IRG/WP 07 -10605

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Foraging Behavior of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) in Response to Borate Treated Wood

Cory E. Campora[1] and J. Kenneth Grace[2]

[1]NAVFAC Pacific, Natural Resources Branch, 258 Makalapa Dr. Suite 100,
Pearl Harbor, HI 96860-3134, USA

[2]Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Honolulu, HI 96822, USA

Paper prepared for the 38th Annual Meeting
Jackson Lake Lodge, Wyoming, USA
20-24 May 2007

IRG SECRETARIAT
Box 5609
SE-114 86 Stockholm
Sweden
www.irg-wp.com

Foraging Behavior of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) in Response to Borate Treated Wood[1]

Cory E. Campora[2] and J. Kenneth Grace[3]
[2]NAVFAC Pacific, Natural Resources Branch, 258 Makalapa Dr. Suite 100,
Pearl Harbor, HI 96860-3134, USA
[3]Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa,
3050 Maile Way, Honolulu, HI 96822, USA (*Correspondence Author*)

Abstract

Foragers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, were allowed to tunnel in two dimensional, sand filled arenas containing Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) wafers pressure treated with disodium octaborate tetrahydrate (DOT) to an average retention of 1.77 % BAE on one side of each arena, and untreated wafers of Douglas-fir on the other side. Arenas were established both in the laboratory and in the field. Initial tunnel formation was unaffected by the presence of borate treated wood. Avoidance of borate treated wood developed after ca. 3-5 days. Termites did not avoid borate treated wood as a result of necrophobic behavior. Termite responses when the locations of the treated and untreated wafers were switched within the arenas indicated that the delayed avoidance was related to the location of the treated wood rather than to recognition of the chemical treatment.

Key Words: *Coptotermes formosanus*, disodium octaborate tetrahydrate, termite foraging behavior, Isoptera, Rhinotermitidae

Introduction

Certain slow-acting toxicants that are initially non-repellent to subterranean termites may induce a gradual avoidance response. This has been noted with foraging termites exposed to sulfluramid (Su et al.1995), hydramethylnon (Su et al. 1982), abamectin (Forschler 1996), and boron salts (Grace and Yamamoto 1994). Termites will initiate feeding on wood treated with the preservative disodium octaborate tetrahydrate (DOT), but cease feeding before damage to the wood exceeds the level of cosmetic surface injury (Grace et al. 1992, Grace and Yamamoto 1994). The cause of this delayed avoidance response to an initially acceptable food item is not known.

One explanation may be the tendency of termites to avoid areas where dead foragers are present. Observing foragers tunneling through agar filled petri dishes, Su et al. (1982) noted that if mortality from exposure to a termiticide occurred at a rate fast enough such that termites died and remained at the site of exposure, then subsequent foragers would avoid that location. Thus, even though the termiticide itself provided no stimulus for avoidance, termites shunned the treated area due to the repellency of the termite corpses. This behavior

---

[1] The text of this paper is reproduced with the permission of the Proceedings of the Hawaiian Entomological Society (2007), from a special issue representing the proceedings of the 2004 Pacific Entomology Conference. Contact the Treasurer for more information on the society or journal: Mr. Dick Tsuda, Hawaiian Entomological Society, c/o Dept. of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Maile Way, Room 310, Honolulu, HI 96822, USA (email: dmtsuda@hawaii.edu).

was termed “necrophobia” and was found to occur with non-repellent termiticides possessing a fast-acting mode of action such as chlorpyrifos and chlordane (Su 1982). Forschler (1996) noted a similar decline in local foraging activity when termite mortality occurred in the vicinity of certain bait toxicants.

A second possible explanation for avoidance of non-repellent, slow acting termiticides is that termites may learn to avoid treated areas as a result of sub-lethal exposure. In theory, individuals that are exposed to a non-repellent termiticide, but do not completely succumb, may be able to formulate a relationship between the negative effects they experienced and the chemical treatment. Su et al. (1995a) termed such a response “associative learning” and defined it as “the ability to form associations between previously meaningless stimuli and reinforcements.” Similarly, Thorne and Breisch (2001) termed the behavior “aversion” and explained it as a “learned response after an experiential association between one or more of the compound’s attributes and a negative impact such as sickness.” Occurrence of associative learning or aversion has been suggested to result in avoidance of feeding on such non-repellent bait toxicants as sulfuramid (Su and Scheffrahn 1991) and hydramethylnon (Su et al. 1982).

