to 36 carbon atoms, and an odd number of carbons predominate. Terminally-branched and internally-branched monomethylalkanes are also prevalent in

insect surface lipids and range from simple compositions, in which only one compound is present, to complex mixtures (Blomquist and Dillwith 1985). In most monomethylalkanes, the methyl branch is located on an odd-numbered carbon atom between carbons 3 and 17. As careful analyses of mono-, di- and trimethylalkanes are made on more organisms, it appears that the methyl groups can be positioned almost anywhere on the chain. <u>n</u>-Alkenes, with one, two, or three double bonds, have been characterized in about one-half of the insect species examined to date. The chain length of cuticular <u>n</u>-alkenes usually ranges from 20 to 37 carbon atoms, with odd-numbered chain lengths predominating. The position of the double bond can be almost anywhere in the chain, but is common at carbon 9 (Blomquist and Dillwith 1985).

#### HYDROCARBONS AS TAXONOMIC CHARACTERS

Moore (1969) was the first to report the composition of cuticular hydrocarbons in a termite, Nasutitermes exitiosus (Hill) (Nasutitermitidae). He found the majority of the hydrocarbons to be paraffins from  $C_{24}$  to  $C_{47}$  with compounds with an odd number of carbons in the parent chain predominating. Blomquist and others (1979) and Howard and others (1978, 1980 and 1982) completely characterized the cuticular hydrocarbons of three termite species. They found that R. flavipes (Kollar). R. virginicus (Banks) (Rhinotermitidae), and Z. angusticollis (Hagen) (Termopsidae) possess drastically different hydrocarbon profiles, and all three of these profiles differ markedly from those reported earlier for N. exitiosus (Hill). Those investigators postulated that hydrocarbons might serve as semiochemical cues for caste and species recognition. On the basis of these early results, Howard and Blomquist (1982) hypothesized that each insect species had a mixture of cuticular hydrocarbons that was peculiar to that species and potentially useful as taxonomic characters.

Insects synthesize most if not all of their complement of cuticular hydrocarbons <u>de novo</u> (Blomquist and Dillwith 1985). We assume this synthesis is genetically controlled and is affected only slightly by environmental parameters. Insect species generally have from 10 to 40 major components in their hydrocarbon mixtures. The relatively large number of possible hydrocarbon components found in the cuticle of insects, ease of chemical analysis and identification of hydrocarbons, and apparent species-specific compositions for many insects make hydrocarbons attractive characters for use in chemotaxonomy (see references in Haverty and others 1989 and Page and others 1990).

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We consider hydrocarbons to be unnecessary for higher order taxonomic separations. Obvious and consistent morphological characters will usually suffice. If there are qualitative differences among species within a genus, a reasonable goal would be to develop a dichotomous key to the species. Useful hydrocarbons should be abundant, not minor components (at least 1 percent, but preferably > 5 percent of the total hydrocarbon mixture). They should also be unique or present in only a few of the species, or conversely, they should be common in most of the species yet completely absent, rare or of insignificant quantities in one or a few. Furthermore, they should have a unique elution time so that they do not coelute with another hydrocarbon in the same species, nor should they elute at a time similar to that of a different hydrocarbon in a different species. From a collection of these hydrocarbons, many different dichotomous keys are possible.

For the most part we have been corroborating existing taxonomies that are based on morphological, genetic and/or behavioral characteristics. For example, we have helped unravel species complexes, identified sibling species and substantiated recent synonymies in cone beetles, genus Conophthorus (Haverty and others 1989, Page and others 1990). Ideally, we should use these chemical characters much as classical taxonomists use morphology, behavior or genetics, i.e., to sort the groups of insects on the basis of chemical characters first, rather than after groups have already been sorted on the basis of existing (nonchemical) character criteria.

That is how we initially started working on the cuticular hydrocarbons of termites. While trying to understand a synonymy of two species of cone beetles, C. ponderosae Hopkins and C. lambertianae Hopkins, we decided to use cuticular hydrocarbons as taxonomic characters. To test our methodology we examined the hydrocarbons of the dampwood termite,  $\underline{Z}$ . angusticollis, which had been characterized in the literature (Blomquist and others 1979). We found our results to be qualitatively similar, yet quantitatively very different from those reported by Blomquist and others (1979). Furthermore, our termite specimens were identified as  $\underline{Z}$ . nevadensis, not  $\underline{Z}$ . angusticollis. Additional collections and analyses led us to identify an "extra" hydrocarbon phenotype of Zootermopsis (Haverty and others 1988), and to find a morphological character for unequivocal identification of the three described species of **Zootermopsis** (Thorne and Haverty 1989).

These serendipitous discoveries have encouraged us to continue our work on termites as well as other groups of economically important forest insects. We have found that characterization of cuticular hydrocarbons often leads to subsequent biological or chemical studies which clarify taxonomic questions.

# CHARACTERIZATION OF CUTICULAR HYDROCARBONS

Detailed descriptions of collection and extraction procedures for specific termite species have been published elsewhere (Blomquist and others 1979, Howard and others 1978, 1982, 1988, Haverty and others 1988, 1990). Cuticular lipids are extracted by immersing 15 to 500 termites (depending on size and total quantity of cuticular hydrocarbon), as a group, in 2 to 10 ml of hexane for 10 min. After extraction, hydrocarbons are separated from other components by pipetting the extract and an additional 2 to 8 ml of hexane through 3 cm of activated BioSil-A in Pasteur pipette mini-columns. Hydrocarbon extracts are evaporated to dryness under a stream of nitrogen and redissolved in 30 to 100 p of hexane for GC-MS analyses. When termites are extracted in the field, the hexane extracts can be allowed to fully evaporate. Dried extracts can then be easily shipped to the laboratory for chromatographic analysis. These field-dried extracts are redissolved in 10 ml of hexane and processed as usual. If termites are frozen, they should be removed from the freezer and allowed to warm to ambient temperature before extraction. After extraction, insects should be stored in 70 percent ethanol for later identification by morphological features and to serve as voucher specimens. All voucher specimens should be deposited in an appropriate reference museum.

All of our extractions were accomplished as described above. Our gas chromatography-mass spectrometry (GC-MS) analyses have been performed on a Hewlett Packard 5890 gas chromatograph equipped with a Hewlett Packard 5970B Mass Selective Detector interfaced with Hewlett Packard Chemstation computer. The GC-MS was equipped with a fused silica capillary column (30 m x 0.2 mm ID, HP-1) and operated in split mode (with a split ratio of 8:1 to 20:1). Each mixture is analyzed by a temperature program from either 150°C, or 200°C to 320°C at 3°C/min with a final hold of 10 to 20 minutes. Electron impact (EI) mass spectra were obtained at 70 eV. n-Alkanes were identified by comparing their retention times and mass spectra with external standards (<u>n</u>-C<sub>22</sub>, <u>n</u>-C<sub>24</sub>, <u>n</u>-C<sub>28</sub>, and <u>n</u>-C<sub>32</sub>). Alkenes and methyl-branched alkanes were tentatively identified by calculating their equivalent chain lengths; mass spectra of methylalkanes were interpreted as described by Blomquist and others (1987).

### SORTING SPECIMENS BY HYDROCARBON MIXTURES

The best example of the use of cuticular hydrocarbons as taxonomic characters for termites is the study we initiated with the dampwood termites, <u>Zootermopsis</u> (Haverty and others 1988). In preliminary work we collected what we tentatively identified as colonies of <u>Z. angusticollis</u> (Hagen) from the vicinity of Burney, Calif., in the Lassen National Forest, and extracted their cuticular hydrocarbons. We identified the same hydrocarbon components as published by Blomquist and others (1979), but in markedly different proportions. This was our first clue that considerable intraspecific variation might exist in the mixtures of cuticular hydrocarbons of <u>Zootermopsis</u>, and prompted us to examine this genus more thoroughly.

The current taxonomy of the genus <u>Zootermopsis</u> recognizes three species: <u>Z. nevadensis</u>, <u>Z. angusticollis</u>, and <u>Z. laticeps</u> (Banks) (Emerson 1933, Sumner 1933, Weesner 1970). We identified four consistent, unique cuticular hydrocarbon phenotypes from the three described species. Phenotype I (<u>Z. nevadensis</u>) contains large amounts (<u>ca.</u> 10 percent of the total hydrocarbon) of the symmetrical dimethylalkane 5, 17-dimethylheneicosane (fig. 1). This dimethylalkane is not found in any other phenotype (table 1).

Phenotype II (Z. angusticollis) has the most complex hydrocarbon mixture, sharing many of the components eluting before n-nonacosane with at least one of the other three phenotypes (Haverty and others 1988). However, phenotype II possesses many unique compounds in quantities greater than 1 percent (table 1): an isomeric mixture of 9- and 11-methylheneicosane; 3,11- and 3, 13-dimethyltricosane; 2- or 4-methyloctacosane and 2- or 4-methylhentriacontane.

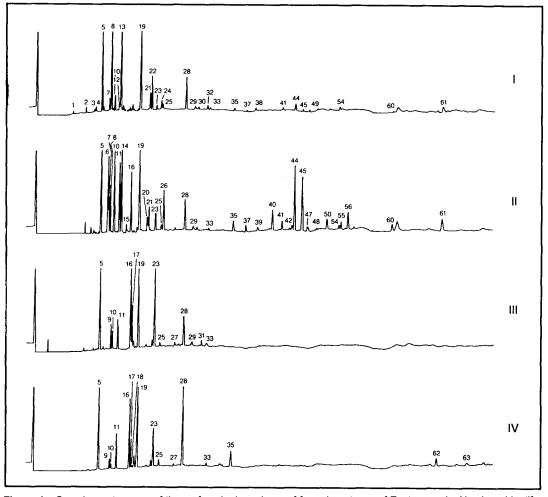
Table 1—Diagnostic hydrocarbons identified from four phenotypes of <u>Zootermopsis</u> (from Haverty and others 1988)

|                                 |                  | Phenotype · |     |     |    |  |
|---------------------------------|------------------|-------------|-----|-----|----|--|
| Hydrocarbon <sup>2</sup>        | ECL <sup>3</sup> | I           | II  | III | IV |  |
| 9-, 11-MeC <sub>21</sub>        | 21.37            | 0           | ++  | 0   | Tr |  |
| 2- or 4-MeC <sub>21</sub>       | 21.62            | 0           | 0   | 0   | ++ |  |
| 5,17-DimeC <sub>21</sub>        | 22.04            | +++         | 0   | 0   | 0  |  |
| 3,11; 3,13-DimeC <sub>21</sub>  | 22.11            | 0           | +++ | 0   | 0  |  |
| <u>n</u> -C23 <sub>23:1</sub>   | 22.70            | 0           | 0   | ++  | ++ |  |
| 3,11-, 3,13-DimeC <sub>23</sub> | 24.10            | 0           | ++  | 0   | 0  |  |
| 2- or 4-MeC <sub>28</sub>       | 28.60            | 0           | ++  | 0   | 0  |  |
| 2- or 4-MeC3 <sub>31</sub>      | 31.75            | 0           | ++  | 0   | 0  |  |
| 7,11-DimeC <sub>35</sub>        | 35.70            | 0           | 0   | 0   | ++ |  |
| 7,13-DimeC <sub>39</sub>        | 38.53            | 0           | 0   | 0   | ++ |  |

<sup>&</sup>lt;sup>1</sup> A triple + indicates > 5.0 percent of the total hydrocarbon component and ++ from 1.0 to 5.0 percent of the total. Trace (Tr) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon was never identified for the phenotype.

<sup>2</sup> Carbon number is the total number of carbons in the parent chain, excluding the methyl groups.

<sup>3</sup> ECL = Equivalent chain length.



**Figure 1—**Gas chromatograms of the surface hydrocarbons of four phenotypes of <u>Zootermopsis</u>. Numbers identify peaks characterized by Haverty and others (1988).

Phenotype III (Z. nevadensis) clearly contains the simplest hydrocarbon profile. Only 10 hydrocarbon components are present in significant quantities (greater than 1.0 percent of the total hydrocarbon fraction). This is the only phenotype we collected from the Pacific Coast with an olefin (tricosene). All of the other major (>5.0 percent) or significant (>1.0 percent) components of Phenotype III are shared with at least one of the other phenotypes (Haverty and others 1988).

Phenotype IV (Z. <u>laticeps</u>) is also fairly simple, with only 13 significant (1.0 percent of total hydrocarbon) components, and is quite similar to Phenotype III. The major differences are the three unique compounds: 2- or 4- methylheneicosane; 7, 11-dimethylpentatriacontane and 7,13-dimethylheptatriacontane (table 1).

Identification of four consistent, unique cuticular hydrocarbon phenotypes from three extant species was in conflict with the species-specific hypothesis put forward by Howard and others (1978, 1982). If cuticular hydrocarbon profiles are truly species-specific, then there is at least one new, undescribed species of Zootermopsis. It appears that Z. angusticollis and Z. laticeps are valid species, but Z. nevadensis is a complex of at least two species or one species with polymorphic hydrocarbon chemistry. Clarification of specific status of each phenotype required many different approaches. The approaches we investigated were correlation of hydrocarbon chemistry and external morphology from an extensive geographic collection (Thorne and Haverty 1989), bioassays of aggressive behavior (Haverty and Thorne 1989), isoenzyme analysis (Korman and others, 1991), and DNA hybridization (Broughton 1989).

# CORRELATION OF HYDROCARBONS WITH EXTERNAL MORPHOLOGY

Determination of <u>Zootermopsis</u> species on the basis of morphology is often equivocal, especially in juvenile or small colonies. However, by sorting specimens on the basis of cuticular hydrocarbon phenotypes, we found a character on the right mandible, the shape and position of the subsidiary tooth, that enables unequivocal diagnosis of <u>Z. angusticollis</u>, <u>Z. nevadensis</u> and <u>Z. laticeps</u> (Thorne and Haverty 1989). This small subsidiary tooth is present at the base of the anterior edge of the first marginal tooth on the right mandible of nonsoldiers of all genera within the Termopsidae. Shape and position of the subsidiary tooth can be consistently and accurately used to diagnose both sexes of all nonsoldier morphs of <u>Z. angusticollis</u> and <u>Z. nevadensis</u>.

Because the subsidiary tooth character does not rely on a series of soldiers (relatively rare in colonies or collections) or alates (only present seasonally and absent from most samples or collections), it can be used to determine virtually any collection of <u>Zootermopsis</u>. Large larvae or nymphs are almost always abundant, and dissection of several individuals will not reduce the value of the collection. The mandible structure does not enable morphological discrimination of the two <u>Z</u>. nevadensis hydrocarbon phenotypes. However, agonistic behavior which involves social interactions between individuals, including fighting, fleeing, or submitting, provides a biological model for species discrimination.

# AGONISTIC INTERACTIONS BETWEEN HYDROCARBON PHENOTYPES

Termites show a wide range of agonistic behaviors when interacting with other termites from a different colony of the same or a different species. Aggressive encounters are often particularly dramatic because of defensive morphological modifications of the soldier caste and effective use of strong mandibles by the pseudergate or worker castes. Interspecific aggression is common in termite-termite interactions. Intraspecific aggression (between colonies) has also been shown in a variety of other termites. A few documented cases exist, however, in which aggression between conspecific termites is absent; i.e., where individuals from different colonies can be mixed with little or no reaction (see references in Haverty and Thorne 1989).

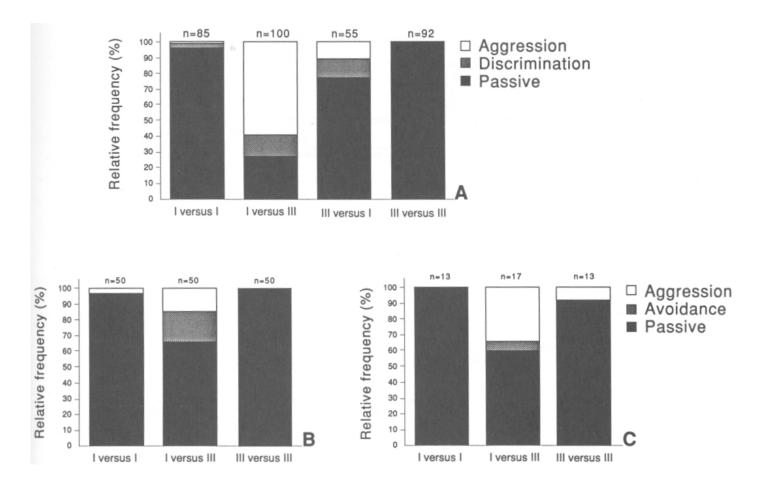
Three experiments were conducted to assess agonistic interactions between hydrocarbon phenotypes (Haverty and Thorne 1989). We hypothesized that meetings between termites from colonies of like phenotypes would be more passive than encounters between individuals of different phenotypes. In the first series of experiments we assessed the agonistic response of soldiers from each of the three hydrocarbon phenotypes with nymphs from each phenotype. In the second we assessed the intra-caste agonistic behavior of either pseudergates or soldiers from hydrocarbon phenotypes I, II, and III. In the third we assessed the intra-caste agonistic behavior of pseudergates or soldiers from hydrocarbon phenotype IV with those like castes from phenotypes I, II, and III.

Our results demonstrated that soldiers or pseudergates seldom attack individuals of the same hydrocarbon phenotype. Phenotype II (Z. angusticollis) is typically aggressive toward phenotype III (Z. nevadensis), but not always aggressive against phenotype I (Z. nevadensis). The variation in response is dependent on which castes are placed in the bioassay arena: soldier-versus-soldier bouts result in consistent aggression, while pseudergate-versus-pseudergate or soldier-versus-nymphs contacts do not. Both pseudergates and soldiers of Z. laticeps (phenotype IV) respond agonistically toward the other three phenotypes.

Although hydrocarbon phenotypes I and III, both <u>Z</u>. <u>nevadensis</u>, are morphologically indistinguishable, agonistic behavioral responses between them are not equivalent to I-versus-I or III- versus-III behavioral responses. We interpret the lack of avoidance or aggressive behavior within each of the two phenotypes of <u>Z</u>. <u>nevadensis</u> and the significant avoidance and aggressive behavior between phenotypes as definite evidence of discrimination between disparate hydrocarbon phenotypes (fig. 2). These agonistic bioassays along with data on distinct hydrocarbon patterns and geographic distributions serve as the basis for designating two subspecies of <u>Z</u>. <u>nevadensis</u>; <u>Z</u>. <u>n</u>. <u>nevadensis</u>, (Hagen) and <u>Z</u>. <u>n</u>. <u>nuttingi</u> (Haverty and Thorne), ssp. nov (Haverty and Thorne 1989).

### HYDROCARBONS OF RETICULITERMES

The morphology of the subterranean termites in the family Rhinotermitidae is quite variable among colonies within the



**Figure 2—**Agonistic behavior between <u>Zootermopsis</u> hydrocarbon phenotypes I and III: A. Soldier (first Roman numeral) vs. nymphs (second Roman numeral) of like or different phenotypes; B. Pseudergates vs. pseudergates; C. Soldier vs. soldier. The number of observations and relative frequency of each class of behavior are shown. Behaviors are passive, avoidance, and aggression.

same species. It is readily acknowledged that the genus Reticulitermes is in need of revision. "The definition of the various species of Reticulitermes and, therefore, the limits of their distributions, is difficult. Certainly this genus is woefully in need of a critical taxonomic study..." (Weesner 1970). We have characterized the cuticular hydrocarbons of "species" of Reticulitermes from the western United States, R. tibialis Banks and R. hesperus Banks, as well as those from two species common to the eastern United States, R. flavipes (Kollar) and R. virginicus (Banks) (fig. 3). Examination of our profiles and those published in the literature reveals 80 hydrocarbons from these species of Reticulitermes from the southern and western United States. Of these hydrocarbons, we consider at least 21 to be of diagnostic value (table 2).

