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NHA Choice test: Feeding dilemma for *Reticulitermes flavipes* (Kollar)

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ABSTRACT

An AWPA laboratory choice test was performed with *Reticulitermes flavipes* (Kollar) to determine the role of feeding stimulation or aggregation by N'N napthaloylhydroxylamine (NHA). Pine blocks were vacuum treated with aqueous concentrations of NHA ranging from 250-10,000ppm and exposed to one gram *R. flavipes* workers in the dual block choice test which included one treated and one untreated block per modified AWPA laboratory test (E1-97) jar. At the end of four weeks, all test blocks were removed and replaced by either filter paper or untreated pine or aspen to observe long-term mortality/survival. Removed blocks were evaluated for mass loss and statisitical evaluation. 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 < 5% at 10,000ppm. Termite mortality at the end of four weeks was observed nearly zero, however, over the next 12 weeks worker mortality reached 100% in certain groups. Filter paper or control pine was supplemented after the 4 week test period. We hypothesize 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, possibly by reduction below toxic levels. Modest accelerated feeding (stimulation) of the control blocks was observed only in the 2600ppm 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%) as previously seen in several no-choice tests.

Keywords: NHA, *Reticulitermes flavipes*, choice test, feeding stimulant

INTRODUCTION

"What causes more damage to homes than storms, fires and earthquakes, yet is rarely covered by homeowners insurance? Surprisingly, the culprits are only about 10mm in length—subterranean termites!" (Dart, A, 2009) 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 USA range as high as $10 billion dollars per year, far exceeding wind, rain and other forms of severe weather damage. Fixed structures, especially structural members can easily be protected by wood preservatives and chemical barriers. But in the last two decades 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 might contain a juvenile growth hormone, a chitin synthetase inhibitor or a non-repellent slow-action bait. (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 includes high repellency and rapid toxicity to feeding termites. The whole concept of termite baiting is to control and remediate an already existent infestation. Therefore, if the termites don't 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 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. 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).

During the mid-1990s the Forest Products Laboratory developed an experimental wood preservative comprised primarily of the naphthalimide derivative: N'N-1,8 napthaloylhydroxlyamine. [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 5000ppm 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, also it was 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 small group of wood preservatives which can double as a termite bait at low concentrations. The advantages of NHA as a wood preservative is that it binds to calcium in wood and resists leaching.

Nitrogen containing compounds are effective as subterranean termite feeding stimulants/aggregants and as masking agents for concealing the presence of other compounds which are repellents to termites, when they are used in low concentrations, less than or equal to about 1000 ppm (0.1%, by weight). The nitrogen containing compounds may be formulated alone, or optionally in a bait or in combination with other compounds effective for controlling or marking 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. The objective of this paper was to test NHA 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.

MATERIALS AND METHODS

Laboratory test methods

An AWPA (E1-96) two-choice test method was selected to compare the feeding and repellent properties of NHA on southern pine blocks. Two similar experiments were conducted, 20 jars with blocks of size 29x2x42 mm (longitudinal/feeders) and the other 33 jars with blocks of size 25x6x25 mm (cross-transverse/squares). The first experiment with the feeder blocks was a two-choice test with treated blocks of NHA at concentrations of 337, 500, 754, and 2604 ppm with 5 replicate jars per concentration. The second experiment (squares) was a two-choice test with treated blocks of NHA at concentrations of 250, 515, 1040, 5337, 8000, 10000 ppm with 5 replicate jars per concentration. In addition, 3 replicate jars each with two untreated blocks were evaluated. Wood blocks were air dried, weighed for mass, and then conditioned to 80%RH. Treated blocks were vacuum treated with aqueous solutions of NHA ranging from 250-10,000 ppms for two rounds of 20 minutes.

Each replicate test vessel contained 50g damp sand, termites and two wood blocks. 500 cc Glass jars (90d x 95h mm; I-CHEM/Fisher Scientific) were seeded with 50 gm sterile sand and 16cc of tap water before adding two pine test blocks (one NHA and one untreated).(see Fig.1) To this basic setup a mass of 1gm live *Reticulitermes flavipes workers was added*. After 4 weeks exposure, both test blocks were removed and dried in a chemical hood and reweighed. (Figure 2) Mass loss was then plotted against NHA ppms (see Fig 3). 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.

