E1-09 Termite Choice Testing: What is it good for?

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ABSTRACT

The prime objective of this investigation was to compare mass loss from termite attack on NHA treated and untreated pine blocks simultaneously in a two-block choice test using *Reticulitermes flavipes* (Kollar). Southern pine blocks were vacuum treated with aqueous solutions of NHA ranging from 250-10,000 ppm and exposed to one gram of *R. flavipes* workers in the dual block choice test which included one treated and one untreated block per the modified AWPA laboratory test (E1-09). At the end of four weeks, all test blocks were removed, weighed and the mass losses statistically modelled using SAS version 9.2. Mass loss was consistently greater in the untreated pair-blocks and ranged from 18-35%. Weight loss in the NHA-treated blocks was highest at low concentrations (18%) and decreased to < 1% at 10,000 ppm. Termite mortality at the end of four weeks was slight or negligible. We conclude that exposure to untreated pine during exposure to the NHA blocks delayed the 100% mortality previously observed in similar no-choice test using similar NHA concentrations of NHA, either by reducing NHA intake below toxic levels or altering gut pH. Modest accelerated feeding (stimulation) on the control blocks was observed only in the 2,600 ppm group. Overall, NHA treatment in pine behaves like a typical repellent wood preservative: as the concentration of preservative increases, feeding and mass loss decreases, however addition of an untreated pine block abrogates the high toxicity of NHA to *R. flavipes* (100%).

Keywords: NHA, *Reticulitermes flavipes* (Kollar), dual choice test, feeding stimulant

INTRODUCTION

Eastern subterranean termites are the single most economically important structural pest in the United States. Each year millions of dollars are spent on prevention, control and repair. In the natural environment subterranean termites provide the valuable service of consuming, digesting and recycling dead and decayed wood and returning valuable nutrients to the forest soil. However, when termites replace forest debris with wood homes and structures like decks or timber bridges the termites penchant for consuming wood becomes a costly problem. Termite damage to homes, wooden structures and businesses continues to be a serious and costly problem exacerbated by global climate change. Damage estimates by the USDA range as high as $7 billion dollars per year (Peterson et al. 2006), far exceeding wind, rain and other forms of severe weather damage in a normal or average year.

Fixed structures, especially structural members can easily be protected by wood preservatives and chemical barriers. In the last two decades, however, a whole new form of protection and remediation has been brought forward in the form of termite baiting systems. Typical termite baits contain wooden interceptors or cellulosic baits which are replaced with a toxicant after the termites discover the untreated bait system. Termite toxicants or insecticides generally contain a juvenile growth hormone, chitin synthetase inhibitor or non-repellent slow-action bait matrix (Haifig et al. 2008). As a general rule, effective wood preservatives, like CCA for instance, would not be considered good candidates for any termite bait. The reasons for this include high repellency and rapid toxicity to feeding termites. The whole concept of termite baiting is to control and remediate an already existing infestation. Therefore, if the termites do not find the bait/toxicant attractive and consume it, the colony will not be suppressed or eliminated from continued consumption of the vulnerable wooden structures in range. At the very least baits should be non-repellent. A weak link in the implementation of effective termite baiting techniques is reliable recruitment of termite foragers to baits. Little is known about how termites locate food sources, but many factors may influence whether foragers find and recruit to a given bait material. These include the abundance and palatability of the bait relative to alternate foods in the habitat, chemical directional clues to the location of food in the soil environment, and the presence of predators and termite competitors at the bait site (Evans and Gleeson 2006, Haifig et al. 2008, Waller et al. 1999).

The vast majority of E1-09 termite tests are "no-choice"; e.g. a single block of treated or untreated wood, designed to evaluate the protective efficacy of potential wood preservatives. The counterpoint of this test represents the "choice test"; e.g. two or multiple blocks of natural or treated wood exposed to termites in a single gallery or test simultaneously to compare feeding.

Choice tests have also been used to evaluate tunnelling through termiticide treated insulation or other building materials (Mankowksi and Grace 2004), feeding responses to commercial wood species (Morales-Ramos and Rojas 2001, 2004) and preference for blue-stained sapwood from beetle-killed trees (Little et al. 2012). A new feeding index including a choice-test has been proposed by Peterson and Gerard (2008).

