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THE ROLE OF FUNGI IN THE DIET OF THE COMMON DAMP-WOOD TERMITE, ZOOTERMOPSIS ANGUSTICOLLIS[1,2]

ESTHER C. HENDEE[3]

INTRODUCTION

Zootermopsis angusticollis (Hagen), the common damp-wood termite, is associated in nature with an abundant and varied fungus flora (Hendee, 1933). Usually the wood which encloses burrows made by termites of this species shows evidence of rot produced by wood-destroying fungi. Although occasionally the termites may enter apparently sound wood, fungus spores and hyphae, which cling to their bodies and are thus carried into the new burrows, soon give rise to fungus mycelium which invades the walls of the burrows. Are these fungi of any benefit to the termites? Cleveland (1924) reports that termites from which the protozoan fauna of the gut has been removed, although unable to digest sound wood, are able to flourish on fungus-digested wood. Cook and Scott (1933) found that microörganisms apparently afford a beneficial supplement to a diet of filter paper or cotton, which is shown to be in itself inadequate for the nutrition of the termites. It therefore becomes of interest to inquire whether normally faunated termites living on their natural diet of wood likewise benefit from the presence of fungi in their food. Roessler (1932) reports a higher rate of growth for termites fed on sound, sterile wood than for those fed on decayed wood. She remarks, however, that an excessive growth of fungi on the decayed wood caused the deaths of many termites. This renders the results of that particular experiment of doubtful significance. The need for further consideration of this problem is therefore evident.

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[3] Research Associate in Zoölogy; resigned June 30, 1935.

In the experiments to be described in this paper termites were placed on various fungus-containing and fungus-free diets in an attempt to provide information bearing upon the following questions: (1) Can termites live and grow normally on fungus-free, sound wood, or is the presence of fungi in the wood essential or beneficial to the termites? (2) If fungus infestation of wood is essential or beneficial in rendering it a suitable diet for termites, is a recent, superficial growth of fungi on sound wood adequate for this purpose, or is the long continued action of fungi, such as that resulting in the production of rot, necessary? (3) Does the fungus-free, sound wood show any advantage over filter paper as a food for termites? (4) Does the total nitrogen content of termites show any significant relation to the presence or absence of fungi in their diet?

MATERIALS AND METHODS

Termites.—Nymphs of the species *Zootermopsis angusticollis* were used in all experiments. They were collected near Berkeley, California. Those used in experiments 1 and 2 were all sibs from one natural colony; those used in experiments 3 to 6 were sibs from another natural colony.

Handling of Termites.—When it was necessary to transfer termites from one container to another, this was accomplished by means of a camels' hair brush or a small piece of paper. Termites were never touched with forceps or other hard instruments. During the course of experiments the termites were kept in darkness at room temperature (18° to 20° C).

Cannibalism Prevented by Isolating Termites.—A difficulty which is encountered in the devising of satisfactory feeding experiments with termites is the fact that termites when kept in groups together practice cannibalism, which makes complete control of their diet impossible. In order to eliminate this difficulty, termites were kept in individual vials during the first feeding experiment. Such isolation, it is realized, is an abnormal condition for a social insect. Nevertheless, the factor of isolation being the same throughout, it is felt that differences observed between results on the various diets are attributable to the effects of the diets and are therefore worthy of consideration.

The duration of experiments with individually isolated termites must probably be limited to the interval between ecdyses, for at ecdysis the termite loses a part of its intestinal fauna of Protozoa which is apparently essential to the termite for the digestion of cellulose (Andrew, 1930; Cleveland, 1924), and the normal methods of refaunation as observed by Andrew, namely proctodeal feeding and cannibalism, would be impossible without contact with other termites.

The Problem of Cannibalism in Group Tests.—For experiments which were to be continued over long periods of time, termites were kept in groups together so that refaunation might occur after ecdysis. It then became necessary to find criteria for judging the effects of the diets which were provided for the termites—criteria which would be significant in spite of the possible occurrence of cannibalism. Obviously, the growth of individual termites in a group cannot be used alone as a criterion in this case, for, even though a group were placed on a deficient or harmful diet, there might still be opportunity for certain individuals to thrive for some time by supplementing the diet through cannibalism or even by wholly refraining from the food provided and subsisting entirely on the bodies of other termites. It is rather the group as a whole and the individuals in relation to the group which must be considered.

Cook and Scott (1933), in studying the nutritional requirements of termites, used viability and group weight as criteria for judging the adequacy of artificial diets on which they fed the termites. They consider that on an adequate diet "cannibalism should not exist and the total group weight should maintain itself if not increase." The present investigation is concerned with factors in the diet which termites choose for themselves in nature. The writer has observed in previous experiments, not reported in this paper, that in groups of healthy nymphs living on the rotten wood in which they have been found in the field cannibalism does occur. The steady decrease in numbers thus occasioned may lead to a decrease in group weight during the first few months but is followed by an increase in group weight when the rate of reproduction by supplementary kings and queens which develop sufficiently exceeds the rate of cannibalism. Therefore, should not the possibility be considered that the natural feeding habits of termites may involve a certain amount of cannibalism? Some necessary supplement to the diet of wood may be thus obtained, or it may afford a method of intestinal refaunation after ecdysis (Andrew, 1930). Considerable numbers of supplementary reproductives are usually present in colonies in nature, and the addition of members to the colony through reproduction probably is quite sufficient to account for the increase in size of the colony in spite of some cannibalism.

In all experiments reported in this paper in which termites were kept in groups, control groups of termites were supplied with a diet of rotten wood similar to that in which they are naturally found. The amount and rate of cannibalism and the gain or loss in group weight and average weight per termite were observed under these conditions. These results

from a diet of rotten wood then being considered as normal, any considerable deviations from them in the results from other diets could be attributed to the factors which had been experimentally varied.

Weights of Termites.—Cook and Scott (1932) have shown that the moisture content and consequently the total or wet weight of termites is rapidly reduced if they are exposed to dry air, or if the moisture content of their food is reduced. In the present experiments both the vapor tension in the containers and the moisture content of the foods were kept as nearly uniform and as constant as possible. Nevertheless, to avoid every possibility of error from fluctuation in moisture content of the termites, only their dry weights were used. The termites were dried to a constant weight in an electric oven at 95° C. Weighings were made with an accuracy of ± 0.1 milligram.

Summary of Criteria.—Briefly, then, the criteria upon which the effects of the various diets have been judged are as follows: (1) for individually isolated termites: (*a*) viability, (*b*) general behavior, (*c*) average dry weight, and (*d*) average nitrogen content; (2) for groups: their general reaction to being placed on a given diet, including (*a*) viability as affected by factors other than cannibalism, (*b*) general behavior, (*c*) group dry weight, (*d*) amount of cannibalism, (*e*) rate of development as indicated by passage through successive instars, (*f*) reproductive activity, (*g*) average dry weight per termite, and (*h*) average nitrogen content. The results from a diet of rotten wood are considered as representative of normal conditions, since that is the diet on which these termites live in nature.

Nitrogen Determinations.—Total nitrogen content of the termites and of the foods was determined by the Parnas and Wagner micro-Kjeldahl process (Pregl, 1930), mercuric sulfate being used as the catalyst. An accuracy of ± 0.002 milligram of nitrogen was obtained by this method. Values for total nitrogen are recorded, however, only to the number of decimal places which are significant when the error due to biological variation in the particular case is taken into consideration. All percentages of nitrogen are on the basis of dry weight.

