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# Effect of Behavior on the Evaluation of Insecticides for Prevention of or Remedial Control of the Formosan Subterranean Termite[1,2]

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**ABSTRACT**

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Laboratory techniques developed to evaluate insecticides for prevention or remedial control of infestations of *Coptotermes formosanus* Shiraki showed that the evaluation could not be based on mortality alone. The behavioral responses of the termites to the insecticides had to be considered. The termites can seal off or avoid treated areas and effectively protect themselves. Factors causing this behavior appear to be the repellency of the insecticide itself or chemical factor(s) associated with dead termites. The insecticides tested could be divided into three groups, depending on the reactions of the termites. Types I and II can be used in preventative treatments. Only type III can be used in remedial treatments.

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There are two major strategies in the management of subterranean termites: prevention by soil treatment and remedial control of active infestations by injecting insecticides directly into infestations. These two strategies require insecticides with almost diametrically opposed characteristics. For soil treatment, a material with a long residual that is highly repellent or kills very rapidly is required. For remedial treatment, however, a material that is not repellent, and that is sufficiently slow acting so that it can be transported in or on the termite throughout the galleries, is required. Affected termites should be healthy enough to leave the treated area, and possibly even recruit other foragers to the area. There should not be an accummulation of dead termites in the vicinity of the treatment.

These characteristics are especially important in insecticides used in the remedial control of species such as the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Colonies of this termite may contain up to $10^6$ individuals and forage as far as 100 m from point to point (Tamashiro et al. 1980). For colonies this large and extensive, there is no way to delineate all of the galleries. Even under ideal conditions, only a small portion of the galleries can be found and treated. In addition, a further complicating factor in remedial treatment is that the termites apparently are able to detect parts of the colony treated with most currently used remedial insecticides and protect themselves by avoiding the treated areas or by sealing off these tunnels. Although it is generally stated that these phenomena do occur, documentation of these behaviors, factors evoking these reactions, and effectiveness of these behavioral patterns in protecting the colony have not been demonstrated.

Any evaluation of insecticides for use against termites, therefore, must include observations of the behavioral responses of the termite to the insecticide, in addition to mortality. The current methods used to evaluate insecticides for termite management cannot be used to evaluate all of the characteristics required of a preventative or remedial treatment insecticide.

Most current methods of evaluating the response of subterranean termites to insecticides utilize soil as the medium to hold the necessary moisture (Hetrick 1952, Osmun 1956, Ebeling and Pence 1957). Their methods, in which the treated and nontreated soils are not completely separated, could allow the vapor phase of insecticides to permeate the nontreated area. Beal and Smith (1971) improved on these methods by using two chambers connected by a glass tube in which the treated soil was placed. Although these techniques were adopted and modified by other researchers (Lewis et al. 1978), they were not suitable for observation because the termites were not visible when they were tunnelling through the medium (Ebeling and Pence 1958, Smith 1979). Repellency also could not be adequately measured by these methods.

An adequate method to evaluate termiticides has to include a device that could maintain high humidity, isolate treated from nontreated substrates, and permit observation of the termites' behavior through the medium. Two devices, one for qualitative assessments and one for quantitative data, were built to examine the response of this termite to several insecticides and to evaluate their potential for use against the Formosan subterranean termite.

## Materials and Methods

### *Preparation of High-Concentration Agar*

Agar was adopted as the medium to replace the soil used in previous methods. Agar provided sufficient humidity and allowed observations of the conditions of the termites, their responses to the insecticides, and their gallery-building behavior. Agar also separated the treated and nontreated areas and prevented even the vapor phase of the candidate termiticides from permeating the untreated areas.

However, a high-concentration agar was needed to prevent cracking of the agar by desiccation. The highest feasible concentration of agar, using the or-

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dinary method of heating the agar on a hot plate while stirring the solution, is ca. 4%. Concentrations more than 4% could not be sufficiently stirred to prevent burning of the agar at the bottom of the vessel. To avoid this problem, a microwave oven was used to prepare high-concentration agars. A fine granular form of agar (Difco) was mixed with water in a glass container and placed in a microwave oven (Panasonic model NE7900). At its maximum setting, the oven generated 8,600 cal/min (ca. 36,000 J/min). It took ca. 9.5 min to prepare 1,000 ml of a 5% agar solution. With this technique, agar concentrations up to 10% could be made.

