1979

Demography of Termite Colonies as Related to Various Environmental Factors: Nutritional Biochemistry and Physiology of Termites

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1975 PROGRESS REPORT

DEMOGRAPHY OF TERMITE COLONIES AS RELATED TO VARIOUS ENVIRONMENTAL FACTORS: NUTRITIONAL BIOCHEMISTRY AND PHYSIOLOGY OF TERMITES

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US/IBP DESERT BIOME
RESEARCH MEMORANDUM 76-42

in

FINAL PROGRESS REPORTS
Process Studies, pp. 95-117

1975 Proposal No. 2.3.3.4

Printed 1979

Citation format: Author(s). 1979. Title.
US/IBP Desert Biome Res. Memo. 76-42.
Utah State Univ., Logan. 23 pp.

Ecology Center, Utah State University, Logan, Utah 84322

ABSTRACT

This year research attention shifted to the desert dry-wood termite, *Marginitermes hubbardi* (Banks), with the idea of trying to evaluate its total role in the Sonoran Desert ecosystem. A beginning was made with a biochemical analysis of one of its preferred host woods, the giant cactus or saguaro (*Carnegiea gigantea* [Engelm.] Britt. & Rose). Extensive feeding trials were also conducted to determine the rate of wood consumption in relation to temperature and feeding group size. It is planned later to measure oxygen consumption and heat production rates so that simple energy budgets can be constructed and, perhaps, the energy flow through a natural population of this termite estimated.

Samples from dead, but termite-free, and infested saguaro skeletons (*C. gigantea*) were analyzed chemically to assess the importance of wood constituents in nest-site selection and suitability. As there was little or no difference in the carbon, nitrogen, extractive, fatty-acid, ash, lignin and calorific content of these samples, it was concluded that some other factor must determine nest-site adequacy. Skeletons which had been dead at least 4 years and which retained a partial covering of dry, loose cortex for protection of alates during entrance-hole excavation, appeared to be the most attractive to *M. hubbardi* for colony initiation.

Groups of *M. hubbardi* fed saguaro wood and maintained at 24, 26, 28, 30 and 32 C consumed an average of 56.78 mg per g termite (fresh) per day. Wood consumption, which was more closely correlated with termite-group biomass than either temperature or colony origin, was predicted by the equation: $Y = -94.98 - 0.0623B + 0.30T^2 + 0.00368BT$; where Y = mg of wood consumed; B = mean daily termite biomass multiplied by the duration (in days) of the experiment; and T = temperature (°C).

INTRODUCTION

To this point in our termite studies we have dealt mainly with field-oriented subjects such as populations, foraging activity and the production and swarming of the winged forms. Much of this work has been accomplished with subterranean termites because their colonies are large, complex and active most of the year. In this final phase introduced here, we have turned to the laboratory to investigate food-energy relationships. The common dry-wood species, *Marginitermes hubbardi*, has proved to be a very suitable animal for these studies, largely because it is so very easy to maintain in culture.

All termites consume cellulosic materials as their primary energy source. The sole nourishment for many species, notably among the Kalotermitidae, is sound, decay-free dead wood which may contain as little as 2.5-3.0% moisture. Other species that nest in the soil forage for dead wood, grass, plant litter and humus. Still other species restrict their diets to dead roots, animal dung or, as among the Macrotermitinae, to fungi cultured on recycled plant material within their nests.

According to Kirk (1971), cellulose is the most abundant of the continuously cycled organic materials. The two groups of organisms capable of digesting cellulose use different digestive strategies. Among the first group are some protozoans, mollusks and arthropods that produce their own cellulolytic enzymes. Members of the second group, which includes ruminants, lack this ability, but possess a rich gut fauna and/or flora which partially degrade cellulose to components that the host can assimilate. There is mounting evidence that termites belong to both groups (LaFage and Nutting, in press). Among the cellulolytic animals, few have been studied more intensively than the termites and their symbiotes, yet we still know very little about their digestive physiology. The lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae) have symbiotic protozoans in the hindgut; the higher termites (Termitidae) do not, but contain symbiotic bacteria instead. Honigberg (1970), LaFage and Nutting (in press) and Noirot and Noirot-Timothée (1969) have written general reviews of cellulose digestion by termites. Lasker (1959) presented a valuable discussion of the problem of defining the relative importance of host and microorganism in cellulose digestion. The ability to utilize cellulose efficiently accounts largely for the widespread success of termites. Emerson (1955) suggested that, because potential food is so abundant, other factors such as the availability of suitable nesting sites may be more restrictive on the growth and dispersal of termite colonies.

Members of the family Kalotermitidae are known as the dry-wood termites because, with few exceptions, their nests are permanently excavated within the wood on which the colony feeds. Mature dry-wood colonies rarely exceed a few thousand individuals (Nutting 1969). In contrast, several rhinotermitid and termitid species routinely have colonies exceeding a million individuals (Wilson 1971). Owing to the fact that kalotermitids can be maintained easily and studied in the laboratory, more is known of their physiology than of the more advanced species, especially the Termitidae. The ecological significance of kalotermitid species remains poorly understood, however. On the other hand, recent investigations, including those by Haverty and Nutting (1975), Haverty et al. (1974, 1975), Johnson and Whitford (1975), LaFage et al. (1973) and Bodine and Ueckert (1975) have done much to clarify the role of subterranean species in arid ecosystems. These studies suggest that subterranean termites may at times consume a large percentage of the annual dead wood and dead grass production. In western Texas, Bodine and Ueckert (1975) have shown that termites compete significantly with large herbivores for grasses. Similar effects on grasslands were noted by Coaton (1951) and Watson and Gay (1970).

M. hubbardi is the most destructive dry-wood termite in the Tucson area. It ranges through central and southern Arizona, south along the coast of western Mexico, at least to the city of Colima. Although the extent of its eastern range in Mexico is unknown, it is generally replaced at higher elevations by *Incisitermes marginipennis* (Latreille). In California, *M. hubbardi* is restricted to a small area along the southeastern border. It is common all along the eastern coast of Baja California. In Arizona it is replaced entirely by *Incisitermes minor* (Hagen) above 1,000 m, while at intermediate elevations, including Tucson (635 m), the two species are sympatric. At Phoenix (275 m) and other low elevations *I. minor* is replaced by *M. hubbardi*, which is considered to be better adapted to high temperature and low moisture.

M. hubbardi consumes a variety of dead woods, including cottonwood (*Populus fremontii* Wats.), paloverde (*Cercidium microphyllum* [Torr.] and *C. floridum* Benth.), ash (*Fraxinus velutina* Torr.), sycamore (*Platanus wrightii* Wats.) and the ribs of the saguaro cactus (*Carnegiea gigantea* [Engelm.] Britt. & Rose).

The overall objective of this study is to measure the ecological significance of *M. hubbardi* in a saguaro forest in the Sonoran Desert. Three types of investigations are planned to answer the basic question. These include studies of saguaro wood composition, nutritional physiology and trophic interactions. Finally, information on saguaro distribution and termite biomass available from other sources can be used to estimate annual energy flow through a population of *M. hubbardi* on a 4-ha plot on the Saguaro National Monument, east of Tucson, Arizona.

OBJECTIVES

During the final two years of research, long-term objectives will be limited to an evaluation of the role of a single species of dry-wood termite in the Sonoran Desert ecosystem with respect to its

1. Capacity for processing dead wood.
2. Nutritive suitability as a prey item.
3. Energy relations and interactions with other trophic levels.

Supporting objectives have guided the research on this dry-wood termite during 1975 as follows:

1. To determine several biochemical characteristics of one of its preferred host woods, the saguaro.
2. To measure its rate of wood consumption with respect to temperature and feeding group size.

METHODS

The Dry-Wood Termite, *Marginitermes hubbardi* (Banks)

This is probably the most common dry-wood species in the Sonoran Desert. It ranges through central and southern Arizona below ca. 1,000 m, adjacent southeastern California, over much of Baja California and the western coast of Mexico south at least to the city of Colima. It attacks dead branches, stumps and logs of many trees in riparian situations, but favors dead paloverde and saguaro skeletons in the desert. It is also a destructive "house termite." The termites used in these studies were collected from dead saguaros found along the roads in the vicinity of the Silverbell site.

The Saguaro, a Preferred Host Wood

The saguaro, largest of the United States cacti, is endemic to the Sonoran Desert (Benson 1969). A healthy, mature plant may attain formidable dimensions, including a height of 13 m, weight of more than 5,000 kg and life span of 200 years (Berry et al. 1960).

After the death of a saguaro, recognized by the total absence of green tissue, the initial stages of decay proceed rapidly. The epidermis and unusually thick, pulpy cortex slough off to expose an inner framework of ligniferous ribs, the secondary xylem. This structure may be called the skeleton, for it provides the sole support of the living plant. The woody skeleton decays much more slowly than the other tissues, requiring 30 years or more to disappear from the desert floor. During certain stages of decay, saguaro skeletons are especially attractive as nesting sites for *M. hubbardi*. Field observations suggest that neither very young nor very old skeletons are potential hosts for termite colonies, although the reason for this is not yet clear. One possible explanation might be that the decay process alters the chemical composition such that the wood is attractive for only a limited period.

To test this hypothesis, three categories of dead saguaro skeletons were selected: A, B and C. **Group A** included plants which were considered too young to support *M. hubbardi* (Figs. 1 and 2). Typically these skeletons remain upright with a firm footing in the soil although the plant may have been downed by wind or lightning. The basal third or more is generally covered with intact, but very dry, cortex; also, in this region an abundance of fibrous material (dried pith) adheres to the ribs. **Group B** skeletons (Fig. 3) retain less or, at times, no intact cortex, less fibrous material and, if standing, a loose and easily disturbed footing in the soil. Quite often they have become dislodged and lie on the soil surface or propped up against some other vegetation. There remains in practically every instance a soil connection with part of the root system. Those skeletons that rest on the surface often have been attacked by subterranean termites at points of soil contact. Group B skeletons frequently contain one or more *M. hubbardi* colonies. **Group C** plants (Figs. 4 and 5) are older and more decayed than the others. They are always prone, rarely retain soil connections with the root system and at times have lost their roots and basal sections completely. In advanced decay (Fig. 5) little structural integrity remains; ribs are fragmented, and the assaults of weather and fungi are extensive. Skeletons in this category never contain active colonies of *M. hubbardi*, but often show evidence that they did at an earlier time.

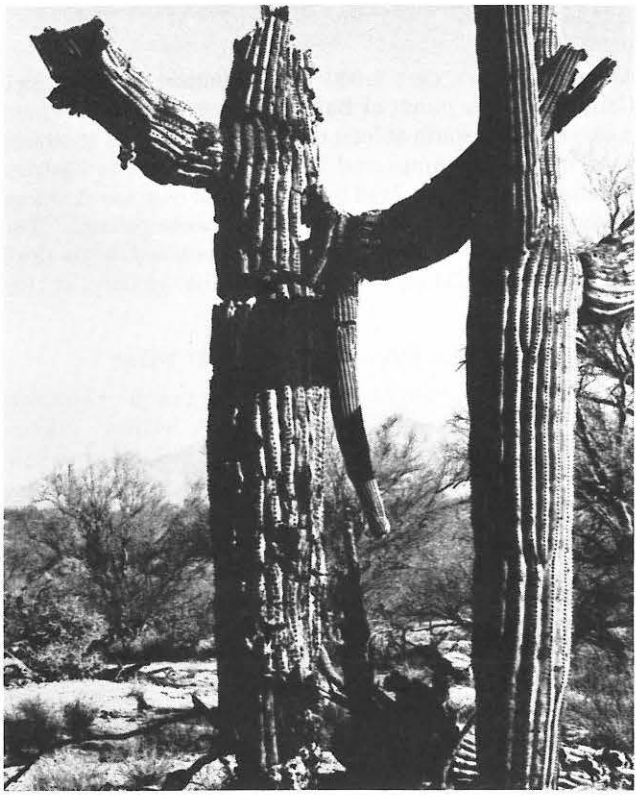

Figure 1. Early Group A saguaro skeleton.

Figure 2. Late Group A saguaro skeleton.

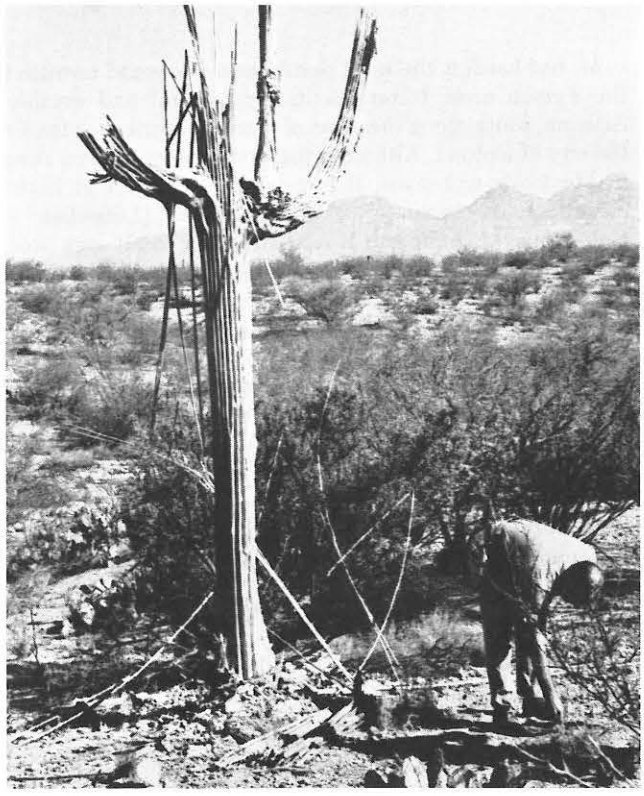

Figure 3. Group B saguaro skeleton.