During the mid-1990s the Forest Products Laboratory developed an experimental wood preservative comprised primarily of the naphthalimide derivative: N'N-1, 8 naphthaloylhydroxlyamine (NHA) (Green et al. 1996, 1997, 2011). Concentrations of aqueous NHA vacuum or pressure treated into southern pine protected the wood and inhibited termite damage in concentrations of 5,000 ppm or higher. We assumed at the time that the mechanism of killing was that of a slow-acting, non-repellent stomach poison (Green et al. 2000, 2001). Shortly thereafter, in conjunction with ARS, it was also discovered that low concentrations (<500 ppm) behaved like a feeding stimulant in a termite bait matrix to eliminate termite colonies in ground (Rojas et al. 2004a). Thus NHA is part of that select and small group of wood preservatives which can function as a termite bait at low concentrations.

Nitrogen containing compounds have been shown to be effective as subterranean termite feeding stimulants/aggregants and as well as masking agents for concealing the presence of other compounds which are repellent to termites, when they are used in low concentrations (less than or equal to about 1,000 ppm or 0.1% by weight). The nitrogen containing compounds may be formulated alone as a bait or optionally in combination with other compounds effective for controlling subterranean termites (Rojas et al. 2004b). This tends to suggest potential for a nitrogen containing compound like NHA to behave as both a toxin and a bait stimulant depending on how it is developed. The objective of this paper was to test treated pine blocks against untreated control blocks in a laboratory two-choice test in order to observe and estimate any overt feeding stimulation or inhibition by NHA.

MATERIALS AND METHODS

***Laboratory Test Methods*:**

An AWPA E1-09 two-choice test method was selected to compare the feeding and repellent properties of NHA on southern pine blocks (AWPA 2012). Two similar experiments were conducted consisting of 20 jars with blocks of size 29x2x42mm (longitudinal/feeders) and the other 33 jars with blocks of size 25x6x25mm (cross-transverse/square termite blocks). The first experiment with the feeder blocks was a two-choice test with treated blocks of NHA at concentrations of 337, 500, 754, and 2,604 ppm with 5 replicate jars per concentration. The second experiment (square termite blocks) was a two-choice test with treated blocks of NHA at concentrations of 250, 515, 1,040, 5,337, 8,000, 10,000 ppm with 5 replicate jars per concentration. Both experiments used untreated wood specimens as the alternative to the NHA treated blocks. In addition, 3 replicate jars each with two untreated blocks were evaluated. Treated blocks were vacuum treated with aqueous solutions of NHA ranging from 250-10,000 ppm for two rounds of 20 minutes. Wood blocks were air dried, weighed and then conditioned to 80% RH.

Each replicate test container had 50g damp sand, 1g *Reticulitermes flavipes* (Kollar) workers and two wood blocks. 500cc glass jars (90d x 95h mm; I-CHEM/Fisher Scientific) were seeded with 50 g sterile sand and 16cc of tap water before adding two pine test blocks (one NHA and one untreated-- Fig. 1). After 4 weeks exposure, both test blocks were removed, dried in a chemical hood and reweighed. Mass loss was then plotted against NHA ppm (Fig. 2). Mortality was estimated and additional cellulosic food sources were added back to the jar—either untreated filter paper or untreated wood. Jars were continually monitored until 90-100% mortality was observed (Note: original wood blocks not put back into jars).

Statistical methods

Since the experiments were run in two separate periods and the block density, surface area and grain direction differed, the two experiments were analyzed in separate statistical models. Statistically, the final weights of the blocks in the feeder experiment were modelled accounting for the two factors treatment and concentration along with their interaction and using initial weights of the blocks as covariates. Since the blocks were paired, this was also included in the model as a random factor. Final weights appeared heterogeneous with variation increasing with weight loss, and this was included in the model by estimating different residual variances for the untreated and treated blocks. The feeder blocks, although having smaller volume, did have initial pre-treatment weights of ~5% greater than the termite blocks. Thus, we have presented results on a percent basis as adjusted by the appropriate initial covariate. The statistical model for the second experiment conducted on the square blocks was similar, except a mean effect type model was used to accommodate the treatment with 3 jars of two untreated control blocks. For this model, pairing was also modelled as well as heterogeneous variation. Mean comparisons between treatment groups were based on the modelled weights adjusted to a common initial weight. The weight losses in the figures were also based on these values. Consumption ratios were also analyzed based on a nonparametric bootstrapping procedure. Statistical modelling was performed in SAS® Version 9.2 (Cary, NC) using mixed modelling procedures (Littell et al. 2006) with graphics from R version 2.11.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Figure 1: Choice test jars with two wood specimens and termites