Biological Variation.—The probable errors due to biological variation of weight and nitrogen content of the termites are recorded in the footnotes of the tables reporting results of the experiments. These errors were reduced to a minimum by choosing for experimental use termites among which the range in initial dry weight was slight and they have been taken into consideration in comparing results for termites on different diets or on the same diet at different times during the experiments.

Diets.—The term "diet" as here used refers to the food upon which the termites were placed. As has already been pointed out, termites which were kept in groups had the opportunity to supplement this diet through cannibalism, and this fact has been considered in judging the effects of the diets which were experimentally provided.

Before being fed to the termites, the filter paper was saturated with distilled water and sterilized at 20 pounds' pressure for 20 minutes. It could not be kept perfectly sterile while the termites were feeding on it, inasmuch as the termites themselves could not be sterilized. It was kept free from any appreciable fungus growth, however, by changing the paper and container at least every third day. That this changing was sufficiently frequent was demonstrated by preliminary tests in which paper was stained and examined microscopically for fungus hyphae at various intervals after termites had been feeding on it. The more frequent changing in experiments 3 to 6 was to avoid the accumulation of fecal pellets in dishes in which considerable numbers of termites were being kept together.

The sound wood was tested for freedom from fungus mycelium by microscopic examination of sections taken at frequent intervals throughout the pieces used. The wood was then cut into small blocks measuring 1 inch by ½ inch by ¼ inch for sound Monterey pine, and 2½ inches by ⅜ inch by ⅜ inch for sound Douglas fir. The blocks were saturated with distilled water, placed in Erlenmeyer flasks, and sterilized in flowing steam at 100°C for 2 hours on 3 successive days. The wood was thus prepared at the time a given series of experiments was begun and was stored in a sterile condition in the stoppered flasks until needed. In experiments in which it was desired to keep the wood used by termites free from fungus growth, the wood was changed at least every second day. Preliminary tests involving the examination of surfaces and of stained sections of blocks on which termites had been feeding showed the wood used to be still free from fungus growth after 3 days' exposure.

Cultures were made from the rotten wood in order to learn whether living fungi were still present and to determine the nature of the fungus flora. The method of making the cultures was the same as that described in a previous paper (Hendee, 1933). Representatives of the following genera of fungi were isolated from the rotten Monterey pine: *Trichoderma, Penicillium, Absidia, Oedocephalum, Haplotrichum,* and *Acrostalagmus.* The first two were the most abundant. None of these fungi is known to be a typically wood-destroying form, so it seems probable that the fungus which had caused the rot had disappeared from the wood and that these were secondary invaders. The rotten Douglas fir con-

tained a *Penicillium* and a white *Poria*. The rot was a cubical brown rot and had probably been caused by the *Poria*, which was generally distributed throughout the wood and present as thin layers of mycelium in the shrinkage cracks. In addition to these fungi, other species of *Penicillia* and *Trichoderma lignorum* (Tode) Harz were isolated from the rotten Douglas fir after the termites had been feeding on it during the course of the experiments. These fungi had doubtless been introduced by the termites.

For the preparation of the diet of sound Douglas fir with *Trichoderma*, flasks were chosen at random from among those containing sound Douglas fir. The wood in these flasks was then inoculated with a suspension of spores of *T. lignorum* in distilled water and incubated at room temperature (18° to 21°C) for from two to three weeks before being fed to the termites. A macroscopically visible growth of fungus mycelium had by that time developed on the surface of the wood. Since the termites ate the surface from the wood rather quickly, the food had to be changed every fourth day to insure the presence of the fungus in the diet at all times. *T. lignorum* was chosen for use in the experiments because it is one of the most commonly occurring species among those found in wood enclosing the burrows of *Zootermopsis angusticollis*. The spore suspensions used in inoculating the wood were prepared from a single spore culture.

The diet of sound Monterey pine with fungi was prepared by placing the termites on damp, sound Monterey pine and allowing the fungi introduced by the termites to grow.

All of the foods were saturated with water when they were placed in the containers with the termites. In instances where the food was not changed daily its moisture content was maintained by frequently adding a few drops of distilled water to it.

EXPERIMENT 1: TESTS WITH ISOLATED TERMITES

One hundred fifty nymphs of the fifth instar were isolated in individual shell vials. Of these nymphs, 50 were fed on each of the following diets:

Fungus-containing, rotten Monterey pine wood; from log in which termites had been found; not changed.

Fungus-free, sound Monterey pine sapwood; unseasoned; taken from near base of tree cut in January; changed daily.

Fungus-free filter paper; Whatman's No. 42; changed every third day.

The initial average dry weight and total nitrogen content of the ter-

mites were determined by weighing and analyzing 36 additional termites of the same instar and size as those used in the feeding experiments. At 21-day intervals, 9 termites were chosen at random from those on each diet and their average dry weight and nitrogen content determined.

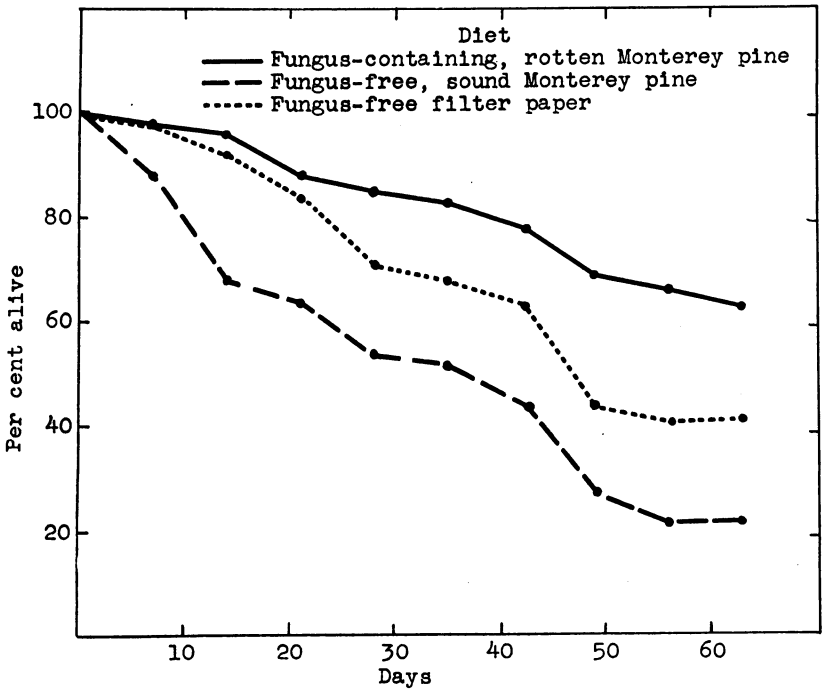

Fig. 1.—Viability of termites in experiment 1. Initial number of termites on each diet was 50. For the first 21 days ordinates are percentages of the initial number. Since 9 termites were removed from each diet group for analysis at 21 days and another 9 at 42 days, ordinates from 21 to 42 days and from 42 to 63 days are percentages of the possible 41 and 32, respectively.