The boron salt disodium ocataborate tetrahydrate (DOT) is similarly categorized as a non-repellent, slow acting toxicant. Tunneling assays using DOT powder mixed with sand demonstrated that DOT did not inhibit tunneling by subterranean termites, but caused the eventual mortality of a substantial number of foragers (Grace 1991). This form of boron is not used commercially for soil treatment, but is commonly used for both preventative and remedial treatment of lumber and wood products intended for use in protected, above-ground exposures (Grace 1997). When treating wood by pressure or diffusion with a preservative, deterrence of feeding is the desired effect. However, even when treated with relatively high DOT concentrations, a small amount of surface (cosmetic) feeding by termites may still occur. Grace et al. (1992) found that wood pressure-treated with DOT to a target concentration of 1.02 % boric acid equivalents (BAE) and exposed for 23 weeks to a field colony of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, remained structurally sound, experiencing only 2.5 % mass loss, although some cosmetic damage did occur. In a further effort to determine the cumulative effect of this “tasting” behavior, Grace and Yamamoto (1994) sequentially exposed wood treated at various high concentrations of DOT to four *C. formosanus* colonies for 10 weeks each. Similar to the previous study, wood treated at the highest concentration of 2.52 % BAE experienced less than 1 % wood loss after the entire 40 weeks, but there was evidence of superficial feeding by all four colonies at the end of each 10-week exposure. These authors hypothesized that exposure to high concentrations of DOT might be causing termites to die in the immediate vicinity of the treated wood, resulting in avoidance by the remaining foragers.

The purpose of our study was to observe the behavior of Formosan subterranean termites as they discovered wood treated with DOT and determine if avoidance to wood treated at commercial rates (American Wood-Preservers Assoc. 2005) developed as a result of either necrophobia or associative learning. Observations of termite foragers were made both in laboratory and field settings using two-dimensional foraging arenas containing treated and untreated wood. This provided a means of establishing where foragers were dying within the gallery system, and examining the corresponding distribution of termite tunnels and the surviving foragers relative to the treated wood.

Materials and Methods

Laboratory assays. Groups of 1500 termites (90% workers, 10% soldiers) collected within the past 24 hours from a field colony (Tamashiro et al. 1973) were each placed in six two-dimensional Plexiglas foraging arenas, maintained under laboratory conditions. The arenas were constructed as described by Campora and Grace (2001), with damp silica sand sandwiched between two Plexiglas sheets creating a 75 by 75 by 0.25 cm tunneling substrate. Termites were placed in a jar connected to the center of the arena, from which they could enter the foraging substrate. Sixteen foraging sites (plastic vials inserted into holes in the arena, each containing a wood wafer) were arranged throughout each arena in a uniform grid pattern (Fig. 1). Foraging sites on one-half of three of the arenas, referred to as treatment arenas, contained Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) wafers pressure-treated with DOT to the commercial retention of 1.77% boric acid equivalents (BAE), while the other half of each arena contained untreated Douglas-fir wafers (see Figure 2). Treated wafers were provided by M. J. Manning, US Borax Inc. (Valencia, CA). All wood wafers in the remaining three (control) laboratory arenas were untreated. Termites were added to center of each arena and allowed to tunnel for 14 days, at which point the wafers were replaced with new wood. Locations of treated and untreated wafers were exchanged in the treatment arenas (Fig. 2). Termites were then allowed to continue tunneling for an additional 7 days, after which the arenas were dismantled, all living termites were counted, and wafers were oven-dried (90° C, 24 hr) and weighed.

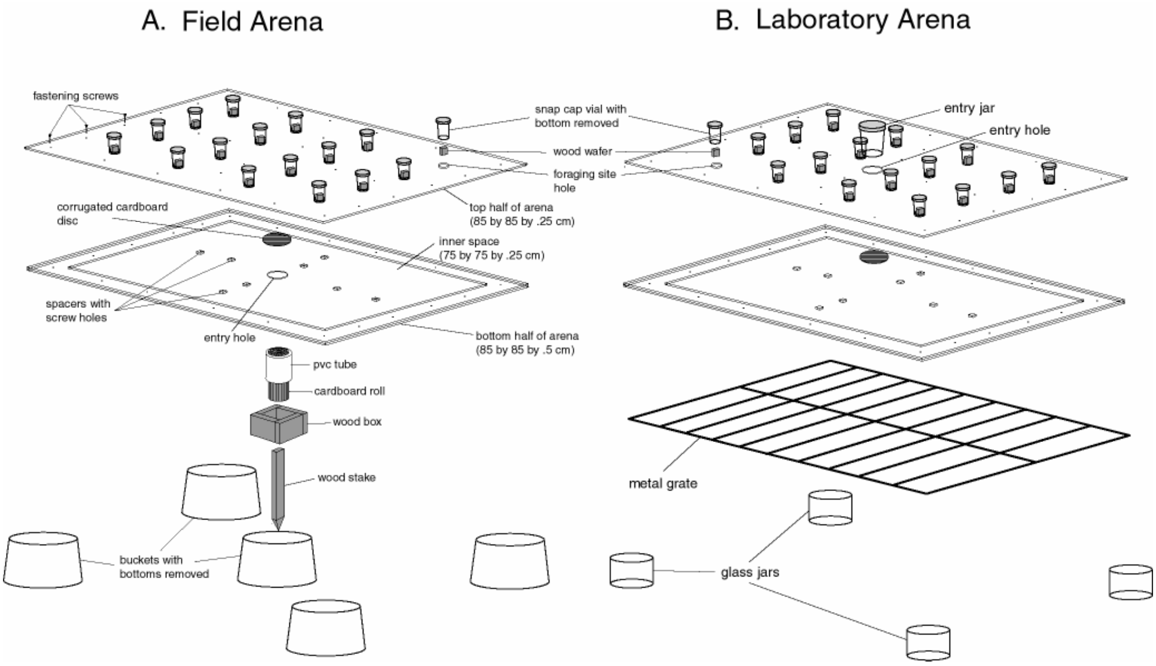

Figure 1. Design of termite foraging arenas for use in the in the field (A) and laboratory (B).

