Influence of a Mineral Insecticide Particle Size on Bait Efficacy Against *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)*

by

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ABSTRACT

We examined the efficacy of termiticidal baits comprised of powdered α-cellulose and a mineral insecticide, cryolite crystals, in laboratory bioassays against pseudergates of Eastern subterranean termites [*Reticulitermes flavipes* (Kollar)]. The influence of cryolite crystal size [0 (control), 0.2, and 20 µm diameter particles] on the overall mortality of termites consuming the bait was determined in forced feeding bioassays. Only baits containing 20 µm diameter cryolite crystals increased termite mortality significantly above the control treatment. An additional study examining feeding on stained bait (control and 20 µm diameter cryolite crystals only) indicated that termites will consume the bait, and that termite mortality from feeding on the bait increases over time.

Keywords: termite control, baits, inorganic pesticides, *Reticulitermes flavipes*

INTRODUCTION

During the past ten years, there has been a change in the types of termiticides available for prevention and control of subterranean termites in the U.S. In addition to the traditional barrier-type repellent termiticides, newer non-repellent termiticides, such as imidacloprid (a neonicotinoid) and fipronil (a phenylpyrazole), have come onto the liquid termiticide market. Termiticides applied as bait products have also entered the marketplace in the past ten years, and have been the subject of considerable research attention (Su & Scheffrahn 1998; Grace & Su 2001). Bait active ingredients

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are usually organic compounds that act either as growth regulators (such as hexaflumuron and diflubenzuron) or metabolic poisons (such as sulfluramid and hydramethylnon). Studies of termite control using inorganic pesticides have been performed with both drywood and subterranean termites (Ebeling & Wagner 1959; Wagner & Ebeling 1959; Roomi *et al.* 1990).

The ability of termites to move soil particles of a particular size has been of interest for many years (Ebeling & Pence 1957) and was used as the basis for a number of termite barriers composed of basalt (Tamashiro *et al.* 1991; Yates *et al.* 2003), granite (French *et al.* 2003), and coral (Su *et al.* 1991; Su & Scheffrahn 1992). These barriers block termite movement by having particles of a size too large for the target termite species to move, and yet packs together well enough to prevent passage around the particles. Ecological studies on movement of soil particles have also been conducted (Laker *et al.* 1982) although mostly with the higher termites (Termitidae).

Cryolite is a solid crystal salt of sodium aluminofluoride [$Na_3(AlF_6)$] with insecticidal properties. Crystals are finely ground into various sizes by the manufacturer prior to inclusion in a formulation sold as a crop protecting spray or dust application. Currently, Kryocide® (Cerexagri, Inc.) is sold to control Lepidoptera and Coleoptera in crucifers and some fruit crops. This paper reports on laboratory studies investigating the potential of cryolite crystals in baits for subterranean termite control.

We examined the effect of cryolite particle size on its acceptability and efficacy in controlling native subterranean termites in several α-cellulose bait formulations. Two initial hypotheses were considered: 1) whether both particle sizes (0.2 and 20 µm diameter) of cryolite crystals would result in the same level of termite mortality when applied at the same concentration (wt/wt) in the bait, and 2) whether termite mortality in the first experiment was due to actual consumption of the bait, or to other factors (such as contact toxicity). These hypotheses were studied using pseudergates of the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar).

METHODS AND MATERIALS

Termites

Lengths (~0.3 – 0.6 m) of fallen pine infested with a termite colony were collected from the John W. Starr State Forest located within 16 km of

Starkville, Mississippi. These bolts were kept in corrugated steel trash cans (~114 L) at ambient temperature (~22-24°C) in the laboratory for less than 3 months prior to the start of the test. The termites from this colony were identified morphologically as *R. flavipes* using the key of Scheffrahn and Su (1994).