Figure 1. Test jars with two wood specimens and termites

Figure 2A Cross sections of all transverse block-pairs after 4 weeks in test #2. NHA (orange) vs untreated pairs. All numbered blocks = NHA, all letter blocks = untreated choice blocks. From 250-10,000ppms. 6 blocks in lower right [CC1-ff) represent untreated control pairs (3) Blocks are paired: above = NHA treated/ under = untreated: eg402/FFF; 174/SS.

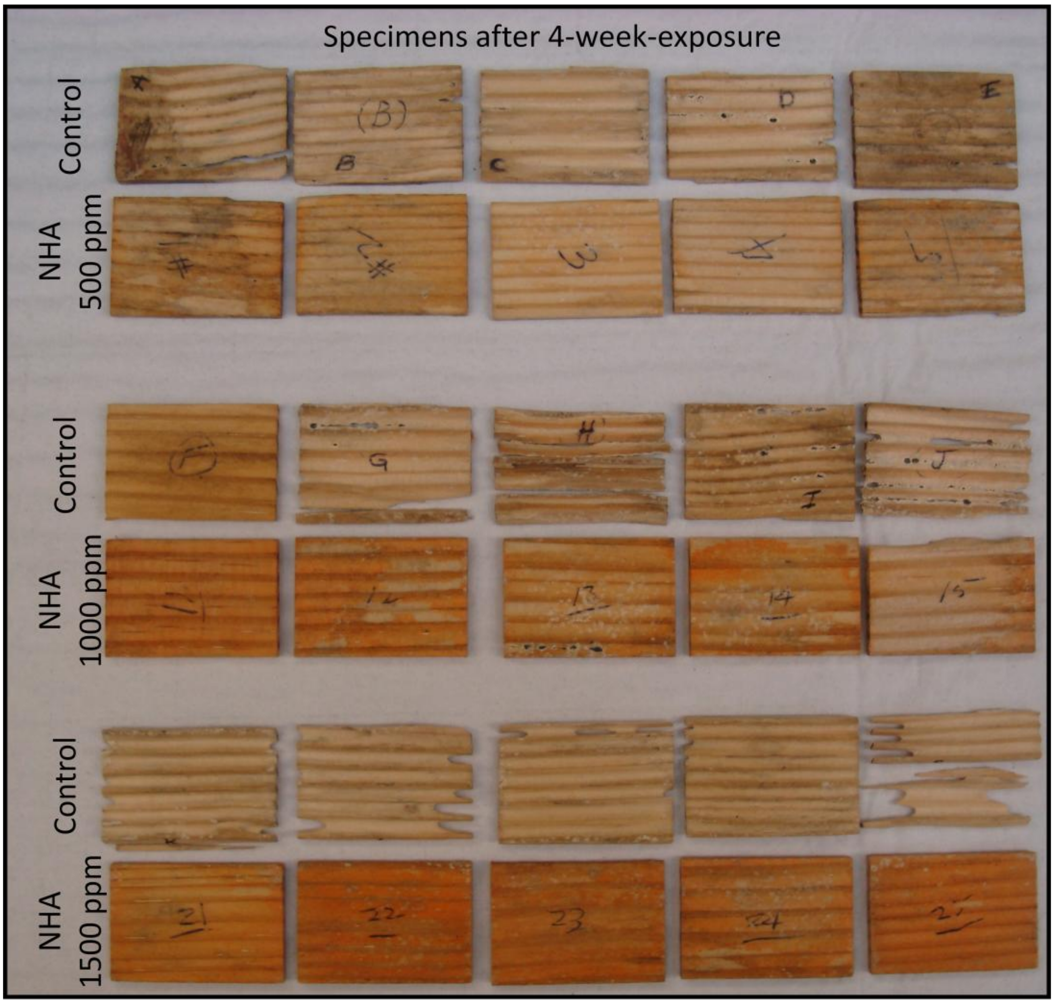

Figure 2B. Target [conc] of NHA-treated and untreated pine feeders after 4-wk exposure
[note: actual [NHA]: blocks 1-5 =337ppm/ 11-15 = 754ppm/ 21-25 = 2604ppm]

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 squares. 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)

RESULTS AND DISCUSSION

Block evaluation

Percent mass loss is shown in Fig 3. 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 decrease down to nearly 0% and the mass loss of the untreated blocks increase to a threshold peak of 34%. All NHA-treated blocks experienced significantly less weight loss than its corresponding untreated pine blocks within the jars (all p-values < 0.001, except at 500 ppm p-value = 0.0218).

