OBSERVATIONS ON THE NATURAL CONTROL OF KANSAS TERMITES WITH SPECIAL REFERENCE TO A BACTERIAL DISEASE AFFECTING *RETICULITERMES TIBIALIS* BANKS

by

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INTRODUCTION

Personal observations made during the past five years have indicated that predators, parasites, and diseases of subterranean termites in Kansas have been responsible for hindering the biotic processes of these insects with an effectiveness worthy of investigation. In numerous instances, species of bacteria, fungi and molds appeared to be the causes of the death of entire colonies, both in the laboratory and in natural habitats.

The present work was begun in the fall of 1941 with a view of compiling some of the general observations on the antagonistic associates of the termites; of investigating the pathogenicity of organisms suspected as causative agents in disease; and to consider the possibility of controlling termite attacks by inoculating natural colonies with a microbic or fungoid pathogen.

The scope of this paper is limited due to the seasonal activity of most available colonies in nature and the fact that a comprehensive study of the many organisms to be considered would require several years of research.

Experimental data have been obtained chiefly from studies of a fungus, Entomophthora sp., and a strain of the bacterium, Serratia marcescens (Bizio), both of which have been found to be pathogenic to Reticulitermes tibialis Banks.

REVIEW OF LITERATURE

Literature on Reticulitermes tibialis Banks

To the termite species Reticulitermes tibialis Banks, Pickens (Kofoid, 1934) gave the common name, "barren-lands subterranean termite", which is generally accepted today. This species was described taxonomically by Nathan Banks (Banks and Snyder, 1920). Banks found that Schwarz (1896), Osten Sacken (1877), and Osborn (1898) had collected the species in Texas, Colorado, and Iowa, respectively, at these early dates.

Snyder (Kofoid, 1934) stated that "This termite of western United States is probably the most widely distributed species of Reticulitermes in the United States". The state records listed by Light (Kofoid, 1934) were Arizona, Arkansas, California, Colorado, Idaho, Illinois, Indiana, Iowa, Michigan, Montana, Nebraska, Nevada, New Mexico, Oklahoma, Texas, Utah (also Lower California). The species range map, however, includes four states which are omitted from the above list, namely, Kansas, Missouri, Oregon and Washington (Kofoid, 1934).

Very little specific work has been reported on the biology of Reticulitermes tibialis Banks. It seems that Light and Pickens (Kofoid, 1934) explained the reason for this as they spoke of the species of American Reticulitermes collectively, stating that "It should be borne in mind, however, that these species are all so closely related that economic findings in relation to one are in the main applicable to the others".

Thus, the literature has tended to consider R. tibialis primarily as an intermediate with characters more or less replicating those of the better known species. The chief biological distinction between this species and other species of Reticulitermes appears to be its ability to withstand more arid conditions. (See Pickens in Kofoid, 1934).

Literature on Biological Control of Termites

Snyder (1935) presented a review of the known enemies of termites throughout the world. He pointed out that "With the exception of the larvae of a conopid fly found parasitic in the heads of one species of termite (Macrotermes gilvus Hagen) in the Oriental region, no insect larvae have been found within the bodies of termites anywhere in the world". He further stated that "These fly larvae apparently do not kill the termites but only cause 'myiesis' or injury".

Since practically all of the successful efforts by workers in the field of biological control have been accomplished by the introduction of internal insect parasites, the elimination of these forms as a known factor in the natural scheme of the Order Isoptera has caused many workers to become skeptical of this type of control for termites. Writing of the protected existence of termites within their earthen runways and galleries in wood, Kofoid (1934) stated that "These habits of life render impracticable the methods used for the control of other insects, such as biological control by the use of enemies". Light (Kofoid, 1934) pointed out that due to their cryptobiotic habit "the possibility of spreading contagious disease, so important in population control elsewhere in nature, is reduced to a negligible minimum among termites".

Although many authors have reportedly observed species of mites, nematodes, fungi, bacteria and molds attacking and destroying termites, they have, with very few exceptions, failed to follow their observations with attempts at isolation and identification of the organisms involved. There are apparently no reports of specific studies of such forms to determine their status as agencies for controlling termites biologically. Merrill and Ford (1916) obtained 100 per cent mortality in 12 days with termites introduced into a moist soil culture of the nematode, Diplogaster aerivora Cobb. Van Zwaluenburg (1928), in reviewing literature on nematode parasitism of insects, pointed out that at least four species are known to be primary parasites of termites, but there is no definite evidence in nature that these parasites are of importance in controlling a termite species.

Nathan Banks (Banks and Snyder, 1920) reported that most of our native termites are infested externally with mites, but, "as usual with mites on insects, they are mostly immature". Snyder (1935) indicated that deaths to termites caused by mites in laboratory colonies are probably due to the larger numbers of mites which develop under such conditions and that in normal situations these organisms which usually occur in the young or migratory stage are not able to kill healthy termites.

Snyder (1935) has also discussed the various forms known to be predacious on termites. These include species of spiders, centipedes, beetles, bugs, flies, ants, wasps, crickets, birds, lizards, toads, wolves, ant-eaters, bears, moles, rats, skunks, apes and man. Termites themselves may be included in this list as they are cannibalistic to a limited extent.

As previously stated, some low forms of plant life are known to be among the enemies of termites. In these, apparently, lie the greatest possibilities for development of a more effective biological check on the increase of termite populations. Literature dealing with these forms is here considered under specific headings.

Literature on Fungus Diseases of Termites

Feytaud and Dieuzeide (1927) listed the following species of Termitaria known to be parasitic on termites: Termitaria coronata on species of Eutermes, Termitaria snyderi on Reticulitermes, and Termitaria thaxteri on Eutermes and Cornitermes. These fungi have been described as externally parasitizing their hosts. Feytaud and Dieuzeide, however, have described a species of Termitaria which proved to be primarily an internal parasite of Reticulitermes lucifugus Rossi in France. Aside from the difference in mode of attack on the host, the species closely resembled Termitaria snyderi which parasitized Reticulitermes in the United States.

Snyder (1935) discussing the relation of fungus diseases to termites, stated that in general they "affect only a small percentage of the entire colony; no epidemics result which kill large numbers of the insects". Sweetman (1936) listed two genera of fungi which have been known to attack termites, namely, Ectomyces and Termitaria. Snyder (1933) reported an observation of Termitaria sp. on the foreleg of the termite Nasutitermes (Nasutitermes) fletcheri Holmgren from the Anamalai Hills, India.

Practically every worker reporting biological studies of termites has noted species of fungi attacking and killing individuals and sometimes whole colonies under laboratory conditions. Williams (Kofoid, 1934) studying the moisture requirements of termites, measured the time required for termites to be killed by desiccation, starvation or fungus attacks, under different soil-moisture conditions. Light (Kofoid, 1934) spoke of the encroachments of fungi in colonies of dry-wood termites as one of the causes for increased numbers of supplementary reproductives within such colonies. In considering the role of supplementary reproductives among Reticulitermes Pickens (Kofoid, 1934) established a large cage with steam pipes for heating. Potential colonies were introduced into this artificial habitat. Pickens stated that "Within a few months fungus, growing luxuriantly because of the constant warmth, wiped out all of the insects so placed". Snyder (1935) stated that "Often a bottle of common termites, when carefully examined, will yield a rare guest of termites, a new fungus disease of termites or an abnormality or 'intercaste', that is, a form intermediate between two normal castes".