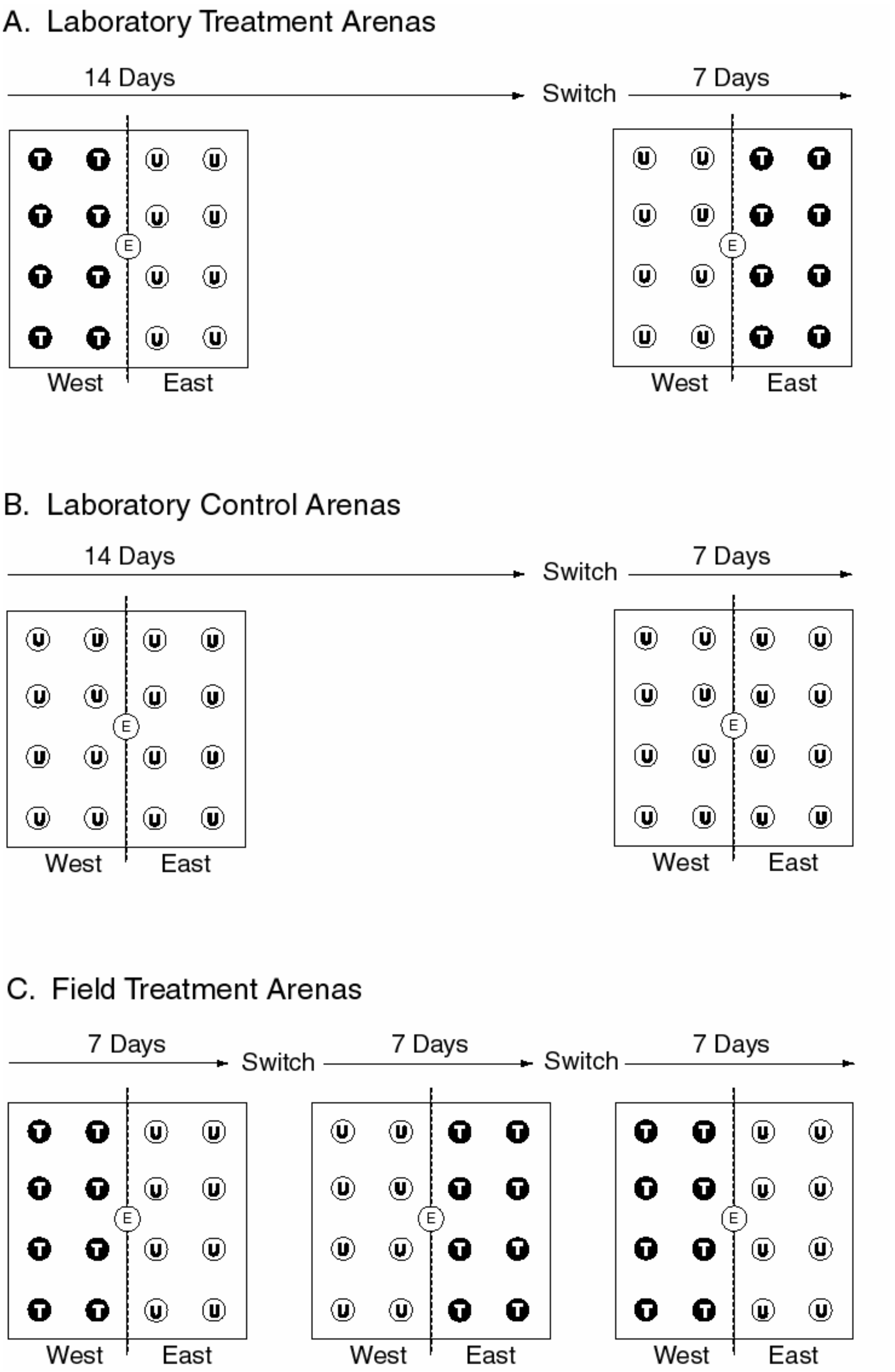

Figure 2. Location and replacement protocol of treated and non treated wood wafers in laboratory treatment (A), laboratory control (B), and field (C) arenas.

