

Three Entomological Laboratory Techniques¹

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- I. Rearing Method for Subterranean Termites John V. Osmun²
- II. Circulation Fans for Fumigation Chambers Harry B. Moore³
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- III. Chambers for Exposure of Roaches to Chemically
Treated Surfaces Frederick P. Diehl⁴ and John V. Osmun

I. Rearing Method for Subterranean Termites

Progress in the study of the response of the subterranean termite, *Reticulitermes flavipes* (Kollar), to insecticides in the soil is dependent on a satisfactory method of rearing and handling these particular insects. Several prerequisites are necessary: an environment with high constant humidity yet without free water, a wood supply free from excessive decay, a source of essential nutrients, rearing chambers which will accommodate large self-sustaining populations, and of major importance, a convenient method for removing the termites for use in experimental studies. Previously reported methods have all had certain limitations, although the use of agar by Light and Weesner (3) did supply a clue for the method finally developed and adopted in this study. After considerable trial and error, including the use of paper toweling and agar, and sawdust and agar, the following technique was adopted:

The equipment used consists of clear plastic crisper dishes 9¼ x 6 x 3¾ inches in size and fitted with clear plastic covers in which two small holes (7 mm. diameter) are drilled to permit the regulation of air exchange. Into each container is poured 250 ml. of 4.5% bacto-agar. After the agar has set, specially cut wood is introduced and placed on pieces of ¼ inch glass tubing to keep it from direct contact with the agar. The wood, aged southern yellow pine, is prepared from 2 x 4 inch boards cut in 6 inch lengths. Into the ends of each are drilled three evenly spaced ⅜ inch holes 3 inches deep. The pieces of wood are then ripped lengthwise cutting through the holes and dividing the wide face into four equal strips. These four are then bundled back together with two rubber bands so their final actual size is approximately 1½ x 3¼ x 6 inches. Each plastic chamber holds three bundles flat along the bottom and a second layer may be used if desired after the termite colony is established and needs more cellulose (Figure 1). The holes hasten termite penetration and acceptance of the wood. Termites are introduced into the chambers from strong, active colonies in the

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field. About 2,000 individuals appear to be the minimum number for a healthy colony in each of these chambers, and several thousand more can be accommodated. Effort is made to include, along with the workers, several mature secondary reproductives or a goodly number of nymphs of the reproductive caste together with a complement of soldiers. When introduced, the termites quickly tunnel the agar and later move from there into the wood. The rearing chambers are kept in a high humidity cabinet at $24 \pm 1^\circ \text{C}$, a temperature set for convenience in connection with the testing temperature rather than necessarily as an optimum for the termites. Maintenance of moisture is not a problem, although

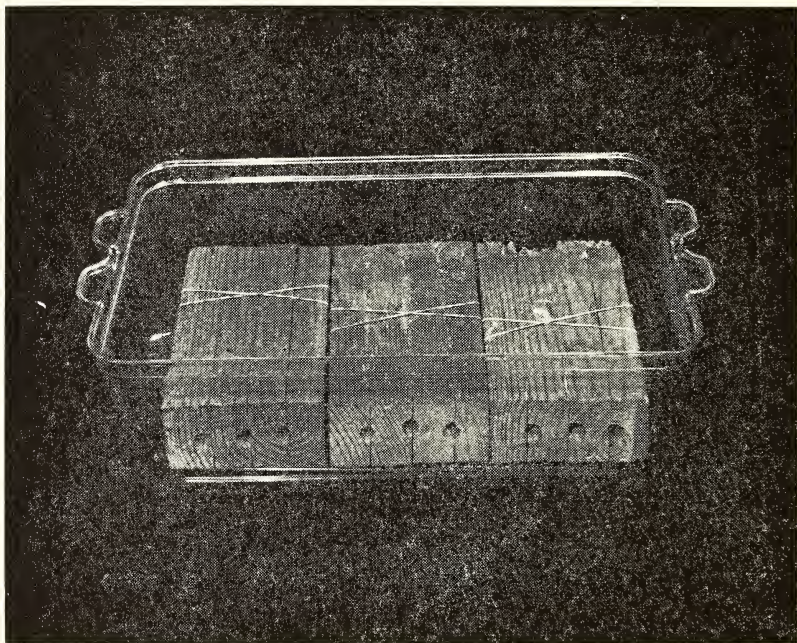


Figure 1

in one colony it was considered necessary to add, several months later, a $\frac{1}{4}$ inch sheet of 4.5% agar placed between the blocks of wood.