RESULTS AND DISCUSSION

Block Evaluation

Mass loss is shown in Fig 2. At the lower ppm of NHA (250-500 ppm), there is some overlap in the mass loss response with a loss of about 10% for treated blocks and 20-25% loss for the corresponding untreated blocks. As the concentration of NHA per block increases, the mass loss of the NHA blocks decreased to nearly 0% and the mass loss of the untreated blocks increased to a threshold peak of approximately 34%. All NHA-treated blocks experienced significantly less weight loss than the corresponding untreated pine blocks within the jars (all p-values < 0.001, except at 500 ppm, p-value = 0.0218).

Given the patterns of consumption, we theorize that there is an initial phase that represents the termite's neutral interest in the NHA blocks which become progressively more repellent and thus promotes increased feeding on the control pine blocks. Simply, as less of the NHA termite block is consumed, control blocks are consumed more aggressively (Fig. 3a, b).

At the mid-range treatment levels of NHA (1,000-3,000 ppm) the mass loss of untreated blocks remains steady at a somewhat higher level while mass loss in the NHA blocks appears to take an exponential decline which continues through 5,000-8,000 ppm. We assume here in phase II that the increasing concentration of NHA is making the majority of workers weaker or sicker, functioning as a non-repellent stomach poison resulting in less interest in feeding. Termite workers might even be partly starving during phase II, as during this phase feeding on NHA blocks is minimal or less than 5%.

Although the first two phases are highly statistically detectable, there is also evidence of consumption differences between the 5,000 to 10,000 ppm groups (p-value = 0.03; Figs. 3a, b). There appears to be a small "operational" weight loss in the NHA blocks at 10,000 ppm, however, visual inspection of those blocks does not support increased surface nibbling. We hypothesize there may be a final phase III that is represented by some increase of feeding on the NHA treated blocks and with slight decreased feeding on the untreated blocks up to over 30% mass loss (at 10,000 ppm). Workers now begin detecting the toxic effects of the NHA and avoid eating these blocks, preferring instead the untreated blocks. In addition, we assume that by some means, the untreated blocks are indirectly abrogating or diluting the toxicity of the NHA blocks. We cannot explain the apparent "tailing off" of feeders seen in the consumption ratio at 1,500 ppm (Fig. 3b).

Mortality of Workers

At the end of 4 weeks, AWPA Standard Method for Laboratory Evaluation of Termite Resistance (E1-09) mortality was visually estimated in all jars as either slight or less than 30%. This result was unexpected in light of previous no-choice tests where concentrations of NHA equal to 0.5% or 5,000 ppm resulted in significant mortality in 3-4 weeks in no-choice testing (Green et al. 1996, 1997). Clearly the addition of the untreated pine block in this choice test prolonged termite survival and effectively delayed or diluted the toxic effects reported previously for NHA blocks alone (Green et al. 1996, 1997), an effect that would not have been observed in a standard, no-choice termite test. Termite mortality can result from direct contact effects, cannibalism, starvation or microbial overload. The abrogation observed may represent the buffering capacity of pine/aspen wood at a pH of 4.66 (Johns and Niazi 1980), or simply reduced feeding below toxic levels. This reduction in mortality in choice testing vs. No-choice testing was also reported by Arch Treatment Technologies in their AWPA data packet from 100% to slight (0-30%) for PTI (AWPA 2007).