*Device for Qualitative Study*

The device used to observe the termites' reactions to the pesticides was composed of a plastic petri dish filled with agar in which were five holes (Fig. 1A). Small air holes were made in the petri cover over the large central hole.

The upper surface of the agar was flat enough so that there was no air space between the cover and agar surface. Clear lacquer was sprayed inside each hole to prevent contamination of the agar by water-soluble insecticides. The petri dish was large enough so that all the termites were not killed by diffusion of the vapors of volatile insecticides when the tunnels reached the treated medium. A 1-g amount of artificial diet similar to that developed by Mauldin and Rich (1975) was placed in each hole. Two of the holes contained treated diets, whereas the others contained untreated diets. The insecticides and the formulations tested were: fenvalerate 2.4E; permethrin 3.2E; resmethrin 2.0E; chlorpyrifos 4.0E; chlordane 8.0E; carbaryl 80W; diazinon 2.0 microencapuslated flowable; pyrethrins 0.62% in oil; and Amdro® (tetrahydro-5, 5-dimethyl-2(1H)-pyrimidinoine{3-[4-(trifluoromethyl)-phenyl]-1-{2-[4-(trifluoromethyl)phenyl]enthenyl}-2-propenylidene} hydrozene) technical. Each insecticide was used at 500, 1,000, and 5,000 ppm, and each concentration was replicated three times.

Three hundred termites (262 workers and 38 soldiers) collected from a field colony were introduced into the center hole. The numbers introduced approximated a 7:1 worker to soldier ratio, which is characteristic for this species in Hawaii. The device with the termites was held at 30°C. Observations were made on whether the termites stopped or continued entering the treated area, and whether the tunnels were sealed or left open.

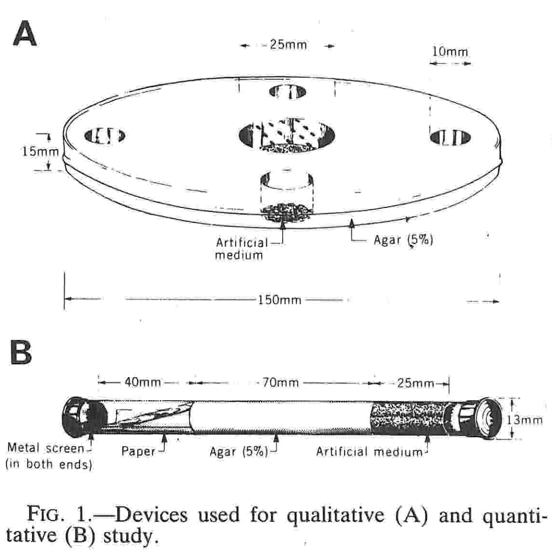


FIG. 1.—Devices used for qualitative (A) and quantitative (B) study.

*Device for Quantitative Study and Analysis of Results*

To quantify avoidance behavior, counts of termites in treated and untreated media at a given instant were required. However, this was not possible in the petri dishes because of the size of dish and the numbers of termites involved. Moreover, in the petri dish the termites were free to tunnel in all directions and did not reach the treated and untreated diets at the same time. Therefore, a glass tube with agar sandwiched between the treated diet and the chamber where the termites were introduced was used (Fig. 1B). A piece of paper was placed in the chamber to provide additional surface area and to serve as food. A total of 150 termites (131 workers and 19 soldiers) were introduced into the chamber. Each end of the tube was stoppered with a polyethylene cork, reinforced with a metal screen. Small holes were punched in the cork for air exchange. The device was stored at 30°C in a horizontal position. The treatments were fenvalerate, chlordane, and Amdro® (each at 100, 500, and 1,000 ppm), and an untreated control. There were 24 units in each treatment, making a total of 240 units.

Six randomly selected units from each treatment were opened at 3, 6, 9, and 12 days after tunnels reached the treated medium. The number of survivors in each tube was counted to ascertain mortality. Abbott's formula was used to correct for natural mortality.