It has been found that maintaining termites in chambers with agar and "clean" wood alone is seldom successful. By introducing a thin layer of soil from the termites' natural environment onto the agar surface, the nutritional requirements other than that of cellulose seem to be satisfied. There is little doubt but what certain fungi must be present in the termite environment to furnish an adequate supply of protein and vitamin (Hendee (2), Hungate (3), Light and Weesner (4), DeLong (1)). Such fungi are present in the soil and in pieces of colonized wood. In colonies established most recently, 75 mg. of live dry yeast was sprinkled over the top of the wood after it was placed in

the chambers. The basis of this action resulted from success experienced with recuperating small, waning colonies held in petri dishes.

The handling technique described has been used not only for maintaining an established colony but also for rearing termites through from eggs. Secondary reproductives are the principal source of eggs, but due to the long period of development it is impractical to count on such methods alone when large numbers of termites are required.

When termites are needed for testing, it has been very convenient to obtain them from the type of rearing chamber described. A bundle of wood is removed and carefully opened along the laminations without necessarily removing the rubber band. From the space occupied, termites are gently shaken into a petri dish. When sufficient termites are collected, the bundles of wood are replaced in the rearing chamber. The termites thus collected in the petri dishes are kept healthy with a little debris from the rearing chambers and a small piece of 4% agar. These termites are held until needed for tests, but for the sake of uniformity they are held in this manner no longer than one week.

During the course of the investigation, termites from three sources were used: a building in Indianapolis, Indiana; a board removed from the ground in Champaign, Illinois; and the experimental termite plots in Allerton Park, Illinois. The only extensive testing has been done with termites from the latter location. They were obtained for the laboratory by driving 2 x 4 x 18 inch stakes into the ground and leaving them for six months. These stakes were then pulled, brought back to the laboratory where the termites were removed and introduced into the rearing chambers described. An initial supply was obtained from a few stakes already in the ground. During the course of tests with topical applications, all three populations were tested to show to the author's satisfaction that no appreciable differences existed with respect to variation in susceptibility to insecticides.

II. Circulation Fans for Fumigation Chambers

Methods for testing the responses of insects to accurately measured quantities of fumigants have frequently been complicated by several factors. These factors include those associated with the desirability of employing relatively small chambers in which a minute amount of gas can be utilized without loss and yet it dispersed uniformly in the atmosphere of the chamber. Pressure cookers with air circulation pumps have been tried, but equipment expense and gas loss reduce their usefulness. Randall et al. (5) describes a method by which weighed amounts of gas were introduced into a bottle which was shaken frequently during the test to help distribute the gas uniformly.

In the method described here, 20-liter flint glass bottles were utilized. These containers are very suitable since they are a convenient size for testing quantities of insects, are air tight except for a small opening in the neck, and can be replicated without excessive need for space or equipment. As used in our research, the bottle opening is plugged with a rubber stopper immediately after the introduction of test cages containing insects and the test quantity of fumigant. The unique

feature is the method of air circulation used to prevent gas stratification. A miniature electric fan is used in each chamber. The fan is powered by a 12-volt, direct current motor of the type used in model airplane and scale model trains. These motors are 1 inch in diameter and $1\frac{1}{4}$ inches long. Each motor is fitted with a four blade aluminum or plastic fan 1 inch in diameter. When used in fumigation tests, the fans are suspended inside the bottles 4 inches from the bottom of the chamber. The wire leads are fitted with soldered loops which allow them to be placed over wire hooks which in turn project through the rubber stoppers and convey the current from a transformer.