The variables measured in the laboratory were tunnel distribution, live forager distribution, dead forager distribution, forager presence on wood, and wood consumption. Tunnel distribution was determined from digital images of the arenas taken at 24 hour intervals. Using ArcView 3.2 GIS software (ESRI, Redlands, California), daily tunnel formation was digitized and quantified by the amount of tunnel surface area in the east and west sides of the arenas. Forager distribution in the arenas was measured from counts of termites made within a 5 by 5 cm grid. Counts were made daily between 3:00 and 5:00 pm. Termites counted consisted of all workers and soldiers within each grid square. To facilitate estimates, termite numbers within each grid square were classified into 6 categories (0 = 0, 1 = 1, 2 = 2 to 12, 3 = 13 to 24, 4 = 25 to 50, and 5 = 51 or more). Because the counts represented snapshots in time of termites as they moved throughout the gallery system, contour maps were extrapolated from the count data in ArcView using the Inverse Distance Weighted method. The volume of the resulting termite density landscape was then calculated for both sides of the arenas. The 5 by 5 cm grid was also used to determine mortality on each side of the arenas. Counts of dead termites were made daily in each grid square, and the totals for each side were compared. Forager presence on wood was measured daily by examining each wafer for the presence of termites. Data were recorded as percent coverage of the wafer by termites to the nearest 10 percent. The coverage on all wafers was pooled and divided by the total possible coverage to obtain the overall percent coverage of wood on each side of the arena. All wafers used in the experiment were pre-weighed, and wood consumption was calculated by weighing the wafers after exposure to termites.

Field assays. Three arenas similar to those used in the laboratory experiment were also placed on plastic supports over an actively foraging *C. formosanus* colony at the Waimanalo Experiment Station of the College of Tropical Agriculture and Human Resources. Each arena was connected to the ground through a roll of corrugated cardboard in a plastic pipe in the center of the arena, such that termite foragers could travel back and forth between the arena and their underground gallery system (Fig. 1). Eight of the 16 wood wafers on one side of each arena were treated with DOT to the same retention as in the laboratory experiment. Seven days after foragers entered the arenas, the wafers were replaced with new wood, and the location of treated wafers was switched to the opposite side of each arena. Wafers in the field arenas were switched again after an additional 7 days such that the new treated wafers were back in the initial locations of the original treated wood wafers. Data was collected from field arenas on tunnel distribution, mortality distribution, and wood consumption.

Analyses. Analysis of variance (ANOVA, General Linear Model, Minitab 2003) was used to determine significant differences between means of the variables measured on each side of the laboratory arenas for each day during the laboratory experiment. Differences in mean tunnel distribution on each side of the field arenas and mean wood consumption on each side of both laboratory and field arenas were also examined using ANOVA (PROC GLM, SAS Institute 1999), with means compared using Tukey's Highly Significant Difference (HSD) test.

Results

General Behavior. Termites in laboratory arenas were generally engaged in one of the four following activities: 1) actively feeding on wood; 2) traveling back and forth within the gallery system, actively excavating sand during tunnel formation and depositing it elsewhere within the arena; 3) traveling singly throughout the gallery system with no apparent goal or destination; and 4) resting in large groups at specific locations within the gallery system. During the first several days, most of the termites visible in the arenas were involved in tunneling, with the remainder of the group remaining in and around the entry jar in the center of the arena. Tunneling activity tapered off after the first week, and most of the termites were found either in resting groups or in the vicinity of the wood wafers. Resting groups tended to be around the perimeter of the center entry jar, but sometimes occurred elsewhere within some of the larger tunnels. Tunneling activity after the first week was sporadic and occurred in localized areas conducted by small groups of workers. There were no significant differences between the distributions of tunnels on either side of the arenas (Fig. 3 - 4), indicating that the presence of DOT treated wood did not deter termite tunneling in the surrounding substrate.

Spatial Distribution of Termites. Termites were initially aggregated in the center of the arenas, with foragers spread throughout the gallery system. However, after ca. 3-5 days, there was an aggregation trend towards the untreated side of the arenas with little foraging in the tunnels on the other side intersecting the borate-treated wafers (Fig. 3). This trend was not significant, however, most likely due to the fact that there were many termites in the portion of the treated (west) sides of the arenas that overlapped with the center entry jar, and there were termites still creating exploratory tunnels on the treated sides that had not yet intersected wood treated with DOT.

Spatial Distribution of Feeding Termites. The distribution of termites in direct proximity to the wood wafers was not as evenly distributed (Fig. 3). In laboratory arenas containing treated wood, there were significantly more termites on the untreated wood compared to the treated wood on 7 of the first 8 days of the experiment. Termites typically abandoned treated wafers ca. 3-5 days after discovery, but the time of discovery of treated wafers varied in each arena. This explains the apparent resurgence of feeding on treated wood on days 9 through 14, seen in Fig. 3. Also confounding our results are apparent feeding preferences between seemingly identical wood wafers. This is demonstrated in the control arenas, where on day 13 there were significantly more termites feeding on the west side of the arena compared to the east side. It is also interesting to note in Figure 3 that in the arenas with treated wood, termites tended to initially feed on treated wood when it was switched to foraging sites that previously contained untreated wood. Due to the small number of arenas used and the variation in foraging between arenas, the number of termites in either side of the arenas was not significantly different after the switch.