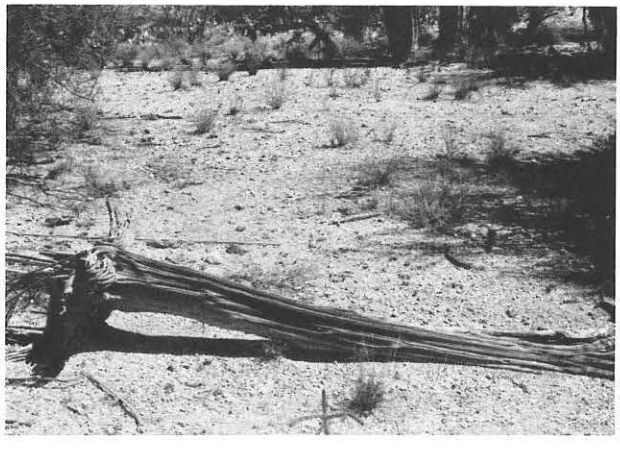

Figure 4. Group C saguaro skeleton.

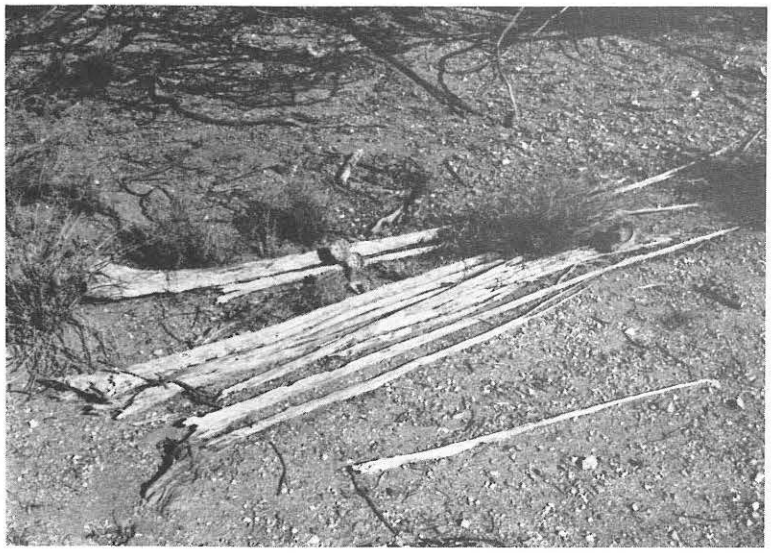

Figure 5. Very late Group C saguaro skeleton.

During late summer 1975, 30 saguaro skeletons, 10 from each group, were collected on or adjacent to the Silverbell site. The basal portion, ca. 0.5 m (Fig. 6), of each specimen was removed, labeled, bagged and returned to the laboratory. Information used in estimating energy flow through a natural population of this termite was later gathered from a 4-ha plot in section 17 of Saguaro National Monument (east; SNME) near Tucson.

Biochemical Studies on Dead Saguaro Wood

All chemical determinations described in this section were performed on wood removed from the 30 skeletons. Prior to analysis, 600-g wood samples were collected from each skeleton and cleaned of any dried pith, cortex or soil. A hatchet was used to split the ribs into slivers small enough to pass through a large Wiley® mill (Arthur H. Thomas Co., Model No. 4) fitted with a 3-mm sieve. The resulting wood dust was further comminuted in a small Wiley® mill (intermediate model) fitted with a 60-mesh delivery unit and 4-oz screw-cap glass jar. The resulting "wood flour" was dried at 105 C for 24 hr.

Extractives—Sixty 20-g wood flour samples, two from each of the 30 skeletons, were subjected to successive solvent extractions in Soxhlet extractors. Individual samples were transferred to dry, single-thickness (123 x 43 mm) preweighed thimbles (Whatman). A preweighed glass wool plug was placed on top of the wood flour to prevent sample loss during extraction and the total preparation was weighed. Samples prepared in this manner were extracted continuously for three successive 6-hr periods with ethanol (95%), ethanol:benzene (1:2, vol/vol) and hot water, respectively. After the thimble had drained and cooled, the entire sample was dried for 24 hr at 105 C, cooled in a desiccator and weighed. Weight loss was considered to represent total extractives and was expressed as a percentage of unextracted, dry, wood flour. Filtrates resulting from these extractions were stored in 1-qt glass bottles. Since it was expected that hot-water filtrates might contain soluble sugars, several samples were tested with Molisch's reagent (a 5% solution of α-napthol in ethyl alcohol). In this test a reddish-violet zone appearing between the sample and an equal volume of concentrated sulfuric acid indicates the presence of carbohydrates in the sample (Oser 1965).

Wood Lipids—The total lipid content of saguaro wood was measured using a modification of the method described by Folch et al. (1957). Thirty 4-g wood samples were homogenized individually in 40 ml of chloroform:methanol (2:1, vol/vol) for 3 min in an omni-mixer (Sorvall Co., Model 17150) on speed 4. The mixture was filtered and the solid residue, resuspended in 40 ml of solvent, was homogenized for an additional 3 min. The mixture was filtered again, washed with 40 ml chloroform:methanol and the filtrates collected in a 500-ml separatory funnel. Thirty milliliters of 0.88% potassium chloride in water were added. The mixture was shaken vigorously and allowed to settle for 24 hr before proceeding. During this time, lipids were partitioned into the lower chloroform layer which was subsequently collected in a tared Nalgene® beaker and dried in a vacuum oven for 48 hr at 40 C. The resulting

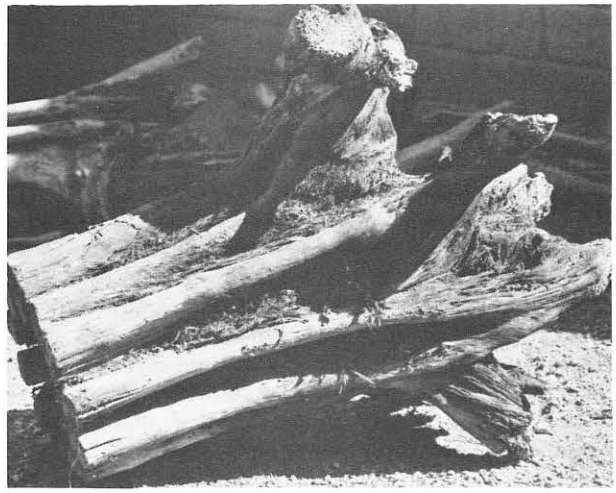

Figure 6. The basal portion of the dead saguaro (*Carnegiea gigantea* [Engelm.] Britt. & Rose).

lipids were desiccated, weighed, resuspended in a volume of chloroform and stored at 4 C for further analysis.

Preparation of Methyl Esters for Fatty-Acid Analysis—Methyl esters of the fatty acids contained in the above extracts were prepared using a modification of the H_2SO_4-CH_3OH procedure described by Rogozinski (1964) as follows: A ca. 50-mg lipid sample (2-3 drops) was placed in a 15-ml centrifuge tube and ca. 2 ml of benzene added. To this was added 4.5 ml of 0.5% sulfuric acid in absolute ethanol (by volume) and a boiling chip. The preparation was capped, heated for 2.5 hr at 90 C on a heating block and allowed to cool. Two milliliters of redistilled petroleum ether were added, mixed thoroughly, allowed to settle and the methyl esters recovered in the ether layer. The fatty-acid methyl esters were stored in redistilled hexane at 4 C.

Purification of Methyl Esters—Fatty-acid methyl esters were separated from contaminates on 1-mm-thick, thin-layer plates (20 x 20 cm) coated with silica gel G-PF 254 (Brinkman Instruments, Inc.). Plates were activated at 105 C for 1 hr prior to streaking. A one-dimensional solvent system consisting of redistilled hexane, ethyl ester and acetic acid (160:40:2, vol/vol/vol) was used to isolate the methyl esters. The plates were sprayed with Rhodamine B (Supelco, Inc.) and read under ultraviolet light. The methyl-ester band was marked, collected in redistilled hexane and stored at 4 C.

Identification of Fatty-Acid Methyl Esters—Fatty-acid methyl esters were identified and measured using a gas chromatograph (Tracor, Model MT220) equipped with a flame-ionization detector. A 6-ft glass column (1/4-inch ID) was packed with 15% EGSS-Y (Applied Science Laboratories, Inc.), a copolymer of ethylene glycol succinate containing methyl-silicone, on 100/120 mesh Chromosorb AW-DMCE (Supelco, Inc.) as suggested by Christie (1973). The carrier gas, nitrogen, was delivered with a head pressure of 40 psi and a flow rate of 70 ml per min. The column was held at 170 C (isothermal) and the detector temperature was 250 C. Straight-chain, even-numbered, methyl esters of fatty acids from C_8 to $C_{18:3}$ (Supelco, Inc.) were used as internal standards. Methyl esters prepared from the saguaro flour were injected into the gas chromatograph in hexane with a 1-μl syringe (Hamilton Co., Model 7101N). Peak areas were measured by triangulation (Bartlet and Iverson 1966) and results expressed as percentages of total fatty acids in the sample.

Carbon Analysis—Two 50-mg samples from each of the 30 skeletons were analyzed to determine their carbon content with a high-frequency induction furnace (Leco, Inc.), according to the method outlined by Allison et al. (1965). Samples were weighed into tared ceramic crucibles and a catalyst containing iron, copper and tin was added. The crucible was inserted into the furnace and burned in a stream of oxygen for 3 min. Carbon dioxide released during combustion was trapped in an Ascarite® bulb from the atmosphere. After every seven determinations, a glycine sample was analyzed to determine recovery efficiency.

Ash Analysis—Three 2-g samples from each of the 30 skeletons were analyzed for inorganic mineral content (ash). Samples weighed into tared ceramic crucibles were burned in a muffle furnace (Thermo Electric Co., Model F-1740) at 550 C for 18 hr. The oven was turned off and allowed to cool before the crucibles were transferred to a desiccating jar containing indicating Drierite®. The ash samples were weighed after an additional 24 hr.

Calorific Content—Two 1-g samples from 21 of the 30 saguaros were ignited in an oxygen-bomb calorimeter (Parr Instrument Co., Model 1221) to measure heat of combustion, i.e., total calorific content. After loading the sample, the bomb was charged with O_2 at 30 psi and immersed in a water jacket. The system was closed and allowed to equilibrate before firing. Corrections for heat generated from the fuse wire and formation of H_2SO_4 and HNO_3 in the bomb were applied to the observed heat rise of the water surrounding the bomb.

An additional series of samples was analyzed to determine if the calorific content of weathered saguaro differed from that in the center of a rib. Results were expressed as kcal/g dry wt.

Nitrogen Content—Wood contains so little nitrogen that conventional Kjeldahl procedures are inadequate, tending to produce inaccurate and inconsistent results. A modified micro-Kjeldahl procedure described by Rennie (1965) is especially well suited for analysis of woody tissue and therefore was used in this study.

Two 200-300 mg wood-flour samples from each of the 30 skeletons were analyzed as follows: The samples were weighed into 100-ml Kjeldahl flasks to which were added ca. 40 mg of mercuric oxide catalyst, 4 g of potassium sulfate and 5 ml of concentrated sulfuric acid. To minimize foaming, 2-3 drops of Dow-Corning antifoam solution were added to each flask. Two blanks were prepared for every 10 unknowns. The flasks were swirled gently and allowed to stand overnight before the Kjeldahl digestion was effected on electric heating racks in a fume hood. The thermostats were set initially as low as possible to minimize frothing and loss of samples from the flask necks. After clearing (ca. 2 hr) the settings were gradually raised to a final position of No. 6. Total digestion required 6 hr. After cooling, but while still warm, ca. 10 ml of water were added to each flask. The addition of water before the preparations reach room temperature is critical because, when completely cooled, the sample becomes solid and extremely difficult to remove from the reaction flask.