RESULTS

Viability.—Viability of termites was decidedly better on the diet of rotten Monterey pine than on that of sound Monterey pine (fig. 1). This was particularly noticeable during the early part of the experiment. At the end of 21 days 12 per cent of the original number had died on the diet of rotten Monterey pine as compared with 36 per cent on that of sound Monterey pine. There were only a few deaths on fungus-free filter paper during the first few weeks but more than on rotten Monterey pine. Later, mortality increased on filter paper.

Appearance and Behavior.—The abdomens of termites which died

while on the diet of sound Monterey pine were abnormally transparent and bloated in appearance. Examination of the intestinal contents after death usually revealed a much reduced protozoan fauna with Protozoa of the genus *Trichonympha* nearly or completely absent. Particles of wood were present in the lumen of the intestine showing that the ter-

TABLE 1

AVERAGE DRY WEIGHT AND NITROGEN CONTENT OF INDIVIDUALLY ISOLATED TERMITES IN EXPERIMENT 1*

Days	Average dry weight per termite in mg	Per cent gain or loss in dry weight	Average nitrogen per termite in mg	Per cent gain or loss in nitrogen	Nitrogen in per cent of dry weight
1	*2*	*3*	*4*	*5*	*6*
Diet of fungus-containing, rotten Monterey pine					
21	3.8	−14.3	0.37	− 5.4	9.9
42	5.2	+19.1	0.45	+14.6	8.5
63	5.3	+19.3	0.46	+18.2	8.8
Diet of fungus-free, sound Monterey pine					
21	3.3	−25.0	0.33	−14.9	10.2
42	3.3	−25.0	0.35	−11.5	10.5
63	3.5	−20.5	0.35	−10.8	9.9
Diet of fungus-free filter paper					
21	2.9	−34.1	0.32	−17.4	11.1
42	3.0	−31.8	0.34	−12.6	11.4
63	3.2	−27.5	0.34	−11.8	10.8

* A sample taken at the beginning of the experiment showed the following: initial average dry weight per termite=4.4 mg±0.2 mg; initial average nitrogen per termite=0.39 mg±0.01 mg; initial nitrogen in per cent of dry weight=9.0±0.1.

mites had been eating about a usual amount. The wood had not, however, been ingested by the intestinal Protozoa to the extent observed in healthy termites. The termites which died on fungus-free filter paper were as a rule shrunken in appearance. They also showed a reduced intestinal fauna. Of the 12 termites which died on rotten Monterey pine, 4 showed a fungus infection. Another which died soon after the experiment was begun had undergone a recent molt and apparently not become refaunated before being isolated. No cause was apparent for the other deaths on rotten Monterey pine.

The living termites were normal in behavior and appearance on the

diet of rotten Monterey pine. Termites on sound Monterey pine were sluggish and comparatively inactive. This became evident within a day after the beginning of the experiment. The bloated appearance of the abdomen mentioned in reference to termites which died on sound Monterey pine was also noticeable in the majority of the surviving termites

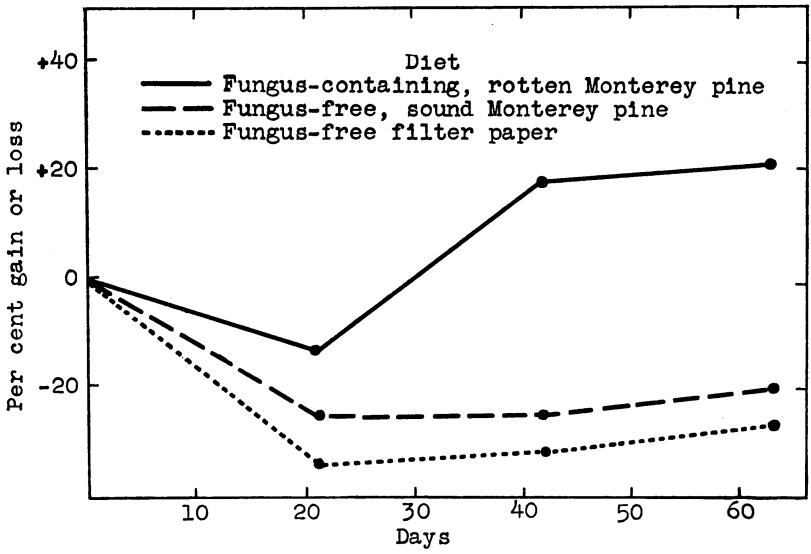

Fig. 2.—Average dry weight per termite in experiment 1. Ordinates represent averages for 9 termites.

on the same diet. The termites on fungus-free filter paper appeared normal during the early part of the experiment, but after the first two weeks became increasingly inactive.

Dry Weight.—Termites on rotten Monterey pine after 21 days showed a loss in average dry weight, possibly owing to a lag in adjustment to the condition of isolation (table 1, col. 3, and fig. 2). Later, however, they gained in weight, showing at 63 days a gain of 19.3 per cent over the initial weight. The termites on sound Monterey pine and those on fungus-free filter paper showed a considerably greater initial loss in weight than those on rotten Monterey pine, and, while they suffered no further loss in weight after the first three weeks, failed to make significant gain during the course of the experiment.

Nitrogen Content.—Termites on rotten Monterey pine suffered a slight initial drop in milligrams of nitrogen per termite, but showed final significant gain in nitrogen (table 1, col. 5, and fig. 3). On sound Monterey pine and on fungus-free filter paper termites showed greater initial

loss in milligrams of nitrogen per termite than those on rotten Monterey pine and subsequent failure to make significant gain. The accompanying loss in body weight, however, was relatively greater than the loss in nitrogen, as is evident from the increase in percentage of nitrogen in relation to body weight (table 1, col. 6). One or more body constituents,

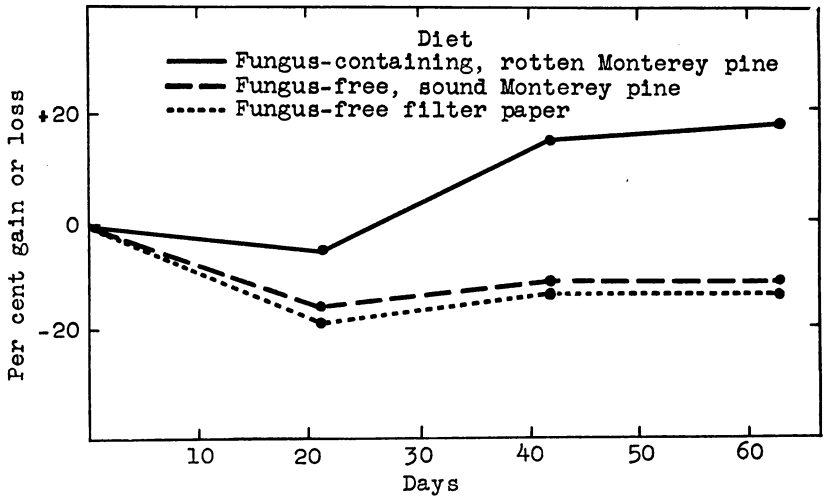

Fig. 3.—Average milligrams of nitrogen per termite in experiment 1. Ordinates represent averages for 9 termites.

presumably fat or carbohydrate or both, decreased more than nitrogen-containing constituents, which shows a nitrogen-sparing action such as is commonly observed in animals during early stages of starvation.

