

**Synergy of Diflubenzuron Baiting and NHA Dusting on Mortality of
*Reticulitermes flavipes***

**F. Green III
R.A Arango
G.R. Esenther**

USDA Forest Products Laboratory
Madison, Wisconsin

**M.G Rojas
J. Morales-Ramos**

USDA-Agricultural Research Service
Stoneville, Mississippi

ABSTRACT

The ability of N’N-naphthaloylhydroxylamine (NHA) to cause mortality in *Reticulitermes flavipes* workers pretreated with the chitin synthesis inhibitor diflubenzuron was tested by adding two NHA dusted workers to 100 (2:100) pretreated workers fed either pure microcrystalline alpha-cellulose or diflubenzuron (0.25%) treated microcrystalline cellulose for 30 days prior to adding the two dusted termites. By day 5 post addition, there was a 50% mortality in the diflubenzuron treated termites only. By day 17, the mean survival of cellulose treated termites was 91 +/-2.8% and in the diflubenzuron fed termites was 1 +/- 1.5%. It has previously been demonstrated that chitin is present in the termite skeleton and in the walls of some internal organs including the digestive track and malpighian tubules. Previous publications showed that an internal chitinous structure known as the peritrophic matrix was adversely affected by the diflubenzuron with these membranes damaged or absent. We propose that the peritrophic membrane is likely to be further damaged by the calcium precipitating agent N,N’ naphthaloylhydroxylamine causing 100% mortality in the diflubenzuron treated termites in 3 weeks.

[The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service. This article was written and prepared by U.S. Government employees on official time, and it is therefore in the public domain and not subject to copyright.]

INTRODUCTION

The Forest Products Laboratory, in partnership with the Entomology Department of the University of Wisconsin-Madison and Alternative Pest Solutions took on the challenge of area-wide, or community wide elimination of *R. flavipes* from a small village in central Wisconsin in the Summer of 2006. The only pesticide tool at our disposal at that time was baiting the village with commercially available diflubenzuron treated alpha cellulose. We purchased and installed 200 bait stations around the community in order to determine the location and extent of the infestation. After one season we removed all un-infested bait stations and moved them to the areas where termite hits were prevalent. After one winter into 2007, one central area originally infested appeared clear of *R. flavipes*. But other pockets of termites, mainly in homes or garages, were identified and persisted relatively unimpeded until 2009. At that juncture, we decided that further use of only the same chitin synthase inhibitor (CSI) treated bait stations was not going to result in the elimination of the remaining colonies so with that in mind we decided to dust termites in these areas with the experimental termiticide NHA. Although we had previously conducted NHA dusting experiments in the laboratory; trap, treat and release had not been tested in the field (Arango et al. 2007, Green et al. 2009, 2010, 2011). Only two sites were selected and in each one only 5-6 grams of *R. flavipes* were dusted with undiluted (neat) NHA and released (TTR) back into the traps from where they were isolated. Within 3 weeks at the next inspection we were startled and amazed by over 90% reduction in foraging termites in the treated areas. Soon those sites were termite free and the village has remained termite free thru 2012 after repeated inspections. At that time we assumed that the elimination of the colonies was due to a weakening of the termites by feeding of diflubenzuron for 3 years prior to the trap, treat and release (TTR) dusting of N’N-naphthaloylhydroxylamine (NHA). The objective of this experiment was to confirm this synergistic relationship in a laboratory test.

AMERICAN WOOD PROTECTION ASSOCIATION

MATERIALS AND METHODS

Termites

In August of 2012, *R. flavipes* workers were collected in corrugated cardboard traps set out in Janesville, WI. They were stored in metal trash cans in a humidified incubator until used.

