# MEASUREMENT OF TERMITE (ISOPTERA: RHINOTERMITIDAE) FEEDING ON PAPER BY VIDEO IMAGE ANALYSIS

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# MEASUREMENT OF TERMITE (ISOPTERA: RHINOTERMITIDAE) FEEDING ON PAPER BY VIDEO IMAGE ANALYSIS

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## ABSTRACT

We describe a method of rapidly measuring the area removed by termites feeding on paper disks by microcomputer controlled video image analysis. The utility of this technique is demonstrated in a short-term (15 day) assay in which *Reticulitermes hesperus* Banks workers fed on filter paper, on rice paper containing a synthetic binder and on rice papers manufactured with natural starch from *Lycoris radiata* (Amaryllidaceae). Termites fed least on rice paper containing natural starch washed only with hot water during the manufacturing process. Greatest feeding occurred on rice paper containing the synthetic binder. Video image analysis is an efficient means of measuring termite feeding activity and comparing feeding on thin substrates of equivalent density and thickness.

Key Words: Termite bioassay, video image analysis, *Reticulitermes hesperus,* rice paper, feeding deterrent, Amaryllidaceae alkaloids.

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## INTRODUCTION

A number of techniques have been employed in laboratory assays to measure the amount of material removed by termites feeding on wood and paper. These range from qualitative indices of feeding damage (McMahan 1966; Beal et al. 1974; Beal 1976) to quantitative measurements, determined either directly on the basis of the weight of the substrate before and after exposure to termites or indirectly by measuring the size of the feeding scars. In tests with wood blocks, direct measurement of oven dried weights of the test blocks is most common (e.g., Su and La Fage 1984), although Reierson (1966), Rust and Reierson (1977) and Rust et al. (1979) used an indirect method of molding modeling clay into the termite feeding scars. Such indirect measurements are readily converted to measures of weight loss. Weight loss over time, adjusted for termite mortality (Su and La Fage 1984), represents the consumption rate. However, calculation of this absolute measure of feeding activity may not always be necessary in comparative assays of suspected behavioral chemicals.

Investigations of the chemical basis of termite food preferences, the resistance of certain woods to termite attack and potential feeding deterrents involve the isolation of chemical compounds and studies of their effects on insect feeding and mortality. These compounds are usually applied to paper disks or filter pads for feeding bioassays because paper is easier to manipulate than α-cellulose or artificial diets (e.g., Howard and Haverty 1979) and feeding is more apparent.

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With these thin substrates, it may be simpler to measure the size of feeding scars than to accurately evaluate weight loss directly.

Weight losses from treated paper disks exposed to termite feeding were used by Rudman and Gay (1963) and by Scheffrahn and Rust (1983) to determine the activity of suspected termite feeding deterrents. However, a problem with the use of substrate weight loss as a measure of termite feeding activity is the hygroscopic nature of paper fibers used as inert carriers of test chemicals. The large surface to volume ratio of paper increases susceptibility to water loss or uptake from the surrounding atmosphere. Impregnation of paper with solvents and test chemicals could have unpredictable effects on hygroscopicity. For example, 7 cm Whatman No. 1 filter papers ($n = 6$, av. wt. $= 347 \pm 13$ mg) to which we applied 1 ml of acetone fluctuated between an average weight increase of $14 \pm 2$ mg and weight loss of $8 \pm 1$ mg over the course of an hour at laboratory conditions (24°C, $35 \pm 1.5\%$ RH) (Grace, unpublished data).

The areas removed by feeding termites from paper disks treated with wood extracts were calculated by Rust and Reierson (1977) by superimposing dots from lettering sheets over the feeding scars in the disks and summing their areas. Disadvantages of this technique are its labor intensive nature and the potential inaccuracies inherent in manually fitting fixed geometric shapes (circles) into irregularly shaped feeding scars.

We describe here a rapid and accurate technique of measuring by video image analysis the area removed by insects feeding on thin substrates such as paper disks. This methodology was originally developed to measure the areas of pathogen-induced leaf lesions (Lindow 1983; Lindow and Webb 1983), and could be implemented in many laboratories on existing video and microcomputer equipment with the addition of the appropriate software.

