Observations on colony collapse in Reticulitermes flavipes (Kollar) in laboratory and field settings in Wisconsin

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Observations on colony collapse in *Reticulitermes flavipes* (Kollar) in laboratory and field settings in Wisconsin

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ABSTRACT

Parallel strategies were designed to eliminate *Reticulitermes flavipes* (Kollar) from a field site in Endeavor, Wisconsin and a simulated field test setup of approximately 20,000 workers in the laboratory. Indoor and outdoor colonies of *R. flavipes* were baited with commercial cellulose monitoring stations and rolled cardboard stations. If the commercial cellulose baits were attacked, they were replaced with termidicidal baits containing 0.25% diflubenzuron (a chitin synthase inhibitor). In active cardboard stations, termites were dusted with N'N-naphthaloylhydroxylamine (NHA) and released back into the colony. Overtime, diflubenzuron gradually suppressed worker activity and termite numbers both in the laboratory and the field. However, sharp reductions (>90%) in foraging workers were observed in both field and laboratory colonies with the addition of dusting with NHA. Termidicidal baits containing 0.5% hexaflumeron were secondarily evaluated in the field as a comparison to diflubenzuron. Observations indicated five notable characteristics or criteria of a colony on the verge collapse in one or both venues: i) increasing soldier to worker ratios >20% in the lab, ii) decreasing overall counts of workers collected, iii) increasing numbers of secondary reproductives captured in hexaflumeron bait cartridges outdoors, iv) increasing susceptibility to mites, and v) higher microbial load including bacteria, fungi and slime molds within the colony. Shortly after these events occur—foraging workers disappeared from both commercial and cardboard stations and the colony was essentially eliminated. Although laboratory results do not exactly mirror field results, observations regarding colony decline in both venues are significant when attempting termite control. We conclude that combinations of termite toxicants are more effective than either one alone, and that the above observations may be used as an indicator of sucessful termite treatment.

Keywords: *Reticulitermes flavipes* (Kollar), termite baiting, diflubenzuron, hexaflumeron, colony elimination, NHA

1. INTRODUCTION

Reticulitermes flavipes (Kollar) is one of the most destructive species, causing significant damage to manmade wooden products (Su and Scheffrahn 1993). Termite damage is more common and costly than that caused by storms, fires and earthquakes and the cost is rarely covered by homeowner's insurance. Repairs, monitoring and elimination of termites can run as high as $40,000-50,000 for a single family home (Dan Rich, personal communication 2009).

Despite extensive research, termite treatment is far from an exact science. For subterranean termites, this is at least in part a result of not being able to observe termite behavior underground, making field results difficult or impossible to interpret. The advent of termite baiting systems, has shifted focus from single structure treatment to community-wide eradication programs in certain situations (e.g. Getty et al. 2000, Kistner and Sbragia 2000; Verkerk and Bravery 2004; Smith et al. 2006). Use of commercial baiting matrices allows for a small window into the colony when stations are active, and is at times the only indication of colony decline. Little has been published regarding field observations during termite colony treatment, but understanding these observations is imperative to understanding the success of an eradication program.

Commercial termite baiting systems are designed to suppress and eliminate termite infestations thus abrogating the estimated $11 billion dollar pricetag placed on termite damage and repair to wood-in-service in the USA annually (Su 2002). There is some disagreement as to whether these commercial systems are up to the task of complete elimination—some advertising suggests that two years may be needed to attract and bait the termites and then an additional 3-8 years is necessary to eliminate the offending colonies(s). Some might say the time and cost of elimination is excessive or a target that is seldom, if ever, effectively achieved. The exact amount of time to eliminate an existing population varies depending on termite species, geographical factors and effectivness of the toxin concentration.

Colony collapse in termites may be compared to colony collapse disorder (CCD) recently noted in honey bees. Current theories about the cause(s) of CCD in honey bees include increased losses due to an invasive mite, new or emerging diseases or viruses, and pesticide poisoning (through exposure to pesticides applied for crop pest control or for in-hive insect or mite control). In addition to these suspects, perhaps the most highly-suspected cause of CCD is a potential immune-suppressing stress on bees, caused by one or a combination of several factors. Stresses may include poor nutrition, drought, and migratory stress brought about by the increased need to move bees long distances to provide pollination services. Researchers suspect that stress could be compromising the immune system of bees, making colonies more susceptible to disease (Ratnieks and Carreck 2010).