After further cooling, the digests were removed and brought to volume in 25-ml volumetric flasks. At this point the samples were stored in a refrigerator at 4 C until they could be distilled. The distillation of ammonia from a 10-ml aliquot of the digest was carried out in a micro-Kjeldahl distilling unit (Arthur H. Thomas Co., Kirk Model) as follows: A 50-ml Erlenmeyer flask containing 10 ml of a 2% boric acid solution and 2-3 drops of modified methyl red indicator (0.125 g methyl red, 0.0825 g methylene blue, 100 ml ethanol) were attached to the delivery tube of the distilling apparatus. Ten milliliters of base solution

(containing 60% [wt/vol] sodium hydroxide [pellet form] and 5% [wt/vol] sodium thiosulfate) were added slowly to the apparatus which held the sample. Heating commenced and continued for 7 min after the first drop of condensate appeared on the cold finger. The boric acid solution, now green, was titrated to its blue-purple end point with standarized hydrochloric acid dispensed from a 10-ml buret. Percent nitrogen in the sample was calculated by the following formulas:

$$\text{nitrogen} = \{[\text{titration (ml)} - \text{blank (ml)}] \times \text{standard factor (mg/ml)}\} / \text{sample wt (mg)}$$

$$\text{standard factor} = (\text{normality of HCl} \times 0.014 \text{ g nitrogen} \times 1{,}000 \text{ mg}) / (1 \text{ meq wt} \times 1 \text{ g nitrogen})$$

Amino Acid Profile—Contrary to most other operations, only one amino acid determination was carried out on the saguaro wood. Amino acid determinations are extremely difficult to perform on woody tissues (LaFage and Nutting, in press). The procedure selected to analyze the sample (B7) was proposed by Scurfield and Nicholls (1970). Seven grams of extractive-free saguaro flour were combined with 700 ml 6 *N* HCl in a 1-liter round-bottom flask. After sitting overnight, the sample was refluxed for 16 hr at 115 C and 15 psi. The hydrolyzate was dried in a rotavapor-flask evaporator (Brinkman Instrument Co., Model R/A) at room temperature. The resulting residue was resuspended in water, filtered and evaporated once more before bringing to volume with citrate buffer, pH 2.2. Amino acids were measured with an automatic amino acid analyzer (Beckman Instrument Co., Model 121). Resulting peaks were identified by comparison with the Beckman amino acid calibration mixture, type 1, and quantities of individual amino acids calculated by a computer program. Results were expressed as percentages of total amino acids present in the hydrolyzate.

Lignin Analysis—Classical lignin analysis (Ritter and Barbour 1935) suffers from two major drawbacks; large samples (2 g) and long reaction times (ca. 15 hr) are required. A rapid (< 1 hr) spectrophotometric method for the analysis of samples containing 3-6 mg of lignin was proposed by Johnson et al. (1961). A total of 50, 10-20 mg extractive-free samples from the three saguaro groups was examined using this method. The saguaro flour was added to 15 x 150 mm test tubes containing 10 ml of a 25% acetyl bromide solution (in acetic acid). A glass marble was placed over the tube opening and the preparation heated for 30 min in a water bath at 70 C. The tubes and their contents were swirled gently at 10-min intervals to ensure that the reagents were mixed well and to promote dissolution of the sample. After cooling in water at 15 C, the reaction mixture was transferred quantitatively to a 100-ml volumetric flask containing 9 ml of 2 *M* sodium hydroxide and ca. 50 ml of acetic acid. One milliliter of 7.5 *M* hydroxylamine hydrochloride was added, the contents mixed thoroughly, cooled and brought to volume with acetic acid. The absorbency of this solution was read at 280 mμ on a grating spectrophotometer equipped with a recorder (Beckman Instrument Co., Model DB-GT) and percent lignin calculated by comparison with the absorbency of a standard prepared from pure lignin. The following equations were used:

$$a_{st} = (A_{st} - A_b)/C$$

$$\text{percent lignin in unknown} = [100V(A_s - A_b)]/wa_{st}$$

where

a_{st} = absorptivity of lignin standard
A_{st} = absorbency of lignin standard
A_b = absorbency of blank
C = concentration of lignin in g/l
V = volume (l) of solution used
A_s = absorbency of sample
w = weight of sample in g

To check the accuracy of this method, three replicates from skeleton A8 were analyzed by the highly reliable H_2SO_4 method (Ritter and Barbour 1935).

Delignification—The quantitative determinatin of holocellulose in wood is possible only after lignin has been removed from the wood (Browning 1969). The chlorite delignification technique described by Ritter and Barbour (1935) was employed to reduce the lignin content of saguaro flour to 2-3% of the original sample weight. Skeleton A8 was selected for a preliminary investigation. Three replicates of extractive-free flour from this sample had an average lignin content of 25.5% determined by the H_2SO_4 method and 28.4% by the acetyl bromide procedure. One gram of extractive-free flour was transferred to a 125-ml Erlenmeyer flask and 32 ml H_2O, 0.1 ml acetic acid and 0.3 g sodium chlorite added. Additions of sodium chlorite and acetic acid in the amounts specified above were made at regular intervals for the duration of the experiment. The reaction was carried out in a water bath at 72 C during periods of 2.25 to 4 hr. The reaction flask was fitted with an externally driven stirrer. After the reaction was completed, the flask was cooled in ice water and the reaction mixture was filtered and washed successively with cold water, acetone and ethyl ether. The preparation was dried at 30 C under vacuum for 12 hr and weighed. The filtrate was tested with Molisch's reagent to determine if the washing had removed any carbohydrates. No attempt was made to analyze cellulose components because delignification had not been entirely successful.

Data Analysis

Wood-composition data were examined where appropriate with a one-way analysis of variance. Treatment means were tested for significance at the $\alpha = 0.05$ level.

Nutritional Physiology

Natural ecosystems are extremely complex associations of living organisms with their nonliving habitat. Very few investigators have estimated energy flow at the community level. Notable exceptions include the studies by Lindeman (1942), Odum and Odum (1955), Odum (1957) and Teal (1957). Darnell (1968) noted that an understanding of energy flow through a community depends to a large extent on an understanding of the energy dynamics of the

individuals comprising that community. Indeed, certain physiological parameters (e.g., total heat production) cannot be measured in the field. Others (e.g., assimilation) are much easier to assess in the laboratory. Engelmann (1961, p. 221) suggests, "There are two aspects to any study of energetics: the field survey and the laboratory experiment." The same author (Engelmann 1966, p. 80) declares, "Paradoxically, a good portion of the data necessary for field estimates must come from laboratory studies." The situation has not changed dramatically during the last 10 years. Although radiotracers appear to offer a promising approach to identifying trophic exchanges in nature (Paris and Sikora 1967), the techniques developed so far are not well suited to dry-wood termites living entirely within wood. It was decided, therefore, that population level estimates on energy flow in *M. hubbardi* would necessarily be projected from laboratory data. The studies described below have provided such data.

Feeding Trials

A complete energetics analysis must include estimates on rates of ingestion, egestion, assimilation, growth, death, numbers, biomass and the calories represented by these figures. Laboratory feeding studies under controlled conditions can provide data on many of these parameters. Brian (1971) suggests that accurate estimates of feeding, assimilation and respiration rates on social insects require that the experimental unit be representative in age and caste structure of the parent society. Wilson (1971) discusses another facet of social insect biology which may affect physiological measurements. As defined by Wilson (1971, p. 297), the "group effect is an alteration in behavior or physiology within a species brought about by signals that are directed in neither space nor time." The density of individuals in a social insect colony is known to have an effect on physiological processes. It was decided, therefore, to measure food intake for differently sized groups of *M. hubbardi*.

Incipient Colonies—*M. hubbardi* alates were collected at lights during dispersal flights in July 1972. Within 2 days they were sexed, paired (one male, one female) and introduced to 41 rearing chambers (100 x 20 mm disposable plastic tissue-culture dishes). Each dish was provided with one to three preweighed and slightly moistened saguaro discs as food. The incipient colonies were held at 32 C and ca. 100% RH until September 3, 1975, when the 16 surviving colonies were dismantled, the termites counted and remaining wood weighed. In addition, the caste of each surviving termite was recorded (A3UNE17).

Intermediate Groups—Numerous saguaro skeletons collected from an area adjacent to the Silverbell site during June and July 1975 contained large *M. hubbardi* colonies (>1,000 individuals) which provided animals for the following experiment. Five groups of 5, 10, 20 and 50 termites (larvae, nymphs and one soldier) were selected from each of three large colonies. Since termites from each colony were represented in every size category experimental unit, variation among the three original termite sources could be ascertained. Termites were allowed to feed on saguaro discs (dried at 105 C for 24 hr) for 90 days in 8-oz wide-mouth glass jars. The following data were recorded for each of the 60 experimental units: initial and final termite biomass; number and caste of survivors after 90 days; ingestion, egestion and the weights of exuviae, carton (a mixture of feces and undigested wood used for construction); dead termites; and unused material. These data and several additional variables calculated from them were examined by an analysis of variance utilizing a randomized complete-block design with a factorial selection of treatments. The survival percentages were transformed to probits before analysis. Treatment means were tested for significance at the $\alpha = 0.05$ level and separated using Student-Newman-Keul's test (Steel and Torrie 1960) or the least significant difference. Where applicable, multiple linear regression equations were developed by the forward stepwise method described by Kim and Kohout (1975).

Daily inspection of the rearing units revealed that mortality occurred predominantly during the early days of the experiment. A biomass-time estimate was therefore required to calculate food consumption and other variables on the basis of response per termite wt per unit time. To be realistic, this estimate should represent average standing crop biomass and the total time of the study. Petrusewicz (1967) and Petrusewicz and Macfadyen (1970) have discussed such a term, "biomass-days" (BT), which they define by the following expression:

$$BT = \sum_{i=1}^{T} B_i$$

or

$$BT = \overline{B} \cdot T$$

where

B_i = daily standing crop biomass
T = time of the study
$\overline{B}$ = average standing crop biomass

$\overline{B}$ is further defined by the expression:

$$\overline{B} = (1/K) \sum_{i=1}^{K} B_i$$

where

K = number of records (days in the study)

Three assumptions were made regarding the behavior of the intermediate-sized feeding groups in order to calculate BT.

1. Mortality occurred only during the first 10 days of the experiment and was linear.
2. Although the group weight changed during this 10-day period due to removal of individuals through mortality,

Table 1. Percentage of saguaro wood (dry wt) removed by successive 6-hr extractions in ethanol (95%), ethanol:benzene (1:2, vol/vol) and hot water[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	10	4.50[a]	0.35	3.12-6.15
B	10	5.25[a]	0.52	2.44-7.73
C	10	4.09[a]	0.31	2.97-6.13

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 2. Percentage of saguaro wood (dry wt) removed by two 3-min extractions with chloroform:methanol solution (2:1, vol/vol)[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	11	2.41[a]	0.18	2.10-3.08
B	10	2.17[a]	0.29	1.32-3.95
C	9	2.95[a]	0.41	1.79-4.99

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by Student-Newman-Keul's Multiple Range Test.

surviving individuals retained their initial weights until the beginning of day 11.

3. Individual weight change over the last 80 days was linear.

The first assumption, though not entirely valid, is largely substantiated by direct observations on the experimental units. Assumption 2, on the other hand, is based on speculation that groups did not settle into a uniform feeding regime for some time after establishment. It is possible that a small weight loss occurred during this period. The assumption is sound when, as in this experiment, weight change is less than twofold (Gordon 1968). Based on these assumptions, a computer program requiring input of only initial and final termite biomass and the number of survivors in each group was used to calculate BT. A number of variables including ingestion, egestion and egg production were subsequently expressed on the basis of response/biomass-days (A3UNE17).

Large Groups—Seven *M. hubbardi* groups containing 490-500 individuals and prepared in the same manner as the intermediate groups were maintained for 90 days at 20, 22, 24, 26, 28, 30 and 32 C. Termites at each temperature were from the same colony and were representative of original caste composition of the colony. The 20 C group suffered excessive mortality from a bacterial infection (probably *Serratia marcescens* Bizio) and was not included in the data analysis (A3UNE17).

RESULTS

Biochemical Studies of Dead Saguaro Wood

Analytical Determinations

After the initial comminution of 600 g of wood collected originally from each saguaro skeleton, only 500 g remained. The second grinding (in the smaller Wiley mill) to produce 60/80 mesh wood flour was very time consuming and therefore continued to produce only ca. 100 g of each sample.

Extractives—Due to the strong retention of ethanol by wood, it was not possible to measure accurately the specific amount of extract removed by individual solvents. The results of 60 saguaro extractions are summarized in Table 1. Three separate samples from the hot-water filtrates showed positive reactions with Molisch's reagent. As cellulose is soluble in neither hot nor cold water (Oser 1965), it is probable that starch, gums, mucilages and monosaccharides were present in these filtrates.

Wood Lipids—Chloroform and methanol in the ratio of 2:1 (vol/vol) is generally considered the most effective, simple solvent system for the extraction of lipids from plant and animal tissues (Christie 1973). However, it was difficult to use this system with samples of less than 4 g, especially when lipid content was low ($<10\%$). In most cases the 4-g saguaro flour samples contained little more than 80 mg of lipid (Table 2).