In addition to mortality, measurements were made of the penetration of the treated medium by the termites. From these measurements, a "percent avoidance," which was defined as "the degree to which the termites avoid the treated medium," was derived in the following manner.

The live termites in the treated medium at the time of observation in each of the unopened units were counted. This number was divided by the estimated number of survivors in the unit. The estimated number of survivors used was the mean number of survivors in equivalent units opened that day. The Mann Whitney U test (Mann and Whitney 1947) was used to compare the fraction of the population in the treated medium with the fraction that was in the untreated medium in the control tube. Percent avoidance was considered to be 0 when there was no significant differences between the two population fractions. When there were significant differences, a modification of Abbott's formula was used to calculate percent avoidance. This was calculated from the formula $A = [(Fn - Ft)/Fn] \times 100$, where: $A$ = percent avoidance; $Fn$ = population fraction found in the untreated medium = $(100\ Nn)/[N(100 - Mn)]$; $Ft$ = population fraction found in the treated medium = $(100\ Nt)/[N(100 - Mt)]$; $N$ = number of individuals introduced; $Nn$ = number of living individuals found in the untreated medium; $Nt$ = number of living individuals found in the treated medium; $Mt$ = percent mortality at the t-th day; and $Mn$ = natural mortality at the t-th day.

## Results and Discussion

### *Qualitative Study*

The data revealed that the nine pesticides could be classified into three types based on the behavioral responses of the termites (Table 1). At high concentrations of type I insecticides, the tunnels to the treated areas were sealed by termites within 1 day after termites reached the treated area. At low concentrations, the tunnels were not sealed but the termites did not enter the area. The insecticide themselves appeared to be repellent. All of the type I insecticides tested were natural or synthetic pyrethroids. Since the tunnels leading to the treated area either were sealed by termites with agar fragments and artificial diet (Fig. 2A) or the termites did not enter the area even if the tunnel was open, contact with the insecticide was minimal. Therefore, only a small proportion of the termites died because of exposure to type I insecticides.

With type II insecticides, the termites which entered the treated area were killed or affected quickly, so that some individuals died in the treated area. However, active individuals were still observed entering the treated area during the initial 2 to 4 days. Subsequently, however, the tunnels were sealed or the termites avoided the treated area. This also seemed to be a concentration related phenomena. Again, because the tunnels were sealed or the termites did not enter the area, most of the test insects survived.

The factor(s) eliciting the avoidance behavior, however, was different from that in type I insecticides. With type II insecticides, the insecticide itself apparently was not repellent since the termites continued to enter the treated area for the first few days (Table 1). The avoidance behavior was not manifested until there were many dead and decaying individuals in the treated area. The reaction, therefore, was against factor(s) emanating from dead and decaying termites, rather than a reaction to the insecticide. With the type II insecticides, the sealed portion of the tunnels contained dead individuals (Fig. 2B), whereas with type I insecticides the sealed

**Table 1.—Effects of various pesticides on *C. formosanus* as measured by the speed at which tunnels reaching the treated area are sealed**

| Type | Insecticide | Concn (ppm) | Effect[a] on day: 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| I | Fenvalerate[b] | 500 | + | | | | |
| | Fenvalerate | 1,000 | + | | | | |
| | Fenvalerate | 5,000 | + + | | | | |
| | Permethrin[b] | 500 | + | | | | |
| | Permethrin | 1,000 | + | | | | |
| | Permethrin | 5,000 | + + | | | | |
| | Resmethrin[b] | 500 | + | | | | |
| | Resmethrin | 1,000 | + | | | | |
| | Resmethrin | 5,000 | + + | | | | |
| | Pyrethrins[c] | 500 | + | | | | |
| | Pyrethrins | 1,000 | + | | | | |
| | Pyrethrins | 5,000 | + + | | | | |
| II | Diazinon[d] | 500 | − | − | − | − | + |
| | Diazinon | 1,000 | − | − | − | + | |
| | Diazinon | 5,000 | − | − | + + | | |
| | Chlorpyrifos[b] | 500 | − | − | − | − | + |
| | Chlorpyrifos | 1,000 | − | − | − | − | + |
| | Chlorpyrifos | 5,000 | − | − | − | + + | |
| | Chlordane[b] | 500 | − | − | + | + + | |
| | Chlordane | 1,000 | − | − | + | + + | |
| | Chlordane | 5,000 | − | − | + | + + | |
| | Carbaryl[e] | 500 | − | + | + | + | + + |
| | Carbaryl | 1,000 | − | + | + | + + | |
| | Carbaryl | 5,000 | − | + | + + | | |
| III | Amdro®[f] | 500 | − | − | − | − | − |
| | Amdro® | 1,000 | − | − | − | − | − |
| | Amdro® | 5,000 | − | − | − | − | − |