During termite treatment in Wisconsin we have noticed similar phenomenon during colony decline such as increased susceptability to fungi or an over abundance of mites (Fig. 1 a&b), where as healthy termite colonies are normally free of microbial overgrowths. We observed termites taken from field bait cartridges and maintained in the laboratory to have a red pigmented bacterial infection on their head and legs upon death, probably from *Serratia marcescens* Bizio, which has been shown to have low pathogenicity in *Coptotermes formosanus* Shiraki with surpressed immune systems (Connick et al. 2001).

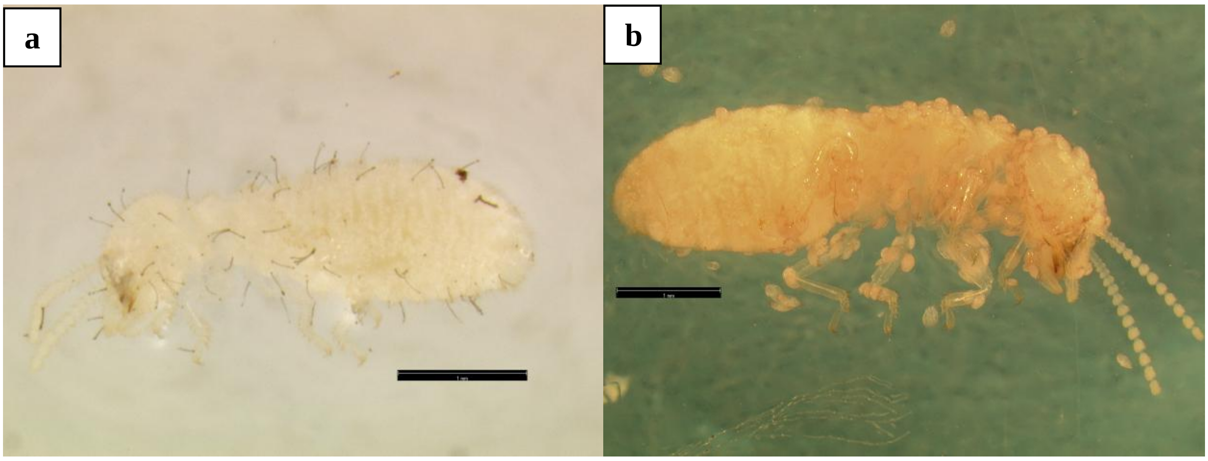

Figure 1: *Reticulitermes flavipes* from hexaflumeron and diflubenzuron cartridges consecutively (scale bar = 1mm); 1a worker termite with potential fungal pathogen; 1b worker termite covered with mites.

In Wisconsin, a community-wide eradication program was developed for the eastern subterranean termite *R. flavipes*, which had become a major invasive pest in the small village of Endeavor in the central part of the state (DeMark and Thomas 2000, Arango and Green 2007). The isolated, and relatively small nature of the infestation made a community-wide program preferable to single structure treatment. Initially, diflubenzuron was chosen as the active ingredient for the baiting cartridges as all pest control operators dealing with termites in the state use this product, including homes already being treated for termites in the village. One season of treatment seemed to have a significant effect on the colony at one location (Appendix 1: zone 1), but little to no effect at other active sites (Appendix 1: zones 2-4).

We decided to use a multi-method, non-traditional approach for treatment of existing termite colonies, with the primary goal of killing and eliminating the infestation (Nibre and Nunes 2007). Certain commercial termite baiting systems containing insect growth regulators clearly suppress the colonies that intercept wood, cardboard or cellulose baits, but suppression may fall short of elimination and requires continuous monitoring and replacement of termite baits over a period of years which adds time and expense. We hypothesized that a combination of baiting and trap-treat and release (Myles 1997) dusting of *R. flavipes* with N‘N-napthalohydroxylamine (NHA) should be able to eliminate colonies or at least suppress them below detectible levels requiring a long interval of rebuilding and recovery of the colonies. Our results show conclusively that field colonies and laboratory colonies can be collapsed by multiple target strategies with specific observations indicating colony decline.

2. MATERIALS AND METHODS

Simulated field test:
A simulated laboratory field trial was initiated in the Fall 2008 designed to parallel or confirm results observed in the field. The intention was to mimic the sharp reduction of termites observed in zone two in Endeavor, WI with a large lab colony of approximately 20,000 workers. A laundry basket (91.4 x 61.0 x 61.0 cm) containing sand, dimensional lumber, as well as dirt and debris from the collection site was seeded with *R. flavipes* from Janesville, WI during the summer of 2008. Monitoring stations at each of the four corners were comprised of rolled corrugated cardboard to serve as collection sites for counting worker and soldier termites. On day one of the lab trial, 650 workers were dusted with NHA (Avocado Research Chemicals Ltd., CAS No. 6207-89-02) and released. On day three another 350 workers were dusted with NHA and released back into the laboratory colony (Phase I). Population size was initially estimated by the Lincoln-Peterson model of mark-recapture using dyed termites. Total termite counts were monitored (November 24-January 23) to determine the effectiveness of NHA dust alone before a single commercial bait station, containing the chitin synthase inhibitor (CSI) diflubenzuron, was added to the center of the colony (Phase II). Termite counts continued to be monitored until there was no more activity observed.