We have collected <u>Reticulitermes</u> in California from areas suspected to have only <u>R</u>. <u>hesperus</u> or <u>R</u>. <u>tibialis</u>, Cloverdale and Owens Lake, respectively. We found that the cuticular hydrocarbon profiles of workers from these two areas are very similar, with the primary difference being one hydrocarbon substitution: 11, 15-dimethylpentacosane in <u>R</u>. <u>hesperus</u> and 2- or 4-methylpentacosane in <u>R</u>. <u>tibialis</u> (table 2, fig. 3). This similarity of hydrocarbon mixtures indicates that these two collections are either the same species or closely related. Our collections of "<u>R</u>.

<u>tibialis</u>" from two different locations in Arizona, Fairbank and Prescott, exhibit extremely different cuticular hydrocarbon profiles which are also different from our collections of either  $\underline{R}$ . <u>hesperus</u> or  $\underline{R}$ . <u>tibialis</u> from California (fig. 3). From these results we infer that there are probably at least three species of <u>Reticulitermes</u> in western North America.

### ORIGIN OF INTRODUCED SPECIES

Another way to use data on cuticular hydrocarbons is to relate the similarity of hydrocarbon profiles to the origin of introduced termite species. To test this approach we examined the cuticular hydrocarbon profiles of four geographically distant populations of the Formosan subterranean termite, <u>Coptotermes formosanus</u> Shiraki, in the United States (Haverty and others 1990). This species is considered to be one of the most voracious subterranean termites and a serious threat to wood in structures throughout its range. It was introduced into Japan before 1600 and subsequently into Hawaii and the mainland United States (Su and Tamashiro 1987). Although many of the infestations in the continental United States were first noticed after 1965, <u>C. formosanus</u> colonies were most likely established after World War II in the port cities of Houston and Galveston, Texas; New

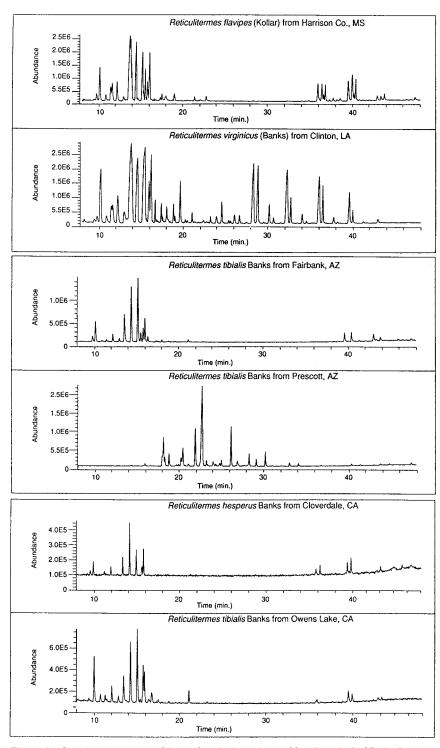


Figure 3—Gas chromatograms of the surface hydrocarbons of four "species" of Reticulitermes.

Orleans and Lake Charles, Louisiana; and Charleston, South Carolina (Beal 1987). There has been a significant "spread" of <u>C. formosanus</u> to other southern port cities including greater Fort Lauderdale, Florida, Memphis, Tennessee, Baton Rouge, Louisiana, and Mobile, Alabama. Similarity or difference in hydrocarbon patterns between the four populations (Hallandale, Florida; New Orleans, Louisiana; Lake Charles, Louisiana; and Honolulu, Hawaii) was determined by stepwise discriminant

analysis to select discriminating variables (proportion of a given hydrocarbon), followed by canonical discriminant analysis to provide two-dimensional displays of the chosen variables. GC-MS analyses of the cuticular wax of representative samples of <u>C. formosanus</u> indicate that all colonies and both castes from all four locations contain the same sixteen hydrocarbon components. We examined this set of quantitative chemical characters to determine the similarity between the hydrocarbon profiles

Table 2—Diagnostic hydrocarbons for four species of Reticulitermes<sup>1</sup>

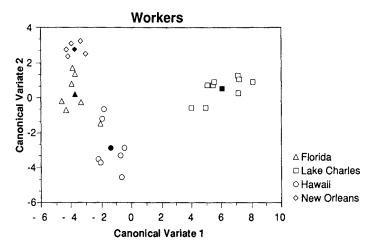
#### Reticulitermes species<sup>2,3</sup>

| Hydrocarbon <sup>4</sup>            | ECL <sup>5</sup> | flav | virg | AZ1 | AZ2 | CA1 | CA2 |
|-------------------------------------|------------------|------|------|-----|-----|-----|-----|
| <u>n</u> -C <sub>23</sub>           | 23.00            | +++  | ++   | +++ | o   | +++ | +++ |
| $C_{25:1}$                          | 24.70            | +++  | +++  | o   | o   | o   | 0   |
| $C_{25:2}$                          | 25.40            | +++  | +++  | O   | o   | o   | 0   |
| 11,15-DimeC <sub>25</sub>           | 25.60            | o    | O    | O   | o   | o   | +++ |
| 2- or 4-MeC <sub>25</sub>           | 25.70            | ++   | ++   | ++  | 0   | +++ | 0   |
| 5,17-DimeC <sub>25</sub>            | 25.90            | 0    | O    | ++  | 0   | o   | 0   |
| $C_{27:1}$                          | 26.70            | 0    | O    | O   | +++ | o   | 0   |
| $C_{29:2}$                          | 28.69            | o    | O    | O   | +++ | o   | 0   |
| $C_{29:1}$                          | 28.70            | o    | O    | O   | +++ | o   | 0   |
| $C_{31:2}$                          | 30.69            | 0    | O    | O   | +++ | o   | 0   |
| 11-; 15-MeC <sub>31</sub>           | 31.40            | 0    | +++  | 0   | 0   | o   | 0   |
| 11,15-DimeC <sub>31</sub>           | 31.64            | 0    | ++   | O   | 0   | o   | 0   |
| 11-; 13-; 15-; 17-MeC <sub>33</sub> | 33.30            | o    | +++  | O   | o   | o   | 0   |
| 11,15-; 11,21-;                     |                  |      |      |     |     |     |     |
| 13,17-DimeC <sub>33</sub>           | 33.57            | 0    | ++   | O   | 0   | o   | 0   |
| 11-; 13-; 15-; 17-MeC <sub>35</sub> | 35.31            | ++   | ++   | O   | 0   | ++  | 0   |
| 11,15-DimeC <sub>35</sub>           | 35.60            | ++   | ++   | O   | 0   | ++  | 0   |
| 7,17-DimeC <sub>35</sub>            | 35.70            | +    | o    | o   | o   | o   | 0   |
| 5,17-DimeC <sub>35</sub>            | 35.80            | ++   | O    | O   | 0   | o   | 0   |
| 11-; 13-; 15-; 17-;                 |                  |      |      |     |     |     |     |
| 19-MeC <sub>37</sub>                | 37.30            | ++   | ++   | ++  | 0   | +++ | ++  |
| 11,15-DimeC <sub>37</sub>           | 37.59            | ++   | +    | 0   | 0   | +++ | ++  |
| 5,17-DimeC <sub>37</sub>            | 37.80            | ++   | o    | ++  | 0   | 0   | o   |

<sup>&</sup>lt;sup>1</sup>Hydrocarbons listed for each species are all from chemical analyses by Page, Haverty, and Escoubas.

within and among the sampled geographic locations. Our underlying assumption was that colonies of <u>C</u>. <u>formosanus</u> with quantitatively similar cuticular hydrocarbon profiles are likely to be more closely related, i.e., originating from the same geographical source, than those that are less similar.

Colonies of <u>C</u>. <u>formosanus</u> from each of the four geographic locations can be separated from colonies from all of the other sites on the basis of concentrations of cuticular hydrocarbon components. Discriminant analysis of the proportions of the cuticular hydrocarbons of workers identified seven hydrocarbon components that reasonably separate the four populations. For the two locations for which we had soldiers, only three hydrocarbon components were necessary. No single component, however, can be used to separate <u>C</u>. <u>formosanus</u> from the four geographic locations. Stepwise discriminant analysis allowed us to use a selection of the hydrocarbon components to effectively distinguish <u>C</u>. <u>formosanus</u> from these geographic locations (fig. 4).



**Figure 4**—Plot of colonies of <u>C</u>. <u>formosanus</u> from four different locations along two axes of canonical discriminant space. Open characters represent actual data points; closed characters represent the mean value for the population.

 $<sup>^2</sup>$  Reticulitermes "species": flav=R. flavipes, virg=R.virainicus, AZ1=R. tibialis from Fairbank, Ariz., AZ2 = R. tibialis from Prescott, Ariz., CA1 = R. hesperus from Cloverdale, Calif., CA2 = R. tibialis from Highway 35 near Owens Lake, Calif.

 $<sup>^3</sup>$  A triple + indicates  $\geq 5.0$  percent of the total, ++ from 1.0 to 5.0 percent of the total and + from 0.5 to 1.0 percent of the total hydrocarbon component. Some trace (tr.) components appear infrequently or consistently in very small quantities (< 0.5 percent of the total). A zero indicates the hydrocarbon was never identified for the phenotype.

<sup>&</sup>lt;sup>4</sup> Carbon number is the total number of carbons in the parent chain, excluding the methyl groups.

<sup>&</sup>lt;sup>5</sup> ECL = Equivalent chain length.

Clearly the population from Lake Charles is very different from those from the other locations. This difference supports the assertion that the introduction of <u>C. formosanus</u> into Louisiana was from two separate sources (La Fage 1987). It also appears that the Honolulu population, at least the insects we sampled from the Manoa Valley in Honolulu, was probably not the original source of infestations in either New Orleans, Lake Charles or Hallandale. The affinity of the infestation from Hallandale, Florida, is least clear. The hydrocarbon profile is significantly different from that of both Honolulu and New Orleans.

Unfortunately, with our present data base, cuticular hydrocarbon profiles have not allowed us to determine whether the more recent infestation in Hallandale, Florida, resulted from international or domestic, maritime or surface commerce. Our results suggest that C. formosanus from Hallandale, Florida, New Orleans, Louisiana, and Lake Charles, Louisiana, are not related to those from Honolulu, Hawaii, and probably originated from other geographical locations.

### CONCLUSIONS

Our experience, thus far, in applying knowledge of cuticular hydrocarbon composition to the identification of termite species has been encouraging. We have used hydrocarbons to sort specimens initially to identify a new diagnostic morphological character for Zootermopsis species. Where morphology cannot be used to separate hydrocarbon phenotypes, we have been able to use inter- and intraphenotype agonistic behavior to corroborate cuticular hydrocarbon phenotypes. Geographical races of Coptotermes formosanus can be distinguished even on the basis of the concentrations of individual hydrocarbon components.

There are still some unresolved questions to be addressed before we consider the use of cuticular hydrocarbons for taxonomic studies of termites. They are: (1) What is the influence of genetics and the environment (e.g., temperature, relative humidity, and diet) on the mixture of hydrocarbons? (2) Are cuticular hydrocarbons, or the other chemicals present in the wax layer, responsible for species or caste recognition? (3) Are hydrocarbon profiles within "good species" qualitatively identical or are there some exceptions to the rules; can different biological species have identical hydrocarbon profiles? (4) What is the rate of change in hydrocarbon profiles as species evolve? Do slight differences in profiles mean that the species are closely related? and (5) Can hydrocarbons, as well as morphology, behavior and genetics, be used to determine phyletic relationships among groups within a genus? Resolution of these questions will greatly advance the precision of cuticular hydrocarbons as chemotaxonomic characters in all insects.

Currently we rely on gross quantities of chemical insecticides applied to the soil for protection of wooden structures from our native subterranean termites. This practice will likely be deemed as unacceptable soon. Future species-specific control strategies for any subterranean termite will require an understanding of the foraging, feeding, and interspecific behaviors of the species to be controlled, and this necessitates accurate identification of the species.

It is readily acknowledged that <u>Reticulitermes</u> is the most economically important genus of termites in the United States and Europe, yet the genus is in desperate need of revision. There should be a significant effort to revise the genus <u>Reticulitermes</u> in North America and two important groups of pantropical termites: <u>Coptotermes</u> and <u>Nasutitermes</u>. These latter two genera are among the most economically important groups in the world. This revision will require collaboration among the termite scientists throughout the world, and will involve studies of morphology, behavior, genetics and, of course, cuticular hydrocarbons. We consider cuticular hydrocarbons to constitute a very useful set of characters that can provide insight into the taxonomy of termites for which classical morphometric analyses have failed.

### **ACKNOWLEDGMENTS**

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# An Assessment of the Potential Uses of Agonistic Behaviors in Termite Control<sup>1</sup>

### Barbara L. Thorne Michael I. Haverty<sup>2</sup>

Abstract: The potential use of termite-termite agonism in pest control is explored and evaluated. Intra- and interspecific encounters among termites from different colonies are known to result in aggressive or avoidance behaviors in a variety of species. Recent studies suggest, however, that intraspecific confrontations only rarely evoke aggressive responses in several economically important termite genera in the United States (Coptotermes, Reticulitermes, and Zootermopsis). Thus the most promising applied use of natural agonistic behaviors among termites appears to be in research on individual- and colony-recognition systems in the Isoptera. Artificial manipulation of recognition and response cues might facilitate novel control methodologies based on masking or interfering with natural stimuli. Directions for future research are discussed.

Insect behaviors are diverse, and knowledge of the behaviors of a species has always been a key element in pest management. Basic knowledge of activity periods, diet preferences, and mating behaviors can be central in determining an approach toward deterring a pest species. Taking advantage of behavioral patterns or interfering with natural repertoires or both can produce very effective control methodologies.

Understanding the foraging behavior of subterranean termites is crucial to prevention of damage to buildings (Beal and others 1989; Su and Scheffrahn 1990). Preventative techniques include proper construction practices to exclude or deter foraging and feeding by subterranean termites and application of large volumes of chemical insecticides as soil drenches to kill or repel foraging subterranean termites. Existing and future methods for remedial control of subterranean termites will also rely on knowledge of the foraging behavior of these insects (Su and Scheffrahn 1990).

Two promising future control methods involve the use of physical barriers and the use of nonrepellent, slow-acting insecticides. Physical barriers will consist of a substrate that has a particle size impenetrable to subterranean termites (Su and Scheffrahn 1990). Nonrepellent, slow-acting insecticides will be used in baits or in baiting systems. Subterranean termites will recruit to the bait stations through natural foraging behaviors and feed on the chemically impregnated wood. It is then hoped that

they will return to their colony and feed their insecticide-laced crop contents to colony mates before dying. Normal foraging behaviors will thus cause an entire colony to be slowly poisoned by toxic baits. Termite colony populations will become depressed, and they will ultimately be eliminated, not simply excluded, from structures.

Another behavior that has recently received attention by termite biologists is agonistic behavior. Agonistic behavior involves social interactions between individuals, including submitting, fleeing and fighting, even to death. Termites show a wide range of agonistic behaviors when interacting with termites from a different colony. In all cases studied to date, termites from colonies of different sympatric species respond aggressively when they meet (Adams and Levings 1987; Clément 1982; Grassi and Sandias 1896-1897; Haverty and Thorne 1989; Ne11968; Springhetti and Amorelli 1982; Thorne 1982; Traniello and Beshers 1985). Intraspecific confrontations also result in clear aggressive or avoidance behaviors in a variety of termite species (Adams and Levings 1987; Andrews 1911; Binder 1988; Clément 1986; Dudley and Beaumont 1889; Howick and Creffield 1980; Jones 1987; Levings and Adams 1984; Nel 1968; Pearce and others 1990; Pickens 1934; Su and Haverty 1991; Thorne 1982). Since many of these termite-termite interactions result in injury or death to some or all of the insects, it is reasonable to evaluate their potential use in control of termites as structural pests.

# CASE STUDY: THE DAMPWOOD TERMITE GENUS Zootermopsis

From an individual termite's viewpoint, the fundamental problem in encounters with other termites is the correct discrimination of colony mates from noncolony mates. We examined intra- and interspecific interactions among all species of Zootermopsis Emerson in the Pacific coastal states and southwest deserts of the United States (Haverty and Thorne 1989). Those results are summarized here, and new data on response of naive soldiers from incipient colonies are reported.

Zootermopsis laticeps (Banks) is morphologically distinct from other members of the genus. This species also has an isolated geographic range in Arizona, New Mexico, and northern Mexico, and lives in dead portions of live trees, usually in riparian situations. In the absence of alates, the morphological characters used to differentiate the described species Z. angusticollis (Hagen) and Z. nevadensis (Hagen) were ambiguous. That imprecision made it impossible to establish valid experimental encounters between colonies. This problem was resolved when Haverty and others (1988) discovered three consistent and distinct cuticular hydrocarbon phenotypes among the two "species," Z. angusticollis and Z. nevadensis, that occur

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along the Pacific Coast. The hydrocarbon separations enabled us to isolate a diagnostic character, shape and position of the subsidiary tooth on the right mandible of nonsoldiers, which established unambiguous discrimination of  $\underline{Z}$ . angusticollis from Z. nevadensis (Thorne and Haverty 1989).

Z. angusticollis shows one distinct hydrocarbon profile throughout its range (labelled phenotype II by Haverty and others [1988]). Two hydrocarbon phenotypes (I and III) are associated with the single morphological species Z. nevadensis, but each chemical phenotype has a discrete geographic distribution and may have temporal isolation in alate phenologies (Haverty and others 1988; Haverty and Thorne 1989; Thorne and Haverty 1989; Weesner 1970). In our experiments we investigated whether intra- or inter-hydrocarbon phenotypes of Zootermopsis colonies show discrimination in behavioral encounters.

In the laboratory we set up pairwise experimental trials in which we placed combinations of various castes from different hydrocarbon phenotypes into petri dish arenas lined with filter paper. Responses were recorded on a ranking system from no noticeable reaction to immediate lethal attack. Observed behaviors were typically either docile or highly aggressive, with little intermediate response.

General results of these experiments are summarized here (for more detail see Haverty and Thorne 1989). In intraphenotype pairings, agonistic reactions among members of any castes are rare, except between some colonies of <u>Z</u>. angusticollis.

Interphenotype interactions frequently stimulate aggressive responses (fig. 1), but behaviors vary depending on which colonies are involved. Z. angusticollis and Z. nevadensis phenotype III, which occur sympatrically throughout much of their range, are more aggressive towards each other than are Z. angusticollis and Z. nevadensis phenotype I, which have an allopatric distribution. The two hydrocarbon phenotypes of Z. nevadensis do show discrimination responses in behavioral encounters, and in that way act like distinct species. The behavioral discrimination was further evidence to justify designation of the Z. nevadensis complex into two subspecies, Z. nevadensis, subsp. nevadensis (Hagen) [phenotype I] and Z. nevadensis subsp. nuttingi (Haverty and Thorne) [phenotype III] (Haverty and Thorne 1989).