Spatial Distribution of Mortality. Dead termites were found on both sides of the arenas (Fig. 3), and were not found in the immediate vicinity of the borate-treated wood. Living termites were observed partitioning off dead termites and avoiding areas of dead termite accumulation. However, these areas were not correlated with the presence of treated wood, until the last days of the experiment when most of termites had died and there were no remaining colony members healthy enough to move corpses. Since sodium borate is a slow-acting toxicant, it is logical to note that exposed foragers would move through the gallery system and would not necessarily die near the treated wood. In fact, during the last week of the experiment there significantly more dead termites on the side of the arena that originally contained untreated wood.

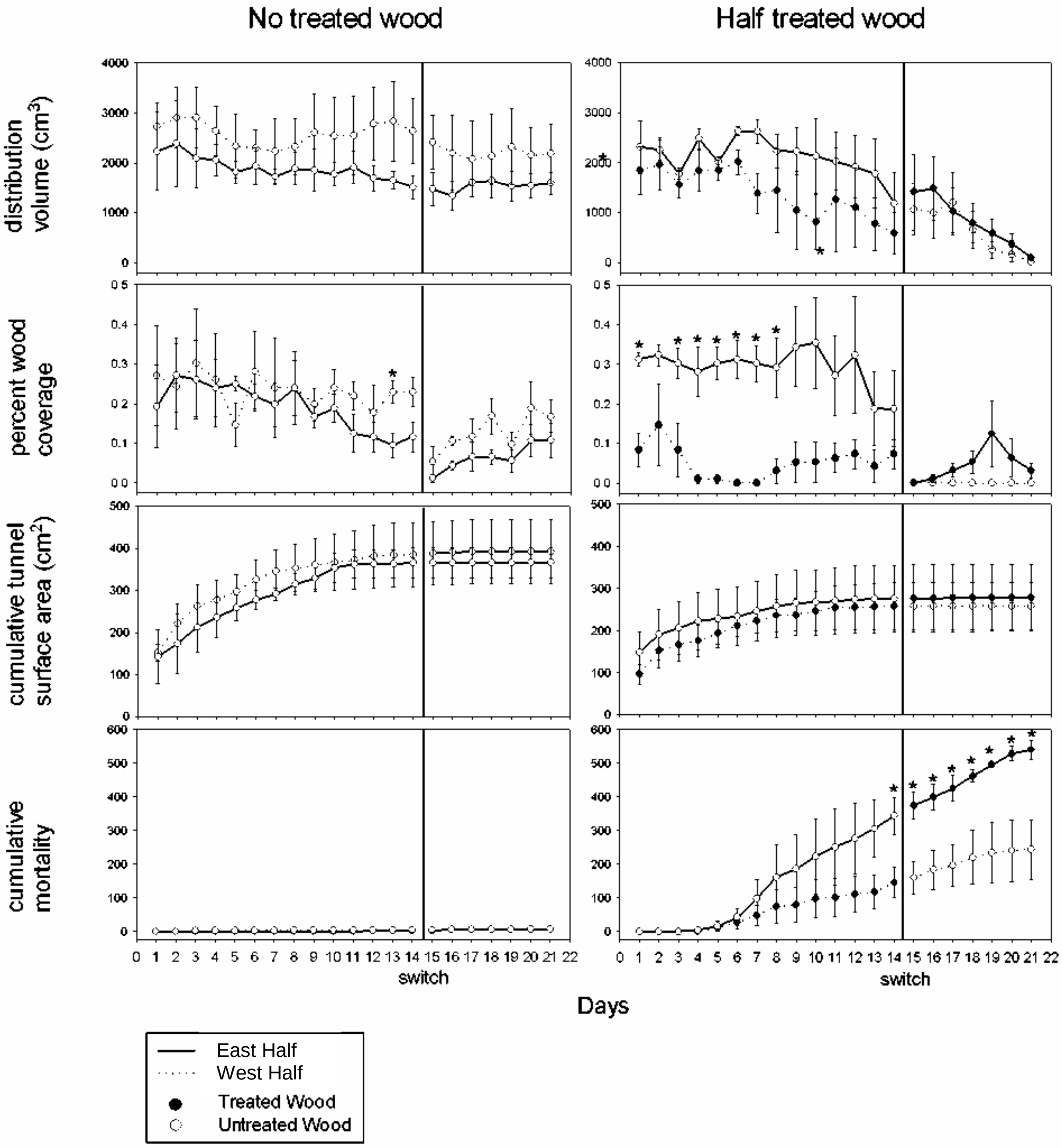

Figure 3. Comparisons of the spatial distribution of termites, feeding, tunneling, and mortality in the east and west halves of foraging arenas. ANOVA, GLM, * = P <0.05.