To illustrate the technique of video image analysis, we describe our evaluation of termite feeding on a Japanese rice paper reputed to resist insect attack (K. Gotoh, pers. comm.). This paper is manufactured with starch from the bulb of *Lycoris radiata* Herb. (Amaryllidaceae) as a binder. The Amaryllidaceae are well known sources of alkaloids, and alkaloids extracted from *L. radiata* bulbs are potent feeding deterrents for the yellow butterfly *Eureme hecabe mandarina* de l'Orza (Numata et al. 1983).

## MATERIALS AND METHODS

Western subterranean termites, *Reticulitermes hesperus* Banks, were collected from infested Douglas fir *(Pseudotsuga menziesii)* framing in a residence in Oakland (Alameda Co.), California, and maintained in a humidity ($94 \pm 5\%$ RH) and temperature ($23 \pm 2$°C) controlled cabinet in our laboratory for two months. Only undifferentiated workers older than the third instar (determined by size) were used in our feeding assays.

Feeding substrates were 25 mm diameter disks cut from Whatman No. 1 filter paper (standard) and from three Japanese rice papers. Two of these rice papers (A and B) are manufactured with natural starch from the bulb of *Lycoris radiata* as a binder, while the third (C) contains a synthetic binder. Rice paper A is reputed to resist insect attack and is manufactured in a traditional manner employing ten hot water washes to extract the *L. radiata* alkaloids and thus reduce the toxicity of the paper to humans. The *L. radiata* starch is washed additionally with acetone during the manufacture of rice paper B.

Six assays were performed with each paper. Each assay consisted of an open 30 ml plastic cup containing 10 mg white sand, 1 ml steam-distilled water, a 25 mm Diam disk and and 30 *R. hesperus* workers. These cups were incubated in a humidity chamber for 15 days. Termites and paper disks were then carefully removed, termite mortality recorded, and each disk brushed clean of sand before video image analysis.

The area of each disk consumed by termite workers was determined with a video image analysis system using an Apple II Plus microcomputer (Lindow and Webb 1983; Lindow 1983) and with a modification of the software Superscan 1.2 (Magnasoft, 5068 Princess Anne Road, La Canada, California 91011). This disk-based software, implemented on an Apple II Plus computer equipped with a single disk drive, color monitor and video analog-digital converter (DS-65, The MicroWorks, Del Mar, CA 92014) examines 32,000 pixels (picture elements) from a video image generated by a black and white video camera (RCA model TC 2011, RCA/Closed Circuit Video Equipment, Lancaster, PA 17604) and records the number of pixels within each of 16 different light intensity ranges. Pixels quantified in different intensity ranges were also represented by different colors in a false color video image reproduced by the program on the Apple II color monitor. This allowed the operator to adjust the video analog-digital converter for maximum resolution of the areas of interest.

To enhance contrast, the white paper disks were examined by video image analysis while on a black velvet background. We used the "slowscan" program option, which quantifies the intensity of each pixel in the image four times and averages the light intensity values. Pixel intensity intervals were assigned to the object within the video image by the method of Lindow and Webb (1983). For example, the mutually exclusive range of pixel intensities representing black background and white rice paper was determined. The number of pixels in the intensity interval representing the paper disk was converted to percent consumption and to units of area ($mm^2$) by comparison to the number of pixels recorded from uneaten control disks and the original disk size (491 $mm^2$).

Nonparametric statistical analysis of disk areas removed by termite feeding was performed with an analysis of variance (ANOVA) of ranked data values and Ryan-Einot-Gabriel-Welsch (REGW) multiple range test, $\alpha = 0.05$ (SAS Institute 1982).

## RESULTS AND DISCUSSION

Successive analyses of video images of rice paper disks with the Apple II image analysis system were highly consistent (Table 1). When three scans were performed on single intact disks, the standard deviation of the mean number of pixels used to estimate the area of the disks in no case exceeded 0.96% of the mean value.