In spite of the many reports of termite mortality resulting from fungus attacks, workers in most cases have confined these reports to casual observations.

Literature on Bacterial Diseases of Termites

Snyder (1935) noted that molds and bacteria appear to be the only effective checks in nature on the increase of termites. Diseases caused by these low forms of plant life have destroyed entire colonies both in nature and in the laboratory. Evidently Snyder did not follow up any of these observations with attempts at isolation and study of the causative organisms.

In a review of the biological associations of bacteria and insects, Steinhaus (1940) has revealed some of the infinite possibilities which confront the entomologist interested in microbiology. Sweetman (1936) also studied the early history, symptoms, specificity and transmission of a large number of the known bacterial diseases of insects. No bacteria causing termite diseases were listed by Steinhaus or Sweetman.

Apparently, the first and only record to date of specific bacterial diseases of termites is the work of DeBach and McOmie (1939). They described the symptoms of two maladies affecting laboratory colonies of Zootermopsis angusticollis Hagen. One of these they ascribed to a strain of Serratia marcescens (Bizio) which caused the head and appendages of the termites to turn red soon after dying from the disease. A species of Bacterium caused the head to turn black. They conducted feeding experiments which showed a 50 per cent mortality due to Serratia marcescens (Bizio) and 22 per cent mortality due to Bacterium sp.

Their inoculation experiments showed 90 to 100 per cent mortality due to Serratia marcescens (Bizio) and 60 per cent mortality due to Bacterium sp.

THE POSITION OF TERMITES IN KANSAS

Distributional records for species of subterranean termites in Kansas are somewhat confusing. Kofoid (1934) in listing termites by states reported Reticulitermes claripennis Banks and Reticulitermes tibialis Banks from this state. However, in a species range map, Kofoid (1934) indicated that three species have been found in Kansas including the two above named and Reticulitermes flavipes Kollar in addition. Furthermore, in a systematic list of the termites of the United States, Kofoid (1934) again indicated that only R. claripennis Banks and R. tibialis Banks occur in the state.

McDonald (1941) stated that Kofoid (1934) also reported Reticulitermes virginicus Banks from Kansas. There is apparently no basis for this addition anywhere in Kofoid's text.

Of 142 different studies of "swarmers" which had emerged from structures showing serious damage by the subterranean termites in central and eastern Kansas during the past five years, 86 cases proved to be Reticulitermes claripennis Banks, and the remaining 56 were Reticulitermes tibialis Banks. Although two other species R. flavipes Kollar and R. virginicus Banks have been reported from Kansas, they have not been observed during this work.

That termites are of wide occurrence in Kansas is evident from the fact that there are only nine counties in the state from which they have not been reported (Fig. 1). The map, Fig. 1, showing the relative importance of subterranean termites by counties is based on a compilation of all available reports and observations on the subject, including: (1) the insect population summaries for Kansas, Smith (1932 and 1934b), and Smith and Kelly (1933, 1935 to 1941); (2) inspection records of two termite control companies; and (3) personal observations.

The map shows that in general termites are more important as a pest in eastern counties. Central counties appear to bear potential dangers. Regions penetrated by the larger streams of the state show a greater percentage of reported infestation. The southeastern border counties are definitely beridden by the pests and it is at this point that the state is closest to the reported range of Reticulitermes hageni Banks and Reticulitermes virginicus Banks which have become well established in southern Missouri and Arkansas.

GENERAL OBSERVATIONS ON NATURAL CONTROL

Predators

A small ant of the genus Cremastogaster has been observed killing subterranean termites. In numerous cases they had apparently eliminated the termites and had taken over their sheltered runways. This occurred most frequently in stumps and debris of open fields where young colonies of termites were, in

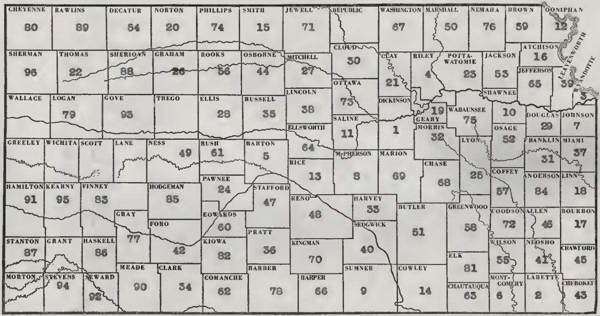

Fig. 1. The numbers indicate the relative importance of subterranean termites in the 96 counties of Kansas from which they have been reported.

some cases, completely destroyed by attacks of this vicious little insect. However, it was not uncommon to find colonies in porch steps and basement window sills from which the termites were retreating in the face of an attack by this ant.

Experiments made by Vaughn (1914) showed that termite killing ants were not common in this locality at that time. Personal observations of the ant-termite conflict and the prevalence of reports on the subject during the past five years have indicated that with the increase of termite numbers, ant enemies of the insect have become an important factor in the natural control of established colonies. Although some ants which fight termites display habits which make them objectionable to householders, the ants' expulsion of termites in some instances has undoubtedly saved considerable repair work.

Among the termite predators is the common black cricket (Gryllus assimilis). It was observed killing workers of small colonies which have been disturbed by the lifting of rocks under which the termites had established themselves. This cricket has also been observed eating swarmers. Vaughn (1914), however, performed some laboratory experiments which showed this Orthopteran to be of minor importance as a natural control.

An unusual observation on termites was made on October 20, 1941. A Mexican bedbug, Triatoma sanguisuga LeConte was observed killing termite workers. It pierced the termites with its stout beak and apparently withdrew their body fluid. A colleague, Albert W. Grundmann, has stated personally that in recent experiments with these bugs he was unsuccessful in getting them to live on various insects which were introduced into their cages. The termite, however, was not used experimentally as food.

A centipede of the genus Scolopendra was found raiding a small colony of Reticulitermes tibialis Banks which were working in the root of a shumac plant under a rock on a sandy hillside. When the rock was disturbed this predator was carrying a worker in its mandibles.

At Manhattan, Kansas, three birds have been seen eating emerging swarmers: The catbird, the robin, and the red-headed woodpecker. Dr. Roger C. Smith has communicated personally his observation of a fourth bird predator, the English sparrow, which has been seen repeatedly devouring the swarming termites on the wing.

Dissection of a common garden toad, Bufo woodhousii woodhousii Girard, which was collected in Barber County, Kansas, and a specimen of Crotaphytus collaris Say, the common collared lizard, collected in Riley County, Kansas, showed that swarming subterranean termites were acceptable in their diets. Both species are known to feed commonly on larger insects.