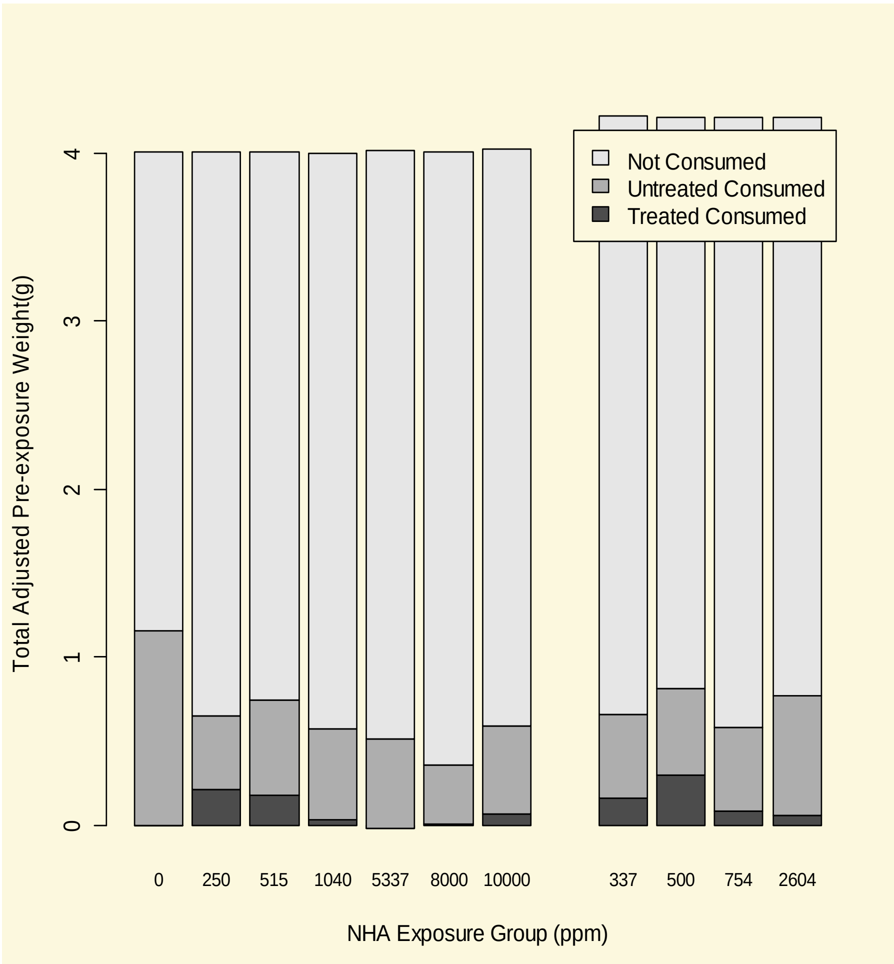

Figure 2: NHA termite choice test showing amount of wood material consumed

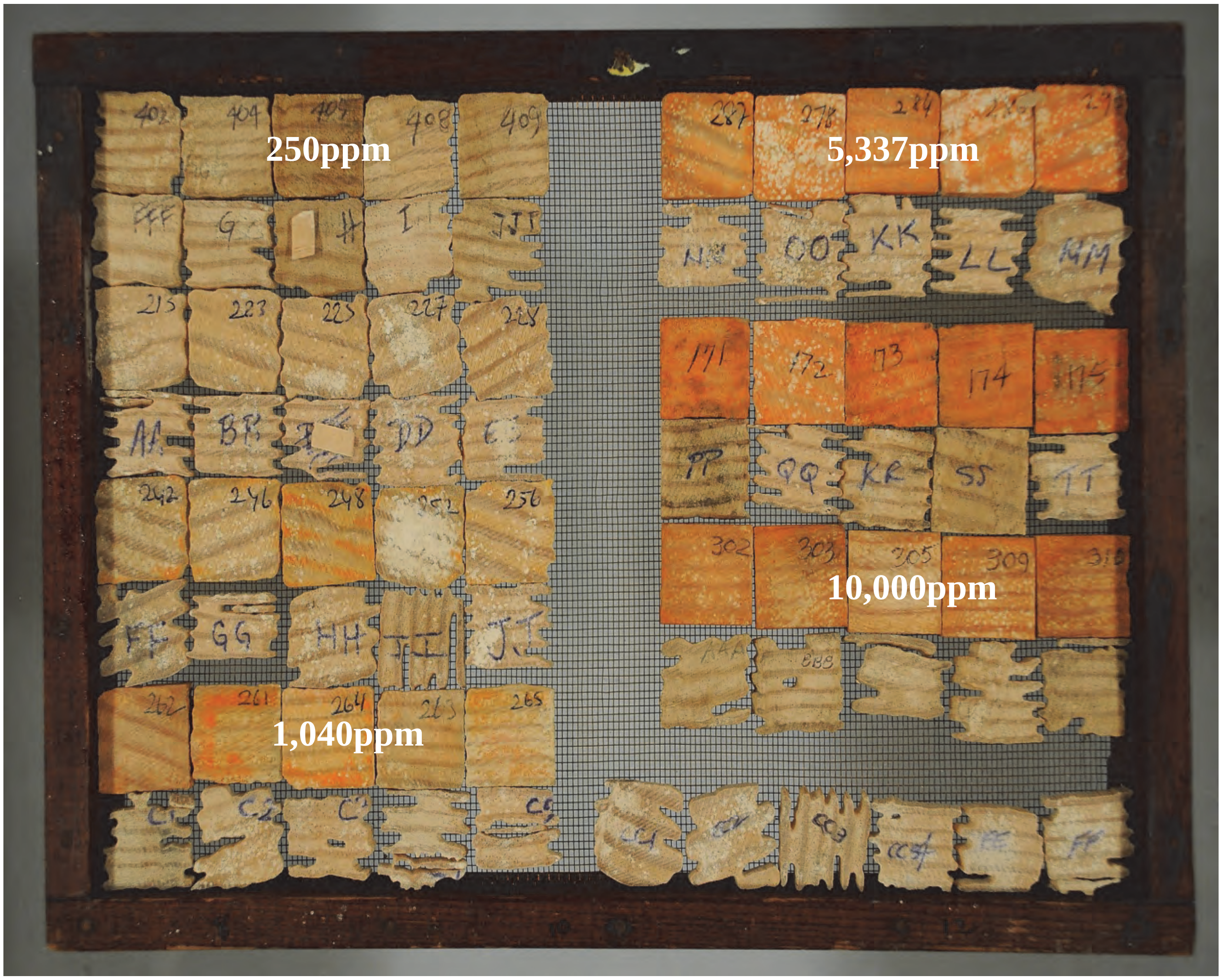

Figure 3a: Termite (square) blocks after choice test (Treated block row above untreated block row for each ppm level)