Treatments

Three grams of workers were placed into a glass jar: I-Chem Series 200 (9.0X9.5cm) with plastic screw topped lids with either an alpha-cellulose pressed bait cartridge or an identical bait cartridge seeded with 0.25% diflubenzuron. After 30 days of feeding 100 treated or untreated workers was put into each of 5 plastic cups (5.3 x 4.0 mm) with a single piece of #1 Whatman filter paper in the bottom to hold moisture. Workers were dusted with undiluted N’N- naphthaloylhydroxylamine and two dusted termites were added to each of the 10 cups of 100 *R. flavipes* workers (ratio 2:100).

Mortality

Termites in each diflubenzuron-treated cup were counted weekly for 4 weeks and mortality recorded as percent of 100.

RESULTS

The results are shown in Figure 1 and Figure 2.

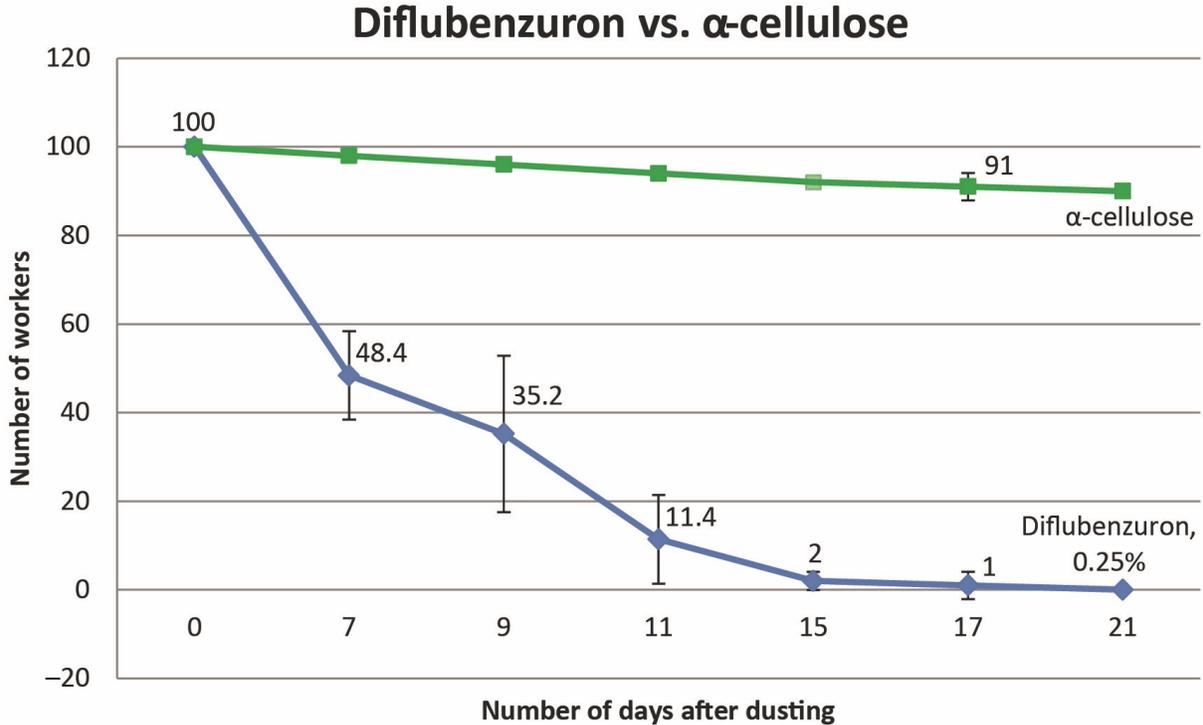


Figure 1: Mortality of *R. flavipes* after adding 2/NHA dusted workers to groups of 100 workers pre-fed for 30 days on either alpha-cellulose or alpha-cellulose plus 0.25 diflubenzuron % baits (mean +/- SD).