Fatty Acids in Saguaro Wood—Fatty acids in saguaro extracts were expressed as percentages of total peak area. The results reported in Table 3 are mean values from duplicate chromatographic analyses on 21 wood samples. Methyl esters with carbon chains less than 12 were unresolved. A large percentage of the fatty-acid methyl esters present in the wood samples consisted of palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2). Three groups of minor components were recognized, including: 1) methyl esters with retention times less than palmitate, 2) those between palmitoleate and stearate and 3) those higher than linoleate.

Carbon Analyses—The carbon contents of 30 saguaro samples, 10 from each of the three age categories, are presented in Table 4.

Ash Analyses—The ash residues from the combustion of saguaro samples from the 30 skeletons, 10 from each category, are shown in Table 5.

Calorific Content—The calorific content (kcal/g dry wt) was determined for 21 of the 30 saguaro skeletons by macrobomb calorimetry and the data summarized in Table 6. Calorific differences between inner and superficial surface wood from the saguaro ribs were of potential interest with regard to both dry-wood and subterranean termite nutrition, as *Gnathamitermes perplexus* (Banks) attacks the weathered surfaces of dead saguaro skeletons.

Table 3. Fatty acids in total chloroform:methanol (2:1, vol/vol) extracts of saguaro skeletons of three age categories. Values are mean percentages of total peak area

		Summary of Means							
Category	No. of samples	Group 1	C16:0	C16:1	Group 2	C18:0	C18:1	C18:2	Group 3
A	5	5.01[a]	31.58[a]	2.56[a]	2.54[a]	27.47[a]	25.79[a]	5.31[a]	0.00[a]
B	8	4.91[a]	30.79[a]	1.71[a]	3.13[a]	23.41[a]	29.31[ab]	6.81[a]	0.00[a]
C	7	4.40[a]	25.00[b]	3.04[a]	2.75[a]	19.48[b]	33.09[b]	11.02[b]	0.14[a]

[a]Each sample value is the mean of 2 replicates.

[b]Means in the same column followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 4. Percent carbon content of saguaro samples representing three age categories[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	10	45.45[a]	0.33	43.35-46.57
B	10	44.96[a]	0.17	44.16-45.58
C	10	46.02[a]	0.17	45.09-46.83

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 5. Percent ash in saguaro samples representing three age categories[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	10	3.83[a]	0.40	1.94-6.24
B	10	3.39[ab]	0.39	1.70-5.72
C	10	2.62[b]	0.21	1.80-4.07

[a]Each sample value is the mean of 3 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 6. Calorific content (kcal/g dry wt) of saguaro samples from the 30 skeletons representing three age categories. Data obtained by macrobomb calorimetry[a]

Category	No. of Samples	Mean kcal/g[b]	SE	Range
A	7	4.43[a]	0.04	4.28-4.56
B	8	4.44[a]	0.03	4.37-4.55
C	6	4.49[a]	0.03	4.37-4.56

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 7. Calorific content (kcal/g dry wt) of wood samples from superficial and inner parts of a dead saguaro rib[a]

Sample	No. of Samples	Mean kcal/g[b]	SE	Range
Surface	5	4.43[a]	0.03180	4.328-4.509
Inner	4	4.63[b]	0.01600	4.604-4.669

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as tested by the t distribution.

The data in Table 7 show that there was a statistically significant difference between the samples from the two regions of the rib.

Nitrogen Content—Rennie (1965) suggested the use of 30-ml Kjeldahl flasks for the digestion of wood flour samples. However, trials with this size flask were unsuccessful due to excess frothing during heating. The addition of antifoaming solution and the use of the 100-ml flasks eliminated much of this problem. The results of the nitrogen analyses are presented in Table 8.

Amino Acid Profile—Total amino acid analyses are often inaccurate measurements even under the best conditions. During hydrolysis with 6 *N* HCl, tryptophan is so labile that it is lost completely. Other amino acids including cystine, threonine and serine, though less labile, are partially destroyed. The addition of sodium thioglycolate to the hydrolysis mixture protects methionine but destroys cystine. It was not added to the single wood sample hydrolyzed.

Scurfield and Nicholls (1970) found that many factors affected the outcome of their amino acid measurements on wood. Among these were the ratio of acid to wood during hydrolysis, humin formation and the loss of amino acids through interaction with wood constituents such as lignin. Also, certain non-amino acid wood components may appear as peaks on the chromatogram. Thirty-three peaks were resolved from the saguaro wood sample. Of the total area resolved, 6.63% was associated with 15 unknowns. The compounds for which authentic standards were available are listed in Table 9.

Lignin Analysis—The results of 50 lignin measurements on saguaro samples from the three groups of skeletons are presented in Table 10. All determinations were carried out on extractive-free samples to avoid contamination by catechol and tannins. The sulfuric acid delignification procedure (Ritter and Barbour 1935) was used as a check on the accuracy of the acetyl bromide determinations. Three H_2SO_4-treated samples from skeleton A8 had average lignin content of 25.00%, a value slightly lower than the mean for

Table 8. Percent nitrogen content of saguaro samples from skeletons representing three age categories[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	9	0.34[a]	0.02	0.29-0.44
B	10	0.39[a]	0.02	0.27-0.55
C	9	0.38[a]	0.02	0.27-0.53

[a]Each sample value is the mean of 2 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 9. The amino acid composition of dead saguaro wood determined by the method of Scurfield and Nicholls (1970). Values represent the percentage of total amino acids in the sample hydrolyzed in 6 *N* HCl in an open tube

Amino Acid	Percentage of Total Amino Acids in the Sample
Lysine	3.13
Histidine	1.19
Arginine	2.81
Aspartic Acid	11.83
Threonine	7.81
Serine	5.36
Glutamic Acid	10.47
Proline	8.42
Glycine	12.17
Alanine	11.26
Cystine	0.15
Valine	7.45
Methionine	0.53
Isoleucine	5.18
Leucine	7.81
Tyrosine	1.33
Phenylalanine	3.11

any four acetyl bromide replicates from Group A skeletons. It may be concluded that the lignin values in Table 10 are slightly high.

Delignification—Saguaro wood is particularly resistant to delignification by the chlorite method described by Ritter and Barbour (1935). This procedure calls for the addition of delignifying reagents to wood samples at 1-hr intervals until lignin content is reduced to ca. 2-3% of the initial sample weight (dry wt). In the first delignification experiment (Table 11) four 1-g extractive-free saguaro samples were subjected to successive additions of solvents at 1, 2, 3 and 4 hr. The sample that received four additions lost the greatest amount of lignin, ca. 50%. In a second experiment (Table 11), solvents were added to the reaction mixture at 15-min intervals. In one trial larger amounts of solvents (+50%) were added. After nine solvent additions, the greatest drop in lignin content was only slightly better (52.5%) than in the first experiment. Since these data were not essential to the overall purpose of the study, no further efforts to determine them were attempted.

Table 10. Lignin content of extractive-free saguaro wood determined by the acetyl bromide method (Johnson et al. 1961)[a]

Category	No. of Samples	Mean %[b]	SE	Range
A	10	29.57[a]	0.76	27.9-33.7
B	10	26.78[b]	0.44	23.9-29.8
C	10	30.97[a]	0.63	28.1-33.3

[a]Each sample value is the mean of 4 replicates.

[b]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Nutritional Physiology

Feeding Trials

Incipient Colonies—The incipient colonies were inspected after 3 yr and 1 mo. The resulting data have been reduced in Table 12. Fecal-pellet production was not measured in this experiment. When the experiment was terminated, the mean number of termites per group was 20.13. It is interesting to find that young and old nymphs together comprise 35.71% of the survivors. Although there were slightly fewer than two reproductives per colony (1.82), one group contained four alates, which demonstrated that dispersal flights can occur in very young colonies. Although three colonies produced supplementary reproductives (one each), all but one colony retained at least one primary reproductive. The amount of wood consumed by each of the 16 young colonies was remarkably similar ($\bar{X} \pm SE = 6.21 \pm 0.42$ g). This corresponds to a daily consumption of 27.17 mg wood/g termite (fresh).

Intermediate Groups—This experiment was the most comprehensive of the feeding trials. The 60 experimental feeding groups provided a vast amount of data which are reported in subsequent tables. While space does not permit the inclusion in text of all data for each feeding unit, several additional tables are available for study on request. Termite response was measured as a function of colony origin, rearing temperature and group size. Biomass-days (BT) or survivors were sometimes used in preference to initial group size as independent variables to assess termite response.

Where appropriate, one-way analyses of variance were performed to identify differences in response at the $\alpha = 0.05$ level. The only significant differences noted among colonies were initial mean weight of individual termites and biomass of dead bodies. These data are summarized in Tables 13 and 14. It was concluded that the three colonies were similar enough so that this variable need not be considered in further analysis.

The only response variable which differed significantly with changes in temperature was the quantity of carton produced. Groups at 24 C produced the least (2.17 mg) and those at 28 C, the most (16.13 mg).

Table 11. Delignification of dead saguaro wood using the method of Ritter and Barbour (1935). All determinations were performed on extractive-free, 60/80 mesh, wood flour dried at 105 C for 24 hr

Sample	Weight, g (dry wt)	Duration, hr	No. Solvent Additions	Wt Loss, g	Percent Lignin Content: Calculated from Wt Loss	Percent Lignin Content: Analysis by H_2SO_4 Method
			Experiment No. 1[a]			
A	1.00	1	1	0.039	24.5	21.6
B	1.00	2	2	0.102	18.2	18.5
C	1.00	3	3	0.123	16.1	15.5
D	1.00	4	4	0.152	13.2	12.3
			Experiment No. 2[b]			
A[c]	1.00	2-1/4	9	0.124	16.0	13.8
B	1.00	2-1/4	9	0.163	12.1	12.5

[a] Solvents added at 1-hr intervals.

[b] Solvents added at 15-min intervals.

[c] Sample B, experiment No. 2 received solvent additions which were 50% larger than those added to Sample A.

Table 12. Final group weight (fresh), total food intake and caste composition of 16 *M. hubbardi* incipient colonies. Termites were maintained for 3 yr and 1 mo at 32 C and ca. 100% RH on saguaro wood

	Group Wt (mg fresh)	Wood Consumed (mg dry wt)	Caste Composition (Mean No. per Group): Primary Reproductives	Secondary Reproductives	Larvae	Young Nymphs	Old Nymphs	Soldiers	Total
Mean	202.99	6.21	1.53	0.19	9.31	3.81	3.38	1.75	20.13
SE	18.99	0.42	0.22	0.10	1.70	0.77	0.77	0.27	2.04
Range	114.8-365.9	3.42-9.10	0-4	0-1	1-23	0-10	0-13	0-3	9-35

Table 13. Dead termites which remained in feeding groups. Data are grouped by colony

Colony	No. of Groups	Mean wt of bodies/group (mg dry wt)[a]	SE	Range
A	20	0.94[a]	0.43	0-7.4
B	20	4.71[b]	1.61	0-27.1
C	20	2.21[ab]	0.78	0-12.0
All	60	2.62	0.64	0-27.1

[a] Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 14. Initial mean weight (mg fresh) of individual termites selected from three natural colonies of *M. hubbardi*

Colony	No. of Feeding Groups	Mean wt (mg fresh)[a]	SE	Range
A	20	10.7[a]	0.20	9.16-12.34
B	20	9.33[b]	0.20	8.08-11.12
C	20	9.64[b]	0.19	8.19-11.58
All	60	9.90	0.14	8.08-12.34

[a] Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

The initial number of termites in a group appeared to affect termite response far more than either colony origin or temperature. One measure of colony success is the total number of offspring (P_r) produced by a feeding group (Table 15). As one might expect, large groups consumed more wood and produced more feces than smaller groups. Table 16 summarizes consumption and fecal-pellet production data for the 60 experimental groups.

When wood consumption and fecal-pellet production are divided by BT, the resulting data (Table 17) look quite different from those in Table 16. On a basis of mg termite/day, fecal production is quite constant among the four groups. Food consumption, however, is higher for individuals in the smallest-sized groups (5).

The data in Table 17 suggest that although individuals in smaller groups consume more food than those in larger groups, they excrete about the same amount of feces; i.e., they assimilate a greater percentage of their food. Apparent assimilation, defined as (consumption — feces)/consumption, was examined by a one-way analysis of variance and the results summarized in Table 18.

Table 15. Mean total offspring (eggs and larvae) produced by intermediate feeding groups during 90 days

Group Size	No. of Feeding Groups	No. of Offspring[a]	SE	Range
5	15	0.00[a]	0.00	0-0
10	15	0.27[a]	0.15	0-2
20	15	2.13[a]	0.76	0-9
50	15	7.27[b]	2.18	0-23
All	60	2.42	0.68	0-23

[a]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

The 60 feeding groups initially contained a total of 1,275 termites. Eight hundred ninety-six (70.3%) of these survived the experiment, during which time 90 new larvae and 55 eggs were added to the population. Survival percentages within the temperature group-size categories (data supplied on request) were analyzed after being transformed to probits. No significant differences were noted among temperatures or group sizes.

