IRG/WP 08-10661

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Transfer of Termiticidal Dust Compounds and their Effects on Symbiotic Protozoa of *Reticulitermes flavipes* (Kollar)

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Paper prepared for the 39th Annual Meeting
Istanbul, Turkey
25-29 May 2008

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ABSTRACT

Dusting of termites in situ has been used as a control measure for decades; however environmental awareness of the toxicity of certain compounds now limits their use (eg arsenical dusts). Our laboratory is in the process of suppressing an isolated colony of *Reticulitermes flavipes* (Kollar) from a small village of 250 homes in mid-central Wisconsin (Endeavor, WI). Initial treatment of the colony involved the use of 200 Microgen bait cartridges containing 0.25% diflubenzuron. The presence of many living termites occupying bait stations throughout the season prompted the addition of various dusting compounds, such as boron, N-N'naptahaloylhydroxylamine (NHA) and other dusts to aide in colony elimination. This laboratory study explores the efficacy of various insecticidal dusts to kill termites as well as to transfer the insecticide to nest mates. Mortality after primary (direct) dusting was found to occur with sodium borax, zinc borate, concrobium polymer (CP) and NHA in 7, 9, 9 and 21 days respectively. However, the ability to cause mortality to 25 un-dusted termites was impaired in the concrobium group and delayed in others. Further tests were performed to determine the mechanism of transfer by examining the survival rates of the symbiotic flagellated protists in the termite hindgut over time after dusting to determine if termiticidal dusts were killing the protozoa necessary for cellulose digestion. Possible mechanisms of mortality are discussed as well as the potential of using dusting compounds in termite treatment or eradication.

Keywords: *Reticulitermes flavipes* (Kollar), insecticidal dusts, symbiotic protozoa, grooming, trophallaxis.

1. INTRODUCTION

Insecticidal dusts have been used against termites for decades (Madden *et al* 2000). The use of arsenic trioxide against termites became widespread in Australia in the first half of the 20th century, but the introduction and subsequent popularity of the organochlorines led to a decline in the use of arsenic (Madden 2001). In the United States, dusting of termites to eliminate colonies fell out-of –favour due to labour intensity and current methods of termite control include improved wood preservatives, soil termiticides, and termite baiting.

Currently, there is a renewed interest in the use of bait and dust toxicants to suppress and even eliminate termite colonies. While the chemical termite barrier is effective in isolating termite activity from structures, the termite colony remains active outside the treated zone (Su and Scheffrahn 1988). The utilization of bait and dust toxicants makes use of termite social behavior, and therefore, does not require determining the location of the nest as termites will distribute the toxicant to other colony members through grooming, contact, cannibalism, and trophallaxis (Ahmed 2000 and 2001). The use of baits and dusts also allows for reduced amounts of insecticide required to treat a termite colony, and therefore reduces the environmental hazard when compared with some other treatment methods (Ahmed 2000). Boron compounds are also thought to have a low mammalian toxicity, thereby making them a more environmentally friendly termite treatment compound.

Recently, Dr. Timothy G. Miles, Director, Urban Entomology Research Project, Toronto, Canada described a simple and more effective method of termite control described as: Trap, Treat and Release (TTR).

> "Both baiting and Trap-Treat-Release are new developments in termite control. Both of these methods use relatively small amounts of chemicals which are aimed at killing large termite populations. We may therefore term these approaches as population-suppression methods (Miles 2001)".

Esenther (1980) utilized a similar technique termed "mark-recapture" in which marked termites were collected in the field, dyed, and recaptured to estimate the size of a subterranean termite colony. Later, Esenther (1985) used this technique to obtain termites in the field for use in laboratory evaluation of transfer of Avermectin BI dust in a simulated and accelerated field test. The successful transfer of the insecticide in these laboratory studies supports the use of dusts in colony elimination.