[a] −, Tunnels open, termites entering the treated area; +, tunnels open but no termites entering the treated area; + +, tunnels sealed.
[b] Emulsifiable concentration.
[c] Oil base.
[d] Microencapsulated flowable.
[e] Wettable powder.
[f] Technical compound.

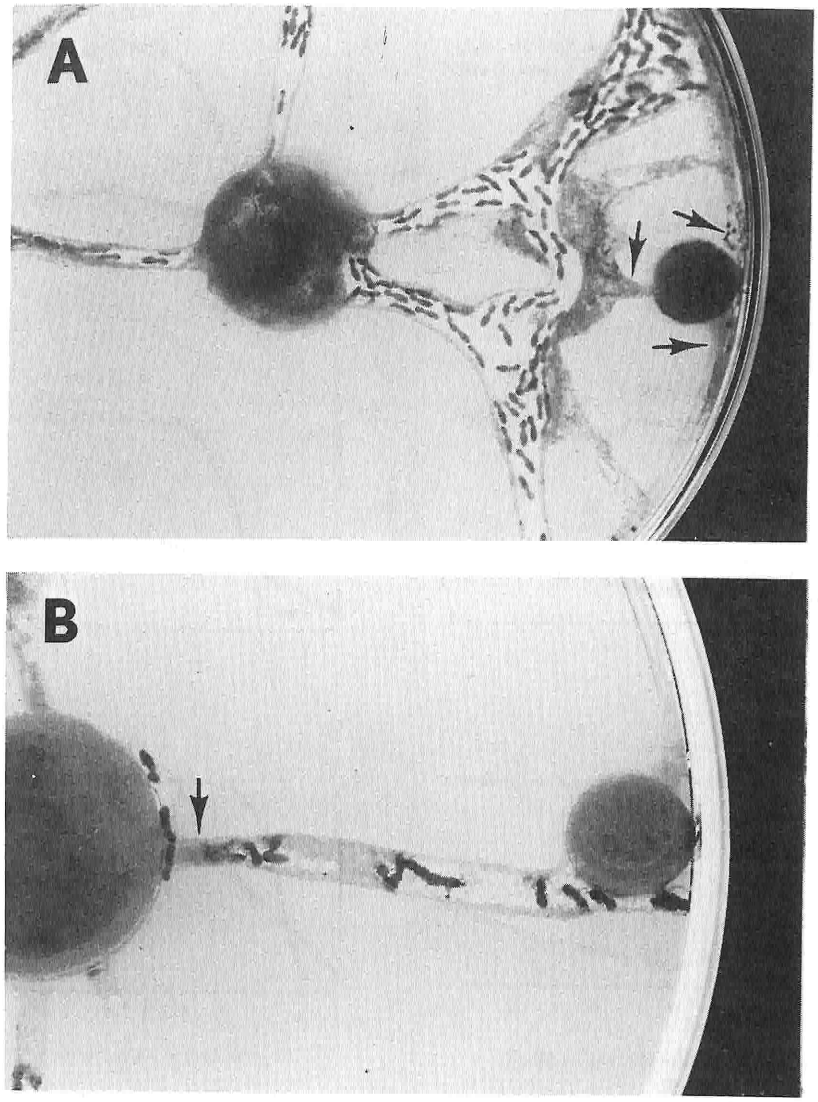


FIG. 2.—Tunnels sealed by termites after reaching fenvalerate-treated (A) and chlordane-treated (B) areas. Arrows indicate portions of tunnels being sealed.

tunnels did not include dead termites. Type II insecticides include some of those most commonly used in or proposed for preventive or remedial treatment.