Field Methods and Observations:
Field trials were initiated with 200 commercial bait stations circumscribing the small village of Endeavor, WI in 2006 (see Arango and Green 2007, Green et al. 2008a). After one year, outer stations were concentrated into the village center and near main sites of termite activity (2007) (Phase I). After one additional year of commercial baiting with diflubenzuron, it was determined that some additional strategy was needed for complete termite elimination. It was decided to employ the trap-treat-release technique of Myles (1997) and the termidicide NHA was selected as the dust after limited success with borate dusting (Phase II). Approximately 5-6 grams of *R. flavipes* trapped in cardboard were dusted and released the same day in October 2008 (Appendix 1: zone 2). In Phase III, starting in 2009, we switched baits to those containing 0.5% hexaflumuron (Appendix 1: zones 3-4), where diflubenzuron had been used previously to note any improvements with a new active ingredient.

Examination of activity in all postitive locations suggested at least five, likely separate colonies/populations in downtown Endeavor. An overview of treatments applied and observations made at each of these zones in the field is presented in Table 1. An illustrated map of each of these locations is shown in Appendix 1.

Table 1: Treatments and observations from proposed zones of termite activity in Endeavor, WI.
*See also Appendix 1.

Zone*	Primary treatment	Secondary treatment	Last date of termite activity	Observed Results
1	Diflubenzuron	NHA dusting	16 September 2008	Baiting in this area began in 2006. After one year of baiting, activity in this area seemed to be eliminated using the baiting cartridges; termites were found to be present again one year later in one of the initially positive stations and were dusted with NHA. No further activity was noted.
2	Diflubenzuron	NHA dusting	17 October 2008	Baiting in this area began in 2007. After two full summers of baiting with diflubenzuron no obvious reduction was noted. ~5,000-6,000 individuals were pulled from a collecting unit and dusted directly with NHA. Three weeks later termite activity was suppressed nearly 90%. Termite activity was eliminated by the following inspection.
3	Diflubenzuron	Hexaflumeron	02 October 2009	Baiting in this area began in 2007. Despite its close proximity to zone 2, NHA dusting in that location did not have an effect on this population. Hexaflumeron stations were added mid-July 2009. On the last date of termite activity there was a predominance of secondary reproductives in the stations. The following inspections showed no further activity.
4	Diflubenzuron	Hexaflumeron	02 September 2009	Baiting in this area began in 2007. Hexaflumeron stations were added mid-July 2009. Nearly one month later there was a predominance of secondary reproductives in the stations for two inspections. Subsequent inspections showed no further activity.
5	Diflubenzuron	None	31 July 2008	Termites in this area were being treated by a PCO for a number of years before this project began using Diflubenzuron. No activity had been noted since 2006, until one station was found to be active around the 31st of July 08. Following inspections showed no further termite activity.

3. RESULTS AND DISCUSSION

Simulated field test:

Results from the laboratory colony examining the effectiveness of NHA + diflubenzuron are given in Figure 2. A sharp decline in absolute worker count was observed on days 3-15—but then counts rose again to over 1000 on days 32 and 50 indicating that the NHA dusting had not outright killed the laboratory colony within 30 days. At this time termite baiting with commercial diflubenzuron was initiated, after which the colony began to decline; absolute numbers dropped and the colony was killed on day 164 (week 23). During the decline of foraging workers captured in cardboard traps, the most noteworthy observation was that the percent soldiers rose from below 5% to nearly 25% during the 23 weeks of the experiment. NHA dusting alone seemed to have an immediate effect on termite numbers, but was not sustained. It appears that NHA may act as a weakening agent that increases susceptibility to diflubenzuron, which is alluded to in the patent by Rojas et al. (2004).