In a series of papers, Grace (1990; 1991) investigated the use of borate compounds (eg boric acid, disodium octaborate tetrahydrate (DOT), and zinc borate) on the ability to kill primary (dusted) and secondary nest mates of *R. flavipes* and *Coptotermes formosanus* Shiraki. In these tests, boric acid was found to cause the most rapid mortality, while the other two compounds killed 100% of termites within 15 days. He also found that treatment of 10% of *R. flavipes* with either zinc borate or DOT resulted in 70-98% mortality of the group (Grace 1991). The ability of *R. flavipes* to tunnel through borate treated sand was also evaluated. Results found that a 10 day exposure caused significant mortality to the termites (Grace 1991).

In this paper we present data that illustrates the length of time it takes for four insecticidal dusts to cause mortality in *R. flavipes* when a) all 50 termites are dusted with a small amount of the insecticide and b) when only 50% are directly dusted. The second aspect of the study was to determine whether or not the dust was causing the elimination of the hindgut symbiotic protists prior to death of the termites. We show that in some cases only the primary dusted termites die whereas in other preparations the morbidity and mortality is transferred to the secondary un-dusted termites by trophallaxis, grooming or other social activities thus killing nest mates at a nearly similar rate as the dusted termites. The latter dusts (ie sodium borax, Zn borate, and possibly NHA) would be more suitable for colony suppression and elimination.

2. EXPERIMENTAL METHODS

2.1 Transfer of Insecticidal Dust to Colony Members

Eastern subterranean termites (*R. flavipes*) were collected using corrugated cardboard bait traps at Janesville, WI during the summer of 2007. Groups of termites (25 or 50) were dusted with a small amount of chemical dust (0.005-0.010g) in a plastic weigh boat. Groups of 50 workers were placed in cylindrical plastic container (50mm d, 40mm h) in a humidified incubator (28°C, 85%RH) with Whitman #1 filter paper as a food source. Groups of 25 dusted workers were added to 25 un-dusted workers as above. Termites were inspected daily for mortality. Chemical powders tested were: sodium borax, zinc borate, NHA and antimicrobial concrobium polymer (Siamons International, Toronto, Ontario).

2.1.1 Transfer of Dust to Un-Dusted Colony Members Through Trophallaxis

Although contact and grooming are thought to be the most efficient mechanism of dust transfer in colony members, the movement of a dusting compound by means of trophallaxis was also evaluated. Ten workers around the fifth instar were dusted with Neutral Red dye (Central Scientific Co., Chicago, IL) and left in a dish overnight so that all the dust was ingested (n=3). The 10 dyed workers were then combined with 10 un-dusted/ un-dyed workers with Whitman #1 filter paper as a food source. Termites were dismantled daily for eight days and termites with a red/ pink color were counted and compared to the number of workers without any coloration.

2.2 Effect of Dusts on Hindgut Symbiotic Protozoa

In order to evaluate the effect of dusts on hindgut protozoa, groups of 25 termites, as above, were dusted with sodium borax, zinc borate, NHA or antimicrobial concrobium polymer, and maintained on filter paper in a humidified incubator. At times 24, 48, 72 hours and 5-7-9 days individual termites were removed and their hindguts examined under a phase contrast Zeiss compound microscope following the methods described by Lewis and Forschler (2006). Viability and type of symbiotic hindgut protozoans present were determined (n=3). Data was recorded to determine termiticidal activity of dusts to hindgut symbionts.

2.3 Dust Injection Assay

Simulated field testing arenas, developed by Esenther (1985) were used to evaluate the potential efficacy of dusts pushed into termite tunnels as well as to determine insecticide repellancy (Figure 1). These tests contained ~ 50 worker termites, placed at the far end of the arena in the nest box and were maintained on Whitman #1 filter paper. Insecticidal dust (~0.005 g) was then pushed through the last tube of the arena with an air can, furthest from the termites. Daily mortality was noted.