Parasites

An unidentified species of nematode has been observed attacking both Reticulitermes claripennis Banks and Reticulitermes tibialis Banks. Observations of the morphology and life history of this form indicate that it is probably the Diplogaster aerivora Cobb which is described in Merrill and Ford

(1916) as being parasitic on Leucotermes lucifugus. Since the generic name Leucotermes has been supplanted by Reticulitermes and the species R. lucifugus is not presently known to exist in Kansas, it is very likely that Merrill and Ford worked with either R. claripennis Banks or R. tibialis Banks. Diplogaster has been found to exist in varying degrees in all the colonies examined. Dissections of 100 specimens of workers from each of 10 colonies of termites have provided the data which are presented in Table 1.

All termites observed in this study were from colonies which had been collected within 24 hours of the time of examination. The termites were dissected under a binocular microscope in cell slides containing a few drops of water. Although the entire specimen was examined in each case, nematodes were found only in the head, usually close to the mouth. The worms observed were all in an immature stage.

Table 1 shows that only 4.5 per cent of the 1000 termites examined were parasitized by the nematode; that Reticulitermes claripennis Banks may be slightly more susceptible to attack than Reticulitermes tibialis Banks; and that this nematode probably occurs very sparsely in most healthy colonies of these species.

Table 1. Percentage of parasitism by nematodes in each of 10 random samples of 100 worker termites from 10 different colonies.

Termite species	Colony No.	No. of specimens examined	No. of specimens with nematodes in their heads	Per cent of parasitism
Reticulitermes tibialis Banks	1	100	4	4
	2	100	3	3
	3	100	6	6
	4	100	5	5
	5	100	2	2
Reticulitermes claripennis Banks	1	100	8	8
	2	100	6	6
	3	100	3	3
	4	100	4	4
	5	100	4	4
Totals		1000	45	4.5

It has been observed further that under laboratory conditions when termites were weakened by disease or extreme changes in environment that the worms, in some cases, have occurred in larger numbers in sick and dying termites and have consumed the bodies of dead termites. In such situations it would be easy for an observer to presume that the termites had been killed by the nematodes. These worms have been observed to complete their life cycle in water cultures and in plates of potato dextrose agar in less than a week, thus indicating that the existence of the immature nematodes in the heads of termites cannot be cases of obligate parasitism.

Observations and study of this worm lead to the conclusion that it was primarily a saprophyte which invaded the domain of subterranean termites to feed on the moist debris of the colony, and was only secondarily parasitic when taken into the mouth of the termite along with food; but attacks by the worm had apparently added to the discomforts of the insects under abnormal conditions such as unfavorable environmental changes and disease. It cannot, from present knowledge, be considered an important factor in natural control.

Two unidentified species of mites have been observed in association with termites. One of these is undoubtedly a member of the family Hoplodermatidae, so-placed because of the movable attachment of its cephalothorax to the abdomen, which characteristic enables members of this family to roll up, concealing the legs (Banks, 1915). It is a large reddish-brown mite and is usually found in its rolled-up position, "playing possum". In no instance has it been observed to attack the termites and its exact relation to the insects was not determined.

The other mite, apparently of the family Tyroglyphidae (Banks, 1915) has been observed to occur in large numbers in a few termite colonies in the laboratory, less frequently in natural habitations. The adults which apparently lived on debris and termite feces were evidently unwelcome guests, because workers and soldier termites fought the mites. On one occasion, an adult mite, while fighting with a worker, had each of its legs severed from its body by the mandibles of the termite.

The "hypopus" form of this mite became attached to the head and limbs of the termites. As many as 10 of these have been observed on one worker termite. This association was apparently a means of transportation for the mite. Numerous "swarmers" have been observed carrying two or three of the "hypopus" stage of this mite. No extensive study has been made of either species, but observations to date have indicated that they are probably of little importance in the natural control of termites.

Observations on Termite Diseases

The appearance of termite disease in laboratory colonies has not been an unusual occurrence. Due to this known susceptibility of termites to maladies apparently caused by bacterial or fungus attacks, the natural habitats of colonies have been watched closely for some indication that such diseases might occur therein.

During the years 1936 to 1941 inclusive, over 600 structures have been inspected with the object of determining the extent of possible termite damage in each and of making comprehensive reports on these inspections to the respective owners. This was accomplished in part by answering some of the requests for such attention which have been sent to the Department of Entomology, Kansas State College, Manhattan, Kansas. Most of the work, however, was done as an employee of termite control operators in Wichita, Kansas, and Tulsa, Oklahoma.

These investigations, which have involved practically every county in eastern Kansas and northeastern Oklahoma, showed numerous cases of severe damage to buildings by subterranean termites, which insects, however, had deserted the scene of their operations. In some instances their runways were being utilized by a small species of ant, but more often the termite abode was free of any apparent cause for the retreat of its original inhabitants. Environmental conditions at the scenes of such observations were not in any obvious way responsible for the absence of the termites. Ten exemplifications of this situation at Wichita, Kansas, in which the damage was not sufficient to warrant immediate repair, were examined at three month intervals over a period of one year. Termites had not returned to the scene of their depredations in any case observed.

Although disease was the suspected cause for these situations, the lack of facilities for conducting experiments at the time of these observations impeded any investigation.

EXPERIMENTS WITH ENTOMOGENOUS FUNGI

A study of the effect of some known entomogenous fungi on termites was begun in February, 1936, and continued into the spring of 1937. Due to extremely dry, hot weather throughout the spring, summer and fall of 1936 plus a severe winter in that year and a late spring in 1937, sizable collections of termites were not available in sufficient numbers to carry on extensive experimentation.

Extreme weather conditions have hindered termite investigation in this locality in several ways. During such periods, these insects, which, under normal conditions, worked close to the earth's surface in practically every convenient habitat, concentrated their efforts on "man-made" structures in which the prerequisites of temperature and moisture are so well maintained. However, in spite of their concentration in such places, the unfavorable weather limited swarming to a negligible minimum, and thereby reduced the chances for contacting a source of supply. When termites were found in a building they were usually inaccessible for laboratory use due to the mortality sustained during removal of the infested timber.

A large number of the termites collected were killed by extreme changes in temperatures in the laboratory. This was especially true in the spring, fall, and winter months when the room was overheated during the day and chilled at night. This condition has been eliminated in recent studies by the use of a constant temperature and humidity apparatus similar to that described by Brindley and Richardson (1931).

The weather conditions during 1936 and 1937 not only limited collection of termites, but it hampered the consideration of the lower plant forms and their position in the natural make up of the colonies. Practically all of the termites collected during this period were of the species Reticulitermes tibialis Banks, which was apparently able to withstand the drouth conditions better than other species which occur in this locality. This species was used exclusively in these first studies.

Material and Methods

The general procedure in obtaining colonies of termites was to wrap termite infested material in moist paper and take it to the laboratory. By spreading a large sheet of paper on a table and gently tapping the termite infested wood, the insects fell onto the paper in considerable numbers with only slight injury to a few of the specimens. By lifting the paper and bending it so the termites were concentrated in its center the insects were easily transferred to a shallow cup.

In order to remove the debris which fell from the wood with the termites, a Petri dish cover was placed over the cup, its top side next to the cup lip. The whole was inverted and placed in an enameled pan and the cup removed. The termites soon slipped from the glass into the pan, leaving all dirt except the few dust particles which were clinging to their bodies. Then the Petri dish cover was lifted from the pan and the termites were gently poured into Petri dishes which had moist filter paper in the bottom and were left for two days so that all injured specimens could be removed before using the group in experiments.