Significantly different ($P = 0.0316$) amounts of termite feeding occurred on the three rice papers (Table 2). Feeding was greatest on the rice paper (C) manufactured with a synthetic binder, and least on rice paper (A) containing *L. radiata* starch washed only with hot water during the manufacturing process. Feeding intermediate to these two rice papers occurred on rice paper B, in which the *L. radiata* starch was washed additionally with acetone during manufacturing.

Table 1. Average size in pixels of intact 25 mm Diam paper disks, each examined three times in succession using an Apple II controlled image analysis system.

| Paper | Replicate | Disk Size ± SD (pixels) |
|---|---|---|
| Rice paper A | 1 | 5433 ± 22 |
| | 2 | 5428 ± 52 |
| | 3 | 5410 ± 3 |
| Rice paper B | 1 | 5465 ± 3 |
| | 2 | 5419 ± 1 |
| | 3 | 5385 ± 18 |
| Rice paper C | 1 | 5394 ± 8 |
| | 2 | 5414 ± 5 |
| | 3 | 5384 ± 19 |
| Whatman No. 1 | 1 | 5415 ± 11 |
| | 2 | 5430 ± 37 |
| | 3 | 5481 ± 37 |

Table 2. Amount removed from 25 mm Diam paper disks by groups of 30 *R. hesperus* workers feeding for 15 days.*†

| | Amount removed by termite feeding (Mean ± SD) | | |
|---|---|---|---|
| Paper | Pixels (No.) | Absolute area ($mm^2$)‡ | % Area |
| Rice paper A | 1946 ± 1754 | 176 ± 158 a | 36 ± 14 |
| Rice paper B | 2233 ± 497 | 202 ± 45 ab | 41 ± 9 |
| Rice paper C | 3870 ± 1284 | 352 ± 117 b | 72 ± 24 |
| Whatman No. 1 | 829 ± 147 | 75 ± 13 | 15 ± 3 |

* Rice paper A is manufactured with *Lycoris radiata* starch washed with hot water, B contains *L. radiata* starch washed once additionally with acetone, and C is manufactured with a synthetic binder.

† Treatment n = 6.

‡ Mean areas of rice papers followed by different letters are significantly different (ANOVA of ranked values, REGW multiple range test, $\alpha = 0.05$). Whatman No. 1 not included in analysis due to greater thickness.

Termite survivorship was slightly higher ($P = 0.0431$) on a filter paper diet than in the rice paper treatments (Table 3). However, survivorship was uniformly high in all treatments, ranging from 85.00% on rice paper A to 93.33% on Whatman No. 1 filter paper, and mean values were not statistically separable ($\alpha = 0.05$). Thus, the observed differences in paper consumption are not attributable solely to differential survivorship.

Our results suggest that feeding deterrency is associated with compounds present in the processed *L. radiata* starch. Results obtained with the thicker Whatman No. 1 filter paper, although not directly comparable to feeding on rice papers, indicate that area analysis would be equally useful in comparing feeding on chemically treated filter papers. This technique could also be applied in tests of longer duration with thicker filter pads or thin wood veneers. Although comparison of the areas of the rice paper disks was sufficient in our assay, and may suffice for many screening purposes, measurements of area can readily be converted to express the weight of material removed, and hence the feeding rate.

Table 3. Average survivorship in groups of 30 *R. hesperus* workers feeding for 15 days.*†

| Paper | Number surviving (Mean ± SD)‡ |
|---|---|
| Rice paper A | 25.50 ± 1.87 |
| Rice paper B | 25.67 ± 2.94 |
| Rice paper C | 27.33 ± 1.51 |
| Whatman No. 1 | 28.00 ± 0.89 |

* See Table 2 for explanation of rice papers.
† Treatment n = 6.
‡ Differences among means are significant (ANOVA of ranked values, $P = 0.0431$), but means are not separable in multiple comparisons (REGW multiple range test, $\alpha = 0.05$).

Area analysis by the use of video images is an efficient method of comparing feeding on thin substrates of equivalent thickness and density. In our short-term bioassay with small numbers of termites feeding on thin paper disks, measurement with an Apple II microcomputer controlled image analysis system appears to be less subject to error than direct weight measurements and is less labor intensive than manual dot-fitting methods.

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