The Great Lakes Entomologist

Volume 15
Number 1 - Spring 1982 *Number 1 - Spring 1982*

Article 3

April 1982

Large-Scale Production of Fungal Bait Blocks for the Attraction of Termites (Isoptera: Rhinotermitidae)

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LARGE-SCALE PRODUCTION OF FUNGAL BAIT BLOCKS FOR THE ATTRACTION OF TERMITES (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT

It has been shown that wood infected with the brown rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. is more attractive to termites than sound wood. For several years, small blocks of infected wood have been used as bait to sample soil sites for the presence of termites. The method described here for bait block production is more suitable than the ASTM method for large-scale work.

In 1961, Esenther and others demonstrated that wood infected with the brown rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.) Murr. (formerly *Lenzites trabea* [Pers. ex Fr.]) was more attractive to the eastern subterranean termite, *Reticulotermes flavipes* (Kollar), than was uninfected wood. Smythe et al. (1967) later showed that the partially decayed wood was also attractive to other species of *Reticulotermes* as well as to *Zootermopsis augusticollis* (Hagen). These studies have since been confirmed and expanded by various workers, including Matsuo and Nishimoto (1974) and Becker and Lenz (1975). It has been known since the original work of Esenther et al. (1961) that the attractive principle could be extracted from wood infected by the fungus.

In 1967, Smythe et al. isolated the trail-following pheromone from *R. flavipes* and *R. virginicus*. Matsumura et al. (1968) studied the pheromone from southern subterranean termites, *R. virginicus* (Banks), and its chemical structure was elucidated (Tai et al. 1968). In a subsequent paper, these workers reported that the pheromone was closely related to and possibly identical with a component of wood rotted by *G. trabeum* (Matsumura et al. 1969). The extract was identified as (Z,Z,E) 3,6,8-dodecatrien-1-ol. Since then, the compound has been synthesized, together with a number of analogues exhibiting varying degrees of attractiveness to termites (Tai et al. 1971). The compounds were shown to be rather unstable.

Certain investigations concerning natural populations of termites, such as that reported by Esenther and Gray (1968), have involved the use of *G. trabeum*-infected wood blocks as "baits," to facilitate the sampling of soil sites for termites. Also, attempts have been made to control populations by the use of poisoned baits, and considerable success was obtained in field trials with mirex (Ostaff and Gray 1975). Since such studies involve large numbers of bait blocks, the production of which can be very time consuming, the present investigation was undertaken to determine if a more efficient and controlled method of production could be devised than that usually used, which is based on the procedure recommended by the American Society for Testing and Materials (ASTM), published in 1976, for testing wood preservatives. This uses "feeder strips" of fungus-infected wood supported by moist sterile soil in screw-capped jars. Small blocks of wood are placed on these, which are in turn infected.

MATERIALS AND METHODS

Preparation of Wood. White pine was sawn into strips 0.8 × 3.0 × 18.0 cm, and placed in supporting racks fabricated from 7/8 × 1/16-inch aluminum stock. A typical rack, shown in Figure 1, measured approximately 16.5 × 18.0 cm, and was made from four strips rivetted together. The two longer sides were constructed from strips previously milled to contain 12 slots measuring 0.9 cm wide by 1.3 cm deep. The wood strips, when placed in a rack, were

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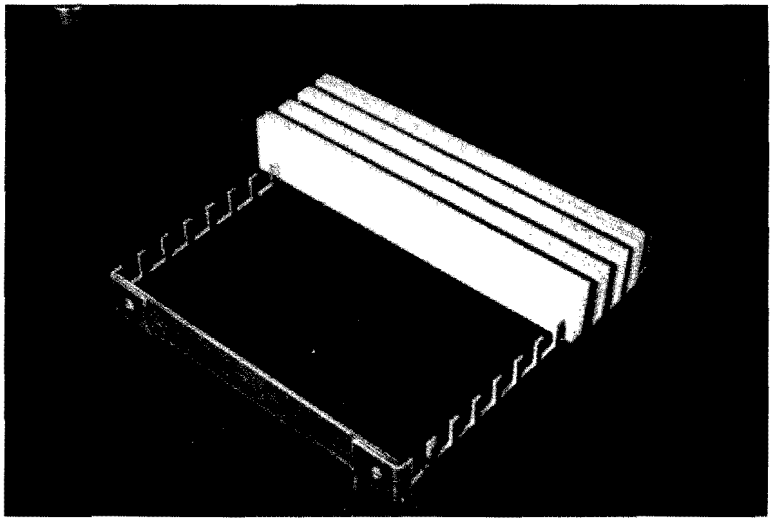

Fig. 1. Aluminum support racks containing uninfected wood strips.

about 0.5 cm apart. Prior to sterilization, the wood was soaked in water overnight and placed in the racks, which were then stacked in polycarbonate animal cages 48 cm long × 27 wide × 16 deep (Fisher Scientific Co., Toronto, Ontario), eight racks per cage. A sheet of aluminum foil was placed loosely over each cage, after adding deionized water to a depth of about 1.0 cm, and cages were autoclaved at 121°C for 30 min. When the autoclaves were opened, the foil sheets were secured in place before cages were removed. When the contents had cooled, the foil was sealed with masking tape to prevent contamination and loss of moisture during storage.