In 1935, Dr. C. L. Lefebvre of the Botany Department had succeeded in isolating from the body of an unidentified termite a culture of fungus belonging to the genus Empusa. He maintained this culture on agar slants in a refrigerator and the cultivation of this material provided one of the organisms used in the series of experiments on termite susceptibility to entomogenous fungi.

Later Dr. Lefebvre also provided the pure cultures of the corn borer fungus, *Beauveria bassiana* (Bals.) Vuill, and the chinch bug fungus, *Beauveria* (*Sporotrichum*) *globulifera* (Speg.) Picard, which were used in these studies.

The genus *Empusa*, according to Beesey (1935), should now be called *Entomophthora* since *Empusa* has been used previously to designate a genus of orchids. This genus is classified in the higher Phycomycetes belonging to the sub-class Zygomycetes, order Entomophthorales. It is a close relative of the molds and often found associated with them in nature. There are about 40 species, all parasitic within insects (Fitzpatrick, 1930).

The genus *Beauveria* which contains the two other fungi used in these experiments belongs to the order Hyphomycetes, the family Mucedinaceae. It is a member of the fungi Imperfecti. The forms are primarily saprophytic.

Species of *Entomophthora* and the species of *Beauveria* considered here have frequently been observed to infect their respective hosts by penetrating the integument of the insects with a growth from the conidia, which have adhered to the insects' body (Lefebvre, 1934; Smith, 1934a; Sawyer, 1929).

Several groups of experimental data have been obtained through various experiments with spore contact and feeding. The procedure in each case has been briefly stated in association with the data presented.

Various kinds of apparatus were used in these attempts to infect termites with the fungi. Small numbers of termites were usually placed in Petri dishes with moist filter paper as food, while larger colonies were kept in wide mouthed bottles prepared with corrugated paper and balsa wood as food. These larger containers were stoppered with corks through which had been placed glass tubes of standard test tube size. This tube was plugged with cotton. When tests were to be made, the Petri dishes were sterilized for one hour in a hot-air oven at 160°C. and the bottles were sterilized in an autoclave for 30 minutes at 15 pounds pressure before colonies of termites were introduced. Shell vials were used in studying the reactions of single termite specimens and damp cells were prepared on microscopic slides for investigating the possibility of spore development in the body fluid of the insects.

The fungi, in each case, were cultivated on potato dextrose agar. Cultures of each organism were maintained on agar in test tubes, Petri dishes, and in 250 cc Erlenmeyer flasks.

Suspensions of spores were made by introducing a small quantity of sterile distilled water into a flask culture which was releasing spores in abundance. By shaking the flask gently, spores were mixed with the sterile water. Such suspensions were always made fresh and immediately transferred into the test jars.

Agar slant cultures were the most convenient sources for securing sterile transfers of the spores which were used in dusting. The plate cultures were used for contact inoculations of the termites by introducing specimens directly onto the sporulating colonies of the fungi.

Presentation and Discussion of Data

In an attempt to infect termites by contact, the following procedure was used in exposing the insects to the conidia of the different fungi: 100 worker termites were introduced into an agar plate in which some colonies of the fungus were producing spores. The termites which were excited by tapping the dish top ran about among the conidiophores and spores. The insects were left in the dish for 10 minutes; then were removed from this dish and placed in another which contained moist filter paper as food.

One hundred other workers were placed in a sterile plate of agar media for 10 minutes then removed to a sterile Petri dish with moist filter paper as food. These were used as controls.

The cumulative data from these tests which are presented in Table 2, show that by contact, termites of the species Reticulitermes tibialis Banks were susceptible to infection by Entomophthora sp. which caused a mortality of 76 per cent in one week. Eight per cent of the specimens were covered by an extensive growth of white conidiophores. These insects died before the growth appeared. Dead specimens on which conidiophores had not developed were sterilized externally by dipping them in 70 per cent alcohol and washing through several changes of sterile water. This removed any spores that might have been adhering externally to the body wall. Normal insects, dusted heavily with conidia, then sterilized in this manner, soon recovered and in no case did they succumb to an attack of the fungus, nor did individuals, so dusted, then sterilized, cause development of the fungus on media when plated.

Some of the dead specimens, which had been sterilized externally, were dissected and a microscopic study of the body fluid revealed many spores which were apparently the zygospores or azygospores of Entomophthora sp., others of these specimens were plated and many colonies of Entomophthora developed on the media.

Table 2 also shows that Beauveria caused only three per cent mortality in the case of B. bassiana and five per cent for B. (Sporotrichum) globulifera. No external growth appeared on any of the dead specimens. Examination by dissection disclosed no visible sign of fungus spores or mycelia within their bodies. However, the insects had apparently carried spores into the dish when they were transferred from the agar culture, as several saprophytic growths of the fungus occurred on the moist filter paper.

Table 2. Per cent mortality of Reticulitermes tibialis Banks in one week from contact exposure with three different entomogenous fungi, and the number of dead on which conidiophores had developed.

Fungus	No. of termites	No. dead in 1 week	No. with conidiophores	Per cent mortality
Entomophthora sp.	100	76	8	76
Beauveria bassiana	100	3	0	3
Beauveria (Sporotrichum) globulifera	100	5	0	5
Control	100	0	0	0

Table 2 further shows that no deaths occurred in the control dish. Thus, although the mortality was extremely low in the case of the two species of Beauveria, it could not be concluded that they died from causes other than some undetermined harmful effect of the fungi.

Feeding tests, so-called because in these experiments the termites were fed filter paper which had been saturated with heavy suspensions of fungus spores, were actually a combination of feeding and contact, since the uncontrollable saprophytic habits of the fungi being studied caused the development of active conidiophores on the filter paper. Five groups of 50 termites each were used in the case of each fungus being tested, and five more groups were established as controls. A few drops of moisture were added to each dish every day for the first week, new spores being introduced with the water in each of the tests. No moisture was added during the second week of observation. In Table 3 is presented the cumulative mortality figures resulting from these tests at the end of one week and two weeks, respectively. These data showed even more conclusively that Entomophthora sp. was pathogenic to the termites. Although the mortality rate was slower during one week than had occurred in the contact tests, a second week of observation showed a mortality of 100 per cent for the two week period.

Table 3. Per cent mortality of Reticulitermes tibialis Banks resulting from confinement with filter paper saturated with heavy suspensions of fungus spores.

Fungus	No. of termites	Cumulative number dead		Cumulative per cent mortality	
		1 week	2 weeks	1 week	2 weeks
Entomophthora sp.	250	72	250	28.8	100
Beauveria bassiana	250	18	54	7.2	21.6
Beauveria (Sporotrichum) globulifera	250	7	22	2.8	8.8
Control	250	2	12	.4	4.8

This increase in death rate during the second week was apparently due to the development of the natural sporulating bodies of the fungus on some of the specimens which had died during the first week and the contact of the termites with the conidia produced by these. In order to determine whether or not this observation was correct, five groups of 10 healthy termites each, were confined separately on moist filter paper in shell vials with one of the dead specimens on which conidiophores were developing. Five other groups of 10 individuals were dusted with spores from media colonies and confined in similar manner. In one week, all 50 termites which had been confined with the dead specimens were also dead. Of the 50 termites which had been dusted, only 16 were dead. This showed that contact with conidia exploded directly from conidiophore growing on the dead hosts were undoubtedly more effective in the infection of healthy individuals than the spores mechanically transferred from media colonies.