Bait Preparation

Two cryolite preparations [50% a.i. for the liquid 0.2 μm (wt/vol.) in distilled water, and 100% a.i. for the powder 20 μm (wt/wt)] were used to formulate the baits for this study. Calculations for the amount of cryolite needed for each formulation took into account the differences in a.i. percentages between formulations noted above. Each particle size (0.2 μm and 20 μm) was tested at 0.5 and 1% (and additionally for the 20 μm size, 2%) a.i. wt/wt in powdered α-cellulose (Alphacel®, ICN Nutritional Biochemicals, Cleveland, OH; average fiber length of 35 μm). All treatments are listed in Table 1. Baits consisted only of cellulose and cryolite formulations. For each bait formulation, 12 g of the cellulose and cryolite preparation was vortexed (Barnstead 16100 mixer) for 2 min in a 125 ml Erlenmyer flask. All bait formulations appeared as a dry powder after mixing. After mixing, 1 g of a dry bait formulation was added to an aluminum foil pan in each test arena (see below).

Forced-feeding study

This study was a simple forced-feeding study, using powdered α-cellulose mixed with two sizes of cryolite preparations (described above). Plastic screw-top jars (8 cm diameter × 10 cm height) were used as arenas. Jars were filled with 150 g of Silica sand (40-100 mesh, Fisherbrand, Fisher Scientific,

Table 1. Treatment list for forced feeding study of cryolite crystals in α-cellulose "baits" against *R. flavipes*.

Treatment	% a.i.	n	Description
A	0	10	α-cellulose powder only
B	0.5	10	0.2 μm cryolite crystals in α-cellulose
C	1.0	10	0.2 μm cryolite crystals in α-cellulose
D	0.5	10	20 μm cryolite crystals in α-cellulose
E	1.0	10	20 μm cryolite crystals in α-cellulose
F	2.0	10	20 μm cryolite crystals in α-cellulose

Pittsburgh, PA) and moistened with 30 ml of deionized water the day prior to test initiation. Jars were stored in an unlit incubator at 25±1°C, ~75 % R.H. A bait formulation was presented in a small aluminum foil pan (Reynold's 650 foil, Reynold's Metals Co., Richmond, VA), formed by folding the edges of a 4.5 × 4.5 cm square up (making the depth of the pan ~0.5 cm). The sides of the pan left ~1-2 mm gaps at the corners that were wide enough for termites to enter. A pan was placed on top of the sand in the center of each jar. Each jar was considered a single experimental unit, and there were 10 replicates of each of the six treatments. Thus the total number of experimental units in the experiment was 60.

On the day of test initiation, termites were extracted from infested wood and counted into 60 groups of 100 pseudergates. An additional group of five pseudergates was used to determine individual body mass. One group of 100 pseudergates was added to each jar in the test such that termites did not fall into the bait. Jars were closed and returned to the incubator. After 21 d, the jars were disassembled, and the number of surviving termites recorded.

Mortality was chosen as the character of interest for these studies instead of consumption of the baits in the jars. Consumption values in jar tests are often questionable when using powdered substances as subterranean termites will occasionally remove the material without actually consuming it (Shelton & Grace 2001).

Stained bait study

Due to concerns regarding the source of the mortality observed in the first study, a second study using similar methods was designed. This study would use a lipophilic stain (Sudan Red 7B) to stain the bait provided to termites in a forced-feeding study. This stain must be consumed for the termites to appear stained, and passage via trophallaxis is thought to be minimal (Su *et al.* 1991). From the results of the first study it was noted that only the 20 µm cryolite particle size was of interest, and thus the stained bait study had three treatments: control (stained cellulose bait only), 1% 20 µm cryolite crystal bait, and 2% 20 µm cryolite crystal bait. All baits were prepared in a similar fashion as before, all percentages as weight/weight, with the exception that the calculations accounted for the addition of Sudan Red 7B applied at 0.5% (weight/weight of the final bait mass) as a solution (using acetone as a solvent). For all baits, cryolite crystals were added to the cellulose first (except in controls), mixed for 2 min on the mixer (as above), then the acetone-stain solution added, mixed again for 2 min, and allowed to dry in a vacuum hood for 4 d.