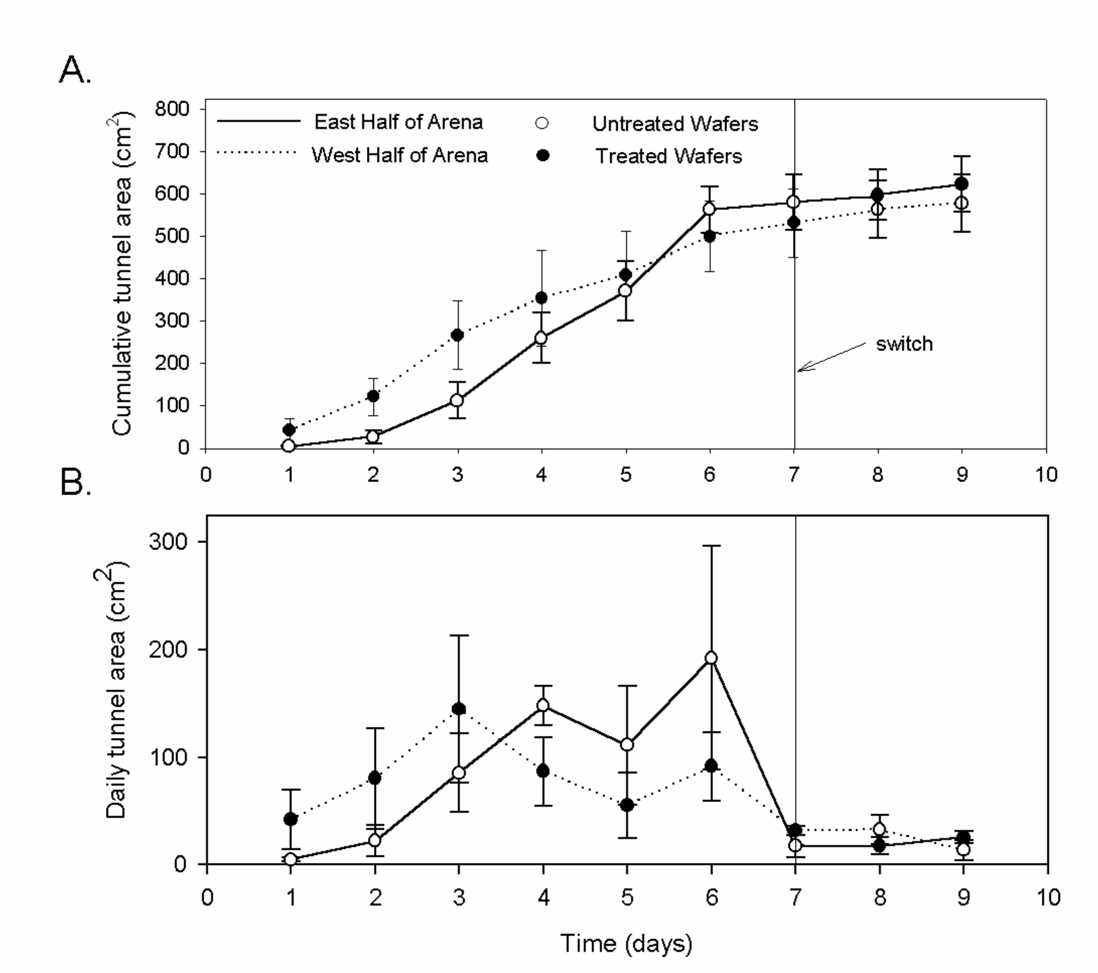

Figure 4. Average cumulative and daily termite tunneling amounts on each side of the field arenas.

Wood Consumption. In the laboratory control arenas, the amount of wood consumed was not significantly different on either side of the arena during the first two weeks, or during the last week. However, termites consumed significantly more untreated wood than treated wood during the first two weeks in laboratory treatment arenas (F = 9.75; df = 1, 4; P = 0.0355), and the amount of feeding that did occur on the treated wood amounted to only slight surface etching (Fig. 5). An even smaller amount of feeding occurred during the last week of the experiment, with no significant difference between sides of the arenas (Fig. 5).

Comparing wood loss between sides of the field arena within each time period, significantly more wood was consumed during the first week on the east side, the side originally containing untreated wood, compared to the west (treated) side (F = 9.69; df = 1, 4; P = 0.0358). There were no significant differences in wood consumption between sides during the second and third weeks (see Figure 5). Comparison of wood consumption across time periods within treatments showed that the mean percentage mass loss from treated wafers was greatest in the second week, but was only significantly greater when compared to the third week (F = 5.06; df = 2, 6; P = 0.0515) (Fig. 5). The greatest amount of untreated wood loss occurred in the first week, and was significantly greater when compared to the third week, but was not significantly greater than the second week (F = 5.70; df = 2, 6; P = 0.0411). There were no significant differences in average wood loss of untreated wafers between the second and third weeks (Fig. 5).