Termites, on becoming infected with Entomophthora sp., became sluggish and weak. A gradual discoloration and swelling of their bodies preceded death. Individuals dead from the disease were usually light brown in color and swollen perceptibly. The internal tissues were broken down and penetrated with mycelia. Only a small per cent of the dead termites were observed to develop conidiophores, but the ones doing so were completely covered by a white furry mass over all the soft parts of the body. A soldier (Plate I, Fig. 1) infected with Entomophthora sp., and on which conidiophores had developed, is typical of this condition. No living specimen has been observed with such growth in evidence.

EXPLANATION OF PLATE I

Fig. 1. A soldier termite which was killed by a fungus disease (Entomophthora sp.) showing the white conidiophores which developed on the soft parts of the body after death.

Fig. 2. Dead and dying termites infected with an unidentified fungus disease.

PLATE I

Fig. 1

Fig. 2

The results of the feeding tests are presented in Table 3, which shows that the species of Beauverie must heve been responsible for some mortality, but the neture of the fatal fungue etteck is unknown. Dead specimens sterilized externally were studied by microdissection but internal structures eppeared normal, no mycelia or spores being found. Other specimens, so sterilized, were plated, but no fungus cultures developed. In an ettempt to explain this situation, the intestinal contents of some healthy termites were removed, pleced on cover slips, and dusted with spores of Beauveria. These cultures were then inverted over e ring cell in which e few drops of water had been placed.

Although severel such cultures were prepared with each species of Beauverie end observed daily for one week, no fungus growth developed in eny case. Examination of smears from these cultures, efter one week, showed that no spores were present. What had happened to the spore inoculation remains unexplained. The possibility of spore digestion by bacteria end protozoa, which were present in the intestinal fluid, was not investigated. Although growths of Beauverie have been observed on e few termites severel days efter death, it appeared thet these exceptional cases were probably saprophytic in nature.

To further investigete termite susceptibility to the entomogenous fungi, 20 colonies of termites consisting of 1500 individuals each were established in jars which had been prepared with balse wood and corrugeted paper as food and sterilized by autoclave, before introduction of the insects. Five each of these colonies were moistened each day for two weeks with heavy spore suspensions of the three fungi being tested, and the five remaining were moistened with sterile distilled water. Due to the impracticability of making a daily count of specimens in colonies thus established, numerical data were not obtained. However, the general observations of mortality over a two week period substantially upheld the data which had been obtained from the previous tests.

Lack of favorable weather conditions and available colonies in nature during the spring of 1937 prevented a study of Entomophthora sp. under field conditions. Studies were discontinued at that time, and the culture was lost before studies were again started in the fall of 1941.

The dead and dying members of a colony of subterranean termites which was collected on March 15, 1942, at Manhattan, Kansas, is illustrated by Plate I, Fig. 2. This photograph was made one week after collection of the colony. Although a few termites were still alive when this photograph was taken, no living specimens could be found 24 hours later.

An isolation from some of the specimens illustrated in Plate I, Fig. 2, appears to be very similar in its development on agar to the Entomophthora sp. which was studied in 1936. This material has not been identified, however, and time has not been available to carry on the tedious process of cultivating enough of the material on agar to warrant establishment of experimental tests.

ISOLATION OF A PATHOGENIC BACTERIUM

Material and Methods

During the past year, a pathogenic strain of the bacterium, Serratia marcescens (Bizio), has been isolated from specimens of the subterranean termite, Reticulitermes tibialis Banks. The type species of this bacterium is known as a common saprophytic form existing rather generally in water, soil, and food.

On August 28, 1941, an unusually large colony of Reticulitermes tibialis Banks was taken from an oak stump on a creek bank about four miles southeast of Garnett, Anderson County, Kansas. Workers, soldiers, brachypterous nymphs, and various other nymphal stages were collected and this wide range of caste development suggested a study of its progress.

The termites were carefully sorted into 10 groups of 1500 each, approximating a ratio of caste distribution in each group equal to that of the natural colony. Portions of the oak stump from which the insects were taken were placed in 10 glass bottles 1 3/4" in diameter and 4 1/2" tall, which were fitted with screw-top metal lids. The wood was moist and required no additional water as the respective groupa of sorted termites were introduced into the bottles. The unsorted insects remaining after the above manipulation appeared to be about equal in number to the 10 prepared colonies. These were placed in a bottle of the same size which had been previously prepared for a laboratory colony with corrugated paper and balsa wood for food. Sufficient water was added to simulate the moisture condition of the other colonies.

Previous studies had shown that it is well to allow a day or two for artificial colonies to absorb the shock of removal from their natural habitat and to observe the mortality resulting from injuries sustained in the transfer.

Twenty-four hours after preparation the one colony, with paper and balsa wood as food, had apparently recovered and were eating the few members which had died. The other 10 colonies were very restless, failing to show the usual composure and purpose which ordinarily dominates the normal society. The dead and dying were completely ignored by healthy individuals. The total number dead in any group, however, did not exceed the expected two or three per cent.

After four days, numerous individuals in each of the 10 groups appeared sluggish, and others were still behaving abnormally. After six days, a greater number of the termites appeared to be suffering from lack of appetite and general sluggishness and the number of dead had increased considerably. Twenty-five of the ailing insects were removed from each bottle and examined externally. A few carried a migratory stage of mites. Dissection of these termites disclosed that a very small percentage were parasitized in the head by immature nematodes. Occurrence of mites or nematodes, however, were too infrequent to explain the general disintegration of the colonies.

Twelve days after collection, approximately half of each of the 10 colonies had died and the dead were strewn about in slimy clusters, some of which were white with a pinkish caste and others reddish brown in color. Thus it appeared reasonable that the mortality must have been due to the effect of some microorganism and that the mites and nematodes were not important.

All 10 of these colonies which had been confined with food from their natural habitat were completely destroyed by October 15, 1941, but the one colony which was fed corrugated paper and balsa wood was still alive after eight months. From these studies it appeared that the cause of the death of this colony of termites may have been present in the food of the termites in their natural habitats.

Further observations, at later dates, have strengthened this hypothesis. Termites of this species collected in Riley County on October 10, 1941, and October 24, 1941, apparently contained individuals which were sick at the time of collection. In each of these colonies, red pigmented termites appeared in the groups in less than 36 hours after removal to the laboratory.

Lack of sufficient moisture might have been a factor as each of these collections was taken from unusually moist environment. Collection methods were similar to those previously described in connection with fungus studies.

Several isolated specimens of termites, red-pigmented in death, were selected from each of the stricken colonies. These were macerated in Petri dishes with distilled water, and a series of dilution plates, using potato dextrose agar, were inoculated from each colony. From six series, 16 pure cultures were started from colonies which varied in appearance on the plates. All of these appeared to be different in the tubes.