With type III insecticides, the termites did not seal off the treated area. They continued to enter the area during the entire experimental period. Although many individuals were killed, they died in different parts of the device. Since there was no accumulation of dead individuals in the treated area and the insecticide was not repellent, they continued to feed on the treated medium. Amdro® was the only type III insecticide of those tested. It is a slow-acting stomach poison.

*Quantitative Study*

The response of *C. formosanus* to the type I insecticides (percent avoidance, percent mortality) is illustrated by fenvalerate (Fig. 3). Mortality was low, and there was no increase in mortality after day 3. In the 500- and 1,000-ppm treatments, the low mortality was due to avoidance, which was almost 100% from day 1. The 15 to 30% mortality caused by these treatments was due to initial contact when the termites investigated the treated medium.

At 100 ppm, percent avoidance declined after day 5. Although this indicated that termites started to penetrate the treated area, it was noted that the walls of the tunnels excavated into the treated medium were covered with agar and fecal material. Apparently, at low concentrations, the effect of the chemical was not strong enough to cause the termites to completely avoid the area. However, the effect was severe enough to cause the termites to line the tunnel so that they did not come into contact with the chem-

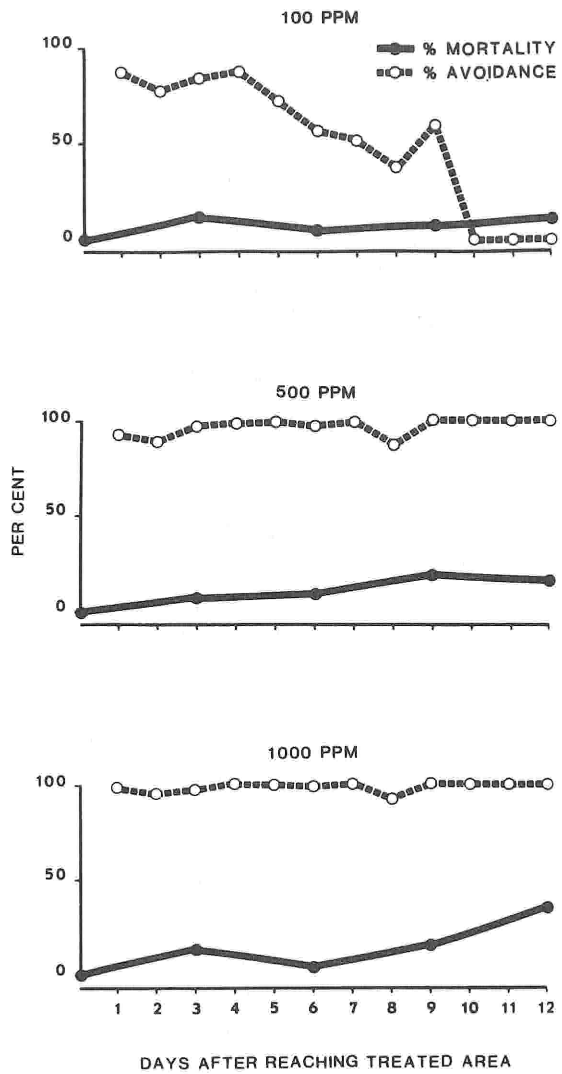


FIG. 3.—Avoidance and mortality curves for fenvalerate at 100, 500, and 1,000 ppm.