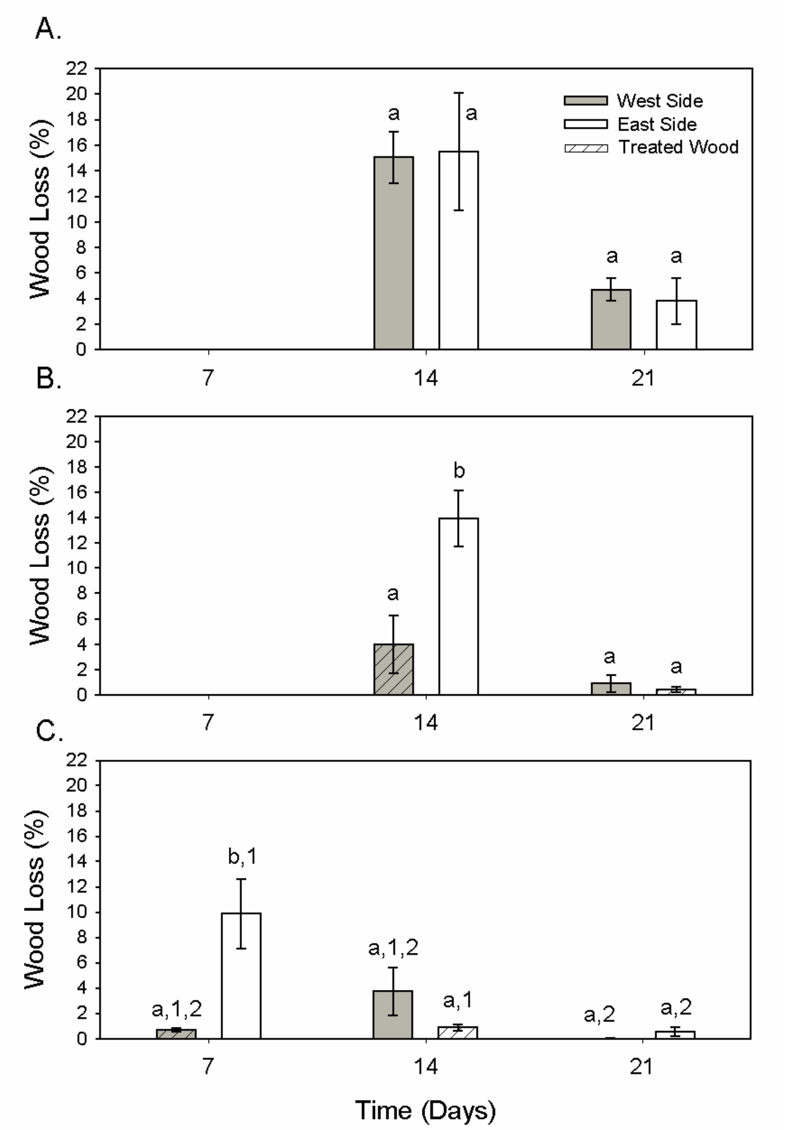

Figure 5. Average percent wood loss from wafers on both sides of laboratory control arenas (A), laboratory treated arenas (B), and field arenas. Means from each side with the same letters are not significantly different (within each time period). Means with the same numbers (C) are not significantly different across time periods (within treatments).

Discussion

Our results suggest that avoidance of borate treated wood by the Formosan subterranean termite does not result from the repellent accumulation of dead foragers in the vicinity of the treated wood. No dead termites were observed in any of the field arenas, and in the laboratory arenas, feeding on treated wood generally ceased before the effects of mortality were observed. Moreover, when dead termites did occur in the laboratory, they were usually not in the vicinity of a treated wafer. This was clearly seen in the laboratory arenas (Fig. 3) where initially termites were equally distributed throughout the entire arena and were present on both treated and untreated wood. After approximately three days, termites stopped any feeding activity on the treated wood wafers, but dead termites were not seen in the arenas until day 5. Once termites started dying, the distribution of both live and dead termites in the arenas shifted to the east side, or untreated side. A three-day lag time between the beginning of exposure of *C. formosanus* to sodium borate and the onset of toxic effects was also observed by Maistrello et al. (2001), when termites were exposed to wood containing 0.1571% BAE.

When the wood wafers were replaced and the locations of treated and untreated wafers were switched, termites returned to feeding at sites where feeding was previously most intense (i.e., those sites that had previously contained untreated wood). In all cases they fed superficially on the newly added treated wood, showing no recognition of the borate treatment. In the field, average consumption of treated wood appeared to be greater during the second week when the treated wafers were placed on the side of the arena that had previously contained untreated wood. The increase was not statistically significant, however, due to variation in feeding rates between the arenas. Nonetheless, these findings suggest that termites did not avoid borate treated wood based on recognition as a result of previous sublethal exposure. Thus, it appears that avoidance is related more to the location of the treated wood than to the chemical treatment itself.