EXPERIMENT 2: GROUP TESTS EXAMINED SERIALLY

Nymphs of the fifth instar were placed in groups of 50 in covered glass containers on the following diets:

Fungus-containing, rotten Monterey pine wood; from log in which termites had been found; not changed.

Sound Monterey pine sapwood on which fungi introduced by the termites were allowed to grow; not changed.

This experiment was carried on simultaneously with experiment 1, and the termites used were of the same initial size and average weight. At 21-day intervals the termites of experiment 2 were counted, and 9 termites chosen at random from each diet group were removed to determine average weight and nitrogen content.

RESULTS

Viability.—Viability was comparable in the two groups. During the 63 days of the experiment, 10 termites besides those taken out for analysis disappeared from the group on rotten Monterey pine, and 13 from the group on sound Monterey pine with fungi. The living termites always appeared active and healthy. No dead bodies were observed. All

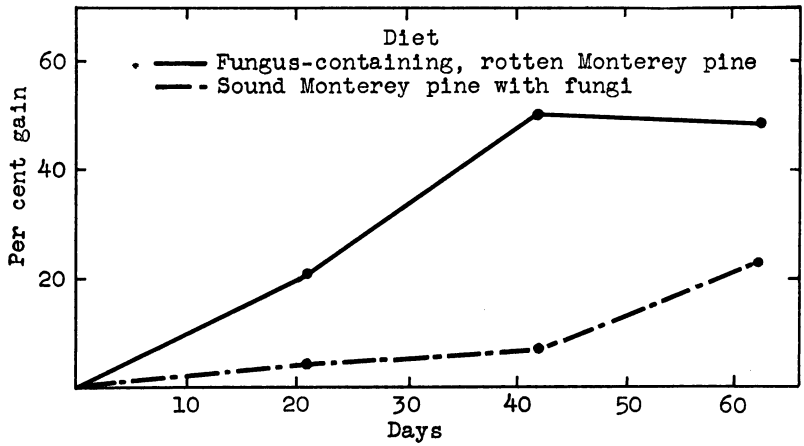

Fig. 4.—Average dry weight per termite in experiment 2. Ordinates represent averages for 9 termites.

deaths were probably due to cannibalistic attack. At least it is certain that the bodies of missing termites had been eaten by surviving termites.

Development.—Development as evidenced by molting to the sixth instar was faster on the diet of rotten Monterey pine than on that of sound Monterey pine with fungi (table 2, col. 4). At 63 days 14 of the 22 termites present on rotten Monterey pine were in the sixth instar as compared with 3 of the 19 on Monterey pine with fungi. In comparing molting on the two diets it should be borne in mind that certain error is doubtless introduced by removal of samples for analysis and by cannibalism. Nevertheless, the two-fold difference in observed progress in molting seems sufficiently great to be significant.

Reproduction.—At 63 days there were 3 supplementary reproductives and 8 eggs on rotten Monterey pine, and 5 supplementary reproductives and no eggs on sound Monterey pine with fungi (table 2, cols. 6 and 7). These differences are probably not sufficiently great to be significant.

Dry Weight.—A gain of 46.6 per cent in average dry weight per ter-

TABLE 2

POPULATION OF GROUPS OF TERMITES IN EXPERIMENT 2*

Days	Total number of termites present	Nymphs of the fifth instar	Nymphs of the sixth instar	Adult soldiers	Supplementary reproductives	Eggs
1	*2*	*3*	*4*	*5*	*6*	*7*
Diet of fungus-containing, rotten Monterey pine						
21	49	48	1	0	0	0
42	33	24	8	1	0	0
63	22	4	14	1	3	8
Diet of sound Monterey pine with fungi						
21	49	49	0	0	0	0
42	32	30	2	0	0	0
63	19	11	3	0	5	0

* All termites were nymphs of the fifth instar at the beginning of the experiment. The initial number on each diet was 50.

TABLE 3

AVERAGE DRY WEIGHT AND NITROGEN CONTENT OF TERMITES KEPT IN GROUPS AND SERIALLY EXAMINED IN EXPERIMENT 2*

Days	Average dry weight per termite in mg	Per cent gain in dry weight	Average nitrogen per termite in mg	Per cent gain in nitrogen	Nitrogen in per cent of dry weight
1	*2*	*3*	*4*	*5*	*6*
Diet of fungus-containing, rotten Monterey pine					
21	5.3	+19.5	0.44	+13.3	8.4
42	6.6	+50.7	0.57	+46.4	8.7
63	6.5	+46.6	0.59	+51.3	9.1
Diet of sound Monterey pine with fungi					
21	4.6	+ 5.5	0.39	0.0	8.4
42	4.7	+ 6.8	0.48	+22.1	9.8
63	5.4	+21.8	0.53	+36.2	9.9

* A sample taken at the beginning of the experiment showed the following: initial average dry weight per termite=4.4 mg±0.2 mg; initial average nitrogen per termite=0.39 mg±0.01 mg; initial nitrogen in per cent of dry weight=9.0±0.1.

mite had taken place in the group on rotten Monterey pine at the end of 63 days, while termites on sound Monterey pine with fungi showed a gain of only 21.8 per cent (table 3, col. 3, and fig. 4).

Nitrogen Content.—The gain in average milligrams of nitrogen per termite during the course of the experiment was 51.3 per cent for termites on rotten Monterey pine and 36.2 per cent for those on sound

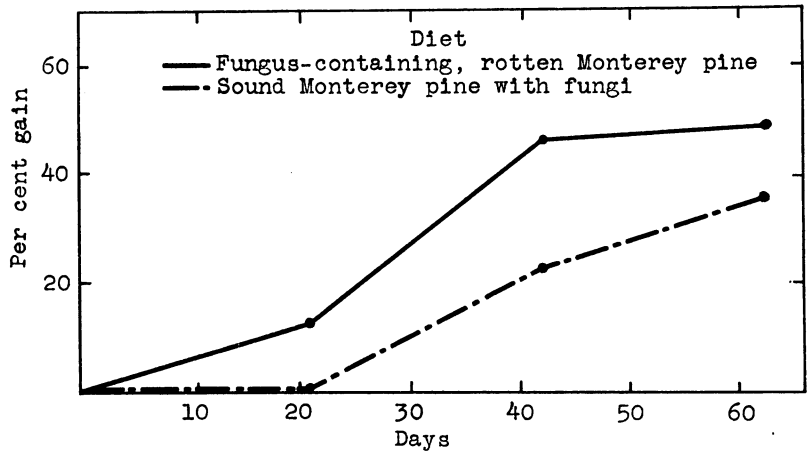

Fig. 5.—Average milligrams of nitrogen per termite in experiment 2. Ordinates represent averages for 9 termites.

Monterey pine with fungi (table 3, col. 5, and fig. 5). It is significant that the difference in rate of gain occurred during the time when the difference in the fungus content of the two diets was greatest. During the first three weeks of the experiment no visible fungus growth had yet appeared on the sound Monterey pine, and during this time the termites on this diet showed no gain in nitrogen, while those on rotten Monterey pine made a gain of 13.3 per cent. During the later weeks of the experiment, when the fungi introduced by the termites had produced a visible fungus growth on the surface of the sound Monterey pine, the termites feeding on this sound wood and fungus made gains in nitrogen comparable to those of termites on rotten Monterey pine.