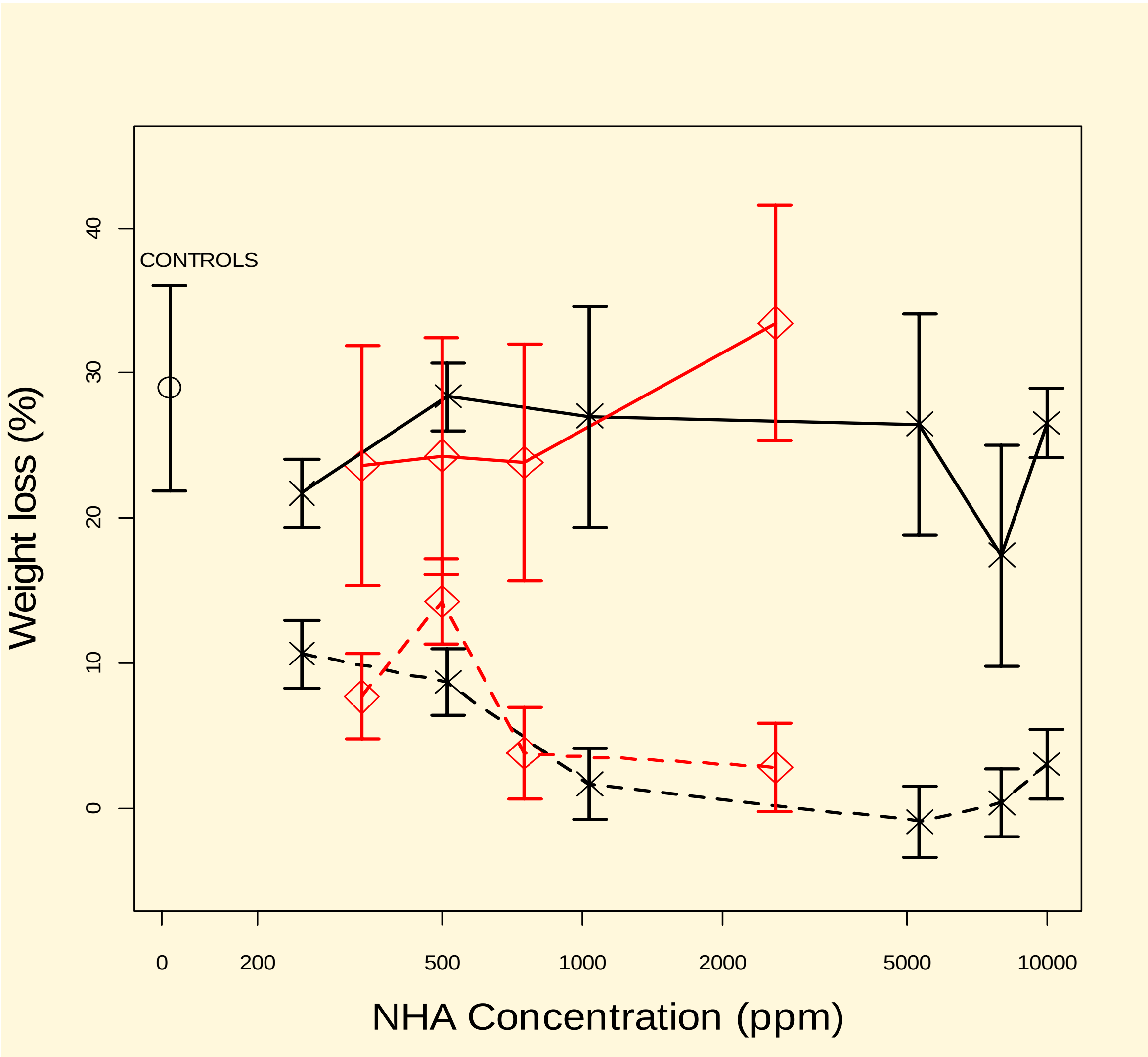

Figure 3. Percent mass loss of untreated blocks (solid lines) and NHA treated blocks (dashed lines). Black lines are termite blocks and red lines are feeder blocks. [note: 3 pair of controls in upper left corner @0ppm] This figure shows that all untreated blocks had at least double the mass loss as the corresponding NHA treated blocks. [shown +/-2*SE]