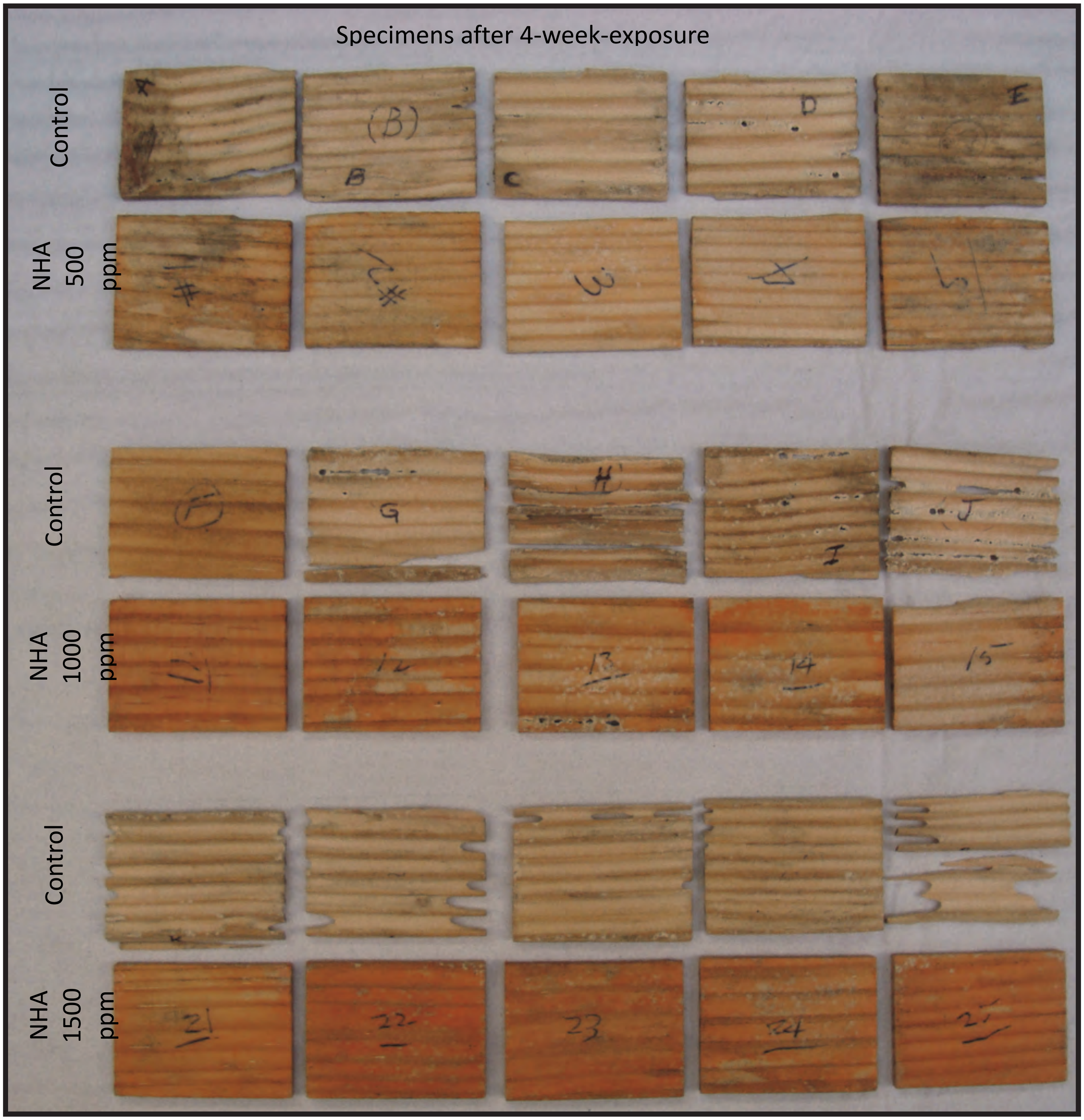

Figure 3b: Target [conc] of NHA-treated and untreated pine feeders after 4-wk exposure
[note: actual NHA conc. for blocks: 1-5 =337ppm/ 11-15 = 754ppm/ 21-25 = 2604ppm]

CONCLUSION

An AWPA standard E1-09 choice termite test was performed using pine with workers of *R. flavipes* in order to compare feeding between the treated NHA block vs. the untreated block (AWPA 2012). NHA-treated blocks were vacuum treated with aqueous NHA ranging in concentration from 250-10,000 ppm. In each of 53 jars the untreated control pine had greater mass loss than the NHA treated block. We conclude that the NHA evoked a feeding deterrent for the treated pine blocks and from 2,500 ppm and above, also acts as a wood preservative by nearly complete suppression of feeding. Little evidence of feeding stimulation was observed in any pair of blocks. The mechanism by which control pine blocks abrogate any toxicity of NHA is unknown, but may relate either to pH buffering by the untreated wood or reduced feeding on treated blocks below toxic levels.