EXPERIMENTS 3 TO 6: GROUP TESTS EXAMINED TERMINALLY

Experiments 3 to 6 were designed to show the effects of the presence of fungi in the diet upon the group of termites living together when the group is considered as a unit, as well as upon the individual termites as

members of the group. They comprised a series of closely related experiments, carried on simultaneously and differing from each other only in the length of time during which each was continued.

In each of experiments 3 to 6 two groups, each composed of 50 nymphs of the fourth instar, were placed in covered petri dishes on each of the following diets:

Fungus-containing, rotten Douglas fir heartwood; from center of untreated railroad tie; not changed; supply replenished as needed.

TABLE 4

TOTAL NITROGEN CONTENT OF FOODS ON WHICH TERMITES WERE FED IN EXPERIMENTS 3 TO 6

Food	Sample 1	Sample 2	Sample 3	Average
	Total nitrogen in per cent of dry weight			
Fungus-containing, rotten Douglas fir	0.08	0.13	0.11	0.11
Sound Douglas fir with *Trichoderma**	0.15	0.13	0.17	0.15
Fungus-free, sound Douglas fir	0.10	0.11	0.11	0.11
Fungus-free filter paper	0.05	0.06	0.04	0.05

* Samples for analysis were taken from surface of blocks covered with mycelium.

Fungus-free, sound Douglas fir heartwood; from summer-felled log; air seasoned for three months prior to use; still fragrant; changed every second day.

Sound Douglas fir heartwood on which there was a recent, superficial growth of the fungus *Trichoderma lignorum;* changed every fourth day to insure presence of fungus.

Fungus-free filter paper; Whatman's No. 42; changed every second day.

The total nitrogen content of these foods as determined by analysis of samples is given in table 4.

The lengths of time during which the termites were fed on these diets were as follows:

Experiment 3..... 28 days
Experiment 4..... 56 days
Experiment 5..... 84 days
Experiment 6.....112 days

At the termination of these periods of time the termites of the respective experiments were killed and their group and average dry weights determined. They were also analyzed for total nitrogen content. The initial group and average dry weight and nitrogen content were determined by

weighing and analyzing two additional groups each composed of 50 fourth instar nymphs. The termites in all groups were counted every eighth day.

In this series of experiments no attempt was made to prevent cannibalism. In instances when termites had been killed or had died but were

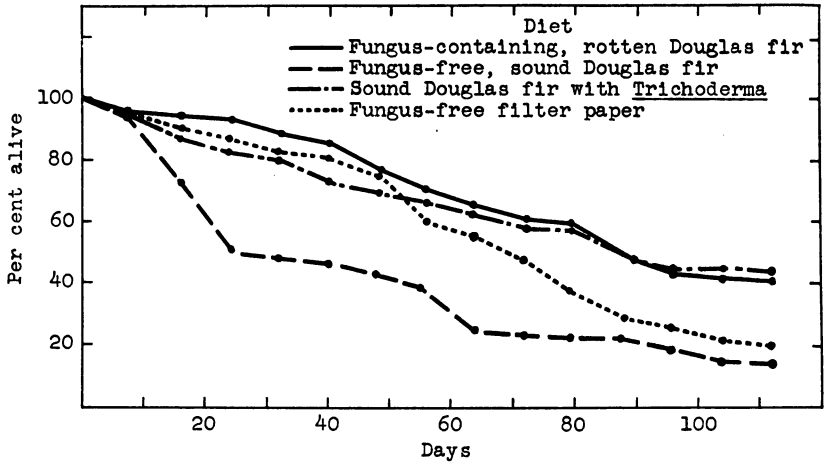

Fig. 6.—Viability among groups of termites in experiments 3 to 6. For the first 28 days ordinates represent averages for 8 groups. At 28 days and at each succeeding 28-day interval the number of groups is decreased by two, these groups having been removed for analysis at those times.

not completely eaten, the dead bodies were removed within 24 hours. Otherwise, they would have afforded a substratum for the development of undesirable microörganisms. A record was kept of the number of bodies removed from each group (table 5, col. 7).

RESULTS

Viability and Cannibalism.—Figure 6 shows the average viability among the groups of termites which were fed on the various diets.

With but one exception, all termites lost to groups on the diet of rotten Douglas fir were eaten by the surviving termites (table 5, cols. 5 and 6). Although the groups were inspected daily, it was only on rare occasions that bodies of victims were observed. These could hardly be called dead bodies for they were always in the process of being literally eaten alive. The antennae on severed heads would still be waving, and headless bodies might still be capable of walking away if released for an instant by the aggressor. Termites thus attacked always appeared to have been healthy, and no evidence for death from any cause other than

TABLE 5

MORTALITY AND CANNIBALISM OBSERVED IN EXPERIMENTS 3 TO 6*

Experiment No.	Days	Group	Number of termites surviving	Total number of termites dead	Number of dead bodies eaten	Number of dead bodies uneaten; removed
1	*2*	*3*	*4*	*5*	*6*	*7*
Diet of fungus-containing, rotten Douglas fir						
3	28	a	48	2	2	0
		b	47	3	3	0
4	56	a	33	17	17	0
		b	39	11	11	0
5	84	a	29	21	21	0
		b	28	22	21	1
6	112	a	22	28	28	0
		b	20	30	30	0
Diet of fungus-free, sound Douglas fir						
3	28	a	29	21	21	0
		b	14	36	18	18
4	56	a	37	13	12	1
		b	29	21	20	1
5	84	a	10	40	28	12
		b	12	38	18	10
6	112	a	4	46	29	17
		b	10	40	28	12
Diet of sound Douglas fir with *Trichoderma*						
3	28	a	43	7	7	0
		b	35	15	15	0
4	56	a	34	16	16	0
		b	35	15	15	0
5	84	a	35	15	15	0
		b	24	26	26	0
6	112	a	25	25	25	0
		b	19	31	30	1
Diet of fungus-free filter paper						
3	28	a	46	4	4	0
		b	44	6	6	0
4	56	a	26	24	17	7
		b	27	23	15	6
5	84	a	21	29	25	4
		b	13	37	23	14
6	112	a	7	43	26	17
		b	13	37	21	16

* Each group consisted of 50 termites at the beginning of the experiments.

cannibalism was ever found among groups on the fungus-containing wood. The one instance when the body was not eaten occurred when a termite had been recently attacked at the time the group to which it belonged was removed for analysis. Occasionally mandibles were found on the bottoms of the dishes containing termites, these indigestible parts having been discarded when the other parts of the bodies were eaten. At the other times all traces of the missing termites had disappeared. The covers of the containers were held firmly in place by rubber bands to make escape impossible, so it seems safe to assume that the completely missing termites had also been eaten.

Certain facts indicate that the cannibalism as observed on rotten Douglas fir may be representative of the normal occurrence of cannibalism among termites in nature: (1) The diet of rotten wood was similar to that available to *Zootermopsis angusticollis* in nature. (2) There was general uniformity in the rate of cannibalism throughout the time the experiments were continued. (3) There was close agreement between the numbers of termites missing as a result of cannibalism in individual groups and the mean number missing at a given time. (4) All termites appeared healthy and showed normal behavior. (5) These results are similar to those obtained for the group on rotten wood in experiment 2, although the termites were of a different age and from a different natural colony, and the food supplied was a different species of wood. (6) The development of supplementary reproductives and the laying of eggs before the close of the series of experiments indicate that an increase in numbers would soon have occurred, as has been the case in other groups which have been under observation for longer periods of time.