The caste composition of each of the 60 feeding groups was recorded (Table 19) at the end of the feeding trials. One-way analysis of variance was used to determine if the percentage of individual castes varied among the feeding groups according to temperature or group size. While no differences in caste composition were noted among the five temperature treatments, group size did influence caste ratios. Larger groups tended to allocate more biomass to nymphal castes and thus maintained smaller percentages of soldiers, supplementary reproductives and larvae than did smaller groups.

Percent biomass gained by a feeding group is one estimate of productivity. These data are given in Table 20. Productivity was noted only in groups which initially contained 50 termites.

Multiple linear regression equations were developed which predict termite food consumption, fecal-pellet production and assimilation rates as functions of biomass-days and temperature (Table 21). They explain more than 89% of the observed variation.

Large Feeding Groups—Although seven temperatures were used to test termite response in large feeding groups, there was only one replicate per treatment and, therefore, no statistical analyses were performed. Tables 22 and 23 summarize the results of this experiment.

DISCUSSION

Biochemical Studies on Dead Wood

The preceding laboratory and field observations strongly suggest that *M. hubbardi* can maintain itself and grow on saguaro wood that macroscopically appears free of decay. Since approximately a third of this study is concerned with differences in the chemical constituents of aging wood, it is necessary to include some commentary on its structure and chemistry. The basic approach to woody tissue analysis has progressed little during the last 20 years and, although much of the methodology has become somewhat standardized, confusion remains because some investigators have been negligent in reporting their laboratory procedures and careless with terminology. The necessity for paying strict attention to details should be especially obvious to those who study organisms which habitually consume woody tissues. Although the composition of wood varies widely according to species, age, tissue and growing conditions, the data in Table 24 provide general values for the major elements and organic compounds as they occur in decay-free wood.

Analytical Determinations

Extractives, Wood Lipids and Fatty Acids—Quantitatively neither the ethanol/ethanol:benzene/hot water nor chloroform:methanol extracts were statistically different for the three saguaro groups (Tables 1 and 2). As one would expect, the compounds extracted by the first solvent system were almost double those removed by the second. It should also be noted that the chloroform:methanol treatments were much shorter (6 min) than those in ethanol/ethanol:benzene /hot water (18 hr). The latter solvents probably removed a number of constituents in addition to lipids. The fact that these compounds were not identified should not suggest that they are unimportant, but rather that their relevance to termite nutrition is as yet unknown or only suspected. Many of these compounds are probably involved in modifying the attractiveness or resistance of wood to termites and thus its suitability as food. Although specific termite attractants such as vanillic acid, p-hydroxybenzoic acid, p-coumaric acid and protocatechuic acid have been isolated from fungi (Becker 1964), none has yet been reported from the higher plants. Becker (1971) has reviewed the studies on substances found in wood which attract xylophagous insects. For example, females of *Hylotrupes bajulus* (L.) and another large cerambycid, *Ergates faber* (L.), are attracted by the $C_{10}H_{16}$ hydrocarbons (α- and β-pinenes), carene and sabinene (Becker 1971). It seems reasonable to expect that similar examples will be found, particularly of the sort which would lead to the establishment of dry-wood termites in suitable dead-wood nesting sites. In general, natural termite resistance is a function of many variables including toxic or repellent chemicals, nutrient imbalance or lack of specific growth factors, adverse physical characteristics and preconditioning.

The lipid content of wood (ether or chloroform:methanol extracts) is complex as well as variable. It contains numerous fats, fatty acids, phytosterols and waxes, all of which may influence wood-termite relationships. Three fatty acids (palmitic, stearic and oleic) in similar, but not quite equal, ratios made up practically all of the FA (78.38-84.84%) in the saguaro extracts (Table 3). The two saturated compounds decreased in relative abundance in Group C plants. Two unsaturated fatty acids (oleic and linoleic) showed a commensurate increase in the C skeletons. Although the reason for this is not entirely clear, it might have been caused by the presence of fungal tissues high in unsaturated FA (Carter et al. 1972) in the older skeletons. It is difficult to relate such slight lipid differences to nutrition. Indeed, an increase in polyunsaturated FA as in Group C saguaros should enhance rather than retard termite growth.

Table 16. Wood consumption and fecal-pellet production by intermediate-sized feeding groups

Group Size	No. of Feeding Groups	Mean Wood Consumption (mg dry wt)[a]	SE	Range	Mean Fecal Pellet Production (mg dry wt)[a]	SE	Range
5	15	236.33[a]	23.28	87-382	31.61[a]	6.19	2.5-73.9
10	15	388.33[a]	34.04	157-597	87.54[a]	10.17	13.6-145.2
20	15	724.60[b]	61.86	277-1037	209.53[b]	17.33	79.5-304.3
50	15	1680.67[c]	141.77	817-2847	611.35[c]	60.27	179.0-1070.9
All	60	756.48	82.85	87-2847	237.51	33.81	2.5-1070.9

[a]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 17. Food consumption and fecal-pellet production divided by biomass-days for the intermediate-sized feeding groups

Group Size	No. of Feeding Groups	Mean Wood Consumption (mg·g fresh termite^{-1}·day^{-1})[a]	SE	Range	Mean Fecal-Pellet (mg·g fresh termite^{-1}·day^{-1})[a]	SE	Range
5	15	294[b]	39	0.05-1.27	0.016[a]	0.003	0.006-0.055
10	15	84[a]	10	0.03-0.43	0.015[a]	0.002	0.008-0.037
20	15	55[a]	2	0.02-0.10	0.016[a]	0.001	0.006-0.026
50	15	45[a]	3	0.02-0.07	0.016[a]	0.001	0.004-0.023
All	60	119	22	0.02-1.27	0.016	0.001	0.004-0.055

[a]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 18. Apparent assimilation of food by the intermediate-sized feeding groups

Group Size	No. of Feeding Group	Mean Apparent Assimilation[a]	SE	Range
5	15	0.876[a]	0.018	0.78-0.99
10	15	0.775[b]	0.018	0.66-0.91
20	15	0.701[c]	0.012	0.62-0.84
50	15	0.641[d]	0.015	0.58-0.84
All	60	0.749	0.014	0.58-0.99

[a]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 19. Final composition of intermediate-sized feeding groups of *M. hubbardi*. Data are presented for temperature and group size categories

	No. of Groups	Eggs	New Larvae	Secondary Reproductives	Larvae	Young Nymph	Old Nymph	Soldiers	Row Total
Temperature, °C:									
				Total Number of Individuals					
24	12	5	0	20	5	138	17	5	190
26	12	12	1	24	9	100	63	9	218
28	12	33	0	22	9	82	31	10	187
30	12	7	28	24	7	100	37	9	212
32	12	33	26	23	14	95	30	13	234
Column Total	60	90	55	113	44	515	178	46	1041
% of Grand Total		8.65	5.28	10.85	4.23	49.47	17.10	4.42	
				Mean No. of Individuals					
24	12	0.42[a]	0.00[a]	1.67[a]	0.42[a]	11.50[a]	1.42[a]	0.42[ab]	15.83[a]
26	12	1.00[a]	0.08[a]	2.00[a]	0.75[a]	8.33[a]	5.25[a]	0.75[ab]	18.17[a]
28	12	2.75[a]	0.00[a]	1.75[a]	0.75[a]	6.83[a]	2.58[a]	0.75[ab]	15.58[a]
30	12	0.58[a]	2.33[a]	2.00[a]	0.58[a]	8.33[a]	3.08[a]	0.75[ab]	17.67[a]
32	12	2.75[a]	2.17[a]	1.92[a]	1.17[a]	7.92[a]	2.50[a]	1.08[b]	19.50
All	60	1.50	0.92	1.87	0.73	8.58	2.97	0.75	17.35
Group Size:									
				Total Number of Individuals					
5	15	0	0	15	2	15	1	4	37
10	15	4	0	27	5	58	4	10	108
20	15	21	11	34	16	136	14	14	246
50	15	65	44	37	21	306	159	18	650
Column Total	60	90	55	113	44	515	178	46	1041
% of Grand Total		8.65	5.28	10.85	4.23	49.47	17.10	4.42	
				Mean No. of Individuals					
5	15	0.00[a]	0.00[a]	1.00[a]	0.13[a]	1.00[a]	0.67[a]	0.27[a]	2.47[a]
10	15	0.27[a]	0.00[a]	1.73[b]	0.33[a]	3.87[a]	0.27[a]	0.60[b]	7.20[b]
20	15	1.40[a]	0.73[a]	2.27[bc]	1.07[b]	9.07[b]	0.93[a]	0.93[c]	16.40[c]
50	15	4.33[b]	2.93[b]	2.47[c]	1.40[b]	20.40[c]	10.60[b]	1.20[c]	43.33[d]
All	60	1.50	0.92	1.87	0.73	8.58	2.97	0.75	17.35
				Mean No. ÷ Group Size					
5	15	0.000[a]	0.000[a]	0.027[a]	0.200[a]	0.200[a]	0.013[a]	0.053[a]	0.493
10	15	0.027[ab]	0.000[a]	0.033[a]	0.173[ab]	0.387[b]	0.027[a]	0.060[a]	0.720
20	15	0.070[bc]	0.037[ab]	0.053[a]	0.113[bc]	0.453[b]	0.047[a]	0.047[a]	0.820
50	15	0.087[c]	0.059[b]	0.028[a]	0.049[c]	0.408[b]	0.212[b]	0.240[a]	0.867
All	60	0.046	0.024	0.035	0.134	0.362	0.075	0.046	0.818

[a]Means in the same column followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 20. Percentage of original biomass (fresh) gained or lost by intermediate-sized groups during the 90-day feeding trial. Data are categorized by temperature and group size

Treatment	No. of Feeding Groups	% Wt Change[a]	SE	Range
Temperature, °C:				
24	12	-30.64[a]	10.23	-4.12 to -100.00
26	12	-8.00[a]	10.46	-42.40 to -100.00
28	12	-24.02[a]	9.05	-7.30 to -100.00
30	12	-19.65[a]	12.64	-28.38 to -100.00
32	12	-25.67[a]	5.17	-6.81 to -59.79
All	60	-21.60[a]	4.36	-42.40 to -100.00
Group Size:				
5	15	-51.95[a]	9.91	-6.81 to -100.00
10	15	-25.73[b]	7.42	-10.14 to -100.00
20	15	-11.05[bc]	3.94	-7.30 to -49.20
50	15	+2.34[c]	5.93	+42.40 to -32.53
All	60	-21.60	4.36	+42.40 to -100.00

[a]Means followed by the same letter are not significantly different at the $\alpha = 0.05$ level as determined by the least significant difference.

Table 21. Equations for predicting wood consumption (mg), fecal-pellet production (mg) and apparent assimilation efficiency by groups of 5-50 *M. hubbardi*; B = biomass-days, T = °C

Response Variable	Equation	R^2[a]
Wood Consumption (mg)	$Y = -94.98 - 0.0623\ B + 0.30\ T^2 + 0.00368\ B \cdot T$	0.961
Fecal Production (mg)	$Y = 53.06 - 0.03\ B - 0.0842\ T^2 + 0.00175\ B \cdot T$	0.944
Apparent Assimilation[b] Efficiency	$Y = 0.914 - 1.99 \times 10^{-5}\ B + 3.166 \times 10^{-10}\ B^2$	0.894

[a]R^2 = coefficient of determination (Steel and Torrie 1960).