In order to determine which of the organisms was causing the death of the termites, a series of feeding experiments was used. Termites were fed white blotting paper which had been saturated with heavy suspensions of the cultures being tested.

The characteristic symptoms of the disease were observed within a few hours. In one of the plates the paper had been saturated with a suspension of red-pigment-forming bacteria. In this plate the termites were extremely agitated. In 24 hours some of them slowed in their movement, became sluggish, and apparently lost their appetite. In 48 hours a few were dead and some others were lying on their backs, feebly working their legs and antennae. In another 24 hours the head and limbs of the dead termites had turned bright red. On the fourth day the specimens which had been first to die were but a mass of red slime. Pure cultures of the red-pigment-forming organisms were isolated from the dead termites.

The mortality occurring in any of the plates where the other organisms had been tested was not large enough to be considered important.

In order to determine the actual death rate of termites from the effects of the disease, several feeding and inoculation tests were made. Sterile Petri dishes were used in each case, with white blotting paper cut to the size of the dish and placed on the bottom as food. Groups of 25 worker termites, which had been immersed in a weak solution of iodine for two minutes, then washed through several changes of sterile distilled water in order to eliminate some of the contamination which might cling to their bodies, were introduced into each dish. Both test and control specimens were so treated. Healthy termites showed no ill effects from such treatment. Termites which had been dipped into heavy suspensions of the bacteria then treated by the iodine wash, were plated and no growth of the bacteria developed, indicating that the iodine bath had a complete sterilizing effect.

In the feeding tests the blotting paper was saturated with a thick suspension of the red pigment by sterile transfer, and all moisture added to the tests throughout the experiment was similarly prepared. Equivalent moisture was added to the controls but only sterile, distilled water was used.

Inoculation experiments by punctures were conducted using a number 00 insect pin which had been flamed and dipped into a butyrous agar growth of the red pigment. Control specimens were pricked with a sterile pin of the same size. The controls apparently suffered no ill effects from this operation. Head inoculations were made by puncturing the head capsule at the vertex while the abdominal inoculations were introduced medio-ventrally in the anterior segments.

Unless otherwise stated the tests reported were conducted under high humidity condidions and at a temperature of about 75^{0}F. They were kept in a constant temperature and humidity apparatus similar to that described by Brindley and Richardson (1931). Normal laboratory colonies of termites flourished under these conditions.

Presentation and Discussion of Data

Several feeding and inoculation tests were observed over a two weeks period. Table 4 shows the cumulative number of dead and the per cent mortality from the various test and control dishes after two days, four days, one week, and two weeks, respectively.

Of the 250 termites used in the feeding tests, 115 were dead in two weeks, showing a mortality of 46 per cent in that period. Only one of the 75 termites used as controls had died, its death having occurred as a result of cannibalistic attack by other termites. A few of the termites in the test dishes were eaten after they had died.

In one week, 100 per cent mortality resulted from both types on inoculation tests, 125 termites being used in each series. Controls with 25 termites each showed one death in the case of head inoculation and two in the abdominal inoculation check in the same period of time. These control specimens apparently died after being attacked by other termites in their groups.

Table 4. The death rate of the termite, Reticulitermes tibialis Banks, due to a pathogenic strain of Serratia marcescens (Bizio).

Type of experiment	Termites used	Cumulative number dead				Cumulative per cent mortality			
		2 days	4 days	1 week	2 weeks	2 days	4 days	1 week	2 weeks
Feeding	250	0	20	84	115	0	8	34	46
Control feeding	75	0	0	0	1	0	0	0	1.3
Head inoculation	125	44	118	125		35	94	100	
Control head inoculation	25	0	0	1	2	0	0	4	8
Abdominal inoculation	125	40	90	125		32	72	100	
Control abdominal inoculation	25	0	0	2	4	0	0	8	16*

*Deaths in controls were due to causes other than the pathogen studied, cannibalistic attacks by other members of the colony being the chief cause observed.

The per cent mortality has not varied considerably from that reported by DeBach and McOmie (1939) in a study of a strain of Serratia marcescens (Bizio) which they found pathogenic to Zootermopsis angusticollis Hagen. They reported 50 per cent mortality in two weeks from feeding. Their tests showed 90 per cent mortality in three days when the termites were inoculated abdominally and 100 per cent mortality in three days by head inoculation.

General observations on the development of this disease among specimens of Reticulitermes tibialis Banks have been as follows: (1) The termites died approximately 24 hours before the characteristic red coloring of the head and limbs began to appear. (2) The diseased termites attempted to seclude themselves from the rest of the group, which was practically impossible, due to the excited running about by the healthy termites. (3) Termites recently dead from the disease were, in some instances, eaten by other members of the colony. When the dead became numerous and individuals started to turn red, the general tendency of the normal insects was to start building little barriers of chewed paper around the disintegrating specimens and to seal the top of the barrier, forming a mound on the paper. (4) The disease apparently killed more termites when they were confined under very moist conditions, and with a decrease in the moisture supply the development of the disease was delayed noticeably. (5) In every feeding test the termite mortality rate approximated the same gradient from the time the first illness appeared until the maximum mortality for the period observed had been reached.

Sick termites, apparently in the last stages of the disease, were bathed in a weak solution of iodine in order to eliminate any bacteria that might have been adhering to the body surface. These termites were plated, and in each case the only growth developing on these plates was the characteristic colonies of Serratia marcescens (Bizio). This showed that the bacteria was undoubtedly the primary cause for the illness and death of the termites, and that it was not occurring secondarily as a saprophyte on insects dying from the pathogenic effect of some other organism.

That the termites were in some way affected by the presence of the bacteria was indicated by the abnormal activity of a group when first introduced into a dish containing the suspension saturated paper. The pigmenting bacteria produce a putrid odor, but since termites in nature are commonly associated with decaying organic matter, it does not seem likely that they could be reacting to this odor. Their undeveloped eyes probably could not distinguish the red coloring.

Further evidence of this reaction against termites affected with this disease was obtained by introducing groups of termites into shell vials containing blotting paper saturated with bacterial suspension. An escape for the termites was then provided through a hole in a cork plug which had been fitted into another vial containing a piece of blotting paper saturated with plain water. The apparatus then consisted of the two vials fitted snugly together with part of the drilled cork in each. In all cases the termites immediately moved into the cork and in a few hours had sealed the end which had opened into the vial with the suspension soaked paper and had connected the water soaked paper to the cork.

Other tests have shown that when soil saturated by bacterial suspension was placed between termites and the only available food supply, the insects penetrated the soil to get to the wood, but many of the individuals of the colony died from the disease soon afterward.

Williams (Kofoid, 1934, p. 42-49) showed in experiments with a humidity gradient apparatus that Reticulitermes tibialis Banks preferred a saturated atmosphere and was, as he stated, "obviously, accustomed to living in an atmosphere having a relative humidity of 100 per cent". Williams also stated that the species thrived in sand or sandy loam saturated with water.