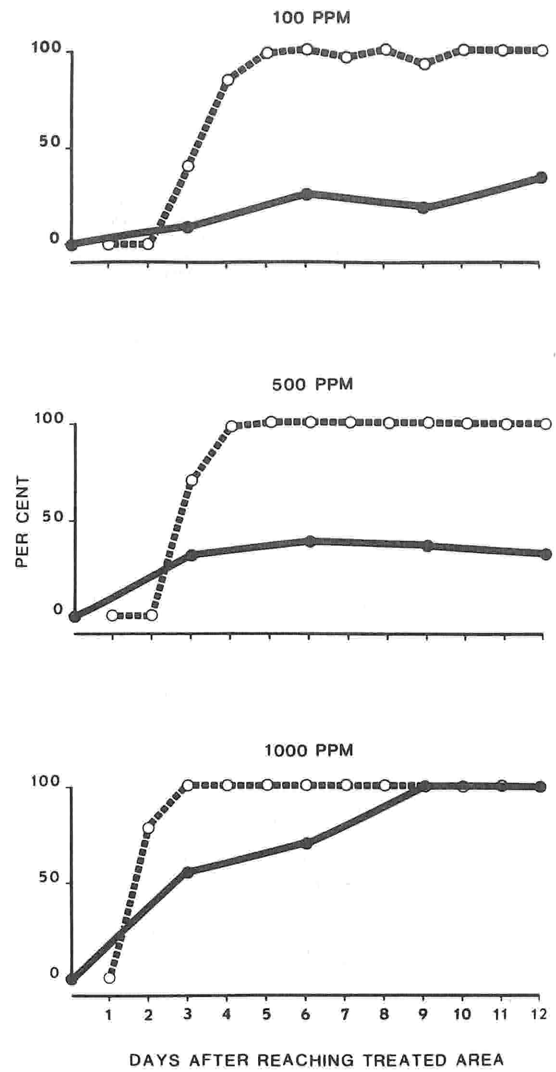


FIG. 4.—Avoidance and mortality curves for chlordane at 100, 500, and 1,000 ppm.

ical as they passed through. They apparently did not feed on the medium.

The response to chlordane exemplified the reaction to type II compounds (Fig. 4). The responses to chlordane at 100 and 500 ppm were similar. For the initial 2 days, termites entered the treated area as frequently as the untreated, but many could not leave the treated area because they were affected by chlordane. After dead termites accumulated in the treated area, the unaffected individuals avoided entering the area. After 5 days, no active termites were seen in the treated area. As a result of this avoidance, mortality remained at ca. 30 to 40%.

In the 1,000-ppm treatment, mortality continued to increase even though termites avoided entering the treated area after day 3. Apparently, at high concentrations of chlordane, the vapor pressure was strong enough to affect all of the individuals in the tube. Mortality reached 100% before the termites could effectively seal off the treated area. However, this was due to the limitation of the size of the device. In the large petri dishes, the termites were able to seal off even higher concentrations of chlordane. Therefore, in the field, where colonies are spread over large areas, they probably could seal off galleries that have been treated before significant portions of the colonies are affected.

Chlordane apparently was not as repellent as fenvalerate, since the termites entered the treated area during the initial 2 days. These observations differ from those noted by Smith (1979) in his soil test, where he found that chlordane was repellent and fenvalerate was not.

Figure 5 shows the effects of the type III compound, Amdro®. Only Amdro® caused mortality to continue to increase, indicating that the termites

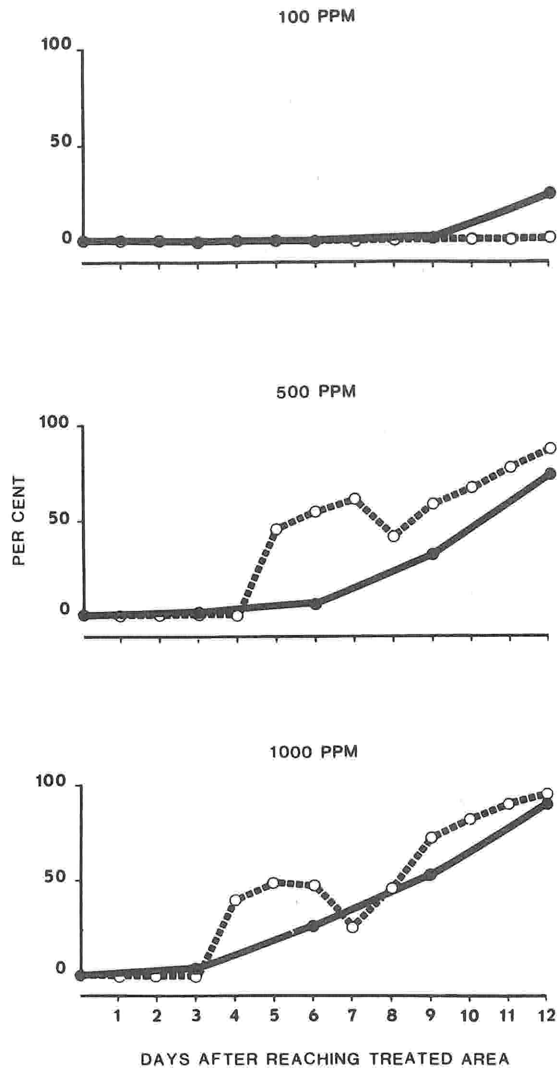


FIG. 5.—Avoidance and mortality curves for Amdro® at 100, 500, and 1,000 ppm.