Supplementing experiments reported in Haverty and Thorne (1989), we have recently conducted a series of comparable trials involving young, naive soldiers from experimentally bred laboratory colonies. In July and August 1987, the wings of mature male and female Z. nevadensis subsp. nuttingi alates from a variety of colonies were removed and the alates were paired in small petri dishes lined with moist sand and containing a piece of Douglas-fir wood. Many of these couples successfully reared offspring. The first soldier differentiated when brood size reached approximately 15-20 individuals. In March 1989 we removed these young soldiers from incipient colonies which still contained both primary reproductives, and introduced each soldier to three nymphs or larvae from a haphazardly selected colony of Z. nevadensis subsp. nevadensis [14 trials] or Z. angusticollis

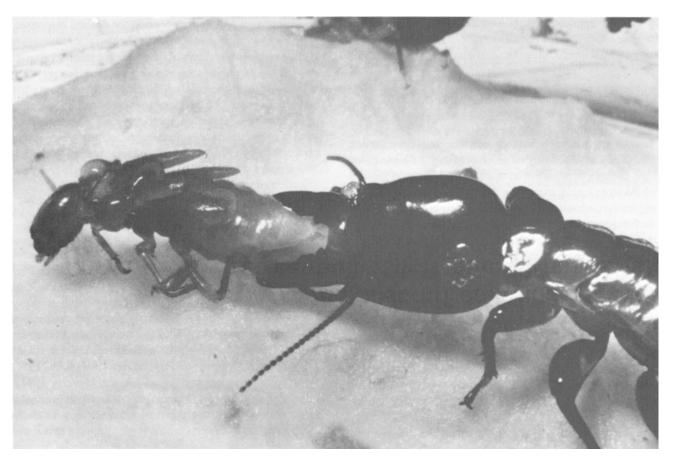


Figure 1— $\underline{\text{Zootermopsis}}$  angusticollis soldier attacking a  $\underline{\text{Z}}$ .  $\underline{\text{nevadensis}}$  nymph.

[10 trials]. Each soldier was used only once (experimental protocol identical to that described for Haverty and Thorne 1989, Experiment 1). In the <u>Z. nevadensis</u> subsp. <u>nuttingi</u> versus <u>Z. nevadensis</u> subsp. <u>nevadensis</u> encounters, naive <u>Z. n.</u> subsp. <u>nuttingi</u> soldiers attacked and killed <u>Z. n.</u> subsp. <u>nevadensis</u> larvae or nymphs in 5 of the 14 trials. In all 14 cases soldiers showed agonistic responses by repeatedly flaring their mandibles. Results of the <u>Z. nevadensis</u> subsp. <u>nuttingi</u> soldier encounters with <u>Z. angusticollis</u> larvae or nymphs were consistent: in each of the 10 cases the soldiers attacked and killed the <u>Z. angusticollis</u>. These data on naive soldiers parallel results from the experiments using soldiers from mature field colonies reported in Haverty and Thorne (1989).

The generally docile reactions observed in the intraphenotype trials raised the possibility that passive fusion might occur between members of two conspecific field colonies whose gallery networks met in a host log. We have demonstrated experimentally, however, that soldiers, nymphs, and pseudergates will kill and consume functional reproductives from a foreign colony, even within the same hydrocarbon phenotype. Thus, complete colony fusion in nature is unlikely (Haverty and Thorne 1989). Aside from Zootermopsis, the only cases of passive coalescence of termite colonies reported in the literature involve Reticulitermes (Rhinotermitidae) (Clement 1986; Jones 1987) and some populations of Coptotermes (Rhinotermitidae) (Su and Haverty 1991; Su and Scheffrahn 1988).

The stimuli responsible for aggressive behaviors in Zootermopsis or other species of North American termites are unknown. Clearly, however, the rapidity, consistency, and efficiency of these aggressive responses are effective in termitetermite interactions. In the remainder of this paper we examine realistic potential uses of these natural behaviors as elements of pest management strategies.

# POTENTIAL APPLIED USES OF TERMITE AGONISTIC BEHAVIORS

Natural aggressive behaviors between termite colonies might be used constructively either in bioassays to learn more about the biology of a particular species or directly in termite control. As an example, in species showing consistent intraspecific aggression, agonistic behaviors could be used to precisely map the foraging territory of a given colony (technique used by Pearce and others 1990). Termites from different locations could be observed in encounters with (A) individuals known to be from the focal colony and (B) members of a geographically distinct, conspecific colony. A passive response to sample A and an aggressive response to B would suggest that termites in the test sample were foragers from the same colony represented in sample A (but responses of termites to neighbors versus strangers has not yet been studied [see Gordon 1989]). An agonistic response to both groups A and B would mean that the termites in the test sample were from a separate (third) colony.

Precise knowledge of the foraging areas of a termite colony would be useful in studies of colony population size, growth rate, foraging range, and the density of colonies within a community. In treatment procedures, knowledge of foraging areas may become crucial as control techniques using bait toxicants are developed. Unfortunately, use of agonistic bioassays in mapping colony foraging areas will not work reliably on any of the two dominant genera of subterranean termite pests in the United States Reticulitermes and Coptotermes), because intraspecific mixtures of these termites are generally docile. (Broad variability in degree of agonistic response among termites from different colonies or populations or both has been noted by Haverty and Thorne 1989; Su and Haverty 1991; Su and Scheffrahn 1988; Thorne 1982.)

Perhaps the most promising and intriguing outcome of the study of termite agonistic behaviors is that such work might ultimately lead to an understanding of the recognition code(s) used by termites to discriminate colony mates from noncolony mates. Recognition may involve cuticular hydrocarbon components (Howard and Blomquist 1982; Howard and others 1980, 1982); other behavioral and chemical cues are likely involved as well. Once the discrimination process is understood, numerous applied approaches could be designed around the theme of masking or interfering with recognition stimuli. Manipulation of recognition and response cues could be used to enable chemically loaded intruders to enter a colony, to potentially instigate a civil war, or to impregnate building materials with an offensive, behavioral stimulus to prevent termite colonization.

Very generally, any stimulus that aggravates termites is potentially useful in termite control. The scientific goal is to find a stimulus that repels termites, or triggers an avoidance response, but it must be a persistent avoidance or repulsion stimulus with little or no habituation. If the termites acclimate to the stimulus, it will no longer elicit the desired response.

Control applications involving behavioral bioassays or live termites as an integral part of the pest management program will require trained operators and, typically, a longer treatment period than with chemical applications. Expense is predicted to be higher because of the labor involved in culturing and sampling termites. Integrated approaches may be beneficial in a variety of circumstances. However, in cases where little or no pesticides are desired in the infested area, control methodologies which make use of natural behavioral repertoires may provide effective alternatives.

### DIRECTIONS FOR FUTURE RESEARCH

### Studies of Additional Taxa

Further studies on patterns of agonistic behavior within and among termite species will broaden our perspective on the potential uses of such aggressive or repulsive responses in termite control. Thus far, agonistic behaviors within and between Zootermopsis species have been studied (Haverty and Thorne 1989). Intraspecific agonism has been studied in Heterotermes aureus (Snyder) (Binder 1988; Jones 1987) and Coptotermes formosanus shiraki (Su and Haverty 1991). The genus Reticulitermes is the most economically important group of wood-destroying insects in the United States, yet little behavioral work has been done on the Reticulitermes species within the United States. This genus should definitely be the target of

future research on intra- and interspecific agonistic behavior. Chemoreception

The neurobiology of chemoreception has never been examined in termites, but this approach should be tractable and could help, for example, in our understanding of the role of cuticular hydrocarbons (or fractions of these lipids) in termite-termite recognition processes. Such studies would help us identify natural, biologically active compounds with potential for practical applications.

### **Detailed Ethological Analyses**

Many of the behaviors between termites of different colonies may be more subtle than overt aggression, avoidance, or apparent tolerance. Fine scale ethological studies of intercolony interactions must be done to investigate the spectrum between fighting and "passive" responses, and isolate the stimuli evoking each category of response.

### Evaluation of Experimental Methodologies

Laboratory results should be confirmed in the field to validate the experimental protocols used to study dynamics between termite colonies. When intra- or interspecific encounters occur between termites in their natural social and physical environment, does one observe the same passive or aggressive reactions which are seen in laboratory trials involving small subsets of the same two colonies?

The methods for studying termite-termite encounters are still being devised and refined. Binder (1988) introduced some innovative approaches in his study of <u>H</u>. <u>aureus</u>. In one experiment he simulated a foraging arena with a balsawood barrier separating termites from separate colonies (dyed to differentiate colony affiliation). A high magnification video camera was used to document movements and behaviors over the 24 hours required for the termites to penetrate the barrier. Similar studies employing video equipment and image tracking software would expand our understanding of termite-termite behaviors. Eventually, standardization of protocols should be considered to facilitate comparisons between studies on related taxa.

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# Factors Affecting the Tunneling Behavior of the Western Subterranean Termite, *Reticulitermes hesperus* Banks<sup>1</sup>

### James L. Smith Michael K. Rust<sup>2</sup>

Abstract: Laboratory studies were conducted to determine factors that affect the tunneling behavior of the western subterranean termite (Reticulitermes hesperus Banks). Soil particle sizes between 2.36 and 0.84 mm prevented tunneling. Exposure to solid layers of calcium, magnesium, or zinc borate did not repel workers, but produced >87 percent kill within 10 d. No tunneling occurred in soils treated with the formulated termiticides tested at concentrations ≥50 ppm. Termites did not tunnel in soils treated with as little as 1 ppm bifenthrin or cypermethrin. Exposure to bifenthrin and cypermethrin deposits immobilized termite workers which prevented them from tunneling.

Termites failed to penetrate a layer of dead termites killed by earlier exposure to chlorpyrifos. Contact with dead termites resulted in 96 percent mortality of the tunneling workers. Workers readily penetrated layers of termites freshly killed by freezing, and some died within 24 h.

At 15°C, the distance tunneled by acclimated termites was significantly greater than those of unacclimated termites. At 21-32°C, the acclimation period did not affect the distance tunneled. Tunneling rates were significantly lower at 15°C than at 21, 27, or 32°C. Tunneling rates at 27°C were significantly greater than at 21 or 32°C.

Most species of subterranean termites live deep within the soil, requiring workers to construct elaborate systems of tunnels to forage for food and water. The competence of a structural subterranean termite pest may, to a

1 Presented at the Symposium on Current Research on Wood-Destroying Organisms and Future Prospects for Protecting Wood in Use, September 13, 1929, Bend, Oregon. great extent, be determined by its foraging behavior. Williams (1977) categorizes foraging in subterranean termites into two basic patterns: species which forage over a long distance to larger, more scattered pieces of litter and species which forage close to the nest, consuming small pieces of litter. *Reticulitermes* spp. in the western United States clearly fall into the first category.

The habit of random foraging may be a distinct advantage for *Amitermes, Heterotermes*, and *Reticulitermes* spp. occurring in open areas or in ranges of dense but widespread vegetation types. Studies by Su and others (1984) suggest that the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) forages in a random network of tunnels. Within small plots, *Heterotermes aureus* (Snyder) was a random forager (Jones and others 1987). Descriptions of tunneling in the western subterranean termite (*Reticulitermes hesperus* Banks) of irregular reticulations with no particular plan also suggest a random foraging pattern (Pickens 1934).

Studies were conducted to determine various abiotic and biotic factors that might affect the random tunneling behavior of *R. hesperus*. The effects of soil particle size, borate compounds, soil treated with termiticides, the presence of dead termites, and temperature on tunneling behavior were studied.

### METHODS AND MATERIALS

### Size of Soil Particles

To determine the optimal particle size, termites were allowed to tunnel in soils with various particle sizes. Glass tubes (9 mm i.d. by 15 cm tall) were filled with soil with particle sizes ranging from 2.36 to 0.84, 0.84 to 0.42, 0.42 to 0.25, 0.25 to 0.18, and 0.18 to 0.15 mm (8 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100 mesh/in.). Twenty *R. hesperus* workers were placed in the bottom of each of five tubes for each range of particle sizes. The distance tunneled by the termites was recorded periodically for 24 h (Smith and Rust in press).

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### Influence of Borate Barriers on Tunneling

Termites were allowed to tunnel in glass tubes packed with a band of solid inorganic borate to determine its effect on their tunneling behavior. Calcium, magnesium, and zinc borate powders (provided by U.S. Borax Research, Anaheim, CA) were used in the study. The bottoms of glass tubes (9 mm i.d. by 15 cm tall) were closed with a cotton plug (1.8 cm long) and filled with 3.9 cm of untreated soil (20-100) mesh), a 1-cm thick layer of borate compound, and 6.5 cm of untreated soil. Cotton plugs (1.8 cm long) were placed in the top of each tube. The tubes were inverted, the cotton plugs were removed from the bottom, and 1.5-ml aliquots of water were added to the soils. This prevented the 3.9-cm untreated layer of soil from being contaminated with borate and termites from encountering any borate before the 1-cm layer. Twenty workers were placed in the bottom of each tube along with a small piece of paper towel to serve as food. The tubes were stoppered and set upright. Termites were allowed to tunnel for 48 h. The number of tubes in which termites penetrated the borate barrier and number of dead termites were counted at 48 h. Termites were removed from the tubes and placed in plastic Petri dishes (35 mm diameter by 10 mm tall) provisioned with a disk of clean paper towel. The dishes and termites were maintained at 25 + 2°C and 93 percent RH. The number of dead termites was counted 7 and 10 days after the termites were removed from the tunneling tubes. Five replicates were used for each of the three different borate compounds.

### Influence of Soil Termiticides on Tunneling

To determine the toxicity and repellency of soils treated with termiticides, termites were permitted to tunnel in glass tubes (9 mm i.d. by 15 cm tall) packed with untreated and treated soil (Smith and Rust in press). A 3.9-cm layer of untreated soil was gently packed into each tube followed by a 7.5-cm layer of treated soil. The tubes were inverted and 1.5-ml aliquots of water were added to the untreated soil. The water was drawn through the treated section with an air vacuum, allowing the soil to be uniformly moistened with minimal contamination of the untreated soils.

Twenty workers were introduced in the bottom of each tube. The distance tunneled by the termites was recorded after 48 h, at which time the termites were removed from the tubes and the number of mori bund and dead insects was counted.

### Avoidance of Dead Termites

To determine the effect of dead termites on the tunneling behavior of R. hesperus, termites were allowed to tunnel in soil and encounter dead termites. Glass tubes (9 mm i.d. by 15 cm tall) were filled with 3.9 cm of soil at the bottom.

Next, a layer of ten dead termites was added, which had been exposed to either soil treated with chlorpyrifos at 500 ppm for 24 h (Smith and Rust in press) or frozen for 24 h. Exposure to treated soil simulates mortality caused by contact with a chemical barrier, and freezing simulates natural mortality. The dead termites were covered with 7.5 cm of soil. Twenty live workers were added to the bottom of each of five tubes per treatment and allowed to tunnel 24 h. At 24 h, the number of tubes in which the termites penetrated the barrier of dead termites and the number of termites rendered dead or mori bund during the tunneling activities were recorded.

### Influence of Temperature on Tunneling

Studies were conducted to determine whether length of acclimation of termites to certain temperatures affected their rate of tunneling. To determine whether acclimation periods were important, 5 lots of 20 termites were held at 15, 21, 27, or 32°C or at room conditions (25°C) for either 12, 24, 36, or 48 h before testing. Glass tubes (9 mm i.d. by 15 cm tall) were filled with 11.4 cm of untreated soil. The soil was moistened with 1.5 ml of water. The tubes were placed in environmental chambers maintained at either 15, 21, 27, or 32°C for 12 h to condition the soil to the appropriate temperature. At the end of each termite acclimation period, twenty workers were placed in each of five tubes per temperature and allowed to tunnel for 12 h. The five tubes with acclimated termites and termites held at 25°C were placed back into the same acclimation chambers maintained at either 15, 21, 27 or 32°C. Distances tunneled were recorded at 12 h. Differences in the distances tunneled from each acclimation period and temperature were analyzed by an analysis of variance (ANOVA) using MSTAT: Micro Computer Statistical Program (Michigan State University, East Lansing), and means were separated by Tukey's HSD at the alpha = 0.05 level (Tukey 1953).

To determine the effect of soil temperature on tunneling rates, workers were permitted to tunnel in glass tubes (9 mm i.d. by 30 cm tall) filled with 25 cm of untreated soil and moistened with 3 ml of water. Groups of termites were acclimated at 15, 21, 27, and  $32^{\circ}\text{C}$  for 48 h. The tubes containing the moist soil were placed in environmental chambers maintained at either 15, 21, 27, or 32°C and allowed to condition for 12 h. After the 48-h acclimation period, twenty workers were placed in each of five tubes per temperature. The five tubes with termites were placed back at the original four acclimation temperatures. Distances tunneled were recorded at 2, 4, 6, 8, 10, 12, and 24 h. Tunneling rates at the four temperatures were determined by regression analysis of variance using MSTAT: Micro Computer Statistical Program (Michigan State University, East Lansing).

#### Size of Soil Particles

Rates of termite tunneling in tubes filled with soils of different particle sizes were as follows:

| <u>Parti cl e</u> | Distance tunneled | Rate ± 95pct    |
|-------------------|-------------------|-----------------|
| size (mm)         | Total cm (h)a     | CLb (cm/h)      |
| 2. 36-0. 84       | 0                 | 0               |
| 0.84-0.42         | 24. 9 (115)       | $0.22 \pm 0.09$ |
| 0. 42-0. 25       | 32. 5 (115)       | $0.28 \pm 0.11$ |
| 0. 25-0. 18       | 28. 8 (100)       | $0.29 \pm 0.13$ |
| 0. 18-0. 15       | 30. 3 (100)       | $0.30 \pm 0.21$ |

- <sup>a</sup> Total distance tunneled in (total cumulative hours)
- $^{\mbox{\scriptsize b}}$  Mean rate with 95 pct confidence level in cm/hr

Consequently, soil with particle sizes  $\geq 0.15$  and  $\leq 0.84$  mm were used in subsequent studies to ensure optimal tunneling activity.

Size of the substrate particles is important in determining whether subterranean termites can tunnel through soil. Ebeling and Pence (1957) showed that soil with particle sizes ranging from 3.35 to 1.18 mm (6-16 mesh/in.) were impenetrable to *R. hesperus*, and soil with particle sizes ranging from 0.3 to 0.15 mm (50-100 mesh/in.) were penetrable. For the Formosan subterranean termite, *C. formosanus*, it was determined that penetration occurred in untreated substrates with particle sizes ranging from 0.8 mm to <0.2 mm (20 to 80+ mesh/in.); particle sizes from 2.4 to 1.7 mm (8-12 mesh/in.) completely prevented penetration (Tamashiro and others 1987).

Substrates with particle sizes  $\geq 3$  mm were easily penetrated by the subterranean termites studied thus far; termites were able to move through the spaces made by these larger particles (Ebeling and Pence 1957, Tamashiro and others 1987). Soils that are impenetrable to termites have particles that are too large for termites to pick up in their mouths and move, yet with spaces between particles small enough so that termite headcapsules will not pass through. The size of particles that are penetrable to termites will vary with each species.

#### Influence of Borate Barriers on Tunneling

R. hesperus workers penetrated 60 percent of the calcium borate barriers and 100 percent of the magnesium borate and zinc borate barriers. There was 42 percent mortality of termites exposed to calcium borate barriers at 48 h, but no substantial mortality with the other two barriers. Termite mortality was 87, 98, and 89 percent in tubes packed with calcium, magnesium, and zinc borates, respectively, 10 d after removal from the tubes. The solid borate layers were not repellent to worker termites, and contact with or ingestion of them caused significant latent mortality.