Arenas for this study were the same as described above, and 10 replicates of each treatment were prepared. On the day of test initiation, ~3500 termites were extracted from a single colony of *R. flavipes* (not the same colony used originally). The termites were counted into 30 groups of 100 workers each, with a group of five termites collected for individual body mass determination. One group of termites was added to each jar, and jars placed into the unlit 25±1°C, ~75 % R.H. incubator. The topics of interest here were mortality and the relative number of stained individuals in each jar. Since mortality was expected in the jars containing cryolite baits, it was decided to breakdown the jars by destructive sampling, three from each treatment on days seven and 14, and the remaining jars on day 21.

Statistical analysis

For the first study, the null hypothesis was that no treatment would have percentage mortality significantly different from any other treatment (including the controls). The alternative hypothesis was that at least one treatment would produce significantly different percentage mortality from another treatment. Mortality data were arcsine-square-root transformed and subjected to a General Linear Model in SAS followed by mean separation using REGWQ ($\alpha = 0.05$; SAS Institute 2001).

The second study was a little more complex due to the time component. Percentage mortality was arcsine-square-root transformed and subjected to a mixed model analysis with time as a covariant (SAS Institute 2001). Means were separated using differences of least-squared means with Tukey-Kramer adjustment (SAS Institute 2001). Percentages of surviving termites that were either stained or unstained were subjected to paired t-tests using SAS (SAS Institute 2001). Comparisons were made within treatments for each week of the study.

RESULTS

Mean mass of individual pseudergates in the forced-feeding study was 2.16 ± 0.09 mg. During breakdown of the experiment, only a single pre-soldier was found (in the 0.5% 20 µm treatment), and no nymphs developed during the test. Mean percentage mortality ranged from 20.00 ± 2.38% in the cellulose-only control to 83.00 ± 2.85% in the 2% 20 µm cryolite treatment (Fig. 1). Results from the general linear model and mean separation indicated that only the three 20 µm treatments resulted in significantly different percentage mortality than control mortality ($F_{5,59} = 68.39$; $P < 0.0001$). Percentage mortality was significantly greater in 20 µm cryolite treatments with greater percentages of cryolite (Fig. 1). All three 20 µm treatments were significantly different from one another and from the 0.2 µm treatments and controls.

For the second study, the mean mass of individuals used was 2.84 ± 0.16 mg. The percentage mortality results are illustrated in Fig. 2. Mortality increased as the experiment progressed ($F_{8,21} = 15.5$; $P < 0.0001$) beginning with no significant differences among the treatments in the first week. However, by the second week the 2% 20 µm cryolite bait treatment had significantly

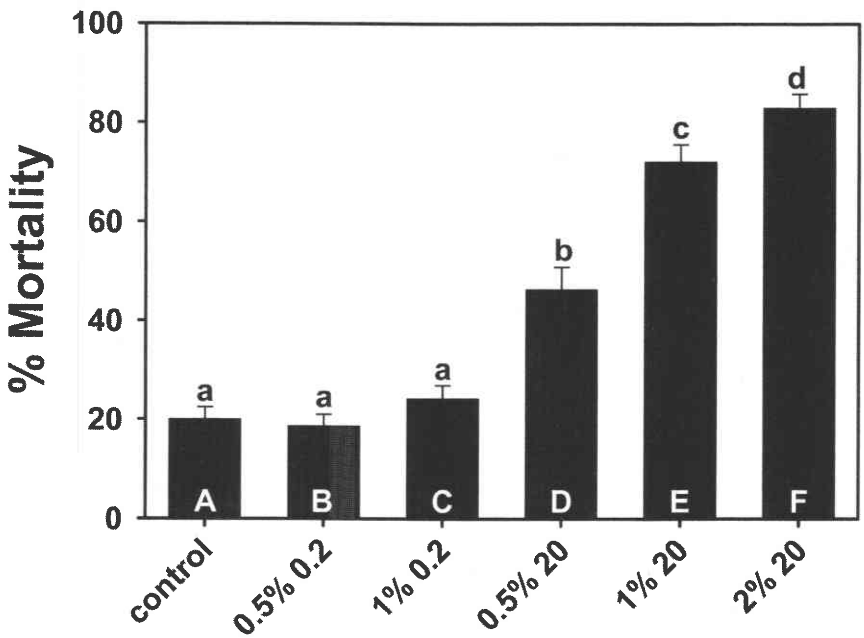

Fig. 1. Mean (±SEM) percentage mortality of *R. flavipes* workers fed α-cellulose baits containing cryolite crystals at various percentage compositions and sizes (in µm). Upper case letters in bars indicate treatments described in Table 1. Bars having the same lower case letter above them are not significantly different (GLM, REGWQ; α = 0.05).

greater mortality than the remaining treatments. In the final week, both cryolite baits had significantly greater mortality than the controls, and were significantly different from one another (with 2% 20 μm cryolite having the greatest mortality).