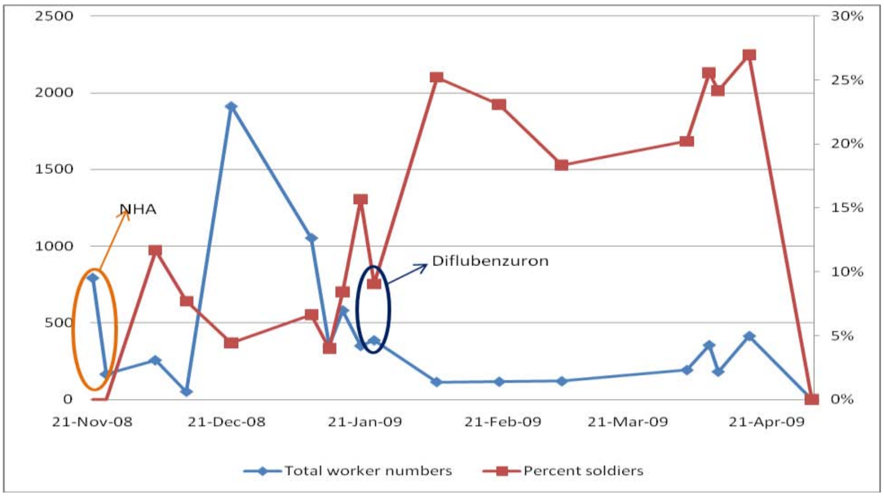

Figure 2: Total number of workers compared with percent soldiers in cardboard monitoring stations (y axis=total number of workers/ z axis=percent soldiers) (from laboratory test)

Field Methods and Observations:

Before the observed results in the field could be analysed, a hypothesized development chart for termites in Wisconsin was created (Fig. 3). Since there are such strong fluctuations in seasonal temperatures in the state, termites are thought to develop differently in Wisconsin compared to areas in the southern United States (Arango et al. 2007). Early instar larvae (1st-3rd) are most prevalent during early spring and are tended by workers which are present year-round. Larvae are thought to molt quickly during this time of development. Production of alates tends to occur within heated structures in late winter to early in the spring and is likely to be predetermined early in the development process. Alates are not known to swarm independent of heated structures in Wisconsin, indicating differentiation may be triggered by temperature or temperature fluctuations. Nymphs are proposed to develop during or after the fourth instar (usually in the spring) with soldier development occurring in the fifth instar or later. Neotenics could develop sometime around or after the sixth instar, with older workers capable of differentiating into neotenics depending on colony requirements. Reproductive forms are not thought to overwinter (Arango et al. 2007).

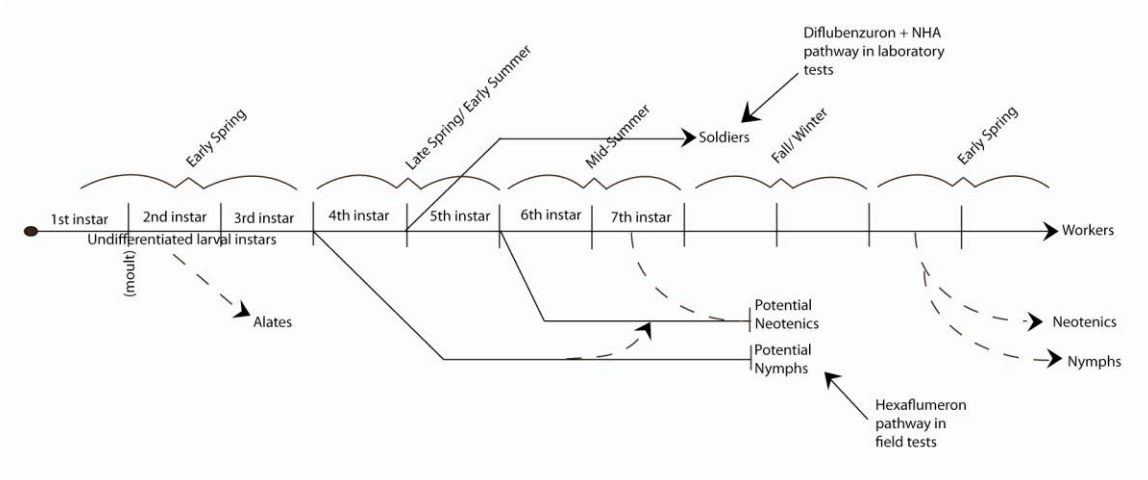

Fig. 3: Hypothetical development pathway for termites in Wisconsin (dashed lines indicate potential developmental pathways depending on colony conditions/requirements)

Results of field trials showed one area of termite activity at our test site to be eliminated over the first winter using only diflubenzuron (Appendix 1: zone 1), however activity returned two years later. Another area was considered ―expanding and active"—in spite of numerous feeding hits on diflubenzuron bait cartridges; it appeared that this area had become just a feeding station (Appendix 1: zone 2). No clearcut explanation accounts for this disparity. Faced with multiple alternatives, we decided to use trap-treat and release (TTR) methods (Myles 1997) and NHA for the dusting treatment. We dusted a captured population of 5-6 grams of *R. flavipes* and three weeks later noted a sharp (>90%) decline in workers observed in all previously actitive bait stations. Three weeks after treatment all bait stations were negative for termite activity, which has held true in all monitoring stations for the past 12 months. We can only surmise that further baiting with diflubenzuron (0.25%) would not have eliminated this colony without dusting with NHA.