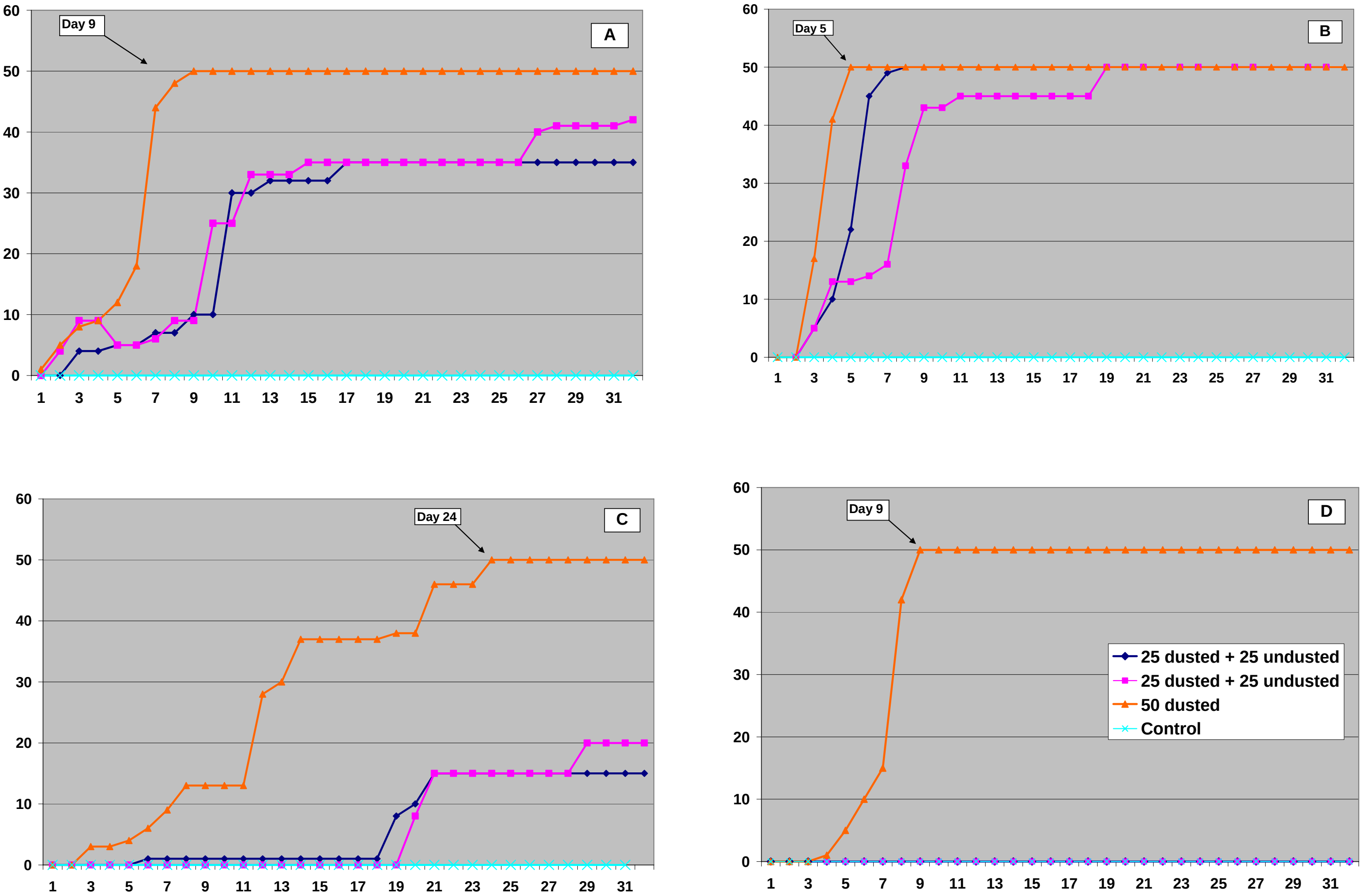
Figure 1: Simulated Field Testing Arena

3. RESULTS AND DISCUSSION

3.1 Transfer of Insecticidal Dust to Colony Members

Results of dusting experiments and mortality of *R. flavipes* are shown in Figure 2. Termite mortality was observed in the Zn borate and sodium borax groups within 2 days. 100% mortality was seen in the 50 dusted sets on day five for sodium borax, day nine in the Zn borate group and on day nine in the Concrobium group. The NHA group reached 100% mortality more slowly on day 24. N'N-napthalimide has previously been thought to act as a non-repellent, slow-acting stomach poison (Green *et al* 2001; Rojas *et al* 2004).