The goal of the second study was to examine whether termites would consume the bait, and this was done by examining termites interacting with stained bait. Paired t-tests of the percentage survivors who were stained *vs.* unstained were performed for each week and treatment separately. In all three weeks, significantly more control termites were stained than unstained (week 1, 2, 3: dF = 2, 2, 3; t = 22.75, 32.38, 46.33; $P = 0.0019, 0.0010, < 0.0001$). For the 1% 20 μm cryolite bait, there were no differences on any week between the percentage of stained and unstained survivors (dF = 2, 2, 3; t = 1.41, -0.49, 1.56; $P = 0.2945, 0.6714, 0.2177$). For the 2% 20 mm cryolite bait, the only significant difference between the percentage stained and unstained survivors

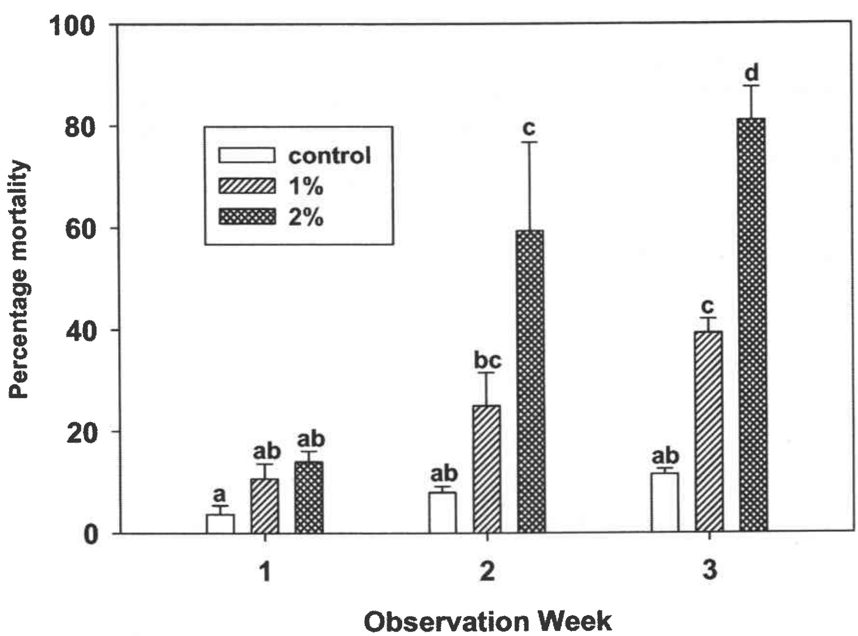

Fig. 2. Percentage mortality by observation week resulting from interaction with stained baits containing 0, 1 and 2% 20 μm cryolite crystals. Bars having the same lower case letter above them are not significantly different [Mixed model, Least Squared Means (Tukey-Kramer adjustment); $\alpha = 0.05$].

occurred during the second week, when the unstained survivors were more numerous (dF = 2, 2, 3; t = -1.02, -9.63, -1.71; P = 0.4157, 0.0106, 0.1853). Figure 3 illustrates the stained *vs.* unstained results for each treatment.

DISCUSSION

The results of the first study indicate that particle size of this mineral insecticide is a significant consideration for controlling termites when incorporated into and applied as a bait formulation. For cryolite crystals, baits containing larger particle sizes (20 μm) were more effective at controlling *R. flavipes* than baits containing the smaller particle size (0.2 μm).