#### Influence of Soil Termiticides on Tunneling

As shown in figure 1, termites did not tunnel into soil treated with bifenthrin or cypermethrin, regardless of concentration. No tunneling occurred in soil treated with chl ordane, chl orpyri fos, fenval erate, i sofenphos, or permethrin at concentrations ≥50 ppm. In addition to preventing tunneling into treated soil, the above soil termiticide treatments also severely reduced tunneling activity in the untreated zone beneath the treated soil (fig. 1). Termites tunneled in soil treated with chlordane, chlorpyrifos, isofenphos, fenvalerate, or permethrin at concentrations ≤10 ppm. The most tunneling occurred in soils treated with chlorpyrifos and isofenphos, whereas very little tunneling occurred in the soils treated with chlordane, fenvalerate, or permethrin at these lower concentrations (fig. 1). After 48 h, termites were removed from the soil-filled tubes. The greatest mortality (76 to 100 percent) occurred in the tubes filled with soils treated with 100 to 500 ppm of bifenthrin or chlorpyrifos (table 1).

Termites did not tunnel in treated soil, especially in soil treated at high concentrations, yet substantial mortality occurred. Insecticide diffused from treated to untreated soil in concentrations high enough to kill or affect behavior. Movement of insecticide seems to be facilitated by water solubility or vapor pressure. Chlordane appeared to move the least distance, while bifenthrin and cypermethrin moved the farthest, because termites tunneled closest to soil treated with chlordane and never

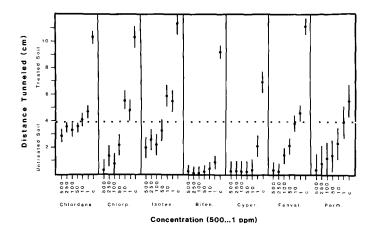


Figure 1--Average distance tunneled by  $\mathcal{R}.$  hesperus underneath treated soils and in soils treated at 500 to 1 ppm with various termiticides. Dashed lines indicate common boundary of treated and untreated soil. Vertical bars represent standard errors of means. Chlorp. = chlorpyrifos, Isofen. = isofenphos, Bifen = bifenthrin, Cyper. = cypermethrin, Fenval. = fenvalerate, Perm. = permethrin, c = control (Smith and Rust in press).

Table 1--Percent mortality of R. hesperus after tunneling 48 h in tubes filled with termiticide-treated soils.  $^{\rm 1}$ 

| Concentration of termiticide (ppm) |      |     |    |    |    |    |
|------------------------------------|------|-----|----|----|----|----|
| Termi ti ci de                     | 500  | 250 | 10 | 50 | 10 | 1  |
| Bi fenthri n                       | 100  | 100 | 76 | 29 | 2  | 9  |
| Chl orpyri fos                     | 90   | 95  | 85 | 51 | 10 | 42 |
| Cypermethrir                       | n 50 | 27  | 1  | 5  | 0  | 44 |
| Fenval erate                       | 32   | 4   | 52 | 0  | 0  | 2  |
| Isofenphos                         | 26   | 39  | 42 | 40 | 0  | 40 |
| Chl ordane                         | 22   | 20  | 4  | 2  | 19 | 21 |
| Permethri n                        | 4    | 6   | 1  | 3  | 3  | 3  |

<sup>&</sup>lt;sup>1</sup> Adapted from Smith and Rust (in press).

approached the soil treated with bifenthrin or cypermethrin (fig. 1). Apparently chlordane is not as repellent as bifenthrin and cypermethrin. At high concentrations less mortality occurred in soils under chlordane-treated soil than in soils under the bifenthrin and cypermethrin treatments, even though termite tunneling was severely reduced. This suggests that biologically active amounts of bifenthrin and cypermethrin were moving longer distances. At low concentrations, no significant mortality was produced underneath bifenthrin and cypermethrin treatments, and very little tunneling was observed in the untreated soil.

We hypothesize that the alpha-cyano pyrethroids produced sublethal effects which may inhibit tunneling, perhaps by temporarily paralyzing termites that contact them. Scott and Matsumura (1983) reported two types of pyrethroid action against cockroaches. Type I action was related to a factor which causes repetitive neuronal discharges, whereas type II action was related to suppressing repetitive discharge and causing immobility. The presence of an alpha-, may lead to type II action. Some pyrethroids with and without alpha-cyano moieties, such astype I and type II action (Scott and Matsumura 1983). This might explain why there was more tunneling in or underneath soils treated with fenvalerate and permethrin than there was underneath soil treated with bifenthrin or cypermethri n.

Factors causing repellency of termites to soils treated with insecticide are unclear. Su and others (1982) classified insecticides as type I or type II, according to behavioral responses of C. formosanus. Type I insecticides (e.g., pyrethroids) killed very few termites because the termites sealed or avoided tunnels that extended to treated areas, and therefore were considered repellent. Type II insecticide treatments (e.g., chlordane and chlorpyrifos) also killed few termites, but survivors avoided the treatment as dead and decaying termites accumulated in the tunnels, not in response to insecticide-treated media. Consequently, type II insecticides were not considered repellent.

In our studies with *R. hesperus* fenvalerate and permethrin performed like type I insecticides, killing very few termites and preventing tunneling in treated soil. The pyrethroids bifenthrin and cypermethrin, however, provided very different effects. They prevented tunneling by immobilizing the insects. Chlordane-treated soils performed as a type I insecticide, and chlorpyrifos did not elicit type I or type II responses. Termites tunneled into soils beneath the chlorpyrifos treatment, resulting in high mortality, but did not tunnel into the treated soil.

When soils were treated with high concentrations of all these termiticides, the amount of tunneling was negligible. Some tunneling occurred when soils were treated with lower concentrations. Biologically active amounts of insecticide move from the upper layers of treated soil to untreated soils below, causing reduced tunneling, mortality, and sublethal effects. This downward movement may increase the effective depth of the primarily surface applications of insecticides. Even though environmental exposure may reduce the initial levels (500 ppm) of soil treatments, it is likely that the soils will retain their repellency for a number of years. There is a considerable amount of research yet to be conducted on the factors that affect longevity and movement of insecticide in the soil, as well as the behavior of termites encountering these termiticide-treated soils.

#### Avoidance of Dead Termites

Workers of *R. hesperus* did not penetrate the layer of dead termites which were killed by exposure to chlorpyrifos, and there was 96 percent mortality from their tunneling efforts. Termites penetrated the layer of termites killed by freezing in 80 percent of the tubes and there was 30 percent mortality. In the untreated controls, workers tunneled to the top of all the tubes with only 5 percent mortality. The layer of termites killed with chlorpyrifos was not penetrated by *R. hesperus* workers; contact with the treated termites was enough to kill

significant numbers of termites within 24 h. The layer of "naturally" killed termites was not completely repellent to tunneling termites, but did produce some mortality. The appearance of dead and decaying termites may lead to the avoidance of treated areas as suggested by Su and others (1982). However, it may also be that toxicant on the dead termites also contributes to avoidance behavior in addition to mortality.

#### Influence of Temperature on Tunneling

Regardless of acclimation periods, the tunneling rates of termites exposed to soil temperatures of 21, 27, and 32°C were not significantly different from the tunneling rates of unacclimated termites at these same temperatures. At 15°C, the distance tunneled by termites acclimated for 12, 24, 36, or 48 h was significantly greater than those tunneled by unacclimated termites (fig. 2).

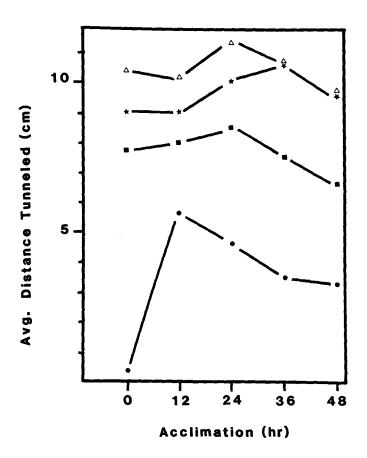


Figure 2--Average distance tunneled by R. hesperus at four different temperatures and five acclimation periods. Circle =  $15.6^{\circ}$ C, square =  $21.1^{\circ}$ C, star =  $26.7^{\circ}$ C, triangle =  $32.2^{\circ}$ C.

At 15°C, termite tunneling rates (0.1 cm/h) were significantly less than at 21, 27, or 32°C (F = 17.65; df = 3; P < 0.01). At 21 and 32°C, there were no significant differences in termite tunneling rates (0.39 and 0.33 cm/h, respectively). Tunneling rates at 27°C were significantly greater than at 15 and 21°C (F = 70.58; df = 2; P < 0.01) (fig. 3).

As termites forage from subterranean galleries where soil temperatures are relatively constant, worker termites will be exposed to elevated temperatures. The rates at which they tunnel will influence the time spent contacting treated soils and the time exposed to elevated and possibly lethal temperatures. The study suggests that termites leaving subterranean galleries do not require acclimation to the elevated surface temperatures, whereas returning termites may require time to acclimate to cooler subterranean conditions.

The above studies show that both abiotic and biotic factors affect the tunneling behavior of *R. hesperus*. Certain physical and chemical barriers have the ability to affect tunneling behavior most drastically. The potential effects of biotic factors such as temperature and of the appearance of dead termites in galleries on the performance of chemical barriers are evident but need further examination.

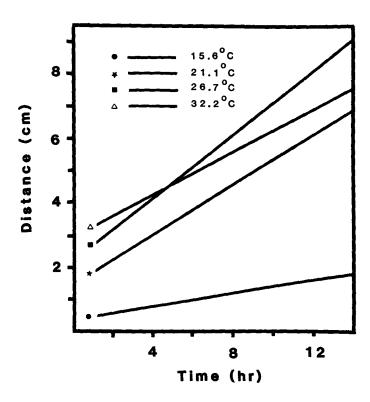


Figure 3--Regression lines of distance tunneled by *R. hesperus* at four different temperatures. 15.6°C, y = 0.44 + 0.10x; 21.1°C, y = 1.46 + 0.39x; 26.7°C, y = 2.21 + 0.49x; 32.2°C, y = 2.98 + 0.33x.

#### **ACKNOWLEDGMENTS**

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# The Potential of Using Acoustical Emission to Detect Termites Within Wood<sup>1</sup>

#### Vernard R. Lewis Richard L. Lemaster<sup>2</sup>

Abstract: Acoustical emission (AE) equipment was used to detect drywood termites <a href="Incisitermes minor">Incisitermes minor</a> in ponderosa pine <a href="Pinus ponderosa">Pinus ponderosa</a> blocks under laboratory conditions. Using a 60 kHz transducer, AE levels were recorded for 0, 5, 10, 15, and 20 termites per block. The association of AE and varying numbers of drywood termites best fit an exponential curve. These data suggest that the detection capabilities of AE increase with increasing numbers of termites. The implications of this finding to the potential use of AE in detecting termites under field conditions are discussed.

In California, as many as 73 species of insects have been reported to cause damage to wooden structures (Ebeling 1975). Of these species, the subterranean termite Reticulitermes hesperus Banks and the drywood termite Incisitermes minor (Hagen) have had the greatest economic impact. Each year, more than 1.5 million inspection reports on termites and other wood-damaging pests are filed with the California Structural Pest Control Board (SPCB). Notices of Corrective Actions against these pests annually exceed 250,000, and the yearly costs of these inspections and control measures exceed \$350 million (Brier and others 1988).

In the past, control of termites within buildings relied heavily on the use of chlordane. However, because of problems involving environmental contamination and pesticide-related injuries, chlordane, along with many other pesticides, are no longer used or registered for control of wood-destroying insects. Since the discontinuance of the use of chlordane for subterranean termite control, recent reports reveal that termite control failures have increased as much as 30 percent (Katz 1989), and often result in additional callback treatments. As the number of callback treatments increases, there is concern that pesticide usage in urban areas, already more

than 10 lb/acre, may also increase (National Academy of Sciences 1980). As a consequence, methods for reliable and accurate detection and control of termites are needed.

Although new research shows promise for the development of nonchemical barriers, biological controls, attractants, and repellents for termites (Rust and others 1988), little has been done to improve inspections of structures. The use of electronic stethoscopes and dogs for detecting termites is gaining in popularity, though their utility has not yet been rigorously tested. Onsite visual inspection is still the dominant detection technique used by the industry. Unfortunately, visual inspection is highly subjective. Consequently, many of the 1,000 complaints filed each year with the California SPCB result in litigation.

Acoustic emission (AE) (the generation, transmission, and reception of energy in the form of vibrational waves in matter) (Kinsler and others 1982) offers a potentially useful innovation for improving the detection of termites. The early applications of AE were for detecting defects and sounds emanating from metals (Dornfeld and Kannatey-Asibu 1980); however, new applications have been developed for machined wood (Lemaster and Dornfeld 1987), and sapwood trunk samples (Haack and others 1988). Although most AE studies are for stored grains and fruit-boring insects (Hansen and others 1988, Webb and others 1988), in Japan the Formosan subterranean termite (Coptotermes formosanus Shiraki) has also been studied (Fujii and others 1990).

Because of the economic losses caused by termites in California, and the need for improved detection of wood-destroying insects, we initiated a pilot study on the use of AE for detecting termites. The insect species studied was the drywood termite <a href="Incisitermes minor">Incisitermes minor</a>. This species was chosen because of its importance as a wood-damaging pest and ease in laboratory rearing.

#### METHODS

A 6-mm diameter hole was drilled at the center of fifty ponderosa pine (Pinus ponderosa Dougl. ex Laws.) blocks (25 mm by 25 mm by 106 mm long). Each block was assigned to one of five groups of 10 specimens each. The first group had 5 drywood termites inserted into each block. The number of drywood termites for the next three groups was increased by a multiple of 5, resulting in 10, 15,

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and 20 termites per block. The last group had no termites placed in the blocks, and served as the control. Each block was inspected using an acoustic emission amplifier (100 dB) and 60 kHz transducer. The emission signals emanating from each block for two 30-second periods were recorded at a threshold voltage of 1.1 volt and were input into a computer for analysis.

#### RESULTS

As the number of drywood termites increased within sample blocks, the AE that were produced increased exponentially (fig. 1). The amount of variance explained by this model was large ( $\rm r^2$  = 0.77). Multiple comparisons revealed significant differences among mean AE counts and drywood termite densities (table 1;  $\rm F_{4,97}$  = 5.57, p < 0.0005). Sample blocks with termites had AE counts three times greater than blocks with 5 termites, six times greater than blocks with 10 termites, three times greater than blocks with 15 termites, and approximately thirty times greater than controls. Blocks with 5, 10, and 15 termites were not significantly different from controls.

#### DISCUSSION

The use of sound vibrations to detect termites in wood is not new. Thirty-five years ago, electronic stethoscopes were first proposed to detect termites (Pence and others 1954). This technology relied on the production of audible sound waves and the ability of the human ear to perceive them. The authors claimed that this device could detect the footfall sounds of a single termite. However, widespread adaptation of this new technology did not occur, possibly because (1) only a limited band of the total sound spectrum could be utilized (e.g., sound in the non-audible frequencies is also produced by termites), (2) large differences exist in hearing ability among individuals, and (3) false readings were produced due to excessive background noise levels.

More recently, workers in Japan have used AE equipment to detect Formosan subterranean termites inside wood (Fujii and others 1990). They demonstrated that AE increased as the number of termites increased; however, the type of association (e.g., linear or nonlinear) was not mentioned. The results of our analysis reveal an exponential relationship between termite number and AE. This finding suggests (1) the sensitivity of AE equipment increases exponentially with increases in drywood termite number and (2) drywood termite behavioral interactions increase exponentially with increases in termite number. However, because closed wooden blocks were used to house termites, it was impossible to tell whether the AEs recorded were derived from feeding, head-banging, walking, or background noise level. Experiments currently under way at the University of California Forest Products Laboratory utilizing a video camera, recorder, AE sensor, and wooden blocks with plexiglass fronts will allow direct observation of

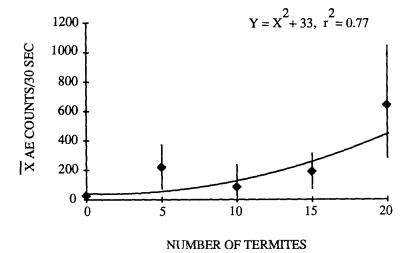


Figure 1-- Nonlinear regression model for mean acoustic emission (AE) counts and number of drywood termites inside ponderosa pine blocks. Vertical bars represent  $\pm$  SD.

Table 1--Mean acoustic emission (AE) counts for five densities of drywood termites inside ponderosa pine blocks.

|         | X AE counts (+SD)/30 sec <sup>2</sup> |  |  |  |
|---------|---------------------------------------|--|--|--|
| Density |                                       |  |  |  |
| 0       | 23.3 (7.5)a                           |  |  |  |
| 5       | 232.1 (163.9)a                        |  |  |  |
| 10      | 104.3 (128.3)a                        |  |  |  |
| 15      | 184.3 ( 92.6)a                        |  |  |  |
| 20      | 631.0 (405.6)b                        |  |  |  |

<sup>&</sup>lt;sup>1</sup>Each mean is based on AE recorded twice from each of 10 pine blocks.

termite behavior during AE readings. These concurrent behavioral and acoustical observations will improve the correlation of AE events and related termite behaviors, as well as outline peak AE activity periods during the day.

Additional areas of study are needed and include characterizing AE from different species of insects and wood and determining the number of sensors needed to inspect large pieces of wood. These later studies will better gauge AE as a diagnostic tool for detecting wood-damaging insect pests. This information is crucial for complying with the appropriate treatment recommendations mandated by the Structural Pest Control Board Act of California.

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# Direct Colony Baiting of Termite Colonies: A Tool for Ecological Studies<sup>1</sup>

#### Don McG. Ewart<sup>2</sup>

Abstract: The benefits of direct colony baiting are described: bait substrates enclosed in polyvinyl chloride tubes are applied in direct contact with the galleries of the termite nest. Attention of researchers is drawn to the potential of this method for species other than the mound-building <u>Coptotermes 1acteus</u>.

In the post-organochlorine era, knowledge of termite biology and behaviour is a precursor to effective control. This paper describes Australian experiences with termite baiting techniques and future directions for baiting research.

#### EXPERIENCE WITH BAITING IN AUSTRALIA

I first used baits to locate termites in 1979, after the method of La Fage and others (1973) as part of a study of the effects of removal of ground-wood by campers in Wyperfeld National Park in the desert of northwestern Victoria. By using rolls of toilet paper as baits and experimentally manipulating the wood supply it was possible to clearly show that fire-wood collection greatly diminished termite numbers (Ewart 1979).

Baiting for termites in Australia has a long history, mostly the work of the CSIRO<sup>3</sup> Division of Entomology in Canberra (Gay and others 1957). One enduring technique is the use of wood dowels to detect termites in trees

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(Greaves 1964). Dowel baits facilitate the detection of termites in living trees, without significant damage to the tree (Greaves and Florence 1966). The technique is simple: a hole is bored in the tree at about chest-height, and a snug-fitting dowel of a palatable eucal ypt species is inserted to at least half the diameter. The dowel is likely to intercept termite galleries towards the center of the tree, where galleries are more abundant in the old heartwood. Within a few days, the dowel may be removed for inspection. The presence of termites may be determined by observing (i) fecal spotting on the bait, or (ii) cavities resulting from consumption of bait as food or excavation to re-open blocked galleries. If a suitable level of activity is detected, the dowel-hole may be treated with an insecticide, typically arsenic trioxide, although organochlorines and other termiticides have been used. The hole represents a potential entry point for other pests (Anon 1987), but this may be prevented by treatment with dieldrin (Greaves and Florence 1966).

Along the Great Dividing Range of eastern Australia and extending to Tasmania grow the tallest flowering plants in the world. These are eucalypts of the subgenus Monocalyptus, fast growing fire-climax trees which typically grow in single-species stands. Their timber, though non-durable, is prized for indoor use and for paper pulp. In the early 1980's I began to examine termite attack on one of these species, Eucalyptus regnans Mueller, the Mountain Ash.