Since continuous circulation of air is detrimental to some test insects, the apparatus has been equipped with a time switch which permits intermittent fan activity. The equipment is illustrated in Figure 2.

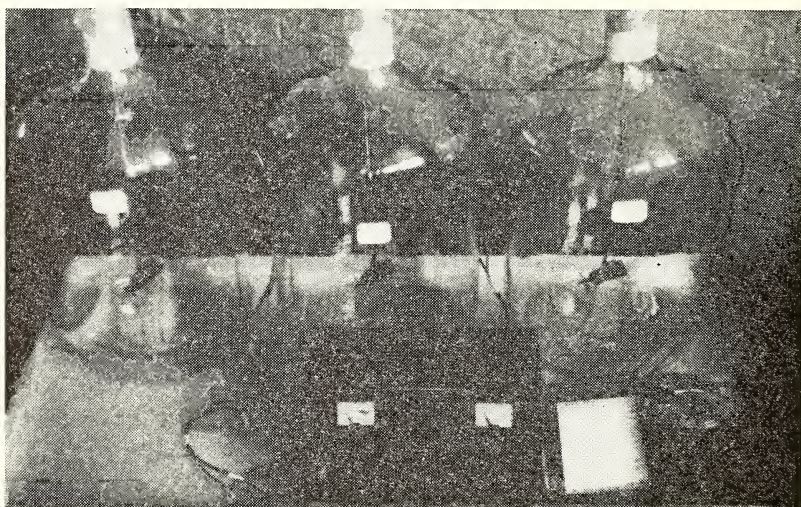


Figure 2

III. Chambers for Exposure of Roaches to Chemically Treated Surfaces

Methods of assaying biologically the insecticidal activity of treated surfaces has never been entirely satisfactory. This has been especially true when roaches have been the test insect involved. Prerequisites for such studies include having a test cage which will confine roaches to the treated surfaces continuously during the exposure period, and will permit observation as well as rapid removal of the insects at the end of the time interval. These observations could be made by using several hundred individual cages with various surfaces and chemical treatments, but the inconvenience and excessive expense would preclude such an approach. A properly designed cage into which could be placed differently treated surfaces would fulfill the needs.

In the course of our study dealing with the exposure of the German cockroach, *Blattella germanica* (L.), to chlordane treated surfaces, it was found that the following technique adequately met the requirements of the test. The basic unit of the test cage consists of two strips of grooved yellow pine, $\frac{1}{2}$ " x 2" x 9". One of the 2" sides of each piece of wood is double-grooved along its length, with the inside distance between the grooves being $\frac{1}{4}$ inch. These two strips serve as parallel sides of a frame holding the test panels. The test surfaces of each cage consist of two 6 x 6" pieces of material, such as glass, metal, plywood, or painted wood (Figure 3). Two such test panels are inserted in grooves of the



Figure 3

2-sided frame in such a manner as to have the treated surfaces face each other. When a roach is placed within the quarter inch space provided, it must crawl on one treated surface or the other. The frames are held together by rubber bands, and the two end openings into the test area are closed with pieces of 2" x 6" observation glass slipped into suitable grooves provided in the frame. Such a device permits the

reuse, retreatment, cleaning, or discard of test panels. A few sets of frames will serve numerous test panels and treatments.

In test situations, the German roaches are anesthetized with CO₂ and placed on a small piece of clean paper. The paper is slipped into the test area and the front observation glass allowed to rest gently on it. When the insects regain consciousness, the paper is quickly withdrawn. At the end of the exposure period, CO₂ is introduced into the chamber and the reanesthetized roaches gently shaken out into holding dishes.

A modification of this test chamber was developed in which a large ¼" thick plastic ring, 6" in diameter, was substituted for the grooved wooden frame. Although this device is somewhat simpler to operate, its use is dependent on the availability of such plastic. Both types of cages permit continuous, natural contact of roaches with insecticide residues, and convenient observation of the toxicological responses.

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