AMERICAN WOOD PROTECTION ASSOCIATION

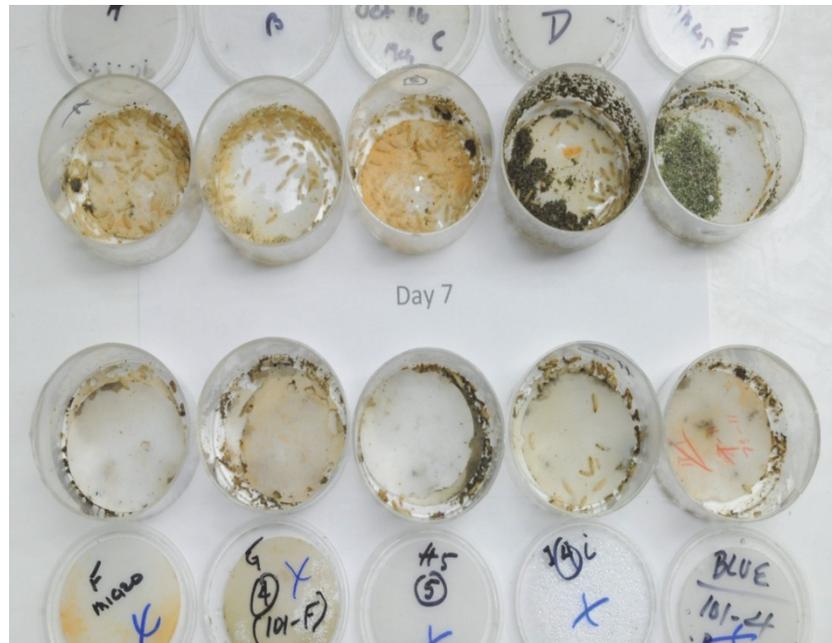


Figure 2: After only 7 days the diflubenzuron groups were decreased by 50% and 98% after 2 weeks and all diflubenzuron treated workers were dead by 3 weeks. Control workers only had 9% mortality in 3 weeks [Top row = pre-fed only alpha-cellulose / bottom row = pre-fed diflubenzuron @ 0.25% for 30 days].

DISCUSSION

It can be readily seen that the diflubenzuron feeding groups (n=5) were weakened or predisposed to death by exposure to the 2:100 NHA dusted termites. Since this NHA-effect took place so quickly, in only 3 weeks, it is unlikely to be an event precipitated by all the living termites molting simultaneously (Evans 2010). Although diflubenzuron, hexaflumuron and other CSI are known to affect ecdysis, disrupting alpha-chitin synthesis during molting (Cabrera and Thomas 2006), this mode of action may be too slow to explain the rapid deaths of *R. flavipes* workers in this experiment. Thus, disruption of the gamma-chitin containing peritrophic membrane in the midgut by minute quantities of NHA is more likely to cause death by interruption of digestion and starvation (Lehane 1997, Evans 2010). In a prior experiment evaluating the ability of NHA to kill *Coptotermes formosanus*, it was the opinion of the authors that the termites succumbed to a slow-acting stomach poison thru starvation (Green et al. 2000). Disruption of the peritrophic membrane could also explain that result of feeding on NHA treated blocks. On the other hand, the groups of termites fed only on alpha cellulose alone had only minimal mortality in the same course of time (9%). Thus, we hypothesize that the likely mechanism of action of NHA was further damage and rupture of the peritrophic membrane as previously described by Morales-Ramos et al. (2006a and 2006b) resulting in the elimination of said colonies of *R. flavipes* in three weeks. We conclude that diflubenzuron baiting followed by NHA dusting in this laboratory setting is identical to that observed in the field where as colonies were suppressed and eliminated in about 3 weeks after dusting. We also conclude that trap, treat and release strategies can augment simple baiting of refractory termite colonies with termiticides (Gautam et al. 2012, Green et al. 2008, Myles T. 2012).

ACKNOWLEDGEMENTS

Special thanks go to Phil Pellitteri, UW-Entomology Extension and Dan Keohane and Randy Kalk of Alternative Pest Solutions for their time and expertise in making this entire project a huge success.