In Victoria, vast even-aged forests of this species, which dated from a particularly bad cluster of wildfires in 1939, were just then becoming of merchantable age. Early logging suggested that the trees contained a higher than expected rate of defect due to termites. Attacks on the heartwood of living trees were by <u>Coptotermes</u> species and a dampwood, <u>Porotermes</u> adamsoni (Froggatt).

Extraction of termites from mountain Ash is laborious and difficult and, unless you are prepared to sacrifice valuable sawlogs, involves coordination with commercial harvesting--following the fallers and collecting wherever possible. This is both extremely dangerous and unproductive.

Given my earlier desert experiences, I preferred to have the termites come to me. I tried a range of baiting techniques with the aim of monitoring foraging activity: transects of rolls of toilet paper, buried wood blocks and dowel baits in standing trees. In Mountain Ash forests, it soon became clear that these termites do not normally forage near the surface of the ground, but are almost entirely restricted to the bole of the tree. My study was stalled for lack of access to the animals and was shortly thereafter dealt a death blow by wildfire.

Wildfire did not necessarily affect the termites, but it drastically changed forest management and meant that the study sites would soon be clear-felled. It was at this rather low point that I first met Dr Jeffrey La Fage whilst he was visiting Dr John French. After some searching discussions and some particularly penetrating questions, I switched to baiting the termites where it was certain that they would be continually present—in their own mounds.

#### MOUNDS OF COPTOTERMES LACTEUS

Nests of <u>Coptotermes</u> <u>lacteus</u>
(Froggatt) are typically formed in wood, usually within the center of a standing tree. The upper surfaces of the nest are always protected by a thick clay wall and, when the parent tree is lost, or the colony grows beyond the limits of the timber, an irregularly shaped mound is formed with the clay wall as the outermost surface (fig. 1). The outer wall is 20 to 60 cm thick and protects an extensive periecie. The spherical endoecie (or nursery) is situated at or below ground level.

Direct baiting of mounds had been tried before without success; the experience then was that baits applied to the wall of the mound were soiled in place, but not attacked (Hunt and Snyder 1931, Gay and others 1955). With corrugated cardboard in a short length of polyvinyl chloride (PVC) tubing, termites can be drawn from the periecie to bait units on the mound surface (French and Robinson 1985).

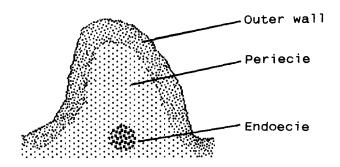


Figure 1--Schematic cross-section of a mound of <u>Coptotermes Lacteus</u>.

This is not an entirely unnatural way of presenting food. Mounds initially form after tree-fall or premature cutting, and <u>C</u>. <u>lacteus</u> readily forage on branches or debris that either contact the exposed periecie or lie on the outer wall. Breaching of the wall or the accumulation of woody debris on the surface of the mound, or both, are not unusual circumstances. The PVC "fistula" bait units (French and Robinson 1985) merely standardize this food source.

#### BALT SUBSTRATES

During trials to identify potential bait substrates, cork (bark of <u>Quercus suber</u> L.) was serendipitously discovered to be highly susceptible to attack (French and others 1986). Indigenous eucalypt timber and exotic softwood (<u>Pinus radiata</u> D. Don), although taken by termites in laboratory colonies (Gay and others 1955, Lenz and Barrett 1984), suffered only minor, erratic attack in the bait units, whereas with cork, a high proportion of baits were reliably attacked (fig. 2).

#### BENEFITS OF DIRECT COLONY BAITING

As a tool for the study of the ecology and biology of termites, direct baiting of termite colonies has many advantages over baits dispersed throughout their foraging territories. Response by the termites is obligatory-the bait substrate (or feeder strip) is applied in direct contact with freshly severed termite galleries. This security of response allows for the use of smaller sample sizes, with consequent economies of effort and expenditure.

Variation in response to baits due to the (mostly seasonal) fluctuations in the

effective size of foraging territories is minimized. Termites (<u>C. lacteus</u> at least) will forage close to the nest at times of climatic extremes.

There can be absolute certainty as to which species and colony are responding to individual baits (and replicates). A major problem with area baitings that use rolls of toilet paper or graveyard stakes is that considerable uncertainties regularly arise as to the identity of species/colonies responsible for a fair proportion of the attack (Ewart 1979, Ferrar 1982, French and Robinson 1980, Usher 1975).

With direct colony baiting, there is no need to employ large-footprint baits to attract termites (Akhtar and Jabeen 1981, French and Robinson 1980, Usher and Ocloo 1974) or for baits large enough to sustain attack for the long exposures typically required to sustain significant attack (Howick and Creffield 1983). Baits may be small, and the tube to the periecie need only be of sufficient diameter to accommodate the expected number of foragers. Thus there are savings to be had from the use of smaller baits, and time savings as a turnover of baits from 7 to 28 days becomes effective.

Wood blocks, stakes and rolls of toilet paper all suffer from the errors as they become cemented to the surface of the soil. Withdrawal of stakes often leaves many termites lost or crushed because of scraping, and inspection of toilet paper may cause the rolls to disintegrate, leaving many termites in

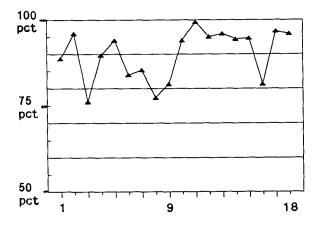


Figure 2--Success rate of cork baits: Percentage of baits with termites present (*n*=80, 20 mounds, 4 baits per mound) at 18 consecutive monthly samples.

fractured galleries on the soil surface. By enclosing the bait in a close-fitting PVC tube, these errors are avoided.

Inspection of the bait creates a clean break of galleries across a small surface area--and the tube may be replaced in exactly the same position, so that the integrity of the galleries is restored. Thus inspection of baits can occur with almost no disruption to the activity of the termites. Swapping of baits takes only a few seconds--one PVC tube is replaced by another containing a new bait block. Thus there is no need to wait for termites to discover the new block, as there has effectively been no interruption of exposure. This is a substantial improvement over the use of stakes, buried blocks, or rolls of toilet paper, because swapping of these baits causes significant damage to galleries and often requires the experimenter to re-excavate or level the surface.

For all but a few species, when it is possible for the experimenter to directly observe foraging termites, it is likely that their normal behaviour has been disrupted by the conditions necessary for observation. The use of direct colony baiting and PVC fistulas enables instantaneous sampling of foraging groups. The bait unit can be removed and replaced before the termites have time to respond. Thus the termites collected by this system represent an instantaneous sample, delimited by an arbitrarily defined, but regularly replicable, break in the galleries at the end of the removable bait unit. The problems of galleries adhering to the bait are overcome by regulating these additions to a constant volume surrounding the bait block. The experimenter may transport the bait tube in a large vial, contents intact, for later dissection in the laboratory.

#### OPPORTUNITIES FOR OTHER SPECIES.

The system I have described is clearly well suited to sampling  $\underline{C}$ . Lacteus in mound populations, but just as it has its roots in the early toilet paper roll studies of the Sonoran Desert (La Fage and others 1973) and  $\underline{C}$ . Formosanus in Cypress stumps (La Fage and others 1973, 1975, 1976), and  $\underline{C}$ . Formosanus in Cypress stumps (La Fage and others 1983), it too can be modified for application to non-mound colonies. Treebased colonies are an obvious opportunity, as are some entirely subterranean species. The method is proven, the opportunities remain.

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## Behavioral Ecology of Subterranean Termites and Implications for Control<sup>1</sup>

#### J. Kenneth Grace<sup>2</sup>

Abstract: Subterranean termites are important structural pests in much of North America, and worldwide. Recent studies of eastern subterranean termite (*Reticulitermes flavipes* [Kollar]) colonies in Ontario, Canada, indicate that these colonies contain greater foraging populations and forage over larger territories than was previously thought to be the case. These results are consistent with those obtained elsewhere with Coptotermes formosanus Shiraki and Heterotermes aureus (Snyder). Implications for the development of baiting techniques for termite control are discussed, and several laboratory investigations of potential bait toxicants and insecticidal dusts are reviewed. Behavioral chemicals are also potentially useful in termite control, and bioassays with tree extractives indicate that semiochemicals affecting termite orientation offer an explanation for the observed pattern of R. *flavipes* infestation in street trees in Toronto.

The eastern subterranean termite (Reticulitermes flavipes [Kollar]) (Isoptera: Rhinotermitidae) has a broad distribution in North America, extending from the southeastern United States to the Great Lakes (Weesner 1970). At the northern edge of this distribution, R. flavipes was first reported at Point Pelee, Ontario, in 1929, and was apparently introduced to Toronto in infested materials brought by ship from the United States about 1935 (Kirby 1965; Urquhart 1953). To date, infestations have been reported from thirty municipalities in southern Ontario, with Kincardine (44°11'N, 81°38'W) representing the northernmost site of established R. flavipes infestations. Outside of buildings, flights of R. flavipes alates are rare in Ontario, and the disjunct distribution of termites in the province results from movement of infested wood (firewood or used lumber). In 1987, a Reticulitermes infestation of several years standing was also discovered in a block of homes in Winnipeg, Manitoba, indicating the potential for further northern distribution of this genus.

#### TERMITE TERRITORIES AND BAITING STRATEGIES

Grace and others (1989) recently evaluated the size of R. flavipes foraging territories and populations at two sites in metropolitan Toronto. These mark-release-recapture studies employed the dve Sudan (Fat) Red 7B (Grace and Abdallay 1989) with methodology similar to that of Su and Scheffrahn (1988) with *Coptotermes formosanus* Shiraki and Jones (1987) with Heterotermes aureus (Snyder). As was the case with these latter two species, eastern subterranean termite colonies were found to cover larger areas and contain greater foraging populations than previously suspected. At one site, the foraging population was estimated at 3.2 million termites. moving over a territory of 1,091 m<sup>2</sup> (Grace and others 1989). Continuing studies at these two field sites and at other locations in Ontario have confirmed the general applicability of these results (Grace 1990a). Additional dyes (Grace and Abdallay 1990a) would be useful in improving the precision of population estimates.

It is interesting to note that the mark-release-recapture studies with R. flavipes were conducted around buildings chemically treated for subterranean termite control, emphasizing that the "chemical barrier" around treated structures has little effect on termite populations outside that narrow band of pesticide-treated soil. As is mentioned elsewhere in these proceedings, the use of toxic baits appears to be the most reasonable method of attacking these populations. In regions where subterranean termites are ubiquitous, such baits would likely be employed to extend the protected area outward from the structures at risk. Baits would have to remain in place following the death of colonies in the baited zone to intercept foragers entering the area from expanding colonies in adjacent areas, as well as foragers from any new alate-founded colonies. However, in northern regions with a disjunct pattern of Reticulitermes infestation and limited occurrence of alate flights. it should be possible to eradicate isolated infestations, with a low probability of re-introduction. Active foraging by colony groups over large areas implies that bait placement is perhaps less critical than would be the case with localized foraging parties, and that a few baits could affect a large termite population impinging upon multiple buildings.

Toxicants intended for use in baits should be nonrepellent and slow-acting (Su and others 1987). Certain borate compounds (Grace 1990b) fit these criteria, although rather high dietary concentrations are required to kill termites. An alternative, although more labor intensive, approach may be to apply borate (or other insecticidal) dusts in a toxic variation of mark-release-recapture methodology. Grace and Abdallay

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(1990b) demonstrated in laboratory assays that coating 5-10 percent of the individuals in groups of 20-40 *R. flavipes* workers with boric acid or barium metaborate dusts resulted in 28-100 percent mortality within 16 days. Subterranean termite foragers collected in traps such as those described by Grace (1989) and Su and Scheffrahn (1986) could be coated with such toxic dusts and released back into the traps to poison other colony members through mutual grooming behaviors. Collections from multiple traps at each site and multiple capture and release cycles could be employed to treat a sufficiently large proportion of the population to kill the entire colony.

An alternative to the use of chemical insecticides in termite baiting systems is the use of microbial biological control agents. such as nematodes or fungi. In Ontario, and other areas of subterranean termite introductions, surveys of the mycoflora associated with termites may reveal potential pathogens. Zoberi and Grace (1990a) isolated 40 fungal species from R flavipes and associated materials. Several of these fungi have been previously reported to be facultative insect pathogens, and a number (e.g., Arthrobothrys oligospora Fresenius, Cunninghamella echinulata Thaxter, and Rhizopus stolonifer Ehrenb. ex Fr.) appear to be detrimental to termite survival in preliminary bioassays. The well-known pathogenic fungus Beauveria bassiana [Balsamo] Vuillemin was also recently isolated from R. flavipes workers infesting a street tree in Toronto (Zoberi and Grace 1990b).

Although attractant or arrestant semiochemicals would be useful in masking otherwise repellent bait toxicants, the high level of foraging activity of R. flavipes at field sites in Ontario indicates that neither decayed wood nor attractant chemicals are necessary prerequisites to implementation of baiting techniques. so long as the feeding substrate or bait toxicant within the collection unit is not repellent. Use of a collection trap is certainly more labor intensive than placement of the toxicantimpregnated decayed-wood blocks originally used to control termites in this manner (Ostaff and Gray 1975). However, it also offers more control possibilities (such as incorporating dusts) and a means of quantifying the decline in the termite population by post-treatment monitoring and mark-releaserecapture methods. Post-treatment bait units, supplemented by wooden stakes, could remain in place both to confirm the efficacy of the control method and as an aid in subsequent inspections of the property for new termite infestations.

#### SEMIOCHEMICALS IN ORIENTATION AND FEEDING

Eastern subterranean termite responses to behavioral chemicals (semiochemicals) may help to explain observed patterns of termite foraging, and may also be useful adjuncts to baiting systems. Low concentrations of the microbicide TCMTB, for example, are repellent to *R. flavipes*, and might protect wood from termite attack more effectively than toxic but nonrepellent preservatives (Grace 1988). Attractants and arrestant semiochemicals, on the other hand, might be used to enhance feeding on baits and to direct foragers towards baits or toxicant-treated soil. *Reticulitermes* workers are able to orient to chemical gradients, both on two-dimensional trails (Grace

and others 1988) and diffused through the soil (Clement and others 1988).

In Toronto and other northern locales, *R. flavipes* infests living trees as well as cellulosic debris and wood in service. Shelter tubes are constructed in bark fissures upward from the base of the tree stem, and termites are active between the inner and outer bark. Although feeding is limited to dead portions of the tree, such as the heartwood and fungus-decayed limbs, some scarring of the bark and sapwood surfaces is also apparent.

In 1980, an inspection of 17,800 street and park trees in metropolitan Toronto revealed termite shelter tubing on 4 percent of those trees (Cooper and Grace 1987). Subsequently, two tree species commonly occurring in Toronto were selected to determine whether chemical factors were involved in the pattern of differential infestation of street trees observed in the 1980 survey. Subterranean termite tubing was found on 19.3 percent of the horsechestnuts (*Aesculus hippocastanum* L.) (Hippocastanaceae) inspected, ranking it as a frequently infested tree species. On tree of heaven (*Ailanthus altissima* [Mill.] Swingle) (Simaroubaceae), however, shelter tubing was noted on only 0.7 percent of the trees inspected, suggesting that it is rarely infested (Cooper 1981).

The bark, sapwood, and heartwood of these two tree species were each extracted in a series of solvents (chloroform:acetone, methanol, acetone, hexane), and the resulting extracts applied to filter paper and exposed to *R. flavipes* workers in no-choice laboratory assays (Grace, in preparation). All extracts were assayed at 4 percent (weight/weight) concentrations, although the yields from extraction ranged from 0.3-10.2 percent (wt./wt.). Only the hexane extract of *A. altissima* heartwood caused significant termite mortality within 15 days. However, the yield of hexane extractives from *A. altissima* heartwood was only 0.3 percent (wt./wt.). Since it was assayed at a 4 percent concentration, this suggests that less mortality might be expected from termite feeding on the natural substrate.

The orientation responses of individual *R. flavipes* workers to these extracts were evaluated in behavioral assays (Grace, in preparation). In these assays, a single worker was placed between a 23 mm diameter paper disk treated with 4 percent (wt./wt) extractives and a second solvent-treated control disk in a small glass petri dish. The position of the worker (in contact with either or neither paper disk) was recorded every 30 seconds over a 20-minute interval. This assay was repeated with 50 workers exposed to each of the 12 extracts from the two tree species, and the proportions in contact with the extract-treated and the control disks compared (paired-comparisons t test). Seven of the 12 horsechestnut extracts elicited significant ( $p \le 0.05$ ) positive orientation responses, and 3 elicited significant negative responses. The opposite trend

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was observed with tree of heaven, for which 7 of the solvent extracts elicited negative responses, and only two elicited positive responses. In addition, the *A. hippocastanum* extractives eliciting the strongest positive orientation responses and the *A. altissima* extractives eliciting the strongest negative responses were present in high concentration in their respective wood matrices. Thus, semiochemical-mediated orientation behavior and initiation of feeding on a particular tree species may explain differential infestation of otherwise equally suitable substrates.

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# Baiting Techniques for Control of *Coptotermes* Species Within Existing Buildings in Australia<sup>1</sup>

#### J.R.J. French<sup>2</sup>

Abstract: Baiting techniques have been developed over the years to control <u>Coptotermes</u> species, the most economically important termite species in Australia. Given the restrictions on organochlorines as termiticides in North America, this paper highlights research that has focussed on baiting programs against <u>Coptotermes</u> as alternatives to current control measures: drenching soil with pesticides or fumigation of entire structures. Details of the baiting procedures, toxins used, and their suitability for use in eradicating <u>Coptotermes</u> infestations in existing buildings are described. Suggestions are offered for the direction in which future baiting techniques may prove productive.

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In Australia subterranean termites cause most of the termite damage to timber in service (French 1986). Of these termites, the most economically important are the Coptotermes species. Inarguably, protection from subterranean termites has come to depend almost entirely on soil barriers (Lenz and others 1988), with the use of toxic, persistent chemicals such as organochlorines and the organophosphate chlorpyrifos. Dusting termites in galleries and sheltertubes with arsenic trioxide is still permitted and is normally the first step in eradicating subterranean termites from existing buildings, followed by a soil treatment.

The risk to the public of these chemicals is underrated, and partially unknown. Yet for the last 20 years these treatments have been standardized and recommended for use by local governments and the pest control industry (see Australian Standard (AS) 2057-1986; AS-2178-1986). The application of these persistent organochlorines as chemical soil barriers has ecological and practical limitations, as was pointed out by Beard (1974). There are many situations within existing buildings that render their use and application impracticable. For instance, termites infesting timber in apartments above the ground floor level are difficult, if not impossible, to control with the conventional chemical barrier treatments.

Many dusting treatments using arsenic trioxide fail to destroy the whole termite colony, because too few termites are "dusted," and these do not return to the nest. Thus, the damage continues. Furthermore, there is a lack of scientific data to indicate precisely how effective all these chemical treatments are in controlling subterranean termites in both the urban and rural environments. Alternative treatments are virtually unknown. The outcome is that the pest control industry in Australia (and the United States) has fewer and fewer options.

Apart from the physical nature of organochlorines, misuse of persistent termiticides by the pest control industry and increased public awareness of persistent home contamination and human exposure to organochlorine termiticides have contributed to these chemicals being severely restricted or banned in some countries around the world. In North America, the main manufacturers have voluntarily ceased production of organochlorines, whereas in Japan, organochlorines are banned, and only the organophosphate, chlorpyrifos (=Dursban), and several synthetic pyrethroids are registered for termite control. French and La Fage (1989) outlined the various treatments used around the world against the most economically important subterranean termite species.