Since observations have shown that a saturated atmosphere was apparently preferred by Serratia marcescens (Bizio), an investigation of the temperature range of both the termite and the bacteria under 100 per cent humidity conditions was undertaken. Colonies of 25 termites, each on filter paper in Petri dishes and agar plates freshly inoculated with Serratia marcescens (Bizio) were introduced into temperature controlled ovens with the water supply maintained to the point where droplets of moisture clung to the dish tops at all times. The observations accumulated from this procedure are tabulated in Table 5, which shows that the temperature ranges of both the termite and the bacteria under these conditions are approximately paralleled between 20°C. and 28°C. and the optimum of each probably occurs between 24°C. and 28°C.

From April 10, 1942, to May 5, 1942, preliminary observations have been recorded on the reaction of natural colonies to inoculation with the pathogenic strain of Serratia marcescens (Bizio).

Three colonies have been inoculated with a suspension of Serratia. The largest colony, located in the greenhouse insectary had been observed attacking 2" by 12" planks laid flat on the soil in soil filled tables. Moisture was abundant as plants situated just above the boards were watered every other day. Inspection of these planks on April 10, 1942, showed that they were each filled with large numbers of termites. The boards were then carefully replaced. On April 15, 1942, only one of the planks was lifted. It appeared the same as it had five days previously. In an attempt to infect the colony, 20 cc of a heavy bacterial suspension was sprinkled over the termites. The next day only a few termites were observed and they were concentrated at one end of the plank away from the center which had been sprinkled. Daily inspections were made each day for two weeks, moving only the plank under which the inoculation had been placed. After the second day, no termites were observed under this plank. At the end of the two week period the bacterium

Table 5. The reactions of Reticulitermes tibialis Banks and the development of Serratia marcescens (Bizio) at different temperatures in saturated atmosphere.

Temperature of saturated atmosphere	Reticulitermes tibialis Banks on filter paper	Serratia marcescens (Bizio) on agar
52°C.	Died in 24 hours	Died in 24 hours
48°C.	Died in 24 hours	No growth
44°C.	Died in 24 hours	No growth
40°C.	Died in 24 hours	No growth
36°C.	Died in 24 hours	Spreading filmy growth in 24 hours slight pinkish tinge
32°C.	Died in 72 hours	Slow normal development
28°C.	Abnormally active but lived throughout 2 weeks observation	Apparently closest to optimun
24°C.	Apparently closest to optimum	Rapid normal development
20°C.	About normal activity	Good growth
16°C.	Activity considerably below normal	Poor growth
12°C.	Sluggish	Poor growth
8°C.	Died in 48 hours	Poor growth
2°C.	Died in 24 hours	No growth

was isolated from the soil under this plank. Each of the other four planks was examined at this time, two of which still harbored termites but they made up a very small proportion of the numbers which had been observed on April 10. From beneath another board about two feet from the area of inoculation, several dead red pigmented termites were collected which were sterilized externally and plated; they soon produced the characteristic colony of Serratia marcescens (Bizio) on agar. One week later a few termites remained under only one plank. On May 5, under this same plank, one small group of termites were still active, these being the only termites observed beneath the planks on the entire bed.

Another colony, located in a stump in a yard at Manhattan, Kansas, was inoculated April 18, 1942. Swarmers had emerged from this stump on March 10, and careful inspection of a runway which occurred on the stump showed that many active workers were present just previous to the inoculation. Into a tiny break in this tube, 5 cc of bacterial suspension were introduced with a hypodermic syringe. This stump, which was not very solid in the ground, was pulled out May 1, and broken up, but termites were not found in any part of the wood or in the soil immediately surrounding it.

Similar hypodermic injection into an infected 4" by 4" stake in a yard about three blocks from where the stump had been treated showed the same results. Temperature was apparently not a factor in any of the cases as active termites were observed in similar habitats on the same day.

These few preliminary observations indicated that inoculations by the bacteria had caused the retreat of active colonies of termites and the death of individuals which were found as much as three feet from an inoculation area. They showed also that the bacteria persisted in the soil for at least two weeks.

The results of these tests offer little more than an indication that further investigation should be made. The main conclusion that can be drawn from these recent observations on the application of a suspension of Serratia marcescens (Bizio) into natural termite colonies is that they indicate the possibilities of biological control of termites by the application of pathogenic microorganisms.

Study of Organism

The pathogenic strain of the red-pigment forming bacterium, Serratia marcescens (Bizio) which is described in this work as effecting the termite Reticulitermes tibialis Banks is apparently the second strain of this bacterium to be described from termites and is the causative agent of what is evidently the first bacterial disease to be described from Reticulitermes.

A complete description of the type species and a discussion of the intricate synonomy which accompanies the history of the organism has been presented by Bergey (1939). DeBach and McOmie (1939) described a strain which they found to be pathogenic to Zootermopsis angusticollis Hagen. The species was described by Topley and Wilson (1938) under the name Chromobacterium prodigiosum, which has apparently been used more than any other synonym in the designation of this species of Serratia.

The pathogen considered in these studies may be described morphologically as a Gram negative rod, less than one micron in length, usually occurring singly but chains of four have been observed. Its flagella have peritrichous arrangement. It does not form spores.

These small, aerobic rods were identified as belonging in the genus Serratia (Bizio) Bergey (1939) on the basis of rapid liquefaction, red pigment production, milk coagulation, and the presence of peritrichate flagella.

Further tests showed that the red pigment was not water-soluble, that gas production from sugars was slight, and that no pellicle was formed on plain gelatin. With this information, according to the system of Bergey (1939), the organism was determined to be Serratia marcescens (Bizio).

Continuing with Bergey's system of classification it was found that this strain of the species varied from the type in its pathogenicity, in its temperature relations, and in some minor cultural differences. Further study showed that it varied from the pathogenic strain described by DeBach and McOmie (1939) only in its temperature relations.

The species determination has been checked by Dr. L. D. Bushnell, head of the Bacteriology Department at Kansas State College. He has stated that the organism should undoubtedly be classified as a strain of Serratia marcescens (Bizio), pathogenic for termites.

In Tables 6, 7, and 8, the data from extensive studies on the relation of temperature to the growth and pigment formation of agar colonies of the bacterium have been presented.

These data show (1) that the bacterial organism grew within the temperature range of 8°C. and 36°C. inclusive; (2) that the maximum temperature for growth was between 36°C. and 40°C.; (3) that the minimum temperature at which growth would occur was between 2°C. and 8°C.; and (4) that the optimum temperature was approximately 28°C. There was a gradient in the pigment production by the organism in either direction from the optimum temperature. This retention in pigment formation was especially evident near the minimum and maximum temperatures for the growth of the organism.

Table 6. Growth in 24 hours, at various temperatures, on potato dextrose agar, of a pathogenic strain of Serratia marcescens (Bizio), isolated from Reticulitermes tibialis Banks.

Temperatures	Diameter in mm	Chromogenesis and morphology of colony
40°C.	0	No growth
36°C.*	16	Effuse, lobate, white growth with slight pinkish tinge throughout
32°C.	7	Pulvinate, bright pink growth with very narrow, white, erose margin
28°C.	14	Pulvinate, radiating from deep red in center through scarlet to white, erose margin
24°C.	13	Pulvinate, bright pink growth with white, erose margin
20°C.	10	Erose, butyrous, white growth with bright pink tinge radiating from center, causing mycolioid appearance
16°C.	7	Pale pink bleb, 2 mm in diameter, surrounded by snowy white, higher at outer margin of white
12°C.	7	Tiny, pale pink spot in center of effuse, slightly iridescent growth
8°C.	2	Bleb, mottled pink and white
0°C.	0	No growth

*At 36°C. the first day's growth was a spreading film-like growth which causes the measurement to be larger than the growth at 32°C.