Two homes in close proximity to this outdoor infestation were positive for *R. flavipes*. Commercial diflubenzuron was not successful at elimination in these locations, only suppression was observed. So after two years, commercial hexaflumuron (0.5%) stations were placed on either side of the positive diflubenzuoron. Termite activity at both sites was eliminated within approximately six weeks. It is possible that hexaflumeron may be enough for colony elimination alone, or that the diflubenzuron acted as a weakining agent, much like NHA plus diflubenzuron. Further tests will be done to explore synergy among the various active ingredients.

The addition of hexaflumeron to the baiting cartridges had an immediate impact on the termite castes seen in the stations, with reproductives made up of nymphal and worker forms comprising ~50% of individuals. Secondary reproductives are not typically abundant in baiting stations, and are not thought to occur in the fall as they were seen here. Based on the proposed development pathway for termites, we hypothesize that larvae and younger workers would be more susceptible to chitin synthase inhibitors, causing mortality, while older individuals may be prompted to differentiate into secondary reproductives. This will require further study. Seasonality may also play a role in this observation as the secondary reproductive forms appeared in the fall, where there would be an abundance of older workers capable of differentiating into a terminal form.

4. CONCLUSION

There appears to be two separate pathways of termite life forms affected dependent on the active ingredient. Diflubenzuron in combination with NHA dust caused an increase in soldier formation or increased presence of soldiers at the stations. The combination of the two active ingredients was effective at eliminating a laboratory colony in addition to controlling termites in the field as seen in zone two (Table 1, Appendix 1). Field observations with hexaflumeron showed an increase in neotenic production, which was never seen in areas treated with diflubenzuron. In both cases, the presence of either caste form may be an effect of the insecticide promoting differentiation into the various castes, or it may potentially be from the lack of workers to feed them, causing them to concentrate nearer the baiting stations. We do not seem to be able to cause an increase in neotenics to occur in the laboratory, which may suggest that the insecticide is having a more colony wide effect than an individualistic effect on caste determination. Another possibility is the neotenic reproductive forms begin moving away from the nest location into the stations which indicates the colony is collapsing. Regardless of mechanism, an over-abundance of secondary reproductives in hexaflumeron stations or soldiers in diflubenzuron stations were definitive signs of termite colony collapse.

Both laboratory and field termite trials should be considered fully successful at eliminating *R. flavipes.* A very active field population in the Village of Endeavor, WI was eliminated—although we are not 100% certain exactly how or why. Clearly, the combination or synergy of NHA dusting of ~5,000-6,000 workers as an addition to the diflubenzuron had a huge impact, as these same locations had been unsuccessfully baited with the CSI: diflubenzuon for over two years. During this period there was no evidence that continued commercial baiting would eliminate this colony. (This same phenomena has been observed in other residential termite locations in Wisconsin). We were forced to employ ―alternate strategies” to invoke colony elimination by dusting with the experimental toxicant, NHA (Nobre and Nunes 2007). It clearly could have depended upon the previous exposure to weakening by diflubenzuron or possibly synergistic effects between the two termidicides. There is no reason to conclude that either bait system is repellent and NHA appears to act as a slow-acting, stomach poison (Green et al. 1997, 2001, 2008b). Because TTR techniques are so labor intensive—we are currently employing the commercially available CSI hexaflumuron at a 0.5% concentration to eliminate two small pockets of *R. flavipes* remaining at two homes in the village. They were termite negative within 6 weeks of installing stations. However, the diflubenzuron proceeded treatment with hexaflumuron. The closed laboratory colony of *R. flavipes* was also successfully eliminated in 23 weeks. We first tried to dust directly with NHA and take advantage of the supposed ―1000 termite multiplier effect” of TTR (see Myles 1997), which was unsuccessful. Subsequent exposure to the commercial diflubenzuron bait system killed the colony 20 weeks later on day 163. Nevertheless, in a closed colony or population of *R.flavipes*, all applied chemical treatments remain for the duration of the experiment—e.g. there is no place for the termites to hide.

5. ACKNOWLEDGEMENTS

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Appendix 1: Zones of potentially isolated areas of termite activity in Endeavor, WI.

41st IRG

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