Although I am aware of the need to refine effective and environmentally safer substitutes for organochlorines and arsenicals in termite control, this paper presents the results of some experiments carried out in southeastern Australia over the past decade, and offers some practical refinements regarding arsenic trioxide treatments, and alternatives (such as bait systems) to the pest control industry. I hope that some of these techniques may be adapted for North American conditions. In Australia, I have concentrated on the use of baits to aggregate termites (in this paper, the term 'termites' refers to subterranean termites' unless otherwise mentioned) in mounds and those that are infesting buildings, and the judicious use of small amounts of toxins to kill these colonies. The toxins referred to in this paper are arsenic trioxide and mirex.

The efficacy of mirex against termites in North America was demonstrated by Esenther and Gray (1968), Beard (1974), Esenther and Beat (1974, 1978, 1979) and Esenther (1979). In Australia, Paton and Miller (1980) found that less than 1 g of mirex was required to kill a colony of the Giant Northern termite (Mastotermes darwiniensis Froggatt), whereas French (unpubl. data) found that less than 0.25 g mirex was effective against a colony of Coptotermes lacteus (Froggatt). By way of contrast, an average 15 square building (1 square = 10 ft²) necessitates about 3 kg of active ingredient (i.e., organochlorine or organophosphate), using the Australian

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Standard, whereas less than 1 g of mirex in wood-decay bait-blocks can suppress an active <u>Coptotermes acinaciformis</u> (Froggatt) colony in a building. Although mirex is considered a carcinogen by some and has been banned in North America, there has been no report of injury to a person by mirex, and the chemical has not been measured in persons with occupational exposure to the compound.

Mirex is currently registered under special permit to licenced pest control operators (PCOs) for controlling  $\underline{M}$ .  $\underline{darwiniensis}$  in north Queensland, the Northern Territory and northwest Western Australia. I am currently negotiating with the Victorian and New South Wales State Governments to allow the use of mirex under a special permit by licenced PCOs.

There are two main areas to apply baits against termites: (a) within and under buildings, and (b) around buildings, in termite mounds and in termite-infested trees. Prefabrication of the bait-containers is similar for both situations. However, whether inside or outside the building, location of active termites is the single most important factor. There is no substitute for a thorough and careful search in and around a building to locate active termite sheltertubes or galleries. Sometimes the termite nest may be located either in an above-ground mound or in a standing tree or stump.

Regardless of the location, the following procedures for aggregating <u>Coptotermes</u> species in bait-containers have been used. Although the application of the toxin may vary, the aim of this control technique is to destroy an active termite colony with a slow-acting stomach poison that will be distributed throughout the colony by foraging termites.

#### ARSENIC TRIOXIDE BAIT CONTAINERS

#### Box-Bait Container

Box-bait containers for use under a suspended floor may be made from 3-ply veneer, and measure  $600 \times 300 \times 200$  mm, with four slits (3 mm wide, 460 mm long) cut into the bottom board. The box is filled with layers of corrugated cardboard wetted with water, and the box sealed with a lid. The box is buried to half its depth into the soil alongside or near active termite sheltertubes on walls or stumps. Water is applied to the soil around the box. After seven days the box container should be inspected for termites. The cardboard should be doused again if no termites present after this period.

#### Pipe-Bait Container

The pipe-bait container consists of a plastic pipe (90 mm diameter, 300 mm long), into which two corrugated cardboard inserts (with corrugations inwards and measuring 60 and 240 mm wide respectively) are placed. A petri dish lid seals the end with the longest cardboard insert. The whole arrangement is buried upright into the soil alongside or near active termite sheltertubes on walls or stumps, to a depth of about 50 mm. The inserts and the ground around the pipe are soaked with water. Inspections for termite activity are made every week, and the system is rewatered if necessary.

#### Conduit-Box Bait Containers in Ground

Plastic conduit (25 mm diameter), with small holes (4 mm diameter) drilled every 100 mm, with a wetted corrugated cardboard insert is buried (ca. 100-150 mm depth) alongside or near sheltertubes, diagonally across the underhouse area, or around the outside of the building. Plastic tee-pieces are inserted about every meter with an upright piece of tubing (200 mm long) onto which is attached a small plastic box (200 x 130 x 100 mm) filled with layers of wetted corrugated cardboard. Before backfilling the conduit-box arrangement into the trench, water is poured the length of the trench. Inspections for termite activity in the plastic boxes may be made every week, and re-watered if necessary.

#### Conduit-Box Bait Container in Tree

In southeastern Australia, old, damaged eucalypt trees are a favored site for Coptotermes colonies. However, it is rarely possible to visually detect an active termite nest within a tree. So, a hole is drilled (ca. 18 mm diameter) into the base of the tree. If the tree is hollow at the center, indicating a possible nest, this is detected when drilling. On withdrawing the drill, wet mud and maybe parts of termites may be seen, indicating an active nest. Often, termite bodies are not found, and this necessitates placing a wood dowel into the drilled hole. Allow about 100 mm to extend beyond the tree trunk so that it may be withdrawn easily. Leave the dowel in position for 2 to 10 h, then remove the dowel. If the dowel cannot be withdrawn easily, and requires a sharp tug, this indicates an active nest. The termites will have "sealed in" the dowel. After the dowel is removed termites are usually found on its surface. At this point, a conduit-box bait-container may be inserted.

The conduit-box bait-container is designed as follows. A plastic conduit (400 mm long, 16 mm diameter) is glued into a plastic rectangular container (300 x 200 x 95 mm) so that it protrudes (200 mm) from one of the longest sides. Small holes (8 mm diameter) are drilled in the conduit within the container, giving termites passing into the conduit access to the interior of the container. A corrugated cardboard insert is arranged within the length of the conduit. Layers of corrugated cardboard completely occupy the interior of the container, and these are slightly wetted with water. A lid is then secured in position.

Termites enter the box-container via the conduit, and can be removed, treated outside the container with arsenic dust (see below for treatment details), and returned to the container. The lid is not replaced; this causes a mass exodus back into the tree. Usually, if a sufficient number of termites are treated, the colony will collapse within 6 to 10 days. Termite activity may be gauged by withdrawing the box-container, and inserting a wood dowel. If after 1 to 2 days the dowel has not been "mudded in," the nest can be considered to have been destroyed. Rarely is it necessary to use more than 3-5 g of arsenic trioxide to eradicate an entire colony.

#### Applying Arsenic Trioxide to Termites in Baits

Irrespective of the design of the bait-container, the general principle in applying arsenic trioxide onto the termites aggregated in the baits is similar. If, on inspecting inside the bait-container, you see several hundred termites walking on the corrugated paper, remove the paper, hold it vertically over a large tray (e.g., a film developing tray is ideal), then give the back of your hand holding the paper a sharp smack. The termites will drop off the paper into the tray. Transfer about 80 percent of the termites from the bait-container, using the papering method, and put them into a container with a tight-fitting lid. Dust them with arsenic trioxide. Take adequate safety precautions by wearing goggles, respirator, and gloves. Rotate the closed container, and ensure that the termites are well covered with arsenic trioxide. The dusted termites are deposited back into the bait-container and onto the untreated 20 percent that are rapidly moving back into the gallery systems. Given that several hundred termites can be dusted in this manner, I would expect a complete collapse of the colony within 6 to 10 days.

After about two weeks, inspect the building for signs of active termites. If none are seen, one can assume the destruction of the colony was successful, and the bait-containers may be withdrawn from the area.

#### "Trojan Termite" Technique

The "trojan termite" technique (coined by the author) refers to the technique of "dusting" several hundred <u>Coptotermes</u> individuals from one colony source with arsenic trioxide and placing them immediately into the gallery systems of <u>Coptotermes</u> termites infesting timbers in a building. The "trojan termites" do not have to be of the same species, but basically should be the same size. After the dusted termites are inserted into the galleries of the "host" colony, the sections that have been opened to allow their placement are left open, to speed up the dispersal of the dusted termites into the gallery systems. Efficacy of this technique ought to be equivalent to bait-container techniques.

#### MIREX-TREATED BAIT-BLOCKS

In the laboratory radiata pine (Pinus radiata D. Don) sapwood stakes (500 x 58 x 18 mm, with the grain running lengthwise) are buried to a depth of 450 mm in moist forest or woodland soil within large containers in the Artificial Fungal Simulator (AFS). Stakes are removed after 12-15 weeks when there is about 15-20 percent mass loss per stake due to wood-decay fungi activity. Adhering soil is removed, and the stakes are sterilized in an autoclave at 120°C for 1 h. After autoclaving, blocks measuring about 58 x 58 x 18 mm are cut from the sterilized stakes. They are impregnated by dip diffusion in a 0.4 percent solution of mirex in acetone for 30 seconds, and the solvent allowed to evaporate. The decayed-wood mirex-treated blocks are then ready for use, and should be kept in plastic bags until required.

#### Placement of Mirex-Treated Bait-Blocks

Mirex-treated wood-blocks are placed either in buildings, outside buildings, or in both locations.

#### In Buildings

Place in Active Galleries--When termites are detected infesting architraves, window frames, mouldings, skirting boards, in flooring, in structural timbers behind panelling, and other timber constructions, the mirex-treated blocks are placed directly in contact with feeding termites. This sometimes requires some "instant carpentry." Often Coptotermes will seal off treated blocks if not placed into active feeding galleries, so other manipulations are necessary. For example, when these termites are detected in skirting boards, breaking open the outer wood and taping the treated blocks to the outer surface do not solve the problem, because, invariably, the termites will seal off the blocks, and no feeding occurs. Rather, cut through and remove the sound skirting board just ahead of the feeding termites. Place the treated blocks in the space left, and cover them with corrugated cardboard and tape.

Assuming termite feeding occurs, allow about 3 to 4 weeks before inspection and retrieval of the partially eaten blocks when no further termite activity can be detected. The blocks are returned to the laboratory and mirex is chemically removed from the partially eaten blocks and used again to impregnate new, wood-decayed blocks. All treated blocks are sealed in suitable containers until used.

Place in Active Sheltertubes--When locating a termite sheltertube, break open a small portion (say about 25 mm) and verify the presence of active termites. If termite soldiers and workers are seen, remove a sufficient amount of sheltertube to allow treated blocks to be inserted between each of the broken ends of the sheltertube. Cover the blocks with corrugated cardboard, and tape in position. When placing several blocks in a sheltertube running vertically up a wall or stump (whether wood or brick construction), tape the blocks onto a suitable length of cardboard before placing between the broken ends of the active sheltertube. Allow 3 to 4 weeks for the termites to feed on the bait-blocks and distribute mirex throughout the colony.

#### Outside Buildings

Place in Active Galleries--When active galleries are found within infested trees, mirex-treated bait-blocks can be placed in conduit-box bait-containers and inserted into the trees (see section on conduit-box bait-container), and termite feeding can occur. Some of the most common places to find termite galleries are under timber structures, such as rail-sleepers and wood beams used in gardenscapes. On turning over these wood structures, the galleries may be seen running along the lengths of the timbers on the soil surface. Mirex-treated blocks can be laid along these galleries and the timber turned back in position. Again, allow up to 4 weeks before inspecting for termite activity. Assuming the blocks are attacked and partially eaten within this period, and no sign of live termites remains, one can assume a total eradication of that particular colony. The blocks may then be retrieved and reconstituted as mentioned previously.

Place in Active Sheltertubes--Careful inspection and digging around the building will often reveal active sheltertubes. Break into the tubes and place the mirextreated bait-blocks between the broken tubes, and cover with corrugated cardboard and some soil to protect the termites from other insect predators, such as ants. Allow 3 to 4 weeks for termites to feed on the bait-blocks. The treatment period is similar to those suggested above.

#### MIREX-SAWDUST-AGAR IN HOLLOW WOOD DOWELS

M. darwiniensis and C. acinaciformis have been successfully controlled using mirex-sawdust-agar in hollow wood dowel baits in Queensland (Paton and Miller 1980) and New South Wales respectively. Small hollow wood dowels are manufactured, usually from hardwood dowelling. These are about 9 mm long, 10 mm in diameter with a 8-mm hole drilled out to a depth of 6 mm. Eucalypt sawdust is normally preferred, and this is impregnated with mirex. Up to 3 percent solutions are prepared for baits fed to M. darwiniensis. The mirex-treated dowels are easily inserted into active termite galleries and sheltertubes, and the contents eaten by the termites. This invariably leads to the death of the colony.

However, as often happens in natural systems, <u>C. acinaciformis</u> in the vicinity of Sydney in New South Wales behave differently than their conspecifics in and around Melbourne, which is about 890 km away. <u>Coptotermes</u> in the Melbourne area have never fed on these baits when applied in and around buildings which have active termite infestations, yet this technique has been very successful in Sydney (J.A. Easey, pers. comm.).

### SUGGESTIONS FOR FUTURE RESEARCH IN BAIT TECHNIQUES

#### Physiological and Behavioral Studies

Given the differences in behavioral response to mirextreated baits of similar composition by <u>C. acinaciformis</u> in different geographic locations, such as between Sydney and Melbourne, it would be useful to understand why there are such differences. This requires long-term studies into the physiology and behavior of this species, which I suggest would be greatly assisted with a strong microbiological emphasis. I cannot envisage the pest control or insecticide industry addressing this question. Rather this work will need to be done by Government agencies and universities. Financial funding could be sought from industry.

#### **Attractancy Studies**

The problem encountered in use of baiting techniques against <u>Coptotermes</u> species has been the inconsistency of bait acceptance. Often baits placed within galleries, and to a lesser extent within sheltertubes, are not eaten and are even bypassed. Thus, it would be advantageous for the pest control industry to acquire baits that are always eaten when contacted. This will require long-term studies on suitable bait substrates, coupled with "attractancy studies."

What is needed is a comprehensive monitoring of the termites' environment with respect to attractants from the wood substrates, attacked and unattacked substrate volatiles, leachates and chemical cues used by termites.

This will call for collaboration with such specialists as biochemists, microbiologists, organic chemists, physicists and statisticians.

#### <u>Development of High-Technological Chemical Dusting</u> Equipment

In Australia the pest control industry still uses simple technology, such as dental puffers, to dust arsenic trioxide onto termites in galleries and sheltertubes. (A dental puffer is a hollow rubber ball, that is filled with a toxin, and a steel tube fitted in place from which to discharge the toxic powder). Although there are few "termite dusts" on the market, the development of our own clathrated inclusion compounds and other forms of encapsulated systems could well indicate a viable option in the near future.

#### Refine the "Trojan Termite" Technique

Include not only other termite species, but other potential slow-acting stomach poisons, such as boron compounds, oxidative inhibitors, insect growth regulators, and so on.

#### Investigate the Gaseous Environment of Termites

Apply gases into termite gallery systems that may cause death or slow collapse. Or "herd" termites into bait containers, and administer a suitable toxin.

#### Use of Repellents

Repellents might be used to aggregate termites into bait containers, or to cause them to move past "pre-treated surfaces" in order to acquire a lethal dose.

#### SOME CONCLUDING REMARKS

The consequences of banning or voluntary withdrawal of organochlorines as termiticides in North America will have far-reaching implications, particularly in termite control measures. In Australia, the restrictions on these chemicals have been greeted with mixed feelings. There is considerable public concern about the use of organochlorines because of the potential for persistent environmental contamination and consequent human exposure. The public perception has been one of health and environmental problems associated with past agricultural use and with the only currently approved use, as termiticides.

The current administrative and legal systems (e.g., building regulations, Australian Standards) still specify organochlorine termiticides. However, events in North America and in Australia have helped to focus attention on pest control management systems that "target" termites more directly, such as baiting, rather than relying solely on chemical soil barriers. The reality of chemical barriers in Australia is that most pest control companies give only a one year warranty on chemical barriers. So it would seem that baiting is rapidly becoming a more acceptable control measure. The bait-block method of control using mirex (Beard 1974, and others) has proved successful in the past, the major drawback being that mirex is no longer registered for that use.

Personally, I prefer the judicious use of such chemicals as mirex rather than the proposed soil chemical treatments using organophosphates and synthetic pyrethroids to eradicate subterranean termites from an existing building. Baiting techniques are not only well-targetted control measures, but are more "user-friendly" than soil chemical barriers. Human and environmental contamination are kept to a minimum given the nature of persistent insecticides. Although no data have been published on the amount of mirex residue to be found in the environment following the death of a termite colony, I would consider it negligible. Our experiments in the field indicate that <u>C. lacteus</u> (Froggatt) mound colonies of between 700,000 and 1 million individuals die within 6 to 10 days after feeding on 10 mirex-treated wood blocks impregnated with a 0.4 percent mirex solution. This represents less than 2 g of active ingredient.

Although I am aware of the history of mirex, we already permit its use under strict situations in Australia (see McFadden 1989). The end-point of our termite control measures is to make sure we kill the insects, and not people! Baiting technology, forming part of an integrated termite pest management strategy, will hopefully ensure this outcome.

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# Population Suppression of Subterranean Termites by Slow-Acting Toxicants<sup>1</sup>

#### Nan-Yao Su Rudolf H. Scheffrahn<sup>2</sup>

Abstract: Historic background and the concept of slow-acting toxicants for population suppression of subterranean termites are reviewed. Information needed for development of bait-toxicants and studies needed to generate such information are summarized.

Current control measures for subterranean termites rely exclusively on soil termiticides, primarily organophosphates and pyrethroids. They are used as toxic or repellent barriers between soil-borne termite colonies and structures requiring protection. La Fage (1986) estimated that soil termiticides are applied at a rate of ca. 390 kg/ha beneath treated structures compared to agricultural rates of 2.17 kg/ha (Pimentel and Levitan 1986). The withdrawal from use of chlorinated hydrocarbon termiticides in 1987 reflects public concern over the use of these persistent chemicals in highly populated urban environments.

Despite the large quantities of pesticides used, soil treatments do not affect termite populations but only provide barriers to separate structures from soil-borne termites. Colonies of subterranean termites, especially those of the Formosan subterranean termite (Coptotermes formosanus Shiraki) which may forage as far as 100 m from the central nest, remain viable near the structures even after treatment (Su and Scheffrahn 1988a). Because of the inability of current control techniques to reduce existing subterranean termite populations, the severity of infestations by C. formosanus in areas such as Honolulu, Hawaii, and New Orleans, Louisiana, has increased in recent years.

Researchers in the early 1900's first observed that the slow-acting arsenic dusts could be used to reduce colony populations of subterranean termites (Randall and Doody 1934). The principle of suppressing colony populations is to provide a means for individual termites to acquire a lethal dose of slow-acting toxicant at a given foraging site. The intoxicated individuals must not be so impaired at the onset of exposure that they cannot move away from the toxicant acquisition site to die. The slow-acting characteristic of a toxicant is particularly important because accumulation of a large number of dead termites at the acquisition site will repel other nestmates from approaching the toxicant (Su and others 1982a). Ideally, the toxicant has to be nonrepellent to termites, or at least be masked by other agents to prevent avoidance behavior by the foraging termites. Under this premise, the toxicant can be incorporated into a bait (feeding acquisition) or tracking powder (contact and grooming acquisition). In this paper, we will primarily discuss the bait con-

Beard (1974) suggested the use of bait toxicants as a possible strategy to eliminate established colonies of the subterranean termites, Reticulitermes spp. Dechlorane (mirex) baits have been used to suppress activity of field colonies of Reticulitermes in the United States (Esenther and Beal 1974, 1978), and to kill field colonies of an Australian subterranean termite, Mastotermes darwiniensis Frogg. (Paton and Miller 1980). Gao and others (1985) also reported successful field control of termite infestations with mirex baits in China.