Given the patterns of consumption, we theorize that there is an initial phase (I) that represent the termites neutral interest in the NHA blocks which become progressively more repellent and thus promotes increased feeding on the control pine blocks. As NHA blocks are consumed less, quite simply, control blocks are consumed more aggressively (Fig. 4) [mass loss: controls UP “^”/ NHA-treated DOWN”v”]

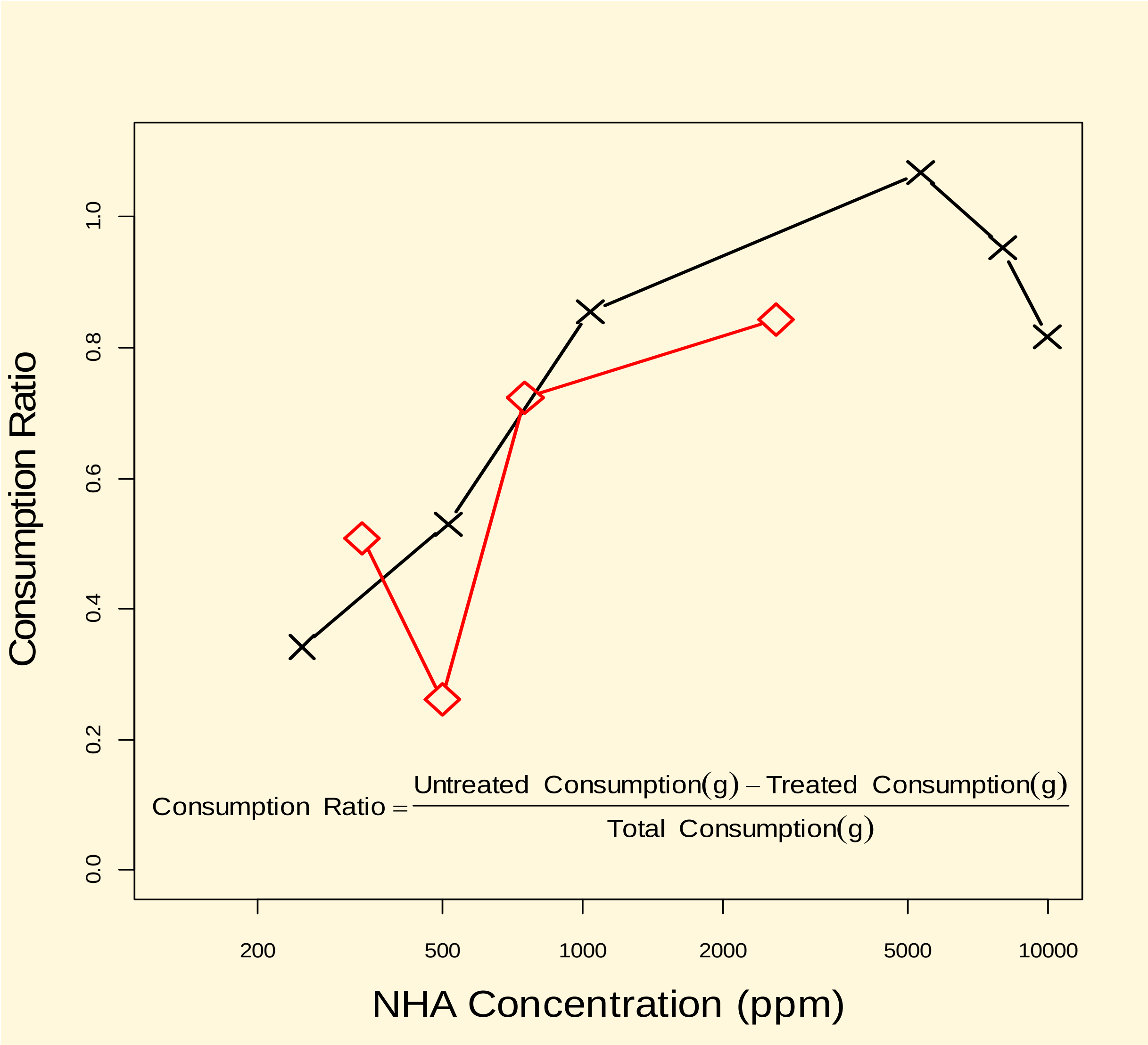

Figure 4. Consumption ratio (consumption difference: untreated minus treated/ divided by consumption total) for both the square termite blocks (black line) and feeder blocks (red line). This figure shows that as the concentration of NHA increases (>>>), this with an increased mass loss in the untreated pine blocks.