Table 7. Growth in one week, at various temperatures, on potato dextrose agar, of a pathogenic strain of Serratia marcescens (Bizio), isolated from Reticulitermes tibialis Banks.

Temperatures	Diameter in mm	Chromogenesis and morphology of colony
40°C.	0	No growth
36°C.	29	Pale purple crateriform center, concentrically ringed with deep purplish red to purplish pink to a narrow, erose margin of rose pink
32°C.*	27	Pulvinate butyrous mass of deep red pigment bordered by radiating rugose ring of bright red, margin light red and slightly erose
28°C.	38	Pulvinate, butyrous mass of deep red in center, margin light red and slightly erose
24°C.	31	Pulvinate, butyrous mass of deep red bordered by radiating rugose ring of bright red, margin light red and slightly erose
20°C.	24	Dark red, pulvinate, butyrous mass in center, concentrically ringed with varying shades of red in radiating rugose growth from dark to light toward erose margin
16°C.	19	Pulvinate mass of butyrous, greyish dark red, surrounded by a ring of deep dark red then bright red to pink to a narrow, white, erose margin
12°C.	14	Scarlet crateriform depression with deep red, raised portion in center, through various shades of red to pink to a narrow white margin
8°C.	5	Bright pink bleb surrounded by radiating rugose white
2°C.	0	No growth

*Note that the normal growth at 32°C. has almost overtaken the abnormal growth at 36°C.

Table 8. Growth in two weeks, at various temperatures, on potato dextrose agar, of a pathogenic strain of Serratia marcescens (Bizio), isolated from Reticulitermes tibialis Banks.

Temperatures	Diameter in mm	Chromogenesis and morphology of colony
40°C.	0	No growth
36°C.	38	Crateriform, slate grey center, 20 mm in diameter, concentrically ringed with pale purple to white to a narrow, erose slightly raised margin of rose pink
32°C.*	42	Pulvinate, red pigmented, mucoid mass with a lighter pigmented, erose, drier margin, the slimy pigment tending to spread over margin; finger-like to wall of dish
28°C.	47	Pulvinate, red pigmented, mucoid mass with a lighter pigmented, erose, drier margin, the slimy pigment tending to spread over margin; finger-like to wall of dish
24°C.	41	Pulvinate, red pigmented, mucoid mass with a lighter pigmented, erose, drier margin, the slimy pigment tending to spread over margin; finger-like to wall of dish
20°C.	35	Contoured, mucoid mass of dark red pigment, varying from dark to lighter shade toward a regular margin
16°C.	28	Scarlet except for 2 mm regular white margin, mycelioid in appearance
12°C.	22	Pink bleb, 6 mm in diameter, apparently covered by white veil continuing as greyish film-like growth to erose margin
8°C.	15	Deep red, butyrous, pulvinate mass, 15 mm in diameter, surrounded by bright red radiating rugose to regular margin
2°C.	0	No growth

*Note that in two weeks the normal situation had been produced, namely, that the growth at 32°C. had become larger than that at 36°C.

SUMMARY AND CONCLUSIONS

1. Five years of observations and study have shown that the two species of subterranean termites, Reticulitermes claripennis Banks, and Reticulitermes tibialis Banks, are apparently the only species of termites of economic importance in Kansas.

2. A fungus, Entomophthora sp. has been found to be pathogenic to Reticulitermes tibialis Banks under laboratory conditions, producing a mortality of 76 per cent in one week from contact-feeding tests. This is apparently the first record of the occurrence of this genus of entomogenous fungi on termites. The symptoms of the disease are described.

3. Tests conducted with the fungus Beauveria bassiana and Beauveria (Sporotrichum) globulifera have shown them to be responsible for the death of small percentages of individuals, but the mode of attack has not been determined. Contact tests showed three per cent mortality due to B. bassiana and five per cent due to B. globulifera in one week. Contact-feeding experiments showed 21.6 per cent mortality due to B. bassiana and 8.8 per cent due to B. globulifera in two weeks.

4. What is apparently the first record of a specific bacterial disease of Reticulitermes is described in this work. The organism causing the disease has been identified as a strain of Serratia marcescens (Bizio) and was isolated from specimens of Reticulitermes tibialis Banks. The disease may be easily diagnosed by the presence of red pigment in infected termites. Experiments in the laboratory have shown a 46 per cent mortality from feeding tests and 100 per cent mortality from inoculation

tests.

5. The temperature ranges in a saturated atmosphere, for both the termites and the bacterial pathogen, have been studied. There is a correlation existing between the two ranges, the optimum ranges being almost parallel at approximately 28°C. Since both apparently flourish under saturated moisture conditions, the temperature relation becomes the controlling factor.

6. Preliminary observations on inoculations of three natural colonies of termites with bacterial suspension have indicated: (1) that the termites were repelled by such inoculation, that some individuals died from the disease, (2) that the disease could be disseminated throughout a colony by sick termites, and (3) that the bacterium persisted in the soil under very moist conditions.

7. A study based on a compilation of all available information on the subterranean termites of Kansas showed: (a) that there are only nine counties in the state from which the insects have not been reported; (b) that the pests are more important economically in central and eastern counties, and (c) that the reported infestations have been generally more numerous from counties through which the major streams take their course.

8. Predacious enemies of subterranean termites in Kansas have been observed as follows: (a) small ants, Cremastogaster sp. attacking workers; (b) the common black cricket attacking

workers and primary reproductives; (c) the Mexican bedbug, Triatoma sanguisuga LeConte attacking workers; (d) a centipede of genus Scolopendra attacking workers; (e) four birds, namely, the catbird, the robin, the red-headed woodpecker and the English sparrow, attacking primary reproductives; (f) the common garden toad Bufo woodhousii woodhousii (Girard) found with primary reproductives in the stomach, and (g) the collared lizard Crotaphytus collaris (Say), also found with primary reproductives in stomach.

9. An unidentified species of nematode which apparently feeds primarily on debris in termite runways has been found to exist as an immature, parasitic form in the heads of a small percentage (an average of 4.5 per cent in colonies observed) of individuals from normal colonies of Reticulitermes tibialis Banks and Reticulitermes claripennis Banks. In laboratory colonies the nematodes occurred in great numbers when the termites were weakened by extreme environmental changes or disease. From present knowledge the nematode cannot be considered as important in the natural control of termites.

10. Two unidentified species of mites have been observed in colonies of subterranean termites but observations have indicated that they are probably unimportant as biological control possibilities.

11. Observations and studies based on a few experiments showed that predators and disease are probably very important in the natural control of termites in Kansas. The introduction

of parasitic organisms to produce epidemics of disease was sufficiently satisfactory to justify the continuation of more extensive experimental investigations on the biological control of termites.

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