On sound Douglas fir with *Trichoderma* viability was nearly as high as on rotten Douglas fir, and, as on the latter diet, all deaths were due to cannibalism.

Viability of termites among groups on fungus-free, sound Douglas fir showed an entirely different picture from that which has just been considered among groups on rotten Douglas fir or on sound Douglas fir with *Trichoderma*. High mortality occurred during the second week on fungus-free, sound Douglas fir (fig. 6). Although dead bodies were often partially eaten, certain observations lead to the conclusion that the majority of deaths were due to some cause other than cannibalism: (1) Dead bodies were often found which were intact and showed no evidence of cannibalistic attack. (2) Termites which were inactive, in some instances even lying on their backs and apparently in a dying state, were observed. (3) Dead bodies were seldom completely eaten. (4)

TABLE 6

GROUP DRY WEIGHT OF TERMITES KEPT IN GROUPS AND EXAMINED TERMINALLY IN EXPERIMENTS 3 TO 6*

Experiment No.	Days	Group	Number of termites surviving	Final group dry weight in mg	Per cent gain or loss in group dry weight	
					Each group	Unweighted mean of groups
Diet of fungus-containing, rotten Douglas fir						
3	28	a	48	196.9	+23.0	+23.6
		b	47	198.7	+24.1	
4	56	a	33	172.1	+ 7.7	+ 9.3
		b	39	177.4	+10.9	
5	84	a	29	146.9	− 8.2	− 6.5
		b	28	152.4	− 4.8	
6	112	a	22	150.1	− 6.3	−10.8
		b	20	135.7	−15.2	
Diet of fungus-free, sound Douglas fir						
3	28	a	29	124.9	−21.9	−39.7
		b	14	68.2	−57.4	
4	56	a	37	125.6	−21.6	−28.1
		b	29	104.8	−34.5	
5	84	a	10	44.6	−72.1	−69.9
		b	12	51.7	−67.7	
6	112	a	4	17.9	−88.8	−81.2
		b	10	45.1	−73.5	
Diet of sound Douglas fir with *Trichoderma*						
3	28	a	43	158.6	− 0.9	− 5.5
		b	35	144.0	−10.0	
4	56	a	34	123.5	−22.9	−18.2
		b	35	138.5	−13.5	
5	84	a	35	143.2	−10.6	−19.7
		b	24	114.2	−28.7	
6	112	a	25	109.1	−31.9	−38.7
		b	19	87.3	−45.5	
Diet of fungus-free filter paper						
3	28	a	46	188.7	+17.9	+ 9.6
		b	44	162.0	+ 1.2	
4	56	a	26	119.9	−25.1	−27.3
		b	27	113.1	−29.4	
5	84	a	21	102.2	−36.2	−49.0
		b	13	61.2	−61.8	
6	112	a	7	38.8	−75.9	−64.0
		b	13	76.7	−52.1	

* Each group consisted of 50 termites at the beginning of the experiments. A sample taken at the beginning of the experiments showed the initial group dry weight to be 160.1 mg±2.1 mg.

Bodies which were in the process of being eaten appeared to be quite dead, in contrast to those which were eaten by termites on rotten Douglas fir as above described. After about the third week the death rate declined and from that time on was not appreciably greater than among groups on rotten Douglas fir, although deaths not attributable to cannibalism continued to occur.

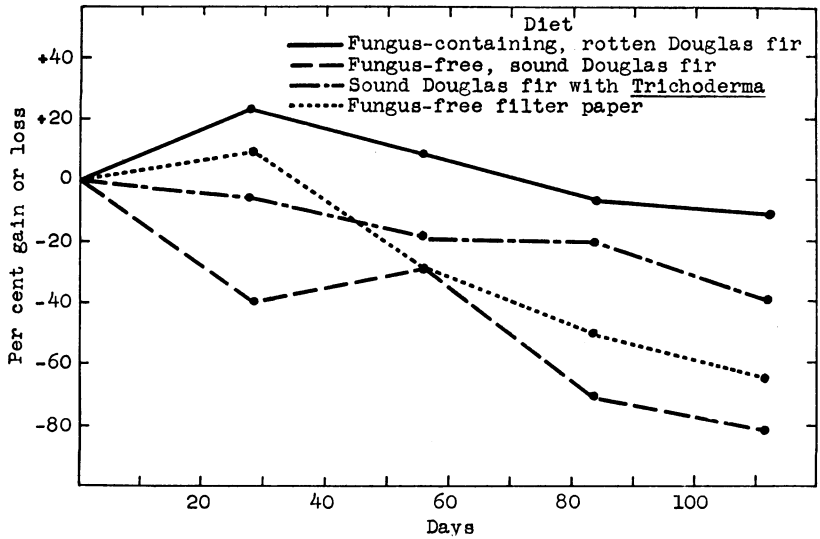

Fig. 7.—Group dry weight of termites in experiments 3 to 6. Each ordinate represents the mean for two groups.

Viability on fungus-free filter paper during the first seven weeks was only slightly lower than on rotten Douglas fir, and the majority of deaths seemed to be due to cannibalism, although a few termites which had molted to the fifth instar remained in a shrunken condition after the molt and soon died. During the eighth week mortality increased. From that time on the death rate remained high; weakened, inactive, and dying individuals were frequently observed; dead bodies were seldom completely eaten and sometimes were left untouched.

Group Dry Weight.—Group dry weight was significantly higher throughout the experiments for termites on rotten Douglas fir than for those on the other diets (table 6 and fig. 7). The groups on rotten Douglas fir which were killed at 28 days and at 56 days, respectively, showed an increase in group weight. At 84 days the group weight was slightly less than at the beginning of the experiment. The tendency towards decrease in group weight occasioned by decrease in numbers through can-

TABLE 7

AVERAGE DRY WEIGHT OF TERMITES KEPT IN GROUPS AND EXAMINED TERMINALLY IN EXPERIMENTS 3 TO 6*

Experiment No.	Days	Group	Final average dry weight per termite in mg		Per cent gain in average dry weight per termite	
			In individual groups	Weighted mean	In individual groups	Weighted mean
Diet of fungus-containing, rotten Douglas fir						
3	28	a	4.1	4.2	+ 28.1	+ 30.0
		b	4.2		+ 31.9	
4	56	a	5.2	4.9	+ 62.8	+ 51.6
		b	4.5		+ 41.9	
5	84	a	5.1	5.3	+ 58.1	+ 64.1
		b	5.4		+ 70.0	
6	112	a	6.8	6.8	+113.1	+112.5
		b	6.8		+111.6	
Diet of fungus-free, sound Douglas fir						
3	28	a	4.3	4.5	+34.4	+40.3
		b	4.9		+52.2	
4	56	a	3.4	3.5	+ 5.9	+ 9.1
		b	3.6		+12.6	
5	84	a	4.5	4.4	+39.4	+36.6
		b	4.3		+34.4	
6	112	a	4.5	4.5	+39.7	+40.6
		b	4.5		+40.9	
Diet of sound Dougals fir with *Trichoderma*						
3	28	a	3.7	3.9	+15.0	+20.9
		b	4.1		+28.4	
4	56	a	3.6	3.8	+13.4	+18.4
		b	4.0		+23.4	
5	84	a	4.1	4.4	+27.8	+36.3
		b	4.8		+48.4	
6	112	a	4.4	4.5	+36.3	+39.4
		b	4.6		+42.8	
Diet of fungus-free filter paper						
3	28	a	4.1	3.9	+28.1	+21.3
		b	3.7		+15.0	
4	56	a	4.6	4.4	+44.0	+35.9
		b	4.2		+30.9	
5	84	a	4.9	4.8	+51.9	+50.0
		b	4.7		+46.9	
6	112	a	5.5	5.8	+73.1	+80.3
		b	5.9		+84.4	