At the mid-range treatment levels of NHA (1000-3000) 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 5000-8000 ppm. We assume here in phase II that the increasing concentration of NHA is making the majority of workers weaker or sicker as a non-repellent stomach poison and less interested in feeding. Termites workers might even be partly starving during phase II. 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 5000 to 10000 ppm groups (p-value = 0.03; Fig 3). 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 10000ppm). 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 can not explain the apparent "tailing off" of feeding seen in the consumption ratio at 10,000 ppm. (See Fig 4).

Mortality of workers

At the end of 4 weeks, AWPA Standard Method for Laboratory Evaluation of Termite Resistance (E1-97) mortality was visually estimated in all jars as either negligible or below 30% , eg 0-30%. This result was unexpected in light of previous no-choice tests where concentrations of NHA equal to 0.1% or 1000 ppm resulted in significant mortality in 3-4 weeks(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). Termite mortality can result from direct toxic 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.

Test…..# of jars......NHAppm…#jars..midterm mortality events………longterm mortality events

Group 1	**5**	**337ppm**	**1@7wks**	**2@17wks**	**2/alive**	**feeders**	**2/90%**	**week 21**
Group 2	5	754ppm	1@16wks	1@17wks	3/alive	feeders	2/90%	week 21
Group 3	5	2604ppm	1@16wks		4/alive	feeders	4/90%	week21
group 4	5	500ppm			5/alive	feeders	4/90%	week21
Contros	3	controls	1@12wks			square	1/90%	week 21
group 5	5	250ppm	1@14wks	start 9/2	4/alive	Termite	4/90%	week21
group 6	5	515ppm	4@12wks	1@13wks	0/alive	Tblocks	XXXX	filterpa
Group7	5	1040ppm	4@12wks	!@16wks	0/alive	Tblocks	XXXX	filterpa
Group 8	5	5337ppm	3@12wks	2@13wks	0/alive	Tblocks	XXXX	filterpa
Group 9	5	8000ppm	5@12wks		0/alive	Tblocks	XXXX	Filterpa
Grp 10	5	10000pp	all alive	0@12wks	5/alive	Blocks	4/90%	Week21

Table 1. Summarizes observed termite mortality/jar after initial 4 weeks, at 12 weeks, up to 21 weeks. Each jar had 2 blocks each. Mortality arbitrarily chosen as<10% survival or 90% dead. Total jars = 53. Total alive @ 12 weeks = 35/53 @ 21 weeks =21/53.

The lowest observed mortality was seen in the four feeder block groups ranging from 337-2604 ppm NHA. Random individual jars appeared to die out from weeks 7-17. The best explanation for this high survival rate is the low concentration of NHA, e.g. below 1000 ppm in three of four groups? More than 40% of the jars (21/53) still had termites surviving after 21 weeks. (See Table 1) A similar biphasic responses has been observed previously in AWPA Data Packages (2007) where mortality increases from 0-30% in 2-choice tests to 67-99% in no-choice tests w/o a second untreated wood block. Thus, decrease in mortality may be partly due to reduced feeding on the insecticide, either NHA or imidacloprid, treated blocks.

The most rapid or highest mortality was observed in higher concentration blocks…eg Groups 6-9. Most of these jars had >90% mortality after 12 weeks incubation. The best explanation for this is that these workers consumed more NHA and the test blocks were replaced by filter paper* instead of untreated blocks either pine or aspen. This unexpected anomaly can be illustrated in Groups 5 and 10, eg the lowest and the highest concentrations of NHA, as untreated aspen blocks were added at 4 weeks causing survival to extend out to 21 weeks. So here again, untreated wood blocks appear to abrogate rapid mortality from NHA. [*pine or aspen wood may abrogate the basic pH effects of NHA@ 8.2; in the original no-choice tests, wet blotter paper was added to prevent starvation, and this paper did not prevent 100% mortality?] Therefore, mortality did not appear to correlate with concentration of NHA in treated blocks. Another explanation is that the relative preference of aspen wood by *R.flavipes* may even further reduce the toxicity of NHA consumption.

CONCLUSION

An AWPA standard E1-97 choice termite test was performed in pine with workers of *R. flavipes* (Kollar) in order to compare feeding between the treated NHA block vs the untreated block. 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 2500ppm up 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 (or aspen) 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?

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