Laboratory studies indicated that hydramethylnon (Amdro $^{\mathbb{R}}$ ), avermectin B<sub>1</sub> (Su and others 1987), A-9248 (Su and Scheffrahn 1988b), sulfluramid (Su and Scheffrahn 1988c), and insect growth regulators (IGRs) such as methoprene, fenoxycarb, and S-31183, have shown delayed toxicity against C. formosanus and the eastern subterranean termite, Reticulitermes flavipes (Kollar) (Jones 1984, Su and others 1985, Haverty and others 1989, Su and Scheffrahn 1989). A field trial with hydramethylnon baits, however, resulted

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## POPULATION SUPPRESSION OF SUBTERRANEAN TERMITES BY SLOW-ACTING TOXICANTS

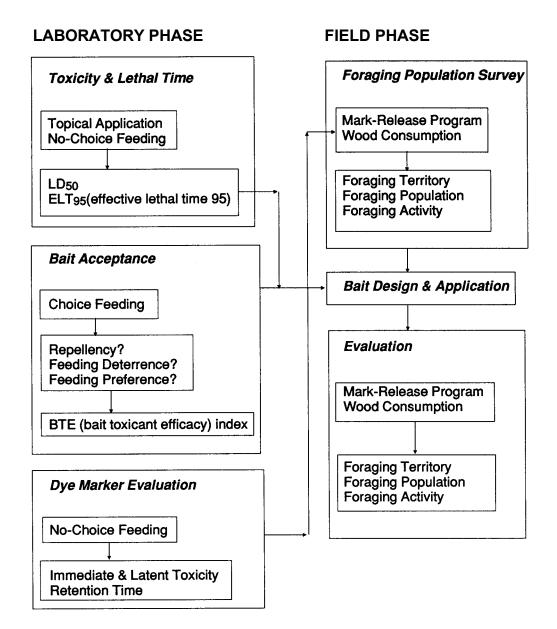


Figure 1--Studies needed to establish information for development of bait toxicants to suppress populations of subterranean termites.

only in partial control of  $\underline{C}$ . formosanus colonies (Su and others 1982b).

INFORMATION NEEDED FOR DEVELOPMENT OF A SLOW-ACTING BAIT

Development of a bait toxicant requires numerous, interconnected laboratory and field studies followed by a rigorous evaluation phase (Figure 1).

#### Laboratory Phase

Toxicity and Lethal Time

Oral or topical  $\rm LD_{\rm so}$  or  $\rm LD_{\rm ss}$  should be calculated to estimate the gross quantity of toxicant needed for control of entire colonies. This information can also be used to compare the relative toxicity among toxicants.

Lethal time of a toxicant is concentration dependent. A bait toxicant should be used at a concentration that causes protracted behavioral impairment and latent mortality so that termites, having fed on bait, will disperse throughout their foraging territories before succumbing to the toxicant. Su and others (1987) defined an effective lethal time 90%  $(ELT_{90})$  as the amount of time required by a fixed concentration of a toxicant to kill at least 90 percent of termites.  $ELT_{90}$  can be used to assess the feasibility of a candidate compound as a bait toxicant and to predict the time frame required for control. Lethal time ( $\mathrm{ELT}_{90}$ ) of candidate toxicants can also be used to compare toxicants.

#### Bait Acceptance

In field trials, a bait must be competitive with other termite food resources. Termites should not be repelled (Su and others 1982a) or their feeding deterred by bait additives (Su and Scheffrahn 1988b). In the past both choice (Su and Scheffrahn 1988b) and no-choice bioassays (Scheffrahn and Su 1987; Grace, in press) were used to determine concentration-dependent feeding on treated substrates. Table 1 summarizes interpretations of no-choice and choice bioassay under various hypothetical experimental outcomes. Although both no-choice and choice feeding bioassays can detect concentration-dependent repellency and feeding deterrence, only a choice bioassay can determine that the toxicant-treated food source is more or less accepted by termites than identical untreated food substrate. Because the behavioral responses of termites toward treated baits are concentration-dependent, it is vital that a choice bioassay be used to determine the acceptable concentration of a candidate toxicant before it is used in field trials.

The two important characteristics, slow-acting and no feeding deterrence, are concentration dependent (Su and others 1987 , Su and Scheffrahn 1988b). A desirable toxicant should be accepted by termites at efficacious concentrations to cause significant delayed mortality. An index combing these three parameters, concentration, delayed mortality and feeding deterrence, therefore, can be used to evaluate the potential of a toxicant. We propose here a Bait Toxicant Efficacy (BTE) index which is defined as the quotient of bait acceptance threshold concentration (BATC) and the threshold concentration to produce significant delayed mortality (DMTC); namely BTE = BATC/DMTC. A toxicant with BTE • 1 (such as pyrethroid, Su and others 1982a) cannot be used in a bait, while BTE > 1 indicates a bait-toxicant potential. Using this criterion, BTE index for the slow-acting A-9248 (dihaloalkyl arylsulfone) against  $\underline{C}$ . formosanus was 3 (600 ppm [BATC]  $\div$  200 ppm [DMTC]) (Su and Scheffrahn 1988c). Similarly, BTEs' for mirex and sulfluramid against  $\underline{C}$ . formosanus were 9 (90 ppm  $\div$  10 ppm) and 2.5 (10 ppm  $\div$  4 ppm), respectively. BTE values for sulfluramid (30 ppm  $\div$  18 ppm) and mirex (15 ppm  $\div$  9 ppm) are identical (1.7) against  $\underline{R}$ . flavipes (Su and Scheffrahn, in press).

#### Dye Marker Evaluation

Dye markers are useful to determine foraging territories and to estimate foraging populations of subterranean termite colonies. There has been criticism of the application of the capturerecapture technique for population estimation of social insects (Ayre 1962). The criticism was based on the argument that individuals in insect colonies do not distribute randomly in space, and thus one of the basic assumptions of mark-recapture model is violated. For example, most of the younger (1-2 instar) termites do not move to distant foraging sites. Baroni-Urbani and others (1978), however, pointed out that the mark-recapture technique is often the only practical method for studying the population dynamics of some insects. This is especially true for the cryptobiotic insects such as subterranean termites.

Su and others (1984) demonstrated that  $\underline{C}$ . formosanus workers chose foraging sites at random; thus all of the released marked foragers should distribute evenly within the population. Moreover, only foragers can be captured using the prescribed trapping system (Su and others 1984, Su and Scheffrahn 1986, 1988a). The mark-recapture technique, thus, is an effective method of quantifying the populations and movements of subterranean termite foragers.

Sudan Red 7B (or Oil Soluble Red 7B) was first used by Lai and others (1983) to estimate the size of colonies of  $\underline{C}$ . formosanus in Hawaii. Laboratory studies showed that Sudan Red 7B was not trophallactically transferred by  $\underline{C}$ . formosanus (Su and others 1983) or  $\underline{R}$ . flavipes (Su and others 1988). This dye was subsequently adopted as the standard dye to determine foraging territory and population of  $\underline{C}$ . formosanus in Florida (Su and Scheffrahn 1988a), and  $\underline{R}$ . flavipes in Toronto (Grace, in press). Jones (in press) also used Sudan Red 7B to define foraging boundaries

Table 1. Potential results of no-choice and choice bioassay and data interpretations.

| Results                     |                                  | Interpretations  |   |  |  |
|-----------------------------|----------------------------------|--|---|--|--|
| Feeding compared to control | Mortality compared<br>to control | No choice bioassay   | Choice bioassay   |  |  |
| _                           | =                                | Not feeding deterrent<br>Not repellent<br>Not toxic<br>(UNDESIRABLE)                                   | Non-preference<br>Not toxic<br>(UNDESIRABLE)              |  |  |
| _                           | >                                | Not feeding deterrent<br>Not repellent<br>Toxicant<br>(DESIRABLE)*                                     | Non-preference<br>Toxicant<br>(VERY DESIRABLE)            |  |  |
| <                           | =                                | Feeding deterrent or repellent Not toxic (UNDESIRABLE)**   | Unpreferred<br>substance<br>Not toxic<br>(UNDESIRABLE)    |  |  |
|                             | >                                | Feeding deterrent or repellent or Mortality due to contact toxicity? or starvation? (VERY UNDESIRABLE) | Unpreferred substance Contact toxicant (VERY UNDESIRABLE) |  |  |
| >                           | =                                | Feeding stimulant Not toxic (Potential additive- masking agent)  | Preferred substance (Potential additive masking agent)    |  |  |
| >                           | >                                | Feeding stimulant Toxicant (VERY DESIRABLE)  | Preferred<br>substance<br>(IDEAL BAIT<br>TOXICANT)        |  |  |

<sup>\*</sup> Theoretically, this scenario should not occur because higher mortality would result in over all lower feeding.

of <u>Heterotermes</u> <u>aureus</u> (Snyder) in Arizona. Sudan Red 7B, however, does cause mortality of <u>C</u>. <u>formosanus</u> (10-15 percent) during staining, and causes a latent mortality of <u>R</u>. <u>flavipes</u> (30-80 percent mortality at 2-8 weeks after being stained with 0.5 percent dye for 10 days) (Su and others 1988).

In a laboratory screening test, we (Su and others, unpublished data) have identified two dyes, Nile Blue and Neutral Red, that have even better potential than Sudan Red 7B as markers for  $\underline{C}$ .  $\underline{formosanus}$  and  $\underline{R}$ .  $\underline{flavipes}$ . Currently, we are

testing the color fastness of these dyes in mixed groups of marked and unmarked foragers.

#### Field Phase

Foraging Population Survey

To adequately evaluate results of field trials, foraging activity, foraging population size, and foraging territory of subterranean termites must be monitored before the introduction of a bait.

Esenther and Beal (1974, 1978) reported

<sup>\*\*</sup> Theoretically, this result cannot be obtained because lower feeding would eventually cause higher mortality by starvation.

successful suppression of field colonies of <u>Reticulitermes</u> spp. with mirex baits. However, the activity of these field populations of <u>Reticulitermes</u> had not been monitored before bait application. It is uncertain whether the field populations were repelled from the test site or whether the populations were indeed suppressed.

Ideally, the colony activity should be monitored for a few years before the application of bait toxicants. The process of marking, counting, releasing, and recapturing is a laborious task. A more sophisticated model than the simple Lincoln index for estimating the foraging population provides more information with the same effort. The weighted-mean model using a multiple mark-release procedure enabled us to obtain estimates with a relatively low standard error (Su and Scheffrahn 1988a). However, the procedure required three months to complete. Moreover, it takes another three months for the last stained termites to totally lose their marking before another cycle of multiple mark-release procedures can be initiated. Because of foraging inactivity during cool months in southeastern Florida, the mark-release program is implemented from April to November. Realistically, therefore, only one mark-release program per colony is possible per annum.

Although the size of the foraging populations can be estimated only once annually, foraging activity can be monitored all year by counting the number of termites in the traps and measuring wood consumption during monthly or bi-monthly trap changes. Although the boundary of a foraging territory is defined before bait application, change in foraging activity and territory should be monitored throughout the bait toxicant evaluation period.

#### Bait Application

The trapping system described by Su and Scheffrahn (1986) can be used for bait field trials. Trap stations within a colony monitoring system should be selected at random to receive toxicant baits, while the others receive similar baits without toxicant. The difference in activity (number of termites and wood weight loss) between treated and untreated stations then can be compared to detect termites' preference in visiting baits with or without toxicant.

In our field trials (Su and others, unpublished data), we use a toxicant bait composed of six pieces of pine (<u>Pinus</u> spp.) board (7 cm by 13 cm by 2 cm thick), three of which were vacuum impregnated

with toxicant while the others were treated with impregnation solvent only. The dry weight of each wood piece was determined before bait assembly. After the baits were exposed to termites, the wood weight loss of toxicant-treated and solvent control boards was compared to ascertain termite feeding preferences.

#### Evaluation

During the field trial, the foraging preference by termites between treated and untreated sites (i.e., number of termites collected per trap) and feeding preference between treated and untreated wood pieces should be monitored. The mark-release program, which would have to be interrupted during the trial because of the potential negative effects of the dye marker, should be reinstituted after the termination of the bait toxicant exposure to determine the effects of the bait toxicant on the foraging populations.

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# How Do We Advise the Pest Control Industry in the Post-Organochlorine Era?<sup>1</sup>

#### J.R.J. French<sup>2</sup>

Abstract: Given the banning of organochlorines for use as termiticides in North America, and with the possibility of such bans occurring in future in Australia and elsewhere, how do we as entomologists advise the pest control industry in termite control? A brief historical review of pesticide bans and the relevance of these bans to the present situation are discussed. Suggestions are offered for entomologists to pursue an integrated pest management approach to termite control based on sound ecological parameters and social priorities.

Before attempting to offer suggestions as how best to advise the pest control industry in the post-organochlorine era, I think it is as fruitful to understand the social and political context in which bans on pesticide use have been imposed historically, as to offer resolutions that may receive the same treatment in the long-term.

So, how did bans on pesticide use come about? Were they imposed by scientists in government and industry? It would seem that was not the case. Before 1962, many scientists were expressing great concern as to the side-effects, the indirect effects, and the long-term effects of organochlorine pesticides. They were not only concerned with the target organisms themselves, but with other organisms, as the pesticides moved through the environment, following food chains as predator ate predator, and acting on people.

Egler (1964) considered that these scientists were ridiculed or ignored or silenced in a variety of ways, even by their own colleagues, and especially by the chemical industry and its chief advocates in the U.S. Department of Agriculture and the U.S. Food and Drug Administration. But their work and concerns were not lost to society. There came a change.

This change came in the form of a book, <u>Silent Spring</u>, written in 1962 by Rachel Carson, a marine biologist. As Ehrlich and others (1977) pointed out, "it is true that there were a few factual errors in <u>Silent Spring</u>, but in many ways Rachel Carson underestimated the hazards of DDT and certain other organochlorines. Nevertheless, she succeeded in awakening the public - and did so in a way that a more technical and highly documented book like Robert L. Rudd's (1964) <u>Pesticides and the Living</u>

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<u>Landscape</u> could not. Rudd, a zoologist, came to many of the same conclusions that were presented in <u>Silent Spring</u>, but because his book was not addressed to the general public it did not engender the level of attack that was directed toward the Carson book."

In the opinion of Ehrlich and others, "no biologist has made a greater contribution to humanity in this country than Rachel Carson. And recent events and government decisions have fully justified her position."

Soon after the U.S. Environmental Protection Agency (EPA) was created in 1970, it was given responsibility for the registration and regulation of pesticides. Litigation by the Environmental Defense Fund produced a court order early in 1971 for EPA to issue public notices of cancellation of the registration of DDT and to review DDT for suspension. The notices of cancellation for DDT, as well as aldrin, dieldrin, mirex, and the herbicide 2,4,5-T, were, of course, appealed. After lengthy hearings, EPA banned DDT for almost all uses in 1972. It found that DDT "posed an unacceptable risk to man and the environment." Restrictions were also placed on mirex. A ban followed a few years later.

Concern was expressed that banning of organochlorines would lead to increased immediate mortality from pesticides because the organophosphate compounds that would replace DDT have a higher acute toxicity even though they are much less persistent and presumably present a relatively small long-term risk. However, about 1969, occupational deaths from pesticides (mainly organophosphates) occurred at the rate of about 12 per year nationally. There was no sign of an increase into the mid 1970's (Ehrlich and others 1977).

The direct threat to humanity by organochlorines is difficult to assess (Anon 1989b). At present, however, it appears that, as alarming as the direct threat may be, the indirect threat is much more serious. These compounds have the potential for irreversibly damaging the capacity of ecological systems to supply those essential services without which civilization cannot persist (Ehrlich and others 1977).

In 1987, the EPA restricted the uses of organochlorines as termiticides, permitting only the use of chlordane as a soil barrier. Alternatives such as the organophosphates (chlorpyrifos [Dursban], and isofenphos [Pryfon 6]) and synthetic pyrethroids (cypermethrin [Demon], permethrin [Dragnet], permethrin [Torpedo], and fenvalerate [Tribute]) have been recommended by the EPA. These are currently the termiticides of choice by the pest control industry in the United States (Mix 1988).

These events have not unfolded in a "neutral environment," but rather, as a result of persistent political challenging by scientists, environmental groups, such as the National Coalition Against the Misuse of Pesticides (NCAMP), consumer and union groups, and the public of

our society's pesticide use. The National Academy of Science in the United States has been concerned with the impact of regulation on the development of new technologies to replace the conventional broad-spectrum chemicals that have dominated the pest control scene since 1950. Whether or not those required technologies will be generated within the chemical industry remains uncertain, and some think, unlikely (see Ehrlich and others 1977).

Now in 1989, we have the voluntary cancellation by the insecticide manufacturers of their organochlorines used as termiticides in the United States. In Australia, there has been mounting public concern over environmental and public health issues surrounding the use of these pesticides. Recent publicity in connection with pesticide residues in beef and an incident at the Streaky Bay School in South Australia following application of aldrin as a termiticide (Anon 1989a) has focussed attention on organochlorines in particular. Currently, all organochlorines are banned in South Australia (Anon 1989b), and in most other Australian states, for all purposes except as termiticides. The question being asked by some scientists and members of the pest control industry in Australia, is -"will organochlorines be banned for all uses, as in the United States?"

Personally, I predict a similar trend in Australia in the not too distant future. There is a strong upswell of feeling by interest groups, such as the Total Environment Centre in Sydney, unionists in all states, and numerous environmental groups, to have these compounds completely banned. There has been wide publicity in the media on the topic, and the public perception has been one of health and environmental problems associated with past agricultural use and with the only currently approved use, as termiticides (Anon 1989b). Notwithstanding the reaction of a pesticide proponent in the pest control industry, terming such critics of pesticide use as "toxic terrorists" (Meadows 1988), the problem of chemical pollution will not readily diminish given current economic values.

### INTERNATIONAL OVERVIEW OF TERMITE CONTROL

In May 1987, the International Research Group on Wood Preservation (IRGWP) conducted a survey of its 55 member countries to ascertain the economically-important termite species in the various zoogeographical regions, their damage ranking to timber-in-service, the chemicals used in control methods, and the status of the termite standards in the respondent countries. Table 1 summarizes the information collected in the survey so far (French and La Fage 1989).

Countries that have standards for chemical control of termites include Canada, France, Greece, India, Italy, Japan, Spain, Australia, and the United States. Nigeria is presently preparing a standard. Organochlorines, organophosphates and synthetic pyrethroids are the most commonly used chemicals. Arsenical preparations are used in Fiji, Greece, Spain, Australia and Uganda. Japan is one of several countries that use gas fumigants as termiticides. Australia, Japan, Canada and the United States also register organophosphates as termiticides.

The methods of application were found to vary considerably between countries. The common practice of applying a chemical barrier over the entire exposed surface area under a pier-type building is carried out in India, Japan, Nigeria and the United States. The treatment of soil adjacent to piers and supports, however, is the preferred procedure for most countries. The issue of compulsory soilbarrier termiticide treatment to new buildings was not directly addressed, though Indonesian authorities stated they do not insist on this approach, unlike most local authorities in Australia. Some countries, such as Fiji and Italy, do not carry out soil-barrier treatments. Treatment of existing buildings is done in most countries only as a consequence of infestation. However, Japan undertakes retreatments every 5-10 years, probably because of the lower persistent organophosphates used. In Australia, many pest control companies carry out yearly soil-barrier treatments using organochlorines, although this is not stipulated in any Australian Standard. However, such "re-treats" are regular business practices in rural areas.