[b]Defined by the equation: $\frac{\text{Consumption} - \text{Feces}}{\text{Consumption}}$

Table 22. Caste composition of six large feeding groups (490-500 individuals) of *M. hubbardi* maintained on saguaro wood for 90 days at ca. 100% RH

Temperature (°C)	Initial No. in Group	Final No. in Group	Production		Caste Composition of Survivors				
			Eggs	New Larvae	Supplementary Reproductives	Larvae	Young Nymphs	Old Nymphs	Soldiers
22	500	444	0	0	4	20	156	250	14
24	500	348	0	0	6	40	126	170	6
26	500	387	0	0	1	1	83	291	11
28	490	334	0	1	9	2	38	268	16
30	500	324	0	0	3	1	87	231	2
32	500	355	13	0	3	2	104	220	13
Column Total	2990	2192	13	1	26	66	594	1430	62
% of Grand Total			0.59	0.05	1.19	3.01	27.10	65.24	2.83

Table 23. Wood consumption, fecal-pellet production, biomass and carton production by six large groups (490-500 individuals) of *M. hubbardi* fed saguaro wood for 90 days. Experimental units were maintained at ca. 100% RH

Rearing Temperature (°C)	No. of Groups	Initial Group No.	Final Group No.	Wt Change of Group (mg fresh)	Wood Consumption (mg·g fresh termite^{-1}·day^{-1})	Fecal-Pellet Production (g)	Carton Production (mg dry wt)
22	1	500	444	+26.8	16.53	5.2170	0.00
24	1	500	348	-613.5	19.44	4.2010	0.00
26	1	500	387	+1251.7	24.48	6.2207	8.10
28	1	490	334	-280.1	23.97	5.3082	201.80
30	1	500	324	+284.9	30.73	8.2760	21.60
32	1	500	355	-808.7	29.93	6.5193	157.50
Mean		498.33	365.33	-23.15	24.18	5.9570	64.83

Table 24. Chemical elements and organic components in sound wood

Element or Component	% Oven-dry Weight	Reference
C	49-50	Tsoumis 1968, modified
H	6	Tsoumis 1968, modified
O	44-45	Tsoumis 1968, modified
N	0.03-0.10	Cowling & Merrill 1966
Ash	0.2-1.0	Tsoumis 1968
Polysaccharides		
α-Cellulose	40-50	Côté 1968; Tsoumis 1968
Hemicellulose (= xylans, mannans, etc.)	15-30	Tsoumis 1968, p. 61
Pectic substances	<1.0	Browning 1952, p. 1189
Starch	0.5-5.0	Wise 1952, p. 644
Gums and mucilages	-	
Lignin	15-35	Stecher 1968, p. 619; Côté 1968
Protein	0.01-0.20	Cowling & Merrill 1966
Extractives	1.0-10.0	Tsoumis 1968

Table 25. Nitrogen content of stem wood of various gymnosperms and angiosperms. The data are for individual samples from a given tree (Cowling and Merrill 1966)

Tree Species	N Content (% by wt)
Gymnosperms	
Abies concolor	0.045
Abies magnifica	0.227
Juniperus virginiana	0.139
Larix occidentalis	0.180
Libocedrus decurrens	0.097
Picea engelmanni	0.118
Pinus contorta	0.071
Pinus echinata	0.130
Pinus elliottii	0.050
Pinus lambertiana	0.124
Pinus monticola	0.113
Pinus palustris	0.038
Pinus ponderosa	0.052
Pinus strobus	0.087
Pinus taeda	0.068
Pseudotsuga taxifolia	0.051
Sequoia sempervirens	0.067
Taxodium distichum	0.057
Tsuga canadensis	0.106
Angiosperms	
Carya ovata	0.100
Castanea dentata	0.072
Juglans nigra	0.100
Liquidambar styraciflua	0.057
Liriodendron tulipifera	0.088
Quercus alba	0.104
Quercus rubra	0.099
Quercus stellata	0.096
Quercus velutina	0.070

Carbon Analysis—Wood contains about 49-50% carbon (Table 24). If, however, other constituents such as nitrogen and ash are abundant, the percentage of carbon may appear lower. The carbon contents of the three saguaro groups were statistically similar between ca. 45-46%.

Ash Analysis—The ash contents of the three saguaro groups (Table 5) were not significantly different. It is doubtful that they have any effect on the presence of colonies in the three groups. Inorganic minerals occur in varying amounts in plant tissues with quantitative and qualitative differences most closely related to soil type. Wise (1952, p. 658) reports that 27 different minerals have been isolated from *Pinus strobus* L. Although the mineral requirements of insects are poorly known, it would appear that *M. hubbardi* has an ample supply of these nutrients at its disposal.

Calorific Content—No difference was noted in the calorific contents of the saguaro groups (Table 6). Although the data in Table 7 indicate a significant difference between the rib interior and the outer weathered surface, the difference, 0.2 kcal/g dry wt, seems too small to reflect a true biological difference. It is possible that energy-rich compounds have been leached from the surface wood. Since termites generally nest in inner areas of the wood, this difference should not have been a factor in determining the presence of *M. hubbardi* in the three saguaro groups. We know of no other calorific values for saguaro wood. For comparison, determinations of gross energy (kcal/g dry wt) were performed on additional desert woods consumed by *M. hubbardi* and other termites found in the IBP sites. These included *Opuntia spinosior* (Engelm. & Bigel.) (4.33), *Cercidium floridum* Benth. (4.30) and *Acacia greggii* Gray (4.55). Since a number of *M. hubbardi* incipient colonies were reared on birch (*Betula* sp.), calorific content was measured and found to be 4.50 kcal/g dry wt.

McBrayer et al. (1974) determined the ash free calorific content of forest floor litter five times during the year. They found that winter litter held substantially less energy (3.9 vs. 4.3 kcal/g dry wt) than spring or summer detritus and concluded that leaching was responsible. Golley (1961) reported a mean calorific content for litter (4.3 kcal/g dry wt) and for standing dead vegetation (4.1 kcal/g dry wt).

Nitrogen Content, Quantity and Distribution in Wood—All living organisms require nitrogen for protein and nucleic acid synthesis. Since the nitrogenous compounds in wood are quantitatively minimal (Table 24), they are of more than casual interest in any consideration of the nutrition of xylophagous organisms.

The nitrogen content of plant materials varies tremendously. Herbaceous tissues normally contain 1-5% nitrogen by weight, whereas woody tissues contain only 0.03-0.10% (Table 25; Cowling and Merrill 1966). Levi et al. (1968) reported carbon-to-nitrogen ratios for several plant materials: tomato foliage (10:1), tobacco stems (55:1), cotton seed hairs (200:1), microbiological agar medium (200:1). In the present study the ratio for saguaro was low, ca. 120:1.

Three factors contribute to the highly variable determinations which have been published on total nitrogen in woody tissues. They are the low nitrogen content, high carbon content and extreme heterogeneity of wood materials. Additional variation stems from the many modifications of the basic Kjeldahl process which have been used almost exclusively for these determinations. In general all Kjeldahl procedures suffer the similar shortcoming of not fully recovering nitrogen bound in N-N or N-O linkages (Rennie 1965). Much of the nitrogen budgeting among termite societies should be reexamined due to the general lack of

Table 26. Nitrogen content of portions of the stem wood of various tree species (after Cowling and Merrill 1966)

Tree Species	Nitrogen Content (% Dry Weight)			
	Cambium	Immature Sapwood	Sapwood	Heartwood
Gymnosperms				
Picea mariana	1.10		0.056	0.059
Picea mariana	1.11	0.27	0.047	0.062
Pinus sylvestris	3.25		0.012	
Pinus	3.33		0.130	
Corsican pine, butt			0.108	0.067
Corsican pine, crown			0.085	0.063
Pinus sylvestris, butt			0.086	0.079
Pinus sylvestris, crown			0.052	
Pinus sylvestris		0.115-0.078	0.047-0.039	0.031-0.047
Angiosperms				
Eucalyptus regnans	2.03	0.52	0.062	0.031
Fraxinus elatior	4.59	0.88	0.22	
Fraxinus sp.	4.70	0.89	0.22	
Ulmus sativa	4.69	0.81	0.27	
Ulmus sp.	4.80	0.83	0.28	

uniform methodology and the current knowledge that the N-O linkage is common in several woody tissues, notably among the Leguminosae.

Early biologists often speculated about the ability of termites to survive and reproduce on diets apparently containing very little or no nitrogen (Cleveland 1925). The specific quantity of nitrogen in wood is most critical for the dry-wood species. After swarming, the reproductives establish their nest in a dead branch or log of limited size which serves not only for shelter but also as the sole source of nutrients. One would think that the composition of the nest material would change qualitatively as well as quantitatively, and eventually limit colony growth (Nagin 1972). Although the relationship between available nitrogen and termite growth is not well understood, we do have data on the distribution of this element in wood (Cowling and Merrill 1966) and some knowledge about the effects of varying nitrogen levels on the development of two xylophagous beetles, *Hylotrupes bajulus* (L.) (Becker 1971) and *Anobium punctatum* De Geer (Bletchly 1969). Despite considerable variation in the literature, Cowling and Merrill (1966) concluded that a higher percentage of nitrogen exists: in angiosperms than in gymnosperms, within the crown of the tree than below it, in sapwood than in heartwood, nearer the cambium than close to the sapwood-heartwood interface, and nearer the pith (in conifers) than in the more recently formed heartwood (Table 26).

The three saguaro groups had a mean nitrogen content (0.37) more than 3.5 times that of the high value for wood shown in Table 24. Wood species which contain unusually high amounts of nitrogen may immediately be suspected of possessing large quantities of alkaloids (Wise 1952). The alkaloids comprise a fairly large heterogeneous group of compounds, all of which contain nitrogen, usually in a heterocyclic ring. Some of the more common alkaloids are: strychnine, quinine, morphine, cocaine, mescaline, ephedrine and nicotine. They are usually found primarily outside the xylem.

Although he did not determine their structures, Caldwell (1966) confirmed the presence of two alkaloids in 3-yr-old saguaro ribs. It seems probable that the high nitrogen content of saguaro is at least partially explained by the presence of these compounds. The question of whether or not they are available to termites or their symbiotes remains unanswered. Although they have received intensive chemical study because of their toxic properties, many of the alkaloids, poisonous to man, appear to have little effect on xylophagous organisms (Robinson 1963). Since nitrogen is an indispensable building block of biological tissues, it tends to remain in a constant state of flux passing from producer to consumer to decomposer. It is surprising that no quantitative differences were noted among the three groups. The high nitrogen level in old (C) saguaro wood suggests that this valuable nutrient is "locked up" for many years in a desert ecosystem where nitrogen may well limit production. Should this be true, the activities of *M. hubbardi*, which enhance nitrogen availability through their feeding habits, may be of great value to the community.

Amino Acid Profile—The quality of nitrogen is also an important factor in nutrition. Scurfield and Nicholls (1970) have isolated and characterized the protein-bound amino acids (PBAA) in the wood of *Eucalyptus* sp. and *Pinus radiata* D. Don. Results of these analyses showed that approximately 50% of the total nitrogen in *P. radiata* was recovered as amino or ammonia nitrogen and included all of the commonly encountered amino acids. Lincoln and Mulay (1929) found a similar situation in pear wood. There were, however, both quantitative and qualitative differences between the wood species tested. Many nonprotein amino acids have been isolated from plant tissues, including gamma-amino butyric acid and beta-alanine (Robinson 1963). Similar data were obtained for saguaro wood.

Lignin Analysis—After cellulose, lignin is the most abundant compound in wood (Kirk 1971). It comprises an entire family of polymeric phenolic compounds which account for most of the methoxyl content of wood. They are resistant to acids, readily oxidized, soluble in hot alkali and bisulfate, and condense with phenols and their compounds (Schubert 1965). Kirk and Harkin (1972, p. 1) describe lignin as "an amorphous, three-dimensional, highly branched aromatic polymer" and suggest that one can best understand its structure from a "biosynthetic viewpoint." As such it is composed of many phenylpropane (guaiacyl) subunits derived from hydroxycinnamyl alcohols. These undergo enzymatic oxidation to form free radicals which subsequently couple with one another at the site of lignification (Kirk 1971).

The resultant lignin is deposited in close association with the cellulose of the plant cell wall to form an intractable

complex which has generally been thought to consist of mutually interpenetrating polymers held together only by physical attraction (Cowling 1961). Kirk and Harkin (1972) have suggested, however, that the association also involves a degree of chemical bonding between lignin and the wood polysaccharides of the cell wall. Regardless of the true nature of this association, it is clear that lignin strengthens the cell wall and protects the cellulose from attack by cellulolytic enzymes. For example, there are microorganisms such as *Bacillus polymyxa* (Praxmowski) Mace that readily attack isolated cellulose, but cannot utilize it in wood at all.

Owing to the many possible linkages between the guaiacyl monomers and the variability among species, a definite structural formula for lignin cannot be given. Approximately 30% of the guaiacyl monomers of softwood lignin appear to contain a free phenolic group, while 70% of the monomers possess carbonyl groups on one of the carbons of the propyl group. Benzyl ether or benzyl alcohol groups are found on 43% of the units. Linkages commonly found between lignin monomers include C-O-C and C-C. The texts by Schubert (1965) and Marton (1966) provide a more complete discussion of the chemistry of lignin. As a group, the lignins are digested less efficiently by the termites and their symbiotes than the hemicelluloses or cellulose (Lee and Wood 1971; Wolcott 1946). Although the lignin content of Group B saguaros was statistically lower than that of A and C (Table 10), the difference was small enough that it was not considered biologically significant. The overall percentage of lignin in saguaro, a hardwood, falls well within the range given in Table 24.

Summary of Wood Analyses

With minor exceptions, the chemical composition of the three saguaro groups was remarkably similar. On the basis of nutritional constituents assayed, we have concluded that they should maintain *M. hubbardi* equally well. As they do not, however, undetermined nutritional components such as those discussed under extractives, environmental parameters or perhaps behavioral factors, must determine the nutritional suitability of saguaro wood.

Although environmental parameters may have influenced the suitability of saguaro skeletons for *M. hubbardi*, it is probable that they would be important only in very old C skeletons which have been reduced in size so as to afford no further protection from temperature and moisture extremes. No data were gathered to substantiate this speculation, however.