* A sample taken at the beginning of the experiments showed the initial average dry weight per termite to be 3.2 mg±0.1 mg. Calculations were made from original data and may differ slightly from those made on the basis of printed figures because of the dropping of insignificant decimal places in the printed table.

nibalism (table 5), had by that time become sufficiently great to outbalance the tendency towards increase through the steady gain in weight of individual termites (table 7 and fig. 8).

Group weight on fungus-free, sound Douglas fir decreased greatly during the first four weeks (table 6 and fig. 7). This was largely a result of the high mortality during this period. At 112 days, group weight on sound Douglas fir showed a loss of 81.2 per cent, a loss approximately eight times as great as that on rotten Douglas fir.

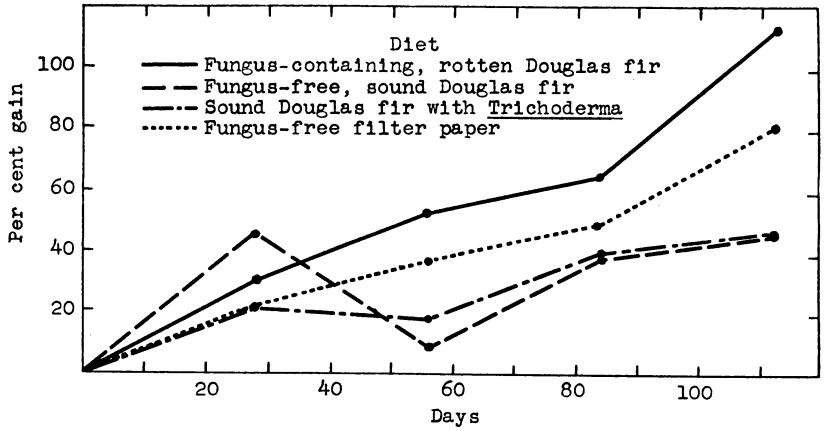

Fig. 8.—Average dry weight per termite in experiments 3 to 6. Ordinates represent averages for all termites weighed at the given time among groups on the respective diets.

Average Dry Weight per Termite.—Average dry weight per termite on rotten Douglas fir increased steadily throughout the experiments, showing a gain of 112.5 per cent at 112 days (table 7 and fig. 8).

On fungus-free, sound Douglas fir termites exhibited a surprising increase in average dry weight during the first three weeks, the same period during which the group weight declined so abruptly. This increase in average weight was probably not altogether due to actual growth of the termites but was occasioned partly by a change in the population of the groups resulting from the death of smaller individuals and the survival of the larger ones. The bodies of dead termites were almost constantly present in the containers, and after the first week an increasing tendency on the part of surviving members to eat these bodies rather than wood was observed. The number of termites eaten in proportion to the number which survived was high in groups on sound Douglas fir during the first few weeks, very much higher than on rotten Douglas fir (table 5), and this excessive cannibalism undoubtedly con-

TABLE 8

NITROGEN CONTENT OF TERMITES KEPT IN GROUPS AND EXAMINED TERMINALLY IN EXPERIMENTS 3 TO 6*

Experiment No.	Days	Group	Final average nitrogen per termite in mg		Per cent gain in average nitrogen per termite		Nitrogen in per cent of dry weight	
			In individual groups	Weighted average	In individual groups	Weighted average	In individual groups	Weighted average
Diet of fungus-containing, rotten Douglas fir								
3	28	a	0.38	0.38	+ 1.6	+ 3.2	9.2	9.2
		b	0.39		+ 4.6		9.2	
4	56	a	0.50	0.47	+ 35.7	+25.7	9.6	9.6
		b	0.43		+ 17.3		9.6	
5	84	a	0.52	0.52	+ 40.0	+40.0	10.2	9.9
		b	0.52		+ 40.0		9.5	
6	112	a	0.62	0.62	+ 66.5	+66.8	9.0	9.1
		b	0.62		+ 67.3		9.1	
Diet of fungus-free, sound Douglas fir								
3	28	a	0.51	0.55	+ 38.9	+ 48.4	11.1	12.2
		b	0.62		+ 67.6		12.7	
4	56	a	0.40	0.46	+ 7.6	+ 25.1	11.1	13.3
		b	0.55		+ 47.8		15.1	
5	84	a	0.76	0.77	+104.3	+108.9	17.0	17.7
		b	0.79		+114.3		18.4	
6	112	a	0.84	0.78	+128.1	+110.5	18.8	17.3
		b	0.75		+102.7		16.6	
Diet of sound Douglas fir with *Trichoderma*								
3	28	a	0.43	0.45	+ 17.0	+22.7	11.7	11.7
		b	0.48		+ 30.0		11.6	
4	56	a	0.48	0.47	+ 28.4	+26.8	13.1	12.3
		b	0.47		+ 25.7		11.8	
5	84	a	0.48	0.55	+ 28.6	+49.9	11.6	12.7
		b	0.67		+ 80.8		14.1	
6	112	a	0.56	0.62	+ 51.1	+68.4	12.8	14.0
		b	0.71		+ 88.4		15.3	
Diet of fungus-free filter paper								
3	28	a	0.43	0.41	+ 15.4	+ 10.5	10.4	10.5
		b	0.39		+ 5.4		10.6	
4	56	a	0.52	0.53	+ 41.4	+ 43.0	11.3	12.7
		b	0.53		+ 44.3		12.8	
5	84	a	0.65	0.67	+ 76.5	+ 82.2	13.4	14.0
		b	0.71		+ 91.1		15.0	
6	112	a	0.79	0.87	+112.7	+133.8	14.2	15.0
		b	0.91		+145.1		15.4	

* A sample taken at the beginning of the experiments showed the following: initial average milligrams of nitrogen per termite=0.37 mg±0.01 mg; initial nitrogen in per cent of dry weight=11.5±0.1. Calculations were made from original data and may differ slightly from those made on the basis of printed figures because of the dropping of insignificant decimal places in the printed table.

tributed to the greater increase in weight of survivors on sound Douglas fir. Later in the course of the experiments, however, the average weight per termite was decidedly lower on sound Douglas fir than on rotten Douglas fir. At 112 days average weight per termite showed a gain of only 40.6 per cent on sound Douglas fir as compared with the gain of 112.5 per cent on rotten Douglas fir.

Average weight per termite on sound Douglas fir with *Trichoderma* showed a gain of 39.4 per cent at 112 days. Although this gain was no greater than that on fungus-free, sound Douglas fir, it was not accomplished through excessive cannibalism nor accompanied by great loss in group weight as was the case on the latter diet. Hence the sound Douglas fir with *Trichoderma* would seem to be in itself a more nearly adequate diet than the fungus-free, sound Douglas fir.