Table l-- The major termiticides used in termite control

| Region        | Country         | Termiticides used <sup>1</sup> |    |    |     |    |       |
|---------------|-----------------|--------------------------------|----|----|-----|----|-------|
|               |                 | oc                             | OP | SP | GAS | AS | О     |
| Ethiopian     | Ghana           | +                              | +  | +  | +   | +  | +     |
|               | Nigeria         |                                | +  |    |     |    | WP    |
|               | South Africa    |                                | +  |    |     |    | WP    |
|               | Tanzania        | +                              | +  |    |     |    |       |
|               | Uganda          |                                | +  |    |     | +  |       |
| Palearctic    | China (Nanjing) | +                              |    |    | +   | +  | Mirex |
|               | France          | +                              | +  | +  |     | +  |       |
|               | Greece          | +                              | +  | +  |     |    |       |
|               | Israel          | +                              | +  | +  |     |    |       |
|               | Italy           | +                              | +  | +  |     |    |       |
|               | South Korea     | +                              | +  |    |     | +  |       |
|               | Spain           | +                              | +  | +  |     | +  |       |
|               | Yugoslavia      | +                              |    |    |     |    |       |
| Oriental      | India           | +                              |    |    | +   | +  | WP    |
|               | Indonesia       | +                              |    | +  |     | +  |       |
|               | Japan           |                                | +  |    | +   |    | WP    |
|               | Phillippines    | +                              |    |    |     |    |       |
| Australian    | Australia       | +                              | +  | +  | +   | +  | Mirex |
|               | New Zealand     | +                              |    |    |     |    |       |
| Hawaii and    | Fiji            | +                              |    |    | +   | +  |       |
| Oceanic Is.   | Hawaii          | +                              | +  | +  | +   |    | NCB   |
| Nearctic      | Canada          | +                              | +  |    |     |    |       |
|               | USA             |                                | +  | +  | +   |    | IGR   |
| Neotropical   | Bolivia         | +                              |    |    |     | +  |       |
| 1.30tt opical | Chile           | +                              |    |    |     | +  |       |
|               | Columbia        | +                              |    |    | +   | +  |       |
|               | Uruguay         | +                              |    |    |     | +  |       |

 $^1$ OC = Organochlorine; OP = Organophosphate; SP = Synthetic pyrethroid; GAS = Fumigation; AS = Arsenicals; O = Other; IGR = Insect Growth Regulator; NCB =Non-chemical termite barrier; WP = Wood preservative treatment.

No country appears to have formulated specialized standards for drywood termite control apart from

recommending fumigants, as in North America and Australia. Yet in the Ethiopian, Hawaiian and Oriental regions, the risk of drywood termite damage exceeds that of subterranean or dampwood termites.

### PROPOSED TERMITICIDES AND TERMITE CONTROL METHODS

Over the years many termiticides and termite control methods have been proposed worldwide, but in this paper the focus is on those applied in developed countries (French 1986, Mauldin 1986, and Lenz and others 1988). Assuming that the organochlorines are to be banned as termiticides, then the proposed termiticides of the future may be divided into two main groups, namely, pretreatments and post-treatments.

#### Pre-treatments

Pre-treatments are complete or partial chemical soilbarrier treatments for buildings under construction. The chemicals proposed are organophosphates, synthetic pyrethroids, brominated hydrocarbons, carbamates, and slow-release formulations of these compounds. An alternative delivery system ("Peacock system") of underslab perforated piping has been promoted in Hawaii, in which termiticides (organophosphates) are pumped in under pressure from an external servicing port. Other pretreatments include physical barriers and wood preservatives (such as boron compounds, copper-chromium-arsenic, light organic solvent preservatives, fluorinated lipids, and sulphones).

#### Post-treatments

Arsenic trioxide, used as a dust, is the only effective termiticidal use of arsenicals in Australia. It is legally and commercially available in Australia and the amount per treatment is small (ca. 3-5 g to eradicate a termite colony of > 1 million individuals).

Mirex, in the bait-block method, is effective when used for remedial treatments directed towards *Coptotermes* and *Reticulitermes* spp. This insecticide is registered for use in three states in Australia against the Giant Northern termite, *Mastotermes darwiniensis* Froggatt, and registration is being sought in Victoria and New South Wales for control of *Coptotermes acinaciformis* (Froggatt).

Fumigants, such as methyl bromide, are used in remedial treatments, particularly against drywood termites. However, exposing localized drywood termite colonies to heat and cold (using liquid nitrogen) may also offer a measure of control (Forbes and Ebeling 1986).

Other potential termiticides may be offered in baitblocks and baiting systems. For example, insect growth regulators, including juvenile hormone analogs and chitin synthesis inhibitors, boric acid, and amdro could be incorporated into bait-blocks. The latter is a non-repellent delayed-action termiticide. If bait systems are used, large aggregations of termites can be exposed to small but direct amounts of termiticides, such as arsenic trioxide, amdro, boric acid, mirex, and inclusion compounds containing toxins, and allowed to return to the colony and so transmit the toxin.

#### ALTERNATIVES TO CHEMICAL TERMITICIDES

#### **Biological Control**

Different methods of biological control exist, and include: bacteria, fungi, protozoa, nematodes, natural predators, natural enemies or competitors, pheromones, attractants, repellents, and insect growth regulators. However, many of these biological control agents are, at present, beyond the scope of practical application for the average pest control operator in Australia. Insect growth regulators and other chemical analogs would seem to have more commercial promise than organisms such as bacteria, fungi, nematodes, and natural predators. I have experimented with the natural enemies or competitors of Coptotermes, and call them "trojan termites." These are termites, usually of the same genus, but different species, that are collected from another colony. They are taken to the infested building, and dusted with a suitable toxin such as arsenic trioxide, then released into the active galleries of the termite species attacking the building timbers. When the two termite groups meet, they fight, and thus help to pass on the poison through the colony originally attacking the building, which subsequently leads to the death of the termite colony.

Although biological control of insect pests is increasingly being used in agriculture due to (1) pesticide resistance, (2) a growing awareness of probable health and environmental risks associated with using chemical pesticides, and (3) the financial costs of chemical control, some barriers do exist in refining such control methods.

Biological control products are usually less stable compared to organic chemicals. Significant evaluation is also required to ensure that live biological controls do not have unintended ecological consequences.

There are large costs in screening potential biological agents prior to obtaining registration. In Australia as of the 1 July, 1989, the Federal Government now charges \$20,000 to register a new chemical product. This will tend to preclude small companies from entering the market, even if their products are environmentally superior to what is already registered.

But there is a balance of forces between what society demands in insecticide selectivity and what society is prepared to pay for, though this balance is changing with increasing restrictions on the use of chemical insecticides. Biological control will probably become more important in the future, with the extent of development determined by economic forces.

#### Physical Barriers

#### Ant caps and shields

Ant caps are metal shields which are placed on the top of each pier beneath the building, and along the top of all foundations. However, because of problems in building construction, such barriers are readily bypassed by subterranean termites. These barriers work fine if the buildings are regularly inspected, because they force the subterranean termites to build shelter tubes over them, thus exposing their presence to the inspector.

#### Non-Chemical Barriers

In 1957, Ebeling and Pence reported on the relationship of particle size to the penetration of subterranean termites through barriers of sand or cinders. This type of non-chemical barrier has recently been perfected in Hawaii to protect structures from attack by the Formosan termite, *Coptotermes formosanus* Shiraki (Tamashiro and others 1987). On the mainland United States, Ebeling and Forbes (1988) have applied for patent protection on the use of sand barriers. Laboratory and field experiments in Australia have shown similar relationships by challenging *Coptotermes* species with granite and basalt substrates (French 1989).

### ADVICE TO THE PEST CONTROL INDUSTRY--A VIEWPOINT

The banning of DDT, for example, would no doubt be a laudable victory in the war to save the environment, but it would be disastrous to mistake such a victory as winning the war. The profound changes necessary to save the environment and society will not be made easily. Powerful opposition can be expected from economic interests. So how can scientists, particularly entomologists, assist in this arena and facilitate a more ecological approach to insect pest management?

First, entomologists need to think and act ecologically. They need to accept that the ideological status of ecology is that of a resistance movement. Its Rachel Carsons and Aldo Leopolds are subversive (as Sears (1964) called ecology itself). They challenge the public or private right to pollute the environment, to systematically destroy predatory animals, to spread chemical residues indiscriminantly, to meddle chemically with food and water; they oppose the uninhibited growth of human populations, the extinction of species of plants and animals, the domestication of all wild places, large-scale manipulations of the atmosphere (e.g., greenhouse effects) or the sea, and most other purely engineering or technological solutions to problems of and invasions into the organic world.

Second, their task is to inculcate basic ecological attitudes in the government, the pest control industry, and the public. They have to teach the population simple but basic ecological notions: a finite world cannot support or withstand a continually expanding population and technology; there are limits to the environmental sinks; ecosystems are sets of interacting entities, and there is no "treatment" which does not have "side effects."

Third, as ecologists, entomologists will have a central role in advising on the management of the environment. Thus, good management will depend on rather long-term studies of particular areas, since ecological situations are dependent both on time and locale. These two features also ensure that there will be a sizeable lag-time between posing the question and receiving the ecological advice, and a major job of the entomologists will be to make the existence of such lags known to the policy-makers. Entomologists will have to apply technology. For example,

integrated pest management systems will surely replace chemical control alone.

Fourth, entomologists need to ensure that the scientific establishment must become more closely regulated by collective decision-making processes. Upgrading the educational system (by words and deeds) and revising the role of mass media would prepare the public to play a more active role in directing scientific activities.

Finally, as Pirages and Ehrlich (1974) concluded, social priorities must be drastically altered to turn science inward to the study of people, their values, and their institutions. This area of inquiry will be vital to future survival, given the need to redirect human aspirations and consumption patterns. If social expectations and behavior are not altered, even the most earnest technological efforts will be unable to keep pace with growing human demands.

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#### **DISCUSSION**

What follows represents the minutes of the workshop on wood-destroying organisms held in Bend, Oregon, September 14, 1989. The theme of the workshop was the state-of-the art of control of wood destroying organisms and the needs for further research on wood-destroying organisms. The agenda for the session was organized by W. Wayne Wilcox and Michael I. Haverty.

### FUTURE OF WOOD PRODUCTS PATHOLOGY

Wayne Wilcox outlined what he thinks are the priorities for education and research on wood products pathology:

- Education of the user (designers, builders, general public or building owners) to reduce the losses due to decay.
- Development of nondestructive techniques to evaluate wood decay in structures (also techniques for diagnosing infestations by active wood-destroying insects in structures).
- Development of new regimes for protecting wood from biodegradation in the absence of toxicants, i.e., biological control and modifications of wood chemistry.
- Evaluation of third-generation wood preservatives, and their effects on nontarget organisms. A variety of compounds or techniques will be used in specific applications. In the future it will be much more expensive to prevent wood decay.
- Increased knowledge of wood-destroying fungi—biological, taxonomic, phylogenetic—and of specialization of fungi in different environments.

Harold Burdsall discussed pathology research at the Forest Products Laboratory, Madison, Wisconsin.

- Institute for Microbial and Biochemical Technology. Investigations of biodeterioration or biodegradation as positive uses of wood-degrading organisms, lignin degradation, wood pulping, pretreatments to reduce the energy needed in pulping, fungi to break down toxic wastes, and fungi to clean up mill effluent.
- Biodeterioration of Wood. Basic biochemical mechanisms of decay, serological examinations, and mechanisms by which enzymes are delivered by fungi taken to wood tissues containing lignin for wood decomposition.
- Center for Forest Mycology Research. Taxonomy of fungi, *Armillaria* root rot, serological investigations of *Phlebia*  for diagnosis of species, and other ways to distinguish species. This group is developing a chicken egg technique as an immunological test for identification of species or

strains. They inject fungal tissue into each breast and each leg of a chicken and repeat the process in 1 week. This results in an increase in diagnostic proteins in the eggs after approximately 3 to 4 weeks.

Elmer Schmidt discussed the most recent (1989) meeting of the International Research Group on Wood Preservation, Stockholm, Sweden. This organization is a forum for new ideas on wood preservation and wood-destroying organisms. The general areas are: (1) biological problems (fungi and insects), (2) testing methods (bioassays), (3) preservation of marine timber, and (4) wood preservation.

### FUTURE OF WOOD PRODUCTS ENTOMOLOGY

Michael Haverty outlined what he thought were the key issues facing researchers in wood products entomology:

- Continued assessment of new soil termiticides with advances in their application for increased efficacy and longer effective life.
- Assessment of non-chemical, physical barriers for protection before construction begins.
- Better understanding of the taxonomy of the economically important termites in the United States, primarily Reticulitermes and Coptotermes.
- Acceleration of research on foraging behavior and related colony demography to increase our understanding of feeding behavior of termites.
- Increasing our understanding of the physiology and behaviors involved in feeding by subterranean termites: attractancy, repellency, and seasonal trends.
- Study of the biochemical mechanisms related to repellency to enhance protection of structures and counter the avoidance of toxic baits. Also continued study of the behavioral/biochemical basis of intra- and interspecific agonistic behavior in termites.
- Continued refinement of the bait/toxicant approach to subterranean termite control based on technological advances from areas above.
- Reassessment of mirex as the toxicant in termite baits.
- Joe Mauldin discussed the use of borates as preventative treatments. Jeff Morrell (Oregon State University), Terry Amburgey (Mississippi State University), and Lonnie Williams (USDA Forest Service, Gulfport) are the primary investigators. Borates can be applied by diffusion into green (wet, moist) wood. High concentrations of borates on the wood surface diffuse into the center of the wood. Pressure treatment may be used after the wood is dry. There is also the possibility of injecting low concentrations of borates into live trees in hopes of protecting wood after harvest. These treatments are effective against termites, beetles, and many fungi. The treatment is tar-

geted for woods which will be protected from moisture, because borates can be leached from the wood. Effects on nontarget organisms are a concern if high concentrations of borates are misused. Borates are the major ingredients in household products such as Visine and 20-mule-team Borax detergent.

Joe Mauldin also discussed research projects by the scientists at the USDA Forest Service laboratory in Gulfport, Mississippi, directed at methods of protecting wood in use.

- Borates (see above).
- Wood extractives (natural preservatives in woods) are being investigated by Skip McDaniel. Chemicals are extracted, isolated, and their toxicity measured. Most of the toxic chemicals identified thus far are terpenes and alkaloids
- Susan Jones is investigating baiting systems in Arizona for Heterotermes and in Mississippi and Florida for Reticulitermes. Some of the borate compounds appear to have promise in bait systems.
- Traditional termite control with soil toxicants is continuing to be developed by Brad Kard. In the laboratory he screens insecticides to find those with promise for long life in soil as termite barriers. He conducts his field studies at test sites all over the United States. Brad is also field-testing plastics with slow-release pesticide and crushed basalt as termite barriers.

Michael Haverty commented on an observation he (and Joe Mauldin and Nan-Yao Su) made in Southeast Asia. In the urban areas in Southeast Asia, the inhabitants have stopped building with wood; they use cement for the load-bearing parts of structures and use wood only in roof structures, window and door frames, or in ornamental situations where it could be easily replaced. Most of the structurally important wood used in Southeast Asia is either naturally durable or pressure treated.

Joe Mauldin was asked for his assessment of the future of soil pesticides. Mauldin emphasized that the USDA laboratory in Gulfport, Mississippi, will continue to conduct research on alternatives to currently registered soil insecticides. In the near future, control of subterranean termites will mostly be accomplished with soil insecticides. Additional areas of subterranean termite control include biological control: fungi, bacteria and nematodes work well in the laboratory, but in the field these agents have not been shown to be effective. Termites apparently have a defense, or way to fend off these treatments, which renders them ineffective in the field. Dogs are also now being used in termite control. The dogs' ability to detect termites in a structure has not been tested, but those marketing these animals as termite detection "devices" claim the dogs are effective at locating termite infestations.

Vemard Lewis inquired about closer scrutiny and a possible ban of fumigants in California. Apparently state regulatory officials are clamping down on use in structures and in wood yards. Ken Grace mentioned that there is a movement to register methyl bromide for fumigation in structures in Canada. Nan-Yao Su reported that Dow Chemical Co. requested tests of sulfuryl fluoride to satisfy the U.S. Environmental Protection Agency. The goal of this research is to quantify the release of this fumigant from treated structures to support reregistration.

John French, Joe Mauldin, Nan-Yao Su, and Ken Grace discussed the future of bait/toxicants for subterranean termite control. Nan-Yao reviewed the basic concept. Over the past 40 years, the public has been relying on widespread use of huge quantities of pesticides in the soil to control subterranean termites. With bait toxicants, termites bring the toxicant back to colony and disperse it to colony mates, thereby causing the death of the entire colony. Until recently it has been difficult to determine whether termites simply move away or are killed by the bait. Nan-Yao feels it is crucial to monitor activity of foraging populations of field colonies by the mark-releaserecapture technique before we apply test toxins. Chemicals under examination currently are: (1) borates, (2) A-9248 (Abbott Laboratories), and (3) sulfluramid (Griffin Corp). A-9248 reduced a C. formosanus colony from 3.6 million to 75,000 termites. Sulfluramid has toxicity, effective lethal time and acceptability to termites comparable to mirex. Sulfluramid is registered for use in baits for cockroaches and fire ants. Nan-Yao wants to emphasize that we are recommending use of bait toxicants to suppress termite populations in urban areas, not in forests.

Michael Haverty discussed his work and that of Susan Jones and Nan-Yao Su on the disruption of caste structure and demography of colonies. Insect growth regulators induce molting of workers or pseudergates to superfluous soldiers and intercastes. The goal of this control strategy is to cause the termite population to crash because of an excess of dependent castes. This strategy works in the laboratory but, so far, has not been demonstrated in the field. *Coptotermes* can tolerate as much as 50 to 60 percent of the population in the soldier caste.

Ken Grace has begun work to quantify *Reticulitermes flavipes* populations in southern Canada. He has determined that the colonies are as large as 3 million with extensive foraging territories. He will soon be moving to the University of Hawaii to study means of controlling the Formosan subterranean termite. He looks forward to a 12-month, warm field season!

John French discussed his work in Australia with mirex in baits. Mirex bait-blocks kill a mound of Coptotermes lacteus in 6 to 10 days. The system appears to be equally effective in buildings. He further emphasized that a bait/toxicant system must be foolproof to be used effectively by pest control technicians. The advantage of these systems is that they would use small amounts of pesticides, localized placements of pesticides, and the unused remnants can be withdrawn and disposed of after control has been achieved. It is sometimes necessary to practice "drastic carpentry" to place termite baits in the foraging path of the termites. Mirex-agar-sawdust baits kill Coptotermes acinaciformis in Sydney but not in Melbourne. There is obviously a behavioral or species difference. Another approach still used in Australia is to puff a toxic powder into termite galleries or wherever the termites aggregate. This strategy relies on the grooming behavior of termites to disperse the toxicant throughout the colony. The use of arsenic trioxide is still allowed in Australia. Inclusion compounds in peanut-shaped molecules

can be used to dust termites. When ingested during grooming the chemical becomes active and kills the termites.

Finally, Michael Haverty made his pitch for chemotaxonomic research on termites. It was the consensus of the group that one of the areas of concern is the Pacific Rim. *Coptotermes* species are probably the most economically important termites

in this area. We need to study this group extensively. Another ecologically important genus of termites is *Nasutitermes*. Our understanding of deforestation will be greatly enhanced by knowledge of the wood-destroying (or wood-recycling) and herbivorous termite species.



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