continued to feed on or contact the medium. At 100 ppm, the termites did not avoid the treated medium. At 500 and 1,000 ppm, there was no avoidance for the initial 4 days, indicating that the compound itself was not repellent. However, as mortality increased, the termites started to avoid the treated area. Avoidance, however, did not reach 100% as occurred in type I and II compounds, so mortality continued to increase. The termites, however, did not enter the treated area as frequently as on previous days. Since the dead individuals did not accumulate in the treated area, and since the compound itself did not seem to be repellent, the reasons for the percent avoidance fluctuating as shown cannot be explained at this time.

The results obtained in these tests showed that mortality alone was not an adequate criterion for assessment of efficacy of termiticides. The behavioral responses of the termites to the insecticides had to be considered. These results showed that type I or II insecticides should be used for preventative treatments since they prevent termites from penetrating the treated area because they are repellent or very fast acting. They should not be used for remedial treatments. Many of the failures in remedial treatment were probably due to the use of type I or II insecticides. For remedial treatments, type III insecticides are required.

Mirex apparently was one such type III insecticide. Esenther and Beal (1974, 1978) reported successful trials to suppress *Reticulitermes* spp. with an attractant-mirex bait. Since mirex no longer can be used, substitutes must be found. Of the insecticides tested, Amdro® appears to be the only insecticide that seems to be potentially useful in remedial control.

## REFERENCES CITED

**Beal, R. H., and V. L. Smith. 1971.** Relative susceptibilities of *Coptotermes formosanus, Reticulitermes flavipes,* and *R. virginicus* to soil insecticides. J. Econ. Entomol. 64: 472–475.

**Ebeling, W., and R. J. Pence. 1957.** Relation of particle size to the penetration of subterranean termites through barriers of sand or cinders. Ibid. 50: 690–692.

**1958.** Laboratory evaluation of insecticide-treated soils against the western subterranean termite. Ibid. 51: 207–211.

**Esenther, G. R., and R. H. Beal. 1974.** Attractant-mirex bait suppresses activity of *Reticulitermes* spp. Ibid. 67: 85–88.

**1978.** Insecticidal baits at field plot perimeters suppress *Reticulitermes*. Ibid. 71: 604–607.

**Hetrick, L. A. 1952.** The comparative toxicity of some organic insecticides as termite soil poisons. Ibid. 45: 235–237.

**Lewis, D. L., G. E. Michaels, D. B. Hays, W. Campbell, and V. Smith. 1978.** Evaluation of the anti-termite activity of hydroxyquinoline and naphthol derivative formulation using *Reticulitermes* in laboratory and field experiments. Ibid. 71: 818–821.

**Mann, H. B., and D. R. Whitney. 1947.** On a test of whether one of two random variables is stochastically larger than the other. Ann. Math. Stat. 18: 50–60.

**Mauldin, J. K., and N. M. Rich. 1975.** Rearing subterranean termites, *Reticulitermes flavipes* and *Coptotermes formosanus,* on artificial diet. Ann. Entomol. Soc. Am. 68: 454–456.

**Osmun, J. V. 1956.** The response of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), to certain insecticides. Ph.D. thesis, University of Illinois, Urbana. 131 pp.

**Smith, V. K. 1979.** Improved techniques designed for screening candidate termiticides on soil in the laboratory. J. Econ. Entomol. 72: 877–879.

**Tamashiro, M., J. R. Yates, P. Y. Lai, J. K. Fujii, and N. Y. Su. 1980.** Size and structure of *Coptotermes formosanus* Shiraki colonies in Hawaii, p. 311. Proc. 16th Int. Cong. Entomol. Kyoto, Japan. 480 pp.