REFERENCES

1. AWPA 2012. Standard method for laboratory evaluation to determine resistance to subterranean termites. AWPA Method E1-09. AWPA Book of Standards, pp. 416-424.
2. AWPA 2007. Data Package for Wood Preservative (PTI) submitted by Arch Wood Protection, Inc, pp.1-41
3. Evans, T.A. and P.V. Gleeson, 2006. The effect of bait design of bait consumption in termites (Isoptera: Rhinotermididae) http://www.termitefree.com/images/Evans.pdf pp. 1-23
4. Green III, F., T.A. Kuster, L. Ferge, T.L. Highly. 1996. Inhibition of wood decay and termite damage by calcium precipitation. International Research Group on Wood Preservation IRG/WP 96-30111.
5. Green III, F., T.A. Kuster, L. Ferge, T.L. Highley. 1997. Protection of southern pine from fungal decay and termite damage with N'N-naphthaloylhydroxylamine (NHA). International Biodeterioration and Biodegradation 33: 103-111.
6. Green III, F., S. Lebow, T. Yoshimura. 2000. Inhibition of termite damage by N'N-naphthaloylhydroxylamine (NHA): *Reticulitermes flavipes* (Kollar) vs. *Coptotermes formosanus* Shiraki. International Research Group on Wood Preservation, Stockholm, Sweden, IRG/WP 00-10354.
7. Green III, F., D.M. Crawford, S. Lebow, T. Yoshimura. 2001. Relative toxicity of N'N-napthaloylhydroxylamine (NHA) against Eastern subterranean and Formosan subterranean termites in southern yellow pine. In: *Proceedings of the 2nd conference on Durability and Disaster Mitigation in Wood-Frame Housing,* Madison, Wisconsin, Forest Products Society, pp. 235-238.
8. Green III F., R.A. Arango, S. Lebow. 2011. Field Testing of the Experimental Wood Preservative N'N-naphthaloylhydroxylamine: Five and eight year results. Proceedings of the Annual Meeting of AWPA, Savannah GA, pp. 192-196.
9. Haifig, I., A.A. Costa-Leonardo, F.F. Marchetti. 2008. Effects of nutrients on feeding activities of the pest termite *Heterotermes tenuis* (Isoptera: Rhinotermitidae). Journal of Applied Entomology 132: 497-501.
10. Johns, W.E. and K.A. Niazi. 1980. Effect of pH and buffering capacity of wood on the gelation time of urea-formaldehyde resin. *In*: Johns, W. E.; Niazi, K. A. Wood and Fiber 1980, 12(4): 255-263.
11. Littell, R.C., G.A. Milliken, W.W. Stroup, R.D. Wolfinger, O. Schabenberger. 2006. *SAS® for Mixed Models, Second Edition*. SAS Institute, Inc. Cary, NC. pp. 814
12. Little, N.S., J.J. Riggins, A.J. London, T.P. Schultz and D.D. Nicholas. 2012. Preference of *Reticulitermes flavipes* (Kollar) for southern pine blue-stained sapwood from beetle killed trees. International Research Group on Wood Preservation, Stockholm, Sweden, IRG/WP 12-10763.
13. Mankowski, M.E. and J.K. Grace. 2004. Response of the Formosan Subterranean Termite (*Coptotermes formosanus*) to Cellulose Insulation Treated with Boric Acid in Choice and No-Choice Tests. International Research Group on Wood Protection, Stockholm, Sweden, IRG/WP 04-10532
14. Morales-Ramos, J.A. and M.G. Rojas. 2001. Nutritional ecology of the Formosan subterranean termite (Isoptera: Rhinotermididae): Feeding response to commercial wood species. Journal of Economic Entomology 94(2): 515-523.
15. Morales-Ramos, J.A. and M.G. Rojas. 2004. Nutritional ecology of the Formosan subterranean termite (Isoptera: Rhinotermididae): Growth and survival of incipient colonies feeding on preferred wood species. Journal of Economic Entomology 96(1): 106-116.
16. Peterson, C., T.L. Wagner, J.E. Mulrooney, T.G. Shelton. 2006. Subterranean termites. USDA Forest Service Home and Garden Bulletin 64, Starkville, MS.
17. Peterson, C.J. and P.D. Gerard. 2008. Two new termite (Isoptera: Rhinotermitidae) feeding indexes for woods of varying palatability. Midsouth Entomologist 1: 11-16.
18. Rojas, M.G., J.A. Morales-Ramos, F. Green. 2004a. Naphthalenic compounds as termite bait toxicants. US Patent: #6,691,453.
19. Rojas, M.G., J.A. Morales-Ramos, D.R. Nimocks. 2004b. Urea and nitrogen based compounds as feeding stimulants/aggregants and masking agents of unpalatable chemicals for subterranean termites, US Patent: #6,824,787.
20. Waller, D.A., S.E. Morlino, N. Matkins. 1999. Proceedings of the 3rd International Conference on Urban Pests; W.H. Robinson, F. Rettich, and G.W. Rambo Eds. pp. 597-600.

Green, F. III, P.K. Lebow and S.Nami Kartal. 2014. E1-09 Termite Choice Testing: What is it good for? Proceedings, 108th Annual Meeting of the American Wood Protection Association, April 29 – May 2, 2012. Nashville, TN. 108:137-143.