Figure 2: Mortality of dusted termite groups; x-axis days; y-axis mortality (x/50): (A) Zinc Boron, (B) Sodium borax, (C) NHA, (D) Concrobium polymer (duplicate sets of 25 + 25 are reported)



The four groups of termites with all 50 workers dusted were similar in that all dusted termites died by day 24. However, the groups became divergent with the 25 dusted + 25 un-dusted termites. The fastest groups to reach 100% mortality were those dusted with sodium borax—ie day 8-18 (Group B). Thus, transfer of the sodium borax was rapid and fatal to 100% termites within 3 weeks. The next highest was Zn borate (Group A) whereas by day 32, approximately 77% of termites were dead. NHA (Group C) had only 35% mortality with little change over the previous week. It appears that neither of the groups of 25 dusted affected the un-dusted group, though because NHA tends to be slow acting mortality may have been delayed past the 30 day mark. The Concrobium group (Group D) was most interesting in that 100% of the original 50 dusted termites died, but 100% of termites were alive at Day 32 in the combined 25 + 25 group. The reason for this phenomenon is unclear, but it may reflect dilution of the concrobium below the toxic threshold.

3.1.1 Transfer of Dusts Through Trophallaxis

All groups started with 50% dyed (red) and 50% un-dyed (white) termites. By the last time point less than 10% of termites remained without noticeable dye coloration (Figure 3 and Figure 4). The results show that un-exposed/ un-dusted termites can be affected by a dusting compound even after it is consumed through grooming by other colony members. These results provide further support for the use of dusting compounds in a colony eradication program by their effective transfer utilizing two different mechanisms of termite social behavior.

Figure 3: Potential transfer of a dust compound utilizing neutral red dye as a marker; Percent red after eight days

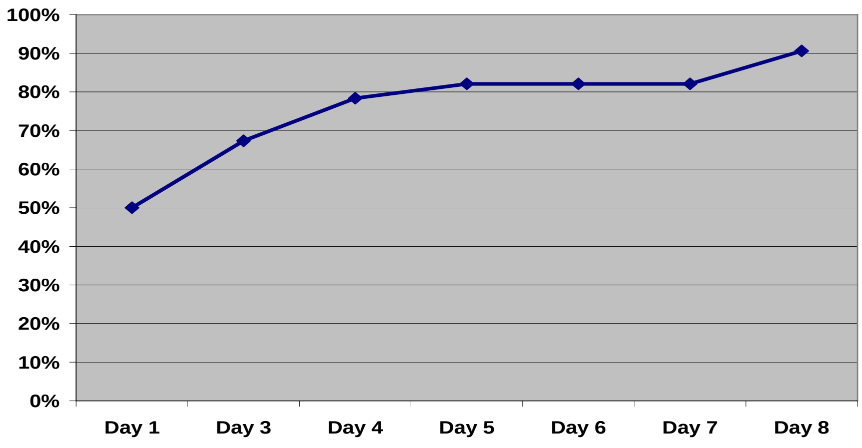


Figure 4: Dyed termite worker

3.2 Effect of Dusts on Hindgut Symbiotic Protozoa

In order to investigate i) possible mechanism of killing in Groups A, B, and C. and ii) possible mechanism of recovery in Group D we examined groups of termites treated with each of the insecticidal dusts to observe the symbiotic biota of the hindgut—more specifically the protozoa flagellate species.

Eleven species of protists are known from the hindgut of *R. flavipes* (Lewis and Forschler 2006). Although species of protists present/absent in the dissected guts were not counted, mortality and general observations were noted. There was some variation among individuals dissected, so conclusions were drawn from sets of three termites for each time period. Some mortality of larger gut protists was seen as early as day one in termites dusted with sodium borax. The most significant mortality of protozoa was seen around day three for sodium borax, with mortality of the termites occurring shortly after. Mortality of NHA dusted protozoa was also seen around day one, but the most significant mortality of the protists occurred between day five and day nine. Termites dusted with concrobium showed a more inconclusive result in hindgut protozoa mortality in that results seemed to vary among individuals and at different time periods. Overall observations showed that larger protists (ie *Spironympha* sp., *Pyrsonympha* sp., *Spirotrichonympha* sp., *Trichonympha* sp.; Figure 5) seemed to be more sensitive to chemical dusts earlier than the other protists. Conversely, *Dinenympha gracilis* Leidy appeared to be the least sensitive organism to the dusts, and was often the only protozoa active at later time periods in dusted termites. Although both sodium borax and NHA appear to act as a hindgut stomach poison, mortality of termites was seen at a later time period in the NHA group, suggesting that sodium borax may cause mortality by means of two different mechanisms (eg external cuticular abrasion).