REFERENCES

1. Arango, R. A., Green, F. III, and G.R. Esenther. 2007. Seasonal response of feeding, differentiation, and growth in the eastern subterranean termite *Reticulitermes flavipes* (Kollar) in Wisconsin. IRG/WP 07-10604. Stockholm, Sweden : IRG Secretariat, 2007: 9 pages
2. Cabrera, B.J. and E.M. Thomas. 2006. Versatility of Baits containing Noviflumuron for Control of Structural Infestations of Formosan subterranean termites (Isoptera: Rhinotermitidae) Florida Entomologist 89:20-31
3. Evans, T.A. 2010. Rapid elimination of Field Colonies of Subterranean Termites (Isoptera: Rhinotermitidae) using Bistrifluron solid Bait Pellets. J. Econ. Entomology 103:423-432.

AMERICAN WOOD PROTECTION ASSOCIATION

4. Lehane, M.J. 1997. Peritrophic Matrix Structure and Function. *Annu. Rev. Entomol.* 42:525-50.
5. Gautam, B. K. , G. Henderson, and R.W. Davis. 2012. Toxicity and Horizontal Transfer of 0.5% fipronil dust against Formosan subterranean termites. *J. Econ. Entomol.* 105(5) 1766-1772.
6. Green, F. III, Kartal, N. , Lebow, P.K, and R.A. Arango. 2011. NHA Choice test : Feeding Dilemma for *Reticulitermes flavipes* (Kollar). The international research group on wood protection, Section 1, Biology, IRG/WP 11-10746, 2011
7. Green, III, F., Arango, R. A. and S.T. Lebow. 2010. Field Testing of the Experimental Wood Preservative N'N-naphthaloylhydroxylamine: Five and Eight Years Results. In: Proceedings One Hundred Sixth Annual Meeting of the American Wood Protection Association, Hyatt Regency Riverfront, Savannah, Georgia, May 23-25, 2010, pp. 192-196;
8. Green, III, F., Arango, R. A. and G. R. Esenther. 2010. Observations on colony collapse in *Reticulitermes flavipes* (Kollar) in laboratory and field settings in Wisconsin. From IRG CD titled 41st iRG Biarritz, May 9-13, 2010 in the 41st annual meeting of the International Research Group on Wood Protection; pp.6.
9. Green, F.III; Arango, R. A. and G.R. Esenther. 2009. Community-wide suppression of *R. flavipes* from Endeavor, Wisconsin - Search for the Holy Grail. IRG2009, Beijing China, International Research Group Wood Preservation: IRG/WP 08-10674, Section 1, Biology, pp. 2-10;
10. Green, F., Arango, R. A. and G.R. Esenther. 2008. Transfer of termiticidal dust compounds and their effects on symbiotic protozoa of *Reticulitermes flavipes* (Kollar) IRG/WP;08-10661. Stockholm, Sweden : IRG Secretariat, 2008: 9 pages.
11. Green, III, F., Lebow, S. T. and T. Yoshimura. 2000. Inhibition of termite damage by N'N-naphthaloylhydroxylamine (NHA): *Reticulitermes flavipes* (Kollar) vs. *Coptotermes formosanus* Shiraki. The International Research Group on Wood Preservation. IRG/WP 00-10354
12. Morales-Ramos, J.A., M.G. Rojas, and H. Sittertz-Bhatkar. 2006a. Peritrophic Matrix of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) *Florida Entomologist* 89(1): 99-101.
13. Morales-Ramos, J.A. , Rojas, M.G. and H. Sittertz-Bhatkar. 2006b. Effects of diflubenzuron on the peritrophic matrix and fat body of Formosan subterranean termite (Isoptera: Rhinotermitidae) workers. *Sociobiology*: 47(3): 667-676.
14. Myles, T. 2012 (personal communication) <http://guelph.ca/living/house-and-home/yard-and-garden/termites/>