The termites on fungus-free filter paper increased steadily in average weight. However, they continually supplemented the diet of filter paper through cannibalistic feeding. At 112 days the gain in average weight per termite on filter paper was 80.3 per cent as compared with 112.5 per cent on rotten Douglas fir.

Nitrogen Content.—The average milligrams of nitrogen per termite increased steadily in groups of termites on rotten Douglas fir (table 8). During the first 28 days the percentage of nitrogen in relation to dry weight decreased from 11.5 to 9.2 and thereafter remained between 9 and 10 per cent. The majority of the termites in groups on rotten Douglas fir had molted to the fifth instar at the end of 28 days, and the value obtained for percentage of nitrogen at that time is in close agreement with the 9.0 per cent recorded for fifth instar nymphs used in experiments 1 and 2.

Termites on sound Douglas fir with *Trichoderma* showed an increase in milligrams of nitrogen per termite similar to that of termites on rotten Douglas fir, although the percentage of nitrogen in relation to weight remained higher.

On fungus-free, sound Douglas fir and on fungus-free filter paper termites showed relatively high gains in average milligrams of nitrogen per termite and high values for percentage of nitrogen. This was probably a result of the large amount of cannibalistic feeding which took place in these groups. The total numbers of termites eaten in the groups of experiment 6 during the 112 days were not greater in groups on fungus-free, sound Douglas fir than in groups on rotten Douglas fir (table 5, col. 6). However, an early decrease in numbers of termites in groups on sound Douglas fir had occurred, succeeded by a death rate about the same as that on rotten Douglas fir. It follows, then, that the amount of

cannibalism per termite among survivors was considerably greater on sound Douglas fir than on rotten Douglas fir during nearly the entire time of the experiments. As is suggested by Cook and Scott (1933), who also obtained high values for percentage of total nitrogen in termites

TABLE 9

POPULATION OF GROUPS OF TERMITES AFTER 112 DAYS OF EXPERIMENT 6*

Group	Total number of termites present	Nymphs of the fifth instar	Nymphs of the sixth instar	Supplementary kings	Supplementary queens	Eggs
1	*2*	*3*	*4*	*5*	*6*	*7*
Diet of fungus-containing, rotten Douglas fir						
a	22	2	12	3	5	86
b	20	0	5	6	9	127
Diet of fungus-free, sound Douglas fir						
a	4	3	0	0	1	0
b	10	8	0	1	1	0
Diet of sound Douglas fir with *Trichoderma*						
a	25	13	9	1	2	2
b	19	12	7	0	0	0
Diet of fungus-free filter paper						
a	7	0	0	0	7	0
b	13	3	5	2	3	23

* Each group was composed of 50 nymphs of the fourth instar at the beginning of the experiment.

which had been feeding on diets high in protein, nitrogen contained in the lumen of the gut may have been in part responsible for these high values.

Development.—Molting to the fifth instar began to take place soon after the experiments were started. At 16 days the average percentage which had molted was 31.8 for those feeding on rotten Douglas fir as compared with 26.1 on sound Douglas fir with *Trichoderma,* 25.9 on fungus-free, sound Douglas fir, and 26.0 on fungus-free filter paper. At 112 days nearly all the nymphs of groups on rotten Douglas fir and

many of those on sound Douglas fir with *Trichoderma* had reached the sixth instar, while none of those on fungus-free, sound Douglas fir had attained that stage of development (table 9, cols. 3 and 4).

Reproduction.—Supplementary reproductives were first observed at 32 days in groups on rotten Douglas fir and on fungus-free filter paper. The proportion of reproductives to the total population was greatest in groups on filter paper, as many as 14 reproductives being present in a group of 39 individuals in one instance. Many of these died however before the experiments were completed. Reproductives developed in but one of the eight groups on fungus-free, sound Douglas fir, the first appearing at 56 days and the greatest number in any group being 3. In all of the groups on sound Douglas fir with *Trichoderma* reproductives were present at some time during the course of the experiments, although they were later in appearing and fewer in numbers than in groups on rotten Douglas fir or on filter paper.

Eggs were first observed at 88 days. They were in the groups of experiment 6, which was the only experiment continued that length of time. The greatest number of eggs were laid in the groups on rotten Douglas fir (table 9, col. 7).

SUMMARY AND CONCLUSIONS

Termites of the species *Zootermopsis angusticollis* were fed individually and in groups on various fungus-containing and fungus-free diets.

The presence of fungi in rotten Monterey pine, or the chemical changes brought about in wood by the fungi, or both factors enabled individually isolated termites to maintain higher viability and to make greater increase in average dry weight and nitrogen per termite on a diet of rotten Monterey pine than on a diet of fungus-free, sound Monterey pine.

The presence of fungi in rotten Douglas fir, or the chemical changes brought about in the wood by the fungi, or both factors enabled groups of termites to maintain higher group dry weight and better viability, to develop more rapidly, and to make greater gains in average dry weight and nitrogen per termite, accompanied by less cannibalism, on a diet of rotten Douglas fir than on that of fungus-free, sound Douglas fir.

The growth of fungi introduced by the termites on sound Monterey pine enabled termites which fed upon it to increase in average dry weight and nitrogen content, but not so rapidly as did termites on a diet of rotten Monterey pine.

The presence of a superficial growth of the fungus *Trichoderma lignorum* on sound Douglas fir led to less cannibalism and higher group dry

weight among termites on such a diet than on that of fungus-free, sound Douglas fir.

Rotten Douglas fir, however, proved to be a better diet for the termites than the Douglas fir which had only the superficial growth of *Trichoderma lignorum*. This was shown by the maintenance of higher group dry weight and by the greater gain in average dry weight per termite on the diet of rotten Douglas fir than on that of sound Douglas fir with *Trichoderma*. Other fungi present in the rotten Douglas fir may have had greater nutritive value or more favorable enzymatic action on the wood than the culture of *T. lignorum*. Furthermore, the chemical changes brought about in the wood through the actual production of rot were probably important in rendering it a suitable diet for *Zootermopsis angusticollis*.

The inadequacy of fungus-free filter paper as a diet for termites was demonstrated by the failure of individually isolated termites to make significant gain in dry weight, and by the high mortality, great loss in group weight, and low gain in average weight in groups of termites on a diet of filter paper as compared with those on their natural diet of rotten wood. These results are in agreement with the observation by Cook and Scott (1933) that termites were unable to live and grow normally on a diet of purified cellulose.

The results of these experiments indicate that fungi play an essential rôle in the natural diet of *Zootermopsis angusticollis*. The fungi offer a source of proteins. They probably supply vitamins which are essential to the normal growth and development of termites. Through the secretion of extracellular enzymes they may render the wood itself more available. It is not known what effect the fungi may have on harmful extractives in the wood. On the diet of fungus-free, sound wood mortality of termites was even higher during the early part of the experimental period than on the more deficient diet of filter paper, while on sound wood on which a growth of fungus had developed viability was good. Whether the termites survived because they were better nourished, or because the fungi had rendered some toxic substance in the wood harmless, or because of both factors is uncertain. It is certain, however, that the differential factor was the presence of the fungus.

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