The mode of action of cryolite crystals is unknown. One possibility is that they may have a physical mode of action by abrading the crop and foregut leading to desiccation. Another possible mode of action of cryolite crystals is fluoride toxicity (Ware 1994). Unfortunately, little data is available on the effects of cryolite against termites, particularly in terms of desiccation studies. A closely related compound, sodium fluoride powder (NaF; technical grade, 95% purity) was examined by Ebeling and Wagner (1959) in the first of two large studies examining "sorptive dusts" against *Kalotermes minor* (Hagen) (now known as *Incisitermes minor*) alates and nymphs. Their studies showed that termites shaken in dry NaF powder (and cleaned afterwards) took anywhere from 4.5-5.1 hrs to obtain 50% mortality for alates. It took greater than 24 hrs of constant exposure to NaF powder to obtain 100% mortality with nymphs (Ebeling &Wagner 1959). Their data, which included many other sorptive dusts, suggested that abrasion was not the likely cause of desiccation with the dusts, but instead the removal of the outer cuticular wax layer was the culprit. They suggest that as a secondary effect after the wax layer has been damaged, water-soluble fluoride (in some of the dusts) would be able to enter the insect acting as an insecticide. However, it must be noted that this NaF powder was not cryolite [$(Na_3(AlF_6)$], and Ebeling and Wagner (1959) were using nearly a 95% a.i. with direct termite exposures not as bait toxicants. Data from our first study suggest that the activity of cryolite crystals may be partly from abrasion, as only the larger size crystals were able to kill termites. The results of the second study indicate that the termites do indeed consume the bait (Fig. 3). However, these results do not prove that abrasion is the only mode of action the crystals may have on termites. While data for topical

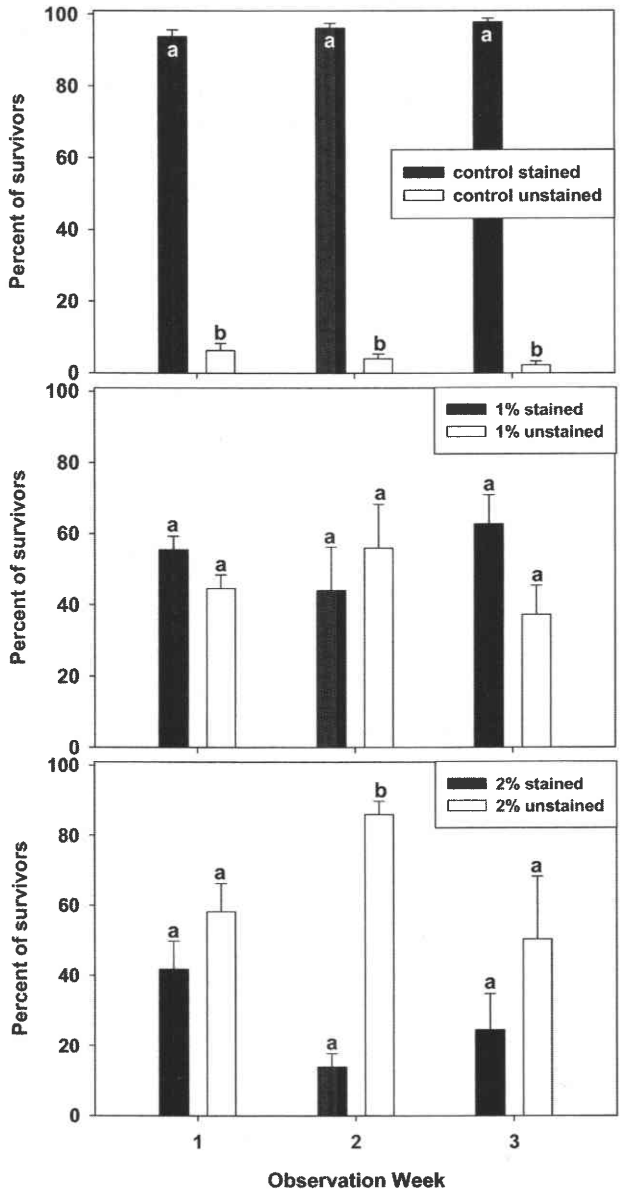

Fig. 3. Percentage of surviving termites that were either stained or unstained for each observation week. Comparisons are made within treatments by observation week. Bars having the same lower case letter above them are not significantly different (within weeks, concentration; t-test; $\alpha = 0.05$).