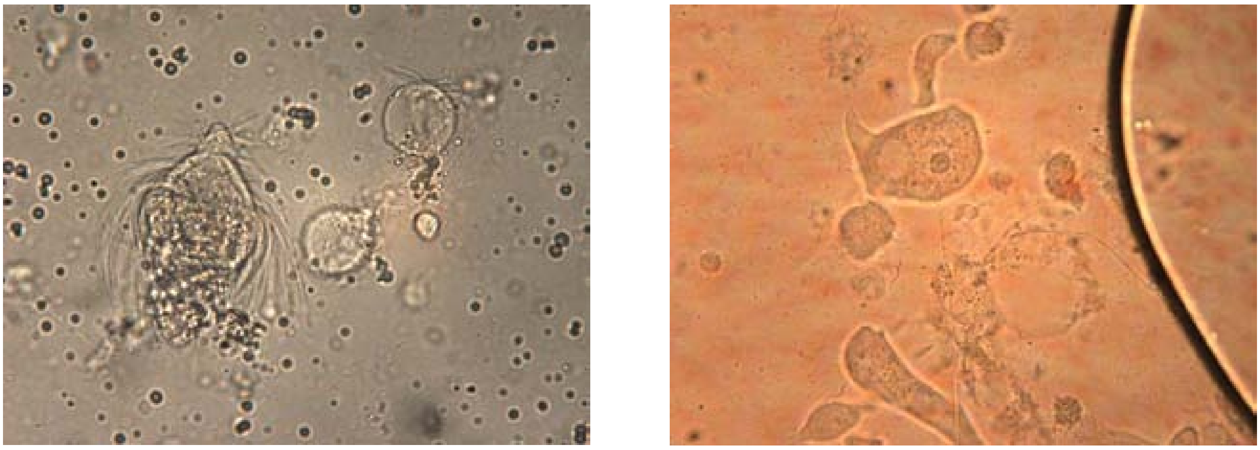


Figure 5: Light microscope images (40X) of hindgut protozoa (Left: *Spironympha* sp.? Right: *Pyrsonympha* sp.? Lewis and Forschler 2006, key)

3.3 Dust Injection Assay

Previous tests using NHA dust in the simulated field testing arena described above had shown 100% mortality within three to four weeks, which is comparable with the results of this study (unpublished result). As observed, NHA is a non-repellent, slow-acting stomach poison in either dust for or as a wood preservative (Green *et al* 2001). Sodium borax dust pushed through the arena caused ~50% mortality of the termites at day five. Unlike NHA and sodium borax, concrobium appeared to act as a repellent as termites did not forage to the dusted end of the testing arena.

4. CONCLUSION

Preliminary results from this laboratory study indicate that morbidity and mortality varied widely in the response of *R. flavipes* to various insecticidal dusts. Although all dusts resulted in 100% mortality of dusted termites (5-24 days), results varied measurably when groups of 25 dusted workers were combined with 25 un-dusted workers. Only borates were capable of 100% mortality of un-dusted workers within the time frame of the study—5 weeks. Although concrobium antimicrobial powder killed 100% of the dusted termites, combination with un-dusted control termites (25) negated or blocked any mortality in the combined groups, possibly by dilution? We conclude that for some products, dilution of dusts by un-dusted/recipient termites at the colony level may abrogate any measurable mortality in primary-dusted termites, and therefore should be an adjunct to other feeding or barrier control methods. However, borate and NHA dusts caused increasing mortality in secondary colony mates, and therefore have an effect on termite population control. We intend to further study the potential of dust transfer in termite treatment as additional commercial dusting compounds become available.

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