Culture Methods. Stock cultures were prepared by inoculating Malt Extract Agar (Difco Laboratories, Detroit, Mich.) slants in 1-oz screw-capped bottles with mycelium from a strain of *G. trabeum* kindly donated by Dr. G. Esenther of the U.S. Forest Products Laboratory, Madison, Wisconsin. These were designated Madison strain 617. Good growth developed in about seven days at 25°C, at which time, by the use of a chromel wire hook, the mycelium was suspended and disrupted in 5.0 ml of sterile Malt Extract Broth removed from flasks which contained 25 ml of medium. The mycelial suspensions were transferred to the flasks, which were then incubated on a reciprocal shaker with gentle agitation at 25°C for seven days. During the incubation period, standard 8-inch square teflon-lined cake pans were sterilized in the autoclave as above, and stored in sealed animal cages for future use.

For bait block production, 400-ml quantities of sterile melted Malt Extract Agar were poured into the cake pans, covered with sterile foil, and allowed to solidify. Flask cultures were transferred to sterile Waring blendor jars and disrupted at low speed for about 2–3 sec, then poured onto the agar surfaces in the cake pans (one culture per pan) and spread with a sterile bent glass rod. The pans were incubated at 25°C under sterile foil, until confluent growth of the fungus developed. At that time, racks of sterile wood strips were transferred aseptically, using surgical gloves, to the pan cultures, forcing the racks into the media until the wood strips contacted the mycelium. Foil-covered pan assemblies were then stacked in sterile animal cages, using wood strips between assemblies for support, at the rate of six units per cage. Sterile water was added to the cage to maintain a high relative humidity, and the cage was covered with foil. Water lost by evaporation during incubation at 25°C was replaced as required. After six weeks of incubation, racks were removed from pans and autoclaved for 20 min to kill the fungus, then dried at 80°C for 24 h, before cutting into 3.0 × 3.0-cm blocks for use as baits.

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DOI: 10.22543/0090-0222.1408

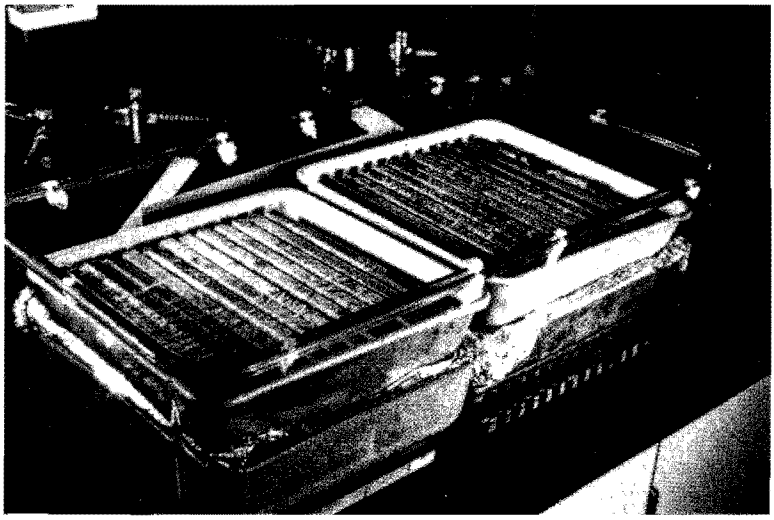

Fig. 2. Opened animal cage unit, showing wood strips infected by *G. trabeum*.

Testing Bait Blocks. To determine if bait blocks were effective attractants, a multiple-choice test using eastern subterranean termites was devised. This was carried out by pouring 4.0% melted agar solution into large thick-walled pyrex storage dishes (20 cm wide × 8 cm deep) to a depth of about 3 cm, and allowing the solution to cool. Just before it gelled, a block of sound pine, one of pine infected with *G. trabeum* by the ASTM method, and one infected by the method described above, were inserted equidistant from each other near the wall of the dish, in a slightly inclined position. Approximately 75% of each block was submerged. Thirty termites from an active colony were transferred to the agar surface of each dish, and the dishes were covered with glass plates. They were then placed in a dark cupboard, and examined weekly for activity.

RESULTS AND DISCUSSION

Figure 2 shows an animal cage culture unit which has been opened after six weeks incubation, with two of the cake pans removed. It can be seen that fungal mycelium has covered the wood strips, and dry weight determinations indicated that a weight loss of from 10 to 15% occurred in that length of time. This is comparable to the growth rate of the fungus found with the ASTM method, and appears to be adequate to elicit the pheromone-like effect previously described. In a separate study to optimize growth rates of the fungus, we were able to increase growth by about 40% using a modification of a medium reported by Levi et al. (1968), in which the carbon and nitrogen sources were supplied by glucose and $NaNO_3$. However, use of this medium did not shorten the required incubation period for bait block production, and the results did not seem to be superior to those obtained with Malt Extract Agar.

The multiple-choice tests indicated that blocks produced by the described method were as attractive to termites as those produced by the ASTM method, judging by numbers of termites seen feeding at random intervals, as well as by the number of shelter tubes constructed to baits and the loss in weight of baits through feeding. In some cases, the blocks produced by the described method appeared to be more attractive than ASTM blocks, and in all cases, fungus-infected blocks were more attractive than sound blocks, irregardless of the method of production.

There is no doubt that the procedure described here is more efficient than the original ASTM method for the production of large number of baits. One animal cage unit can provide 432 wood baits, which would require 216 screw-capped jars using the standard method. The US Forest Products Laboratory (Madison) uses a modification of the ASTM method, in which wood strips rather than blocks are placed on feeder strips (G. R. Esenther, pers. comm.), greatly increasing the efficiency. However, the author believes that the method described above provides a more controlled procedure for fungal growth.

ACKNOWLEDGMENTS

The author wishes to thank the Ontario Ministry of the Environment for providing funds which supported this project, and acknowledges the technical assistance of Mr. Bogdan Zawadzki.

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