There is some reason to implicate the nest site exploration strategy of *M. hubbardi* with its avoidance of A skeletons. Earlier studies (Wilkinson 1962; Usher 1974) suggest that some kalotermitids and termitids preferentially select cracks for entry into wod. Other species, *C. brevis* (Coaton 1948) and *I. minor* (Harvey 1934), tend to excavate entrance holes on smooth surfaces.

Based on several casual and one documented observation, we submit that *M. hubbardi* alates employ a combination of the above strategies for selecting their nesting sites. It has already been stated that Group A saguaros are generally covered by intact, but dry, cortex over much of their surfaces. Saguaro No. 90, plot F2, SNME, died from bacterial necrosis during 1971. On close inspection three *M. hubbardi* entrance holes were found ca. 15 cm below the upper margin of the dried cortext and ca. 46 cm above ground level. It appeared that alates entered the space between the cortext and skeleton, worked their way downward and under the protection of the cortex began the process of excavating the entrance hole. Such a strategy would at least partially explain the absence of termites from very young skeletons which are more fully covered by cortex. Moreover, very old skeletons which lack cortex completely would not provide any protection during the critical excavation period. *M. hubbardi* flights occur during the early evening hours from late June through early September. During this period a vast number of insect and vertebrate predators are abundant and active.

Although the maximum longevity of kalotermitid colonies is not known with certainty (Wilson 1971), Grassé (1949) speculated that dry-wood colonies generally die after 10-15 yr as a result of declining egg production. Field observations tend to substantiate this theory. Moreover, it appears that *M. hubbardi* colonies are in fact rather short lived. No skeleton on plot F2 of SNME had been attacked until it had been dead at least 4 yr. These data, though not conclusive, suggest that *M. hubbardi* colonies may live as few as 2-5 yr. Under favorable laboratory conditions *M. hubbardi* colonies matured, i.e., produced new alates, in ca. 3 yr. Regardless of age, field-collected *M. hubbardi* colonies contained more than 95% nymphal stages. This suggests a tendency to produce reproductive forms very quickly which are capable of dispersing and forming new colonies. Nagin (1972) speculated that kalotermitid colonies might die as a result of failing to produce supplementary reproductives and declining egg production potential when colonies enter a physiological state associated with maturity. Although it was not entirely clear whether these physiological changes were endogenous or exogenous, the net result was the appearance of large numbers of pre-alate forms, i.e., nymphs. A parallel phenomenon is known in certain flowering plants which, when faced with potentially lethal environmental stress, allocate much of their metabolic activity to seed production.

Nutritional Physiology

Feeding Trials

Becker (1969), Gay et al. (1955) and Haverty (1974) are but a few who have reviewed the methods for testing termites in the laboratory. It is particularly disturbing that in spite of their recommendations, no uniform methodology or standards for expressing results have been adhered to. Haverty (1974) reported that a large number of variables can affect wood-consumption rates. Among these he noted wood species and hardness, toxic substances and other feeding inhibitors or deterrents, presence or absence of fungi and degree of decay, moisture content and temperature. In the present study, group size, temperature and colony origin were the variables used to test termite response.

Few authors have recognized the effect of group size on termite response. Nel et al. (1971) found that the relationship between brood production (P_r) and colony size (50-600 individuals) of *H. mossambicus* (Hagen) was related in a linear fashion during the first 116 days. After 168 days, however, groups containing more than 200 termites showed a negative curvilinear response due, in the author's opinion, to competition for space. McMahan (1962) and others have noted changes in caste ratios and oviposition rates as incipient kalotermitid colonies increased in size. Gay et al. (1955) reported no significant differences in survival among laboratory test groups of 2,500, 5,000 and 7,500 workers of *Nasutitermes exitiosus* (Hill). None of the above, however, evaluated wood-consumption rate as a function of colony size.

The observed patterns of growth (Δ biomass) for the *M. hubbardi* feeding groups were consistent with Nagin's (1972) concept of differing physiological states in developing colonies. According to his theory, very young colonies allocate energy reserves to the production of large larval populations which tend to accumulate biomass very quickly. Mature colonies, on the other hand, show less or no growth (P_r or P_s) as they mark time awaiting the proper environmental cues for dispersal flights. The observation that the larger intermediate- and large-sized feeding groups showed little or no production while maintaining large nymphal populations is consistent with Nagin's reasoning. McNeill and Lawton (1970) examined the relationship between production (P) and annual respiration (R) (kcal $\cdot m^{-2} \cdot yr^{-1}$) for 53 animal species. Their regression analyses demonstrated convincingly that long-lived poikilotherms that maintain large populations of old individuals (>2 yr) generally experience one or more high respiratory-cost, nonproductive periods. Consequently, they display low annual production efficiency, i.e., P:R ratios.

According to Nutting (1969) the initial rate of population growth among the kalotermitids is invariably slow. At the end of the first year, such colonies generally contain fewer than 60 larvae and nymphs and 0-3 soldiers. To my knowledge comparable data are not available for 3-yr-old colonies.

McMahan (1966) recognized that past feeding experience might influence the selection of habitat, survival and offspring production by dispersing alates of *C. brevis* (Walker). More recently Honigberg (1970) reported that gut faunules of the same termite species may differ in composition and metabolic patterns. For these reasons I examined colony origin as a factor in the feeding trials of the intermediate-sized groups. No significant differences were noted other than the initial mean wt (Table 14) and the accumulation of dead bodies (Table 12). The absence of additional differences should be accepted with caution in that the colonies from which the termites were obtained were collected within a mile of each other and thus probably represent a single breeding population. As noted in the introduction, *M. hubbardi* enjoys an extensive range from California and Arizona south to Colima, Mexico.

Surprisingly, we were not able to detect a significant temperature effect on *M. hubbardi* wood-consumption rates. This was especially disturbing as very strong relationships between temperature and oxygen consumption are common. Becker (1969) noted a similar feeding response by *Heterotermes indicola* (Wasmann) which maintained essentially constant food intake over the temperature range 20-32 C. Nevertheless, additional experiments encompassing wider temperature ranges and more replicates must be performed before it can be concluded that *M. hubbardi* is insensitive to changing temperatures. Although there were too few replicates for a statistical analysis, the rate of wood consumption did appear to respond to temperature for the six large groups (Table 23). Temperature did not, however, affect survival, productivity (Δ biomass), fecal-pellet production, assimilation efficiency, carton formation or caste composition. Egg production was restricted to the 28 and 32 C groups.

The most pronounced differences in response were due to the groups' sizes. The intermediate-sized groups (5, 10, 20, 50 individuals) were influenced by this variable in practically every response category (Tables 15-21). The following generalizations apply to the three feeding experiments collectively. Although similar in number, the incipient and smaller intermediate-sized groups (5, 10) differed greatly in ultimate caste composition. Whereas the former began as pairs of primary reproductives capable of immediate egg production (82% were alive after 3 yr), the latter were established only with larvae, nymphs and soldiers. Any reproductives which appeared arose gradually as replacements (SR) from larvae or nymphs. During the relatively short duration of these trials (90 days) it is doubtful that the smaller intermediate groups (5, 10) had enough time to produce both SR and brood. On the other hand, groups of 20 and 50 were in a better position to do this, as their larger foraging populations allowed newly formed SR to devote more of their energy to production. This speculation is at least partially substantiated by the data in Table 19, which show that although the mean number of SR per group divided by the initial group size was statistically larger for groups of 5 and 10 than for the 20 and 50, the smaller groups produced significantly fewer offspring (P_r). In terms of colony maturity, we speculate that the 20- and 50-termite groups correspond most closely to natural colonies 2 to 5 years of age in which one or both of the primary reproductives have been replaced by SR.

The ratio of soldiers to nonsoldiers was lowest (2.83%) in the large groups and highest (8.69%) in the incipient colonies. The mean percentage for the intermediate-sized groups was 4.42. The groups of 20 and 50 did not differ statistically with respect to soldier numbers which, as one might expect, were higher than in the smaller groups (Table 19). Although the proportion and occurrence of a particular caste may achieve a reasonably constant, species-specific ratio in mature colonies (Haverty et al., unpubl. data), it is doubtful that the same ratio would apply to incipient or developing colonies. The one or two soldiers which are produced in the first *M. hubbardi* brood appear to meet the colony's needs for the first 3-5 yr or until the population exceeds 50-60 individuals. Nutting (1969) found that ratios of soldiers in 10 *M. hubbardi* colonies commonly varied from 1:13 in a

colony of 68 individuals to 1:68 in one of 1,477. In his largest group (3,119) the soldier percentage was about one-half that of the 500-termite groups in this experiment (1.50 vs. 2.83%). Large groups appear to require fewer soldiers than do small groups.

With the possible exception of predation by ants, *M. hubbardi* is well protected within its woody nest and thus probably requires the services of a rather small soldier population. This is in contrast to the surface-feeding termite *Tenuirostritermes tenuirostris* (Desneux) in which foragers are accompanied by a great number of nasutes.

As expected, larvae constituted the largest percentage (46.24) of the survivors in the incipient colonies and the lowest (3.10) in the large groups. This is also consistent with Nagin's (1972) contention that mature colonies develop and maintain a large proportion of subimaginal forms at the expense of the younger, undifferentiated castes. A relatively constant proportion of larvae (8.58%) was noted among the intermediate-sized groups. Nymphs made up 36% of the incipient groups, 67% of the intermediate groups and 92% of the large groups. Again this substantiates our contention that *M. hubbardi* colonies rapidly attain a "waiting" posture in anticipation of dispersal flights.

Wood-consumption rates by the different groups were of considerable predictive interest as they were strongly correlated with biomass-days (BT) and, to a much less extent, temperature. In the development of multiple linear regression equations (Table 21), the only variables included in the final prediction model were those which were shown by orthogonal contrasts to increase R^2's at least 5%. The first equation in this table explains 96% of the observed variation in wood consumption. While more than 80% of that variation was explained by the linear component of biomass-days, the temperature component explained only 5.5%. It was included only because it was easy to measure and met the minimum requirement for acceptance.

Because they produce little or no cellulase, most metazoan soil organisms are generally inefficient processors of woody detritus. This is also true of many herbivorous insects such as the larvae of Lepidoptera. In contrast a few animals, including the silverfish, *Ctenolepisma lineata*, land snails of the genus *Helix*, ruminants and termites, are surprisingly efficient cellulose assimilators. Lasker and Giese (1956) reported that *C. lineata* absorbed 71.7-87.0% of a pure cellulose diet. The literature reports on assimilation efficiency by laboratory colonies of termites have been summarized by Wood (in press). For the termite species and four species of wood listed, efficiencies vary from 54-61% (dry wt). This may be compared with a dairy cow (a ruminant) which digested 72% of a ration of dry grass (Maynard 1937). *M. hubbardi* apparent assimilation efficiency was highest among the intermediate groups (64-87%) and lowest among the groups of 500 (53%).

Assimilation efficiency, (C — FU)/C, was best estimated by the linear (54.7%) and quadratic (25.2%) components of biomass-days. Temperature was not a significant factor based on the above criterion. Prediction models were developed only for the intermediate-sized groups. Based on the equation for wood consumption (Table 21), an *M. hubbardi* colony containing 1,000 individuals, each weighing ca. 12 mg, would consume only 178.58 g of wood annually if maintained at a constant 28 C (Table 23). It is unlikely that *M. hubbardi* colonies reach these sizes in fewer than 5 yr -- Nutting (1969) found a large *M. hubbardi* colony of 3,119 individuals in a cottonwood fence-post set in the soil no earlier than 1960.

LITERATURE CITED

Allison, L. E., W. B. Bollen, and C. D. Moodie. 1965. Total carbon. Pages 1346-1366 *in* C. A. Black, ed. Methods of soil analysis. Part 2. Monogr. No. 9. Amer. Soc. Agron., Madison, Wisc.

Bartlet, J. C., and J. L. Iverson. 1966. Estimation of fatty acid composition by gas chromatography using peak heights and retention time. J. Assoc. Offic. Anal. Chem. 49:21-27.

Becker, G. 1964. Termiten-anlockende Wirkung einiger bei Basidiomyceten-Angriff in Holzentstehender Verbindungen. Holzforschung 18:168-172.

Becker, G. 1969. Rearing of termites and testing methods used in the laboratory. Pages 351-385 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Vol. 1. Academic Press, New York.

Becker, G. 1971. Physiological influences on wood-destroying insects of wood compounds and substances produced by microorganisms. Wood Sci. Technol. 5:236-246.

Benson, L. 1969. The cacti of Arizona. 3rd ed. Univ. Ariz. Press, Tucson. 218 pp.

Berry, J. W., H. Anmin, and C. Steelink. 1960. Constituents of the saguaro (*Carnegiea gigantea*). I. Proximate analysis of the woody tissues. J. Org. Chem. 25:1267-1268.

Bletchly, J. D. 1969. Seasonal differences in nitrogen content of Scots Pine (*Pinus sylvestris*) sapwood and their effects on the development of the larvae of the common furniture beetle (*Anobium punctatum* DeG.). J. Inst. Wood Sci. 4:43-47.