application of cryolite crystals to termites are unavailable, a study of topical application of this insecticide to third instar larvae and adults of *Coleomegilla maculata lengi* Timberlake (Coleoptera: Coccinellidae) provides some insight (Lucas *et al.* 2004). In their study, cryolite crystals (as Kryocide®, same as this study) applied even at 10x the foliar field rate was unable to produce enough mortality to develop LD_{50} estimates for either life stage of *C. m. lengi*; their Fig. 1 indicates less than 20% mortality at six days from any concentration (Lucas *et al.* 2004). Thus, it seems unlikely that mortality observed in our study was the result of cuticular contact with the material, and more likely was the result of consumption.

One question about the potential use of cryolite as an a.i. for termiticidal baits is whether termites actually eat the bait, or are killed via contact toxicity. Results of the second study indicate that the bait itself was consumed during the experiments (Fig. 3). During preliminary investigations, attempts to stain the cryolite crystals alone were unsuccessful. Given this observation, it is very likely that only the cellulose in the bait (which was the majority of the bait by weight) held any stain. This means that although it can be said from the staining of the surviving termites that the bait as a whole was consumed, it has made it impossible to determine if the crystals specifically were consumed. With this in mind, in Fig. 2 it can be seen that the control mortality is fairly low compared with that of the treatments where the bait contained cryolite crystals. In fact, by the final week of the experiment, both cryolite-containing bait treatments had significantly greater mortality than the controls. If termites were selectively avoiding the crystals within the baits as they fed (somewhat unlikely given the size), then there would be very little difference in mortality among the three treatments. The results of this test suggest that the termites are consuming the crystals along with the cellulose of the baits.

In the second study, surviving termites were stained greater than 90% of the time [range: 93.7 ± 1.92 (week 1) to 97.75 ± 1.03 % (week 3)] in control replicates. The percentage of stained survivors goes down to roughly half this amount for the 1% cryolite bait [range: 44.0 ± 12.3 (week 2) to 62.75 ± 8.19 % (week 3)], and roughly a third of this for the 2% cryolite bait [range: 13.96 ± 3.74 (week 2) to 41.78 ± 8.07 % (week 1)]. These data indicate that the termites may be associating mortality with the bait and possibly reducing their consumption in the 1% and 2% cryolite bait treatments. This is supported by the reduced percentage of stained survivors over the weeks in the 2% treatment (week 1 having the highest percentage of stained survivors). This association may be tied to the concentration of the cryolite crystals in the bait, as the 1% cryolite bait treatment, while having fewer stained individuals than the controls, seemed to be relatively stable in that percentage over the course of the study (Fig. 3). While the obvious suggestion is to use less than 2% a.i. in future studies, the first study indicates that reduction to less than 0.5% may reduce termite mortality too far (Fig. 1).

This study is considerably different from other research examining acceptability of particle sizes (Ebeling & Pence 1957; Tamashiro *et al.* 1991; Su *et al.* 1991; Su & Scheffrahn 1992). Particle size acceptability in termite control has been more of a consideration for physical barriers than for any other type of control method. Both field (Su & Scheffrahn 1992) and laboratory (Su *et al.* 1991) studies have examined the particle size ranges necessary for exclusion of *R. flavipes* foragers. In both studies, particle sizes ranging from 1.18 to 2.80 mm were most effective in preventing termite passage. Obviously, the a.i. particles used in the current study are an order of magnitude smaller than those necessary for physical barriers. Data from this study have indicated that for solid active ingredients, particle size can be an important consideration particularly for bait applications.

This paper has presented data indicating that particle size for a solid a.i. included as part of a subterranean termite bait matrix can be an important consideration in designing bait formulations. Further research is needed on the crystals of the mineral insecticide cryolite, particularly multiple choice feeding tests to examine the possible repellency issue, for use as a control measure against subterranean termites.

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