Bodine, M. C., and D. N. Ueckert. 1975. Effect of desert termites on herbage and litter in a shortgrass ecosystem in West Texas. J. Range Manage. 28:353-358.

Brian, M. W. 1971. Ants and termites. Pages 247-261 *in* J. Phillipson, ed. Methods of study in quantitative soil ecology: population, production and energy flow. Blackwell Sci. Pub., Oxford and Edinburgh.

BROWNING, B. L. 1952. Introduction and miscellaneous determinations. Pages 1119-1137 *in* L. E. Wise and E. C. Jahn, eds. Wood chemistry. Vol. 2. Reinhold Publ. Corp., New York.

BROWNING, B. L. 1969. Wood chemistry. Pages 3-12 *in* K. W. Britt, ed. Pulp and paper technology. 2nd ed. Van Nostrand Reinhold Co., New York.

CALDWELL, R. L. 1966. A biochemical investigation of callus tissue in the saguaro cactus [*Carnegiea gigantea* (Engelm.) Britt. & Rose]. Ph.D. Diss. Univ. Ariz., Tucson. 121 pp.

CARTER, F. L., L. A. DINUS, and R. V. SMYTHE. 1972. Effect of wood decayed by *Lenzites trabea* on the fatty acid composition of the eastern subterranean termite, *Reticulitermes flavipes*. J. Insect Physiol. 18:1387-1393.

CHRISTIE, W. M. 1973. Lipid analysis. Pergamon Press, Oxford. 327 pp.

CLEVELAND, L. R. 1925. The ability of termites to live perhaps indefinitely on a diet of pure cellulose. Biol. Bull. (Marine Biol. Lab., Woods Hole) 48:289-293.

COATON, W. G. H. 1948. *Cryptotermes brevis*, a new woodborer problem in South Africa. Bull. Dep. Agr. Forest. S. Afr. 290:1-18.

COATON, W. G. H. 1951. The snouted harvester termite; natural mortality as an aid to chemical control. Farming in S. Afr. 26:263-367.

CÔTÉ, W. A., JR. 1968. Chemical composition of wood. Pages 55-78 *in* F. F. P. Kollman and W. A. Côté, Jr., eds. Principles of wood science and technology. I. Solid wood. Springer-Verlag, New York.

COWLING, E. B. 1961. Comparative biochemistry of the decay of sweetgum sapwood by white-rot and brown-rot fungi. U.S. Dep. Agr. Tech. Bull. No. 1258. 79 pp.

COWLING, E. B., and W. MERRILL. 1966. Nitrogen in wood and its role in wood deterioration. Can. J. Bot. 44:1539-1554.

DARNELL, R. M. 1968. Animal nutrition in relation to secondary production. Amer. Zool. 8:83-93.

EMERSON, A. E. 1955. Geographical origins and dispersions of termite genera. Fieldiana: Zool. 37:465-521.

ENGELMANN, M. D. 1961. The role of soil arthropods in the energetics of an old-field community. Ecol. Monogr. 31: 221-238.

ENGELMANN, M. D. 1966. Energetics, terrestrial field studies and animal productivity. Pages 73-115 *in* J. B. Cragg, ed. Advances in ecological research. Vol. 3. Academic Press, New York.

FOLCH, J. M., M. LEES, and G. H. S. STANLEY. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497-509.

GAY, F. J., T. GREAVES, F. G. HOLDAWAY, and A. H. WETHERLY. 1955. Standard laboratory colonies of termites for evaluating the resistance of timber, timber preservatives, and other materials to termite attack. Commonwealth Sci. Ind. Res. Organ. Bull. No. 277. Commonwealth Sci. Ind. Res. Organ., Melbourne.

GOLLEY, F. B. 1961. Energy values of ecological materials. Ecology 42:581-584.

GORDON, H. T. 1968. Quantitative aspects of insect nutrition. Amer. Zool. 8:131-138.

GRASSÉ, P.-P. 1949. Ordre des Isopteres ou Termites. Pages 408-544 *in* P. -P. Grassé, ed. Traite de zoologie. Vol. IX. Masson et Cie, Paris.

HARVEY, P. A. 1934. II. Life history of *Kalotermes minor*. Pages 217-233 *in* C. A. Kofoid et al., eds. Termites and termite control. 2nd ed. Univ. Calif. Press, Berkeley.

HAVERTY, M. I. 1974. The significance of the subterranean termite, *Heterotermes aureus* (Snyder), as a detritivore in a desert grassland ecosystem. Ph.D. Diss. Univ. Ariz., Tucson. 89 pp.

HAVERTY, M. I., J. P. LAFAGE, and W. L. NUTTING. 1974. Seasonal activity and environmental control of foraging of the subterranean termite, *Heterotermes aureus* (Snyder), in a desert grassland. Life Sci. 15:1091-1101.

HAVERTY, M. I., and W. L. NUTTING. 1975. A simulation of wood consumption by the subterranean termite, *Heterotermes aureus* (Snyder), in an Arizona desert grassland. Insectes Sociaux 22:93-102.

HAVERTY, M. I., W. L. NUTTING, and J. P. LAFAGE. 1975. Density of colonies and spatial distribution of foraging territories of the desert subterranean termite, *Heterotermes aureus* (Snyder). Envir. Entomol. 4:105-109.

HONIGBERG, B. M. 1970. Protozoa associated with termites and their role in digestion. Pages 1-36 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Vol. 2. Academic Press, New York.

JOHNSON, D. B., W. E. MOORE, and L. C. ZANK. 1961. The spectrophotometric determination of lignin in small wood samples. Tappi 11:793-798.

JOHNSON, K. A., and W. G. WHITFORD. 1975. Foraging ecology and relative importance of subterranean termites in Chihuahuan Desert ecosystems. Envir. Entomol. 4: 66-70.

KIM, J-O, and F. J. KOHOUT. 1975. Multiple regression anlaysis: subprogram regression. Pages 320-367 *in* N. H. Nie et al., eds. Statistical package for the social sciences. 2nd ed. McGraw-Hill Book Co., New York.

KIRK, T. K. 1971. Effects of microorganisms on lignin. Annu. Rev. Phytopathol. 9:185-210.

KIRK, T. K., and J. M. HARKIN.1972. Lignin biodegradation and the bioconversion of wood. Amer. Inst. Chem. Eng., Annu. Meeting, Paper No. 30a. 11 pp.

LAFAGE, J. P., and W. L. NUTTING. Nutrient dynamics in termites. *In* M. V. Brian, ed. Production ecology of ants and termites. Cambridge Univ. Press, London. (In press)

LAFAGE, J. P., W. L. NUTTING, and M. I. HAVERTY. 1973. Desert subterranean termites: a method for studying foraging behavior. Envir. Entomol. 2:954-956.

LASKER, R. 1959. Cellulose digestion in insects. Pages 348-355 *in* D. L. Ray, ed. Marine boring and fouling organisms. Univ. Wash. Press, Seattle.

LASKER, R., and A. C. GIESE. 1956. Cellulose digestion by the silverfish *Ctenolepisma lineata*. J. Exp. Biol. 33:542-553.

LEE, K. E., and T. G. WOOD. 1971. Termites and soils. Academic Press, New York. 251 pp.

LEVI, M. P., W. MERRILL, and E. B. COWLING. 1968. Role of nitrogen in wood deterioration. VI. Mycelial fractions and model nitrogen compounds as substrates for growth of *Polyporus versicolor* and other wood-destroying and wood-inhabiting fungi. Phytopathology 58:626-634.

LINCOLN, F. B., and A. S. MULAY. 1929. The extraction of nitrogenous materials from pear tissues. Plant Physiol. 4: 233-250.

LINDEMAN, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23:399-418.

MARTON, J. 1966. Lignin structure and reactions. Advances in Chemistry Series 59 (R. F. Gould, ed). Amer. Chem. Soc. Pub., Washington, D.C. 267 pp.

MAYNARD, L. A. 1937. Animal nutrition. McGraw-Hill, New York. 483 pp.

MCBRAYER, J. F., D. E. REICHLE, and M. WITKAMP. 1974. Energy flow and nutrient cycling in a cryptozoan food-web. Env. Sci. Div., Oak Ridge Nat. Lab. Pub. No. 575. Nat. Tech. Inf. Serv., Oak Ridge, Tenn. 78 pp.

MCMAHAN, E. A. 1962. Laboratory studies of colony establishment and development in *Cryptotermes brevis* (Walker) (Isoptera: Kalotermitidae). Proc. Hawaiian Entomol. Soc. 18:145-153.

MCMAHAN, E. A. 1966. Studies of termite wood-feeding preferences. Proc. Hawaiian Entomol. Soc. 19:239-250.

MCNEILL, S., and J. H. LAWTON. 1970. Annual production and respiration in animal populations. Nature (London) 225:472-474.

NAGIN, R. 1972. Caste determination in *Neotermes jouteli* (Banks). Insectes Sociaux 19:39-61.

NEL, J. J. C., P. H. HEWITT, and L. JOUBERT. 1971. Growth in relation to colony size in the harvester termite *Hodotermes mossambicus* (Hagen). Phytophylactica 3:1-6.

NOIROT, C., and C. NOIROT-TIMOTHÉE. 1969. The digestive system. Pages 49-88 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Vol. 1. Academic Press, New York.

NUTTING, W. L. 1969. Flight and colony foundation. Pages 233-282 *in* K. Krishna and F. M. Weesner, eds. Biology of termites. Vol. 1. Academic Press, New York.

ODUM, H. T. 1957. Trophic structure and productivity of Silver Springs. Ecol. Monogr. 27:55-112.

ODUM, H. T., and E. P. ODUM. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol. Monogr. 25:291-320.

OSER, B. L., ed. 1965. Hawk's physiological chemistry. 14th ed. McGraw-Hill Book Co., The Blakiston Div., New York. 1472 pp.

PARIS, O. H., and A. SIKORA. 1967. Radiotracer anaysis of the trophic dynamics of natural isopod populations. Pages 741-771 *in* K. Petrusewicz, ed. Secondary productivity of terrestrial ecosystems. Vol. 2. Państwowe Wydawnictwo Naukowe, Warszawa and Kraków.

PETRUSEWICZ, K. 1967. Concepts in studies on the secondary productivity of terrestrial ecosystems. Pages 17-49 *in* K. Petrusewicz, ed. Secondary productivity of terrestrial ecosystems. Vol. 1. Państwowe Wydawnictwo Naukowe, Warszawa and Kraków.

PETRUSEWIZ, K., and A. MACFADYEN. 1970. Productivity of terrestrial animals. F. A. Davis Co., Philadelphia. 190 pp.

RENNIE, P. J. 1965. The determination of nitrogen in woody tissue. J. Sci. Food Agr. 16:629-638.

RITTER, G. J., and J. H. BARBOUR. 1935. Effect of pretreatments of wood on the lignin determination. Ind. Eng. Chem. Anal. Ed. 7:238-240.

ROBINSON, T. 1963. The organic constituents of higher plants, their chemistry and interrelationships. Burgess Publ. Co., Minneapolis. 306 pp.

ROGOZINSKI, M. 1964. A rapid quantitative esterification technique for carboxylic acids. J. Gas Chromatogr. 2: 136-137.

SCHUBERT, W. J. 1965. Lignin biochemistry. Academic Press, New York. 131 pp.

SCURFIELD, G., and P. W. NICHOLLS. 1970. Amino-acid composition of wood proteins. J. Exp. Bot. 21:857-868.

STECHER, P. G., ed. 1968. The Merck Index, an encyclopedia of chemicals and drugs. 8th ed. Merck and Co., Inc., Rahway, N.J. 1713 pp.

STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York. 481 pp.

TEAL, J. M. 1957. Community metabolism in a temperate cold spring. Ecol. Monogr. 27:283-302.

TSOUMIS, G. 1968. Wood as raw material. Pergamon Press, Oxford. 276 pp.

USHER, M. B. 1974. Runway building and food exploration strategy of *Pseudacanthotermes militaris* (Insecta: Isoptera). Biotropica 6:154-157.

WATSON, J. A. L., and F. J. GAY. 1970. The role of grass-eating termites in the degradation of a mulga ecosystem. Search 1:43.

WILKINSON, W. 1962. Dispersal of alates and establishment of new colonies in *Cryptotermes havilandi* (Sjöstedt) (Isoptera, Kalotermitidae). Bull. Entomol. Res. 53:265-286.

WILSON, E. O. 1971. The insect societies. The Belknap Press of Harvard Univ. Press, Cambridge, Mass. 548 pp.

WISE, L. E. 1952. Miscellaneous extraneous components of wood. Pages 638-688 *in* L. E. Wise and E. C. Jahn, eds. Wood chemistry. Vol. 1. Reinhold Publ. Corp., New York.

WOLCOTT, G. N. 1946. Factors in the natural resistance of woods to termite attack. Caribbean Forest. 7:121-134.

WOOD, T. G. The food and feeding habits of termites. *In* M. V. Brian, ed. Production ecology of ants and termites. Cambridge Univ. Press, London. (In press)