A potential explanation for this could lie in the chemical recruitment system utilized by termites during foraging. Subterranean termites orient themselves throughout tunnel systems using trail pheromones deposited by their sternal glands (Luscher and Muller 1960). Trail pheromones play an important role in directing foragers to feed in certain areas (Tschinkel and Close 1973). There is evidence that trail pheromones consist of an ephemeral recruiting component and a more stable orientation component (Hall and Traniello 1985). The strength of the ephemeral component and subsequent recruitment is reinforced or diminished by the quality of the food resource (Traniello and Leuthold 2000). Therefore, if the signal is strong, many termites can be induced to feed at a location, and if the signal is weak, termites may not follow it all. If foragers feeding on treated wood become sickened and later die after feeding, the ephemeral recruiting component can undergo a diminishing rate of reinforcement. As fewer termites travel to the site, there is less pheromone deposited and consequently less foraging traffic until all that remains is the stable orientation component. Rickli and Leuthhold (1987) found that for the harvester termite, *Trinivitemes geminatus*, information provided by trail pheromones was the dominant influence on a termite as it chooses where to go within a network, but that also the amount of activity by termites within the network played a role. This may explain why termites in this study stopped visiting sites containing borate treated wood over time, but resumed feeding on it when it was moved to areas that previously contained untreated wood.

We conclude that initial tunnel formation by a foraging group of Formosan subterranean termites is unaffected by the discovery of borate treated wood. It is unclear however, what the long-term effects of discovered borated treated wood are on a colony as it expands its foraging territory. Additionally, we found that Formosan subterranean termite foragers do not avoid borate treated wood as a result of necrophobic behavior nor from a learned response to borate treated wood. It rather appears that avoidance of the slow acting toxicant may be a behavior mediated by the decreasing amounts of trail pheromone due to mortality within the foraging group. The gradual aggregation of termites in the portion of the arenas containing untreated wood, and the delayed return to the area formerly occupied by treated wafers when the locations of treated and untreated wafers were switched suggests that termites are mapping the resources within their gallery system with respect to their acceptability. That is, they are avoiding the location of the toxic resource rather than recognizing any attributes of the toxic treatment itself. This mapping could conceivably result from changes in traffic within the tunnels due to mortality or sublethal effects reducing the quantity of trail pheromone (or other chemical cues) leading to or deposited near the toxic resource.

Acknowledgments

We are grateful to M. J. Manning and J. R. Yates III for helpful reviews of earlier drafts of the manuscript. This research was supported by USDA-ARS Specific Cooperative Agreement 58-6615-4-237, and McIntire-Stennis funds for forestry research administered by the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

References

American Wood-Preservers' Association. 2005. Book of standards. AWPA Standard P5.

Campora, C.E., and J.K. Grace. 2001. Tunnel orientation and search pattern sequence of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology 94: 1193-1199.

Forschler, B.T. 1996. Baiting *Reticulitermes* (Isoptera: Rhinotermitidae) field colonies with abamectin and zinc borate-treated cellulose in Georgia. Sociobiology 28: 459-483.

Grace, J. K. 1991. Response of Eastern and Formosan subterranean termites (Isoptera: Rhinotermitidae) to borate dust and soil treatments. J. Econ Entomol. 84: 1753-1757.

Grace, J. K. 1997. Review of recent research on the use of borates for termite prevention. 2nd Intl. Conf. On Wood Protection with Diffusible Preservatives. Proc. 7284 Forest Prod. Soc., Madison, WI, pp 85-92.

Grace, J.K. 2002. Hawaiian experience with treated building components. Enhancing the Durability of Lumber & Engineered Wood Products. Forest Products Society, Madison, Wisconsin. Pp. 305-309.

Grace, J.K., and R.T. Yamamoto. 1994. Simulation of remedial borate treatments intended to reduce attack on Douglas-fir lumber by the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology 87: 1547-1554.

Grace, J.K., R.T. Yamamoto, and M. Tamashiro. 1992. Resistance of borate treated Douglas-fir to the Formosan subterranean termite. Forest Products Journal 42(2): 61-65.

Hall, P., and J. F. A. Tramiello. 1985. Behavioral bioassays of termite trail pheromones. Recruitment and orientation effects of cembrene-A in *Nasutitemes costalis* (Isoptera: Termitidae) and discussion of factors affecting termite response in experimental contexts. J. Chem. Ecol. 11: 1503-1573.

Luscher M., and B. Muller 1960. Ein spurbilendes Sekret bei Termiten. Naturwiss. 21: 503.

Minitab. 2003. Release 14. State College, PA

Maistrello, L., G. Henderson, and R. A. Laine. 2001. Effects of nookatone and a borate compound on Formosan subterranean termite (Isoptera: Rhinotermitidae) and its symbiont protozoa. J. Entomol. Sci. 36: 229-236.

Rickli, M., and R. H. Leuthold. 1987. Spacial organisation during exploration and foraging in the harvester termite, *Trinervitermes geminatus*. Revue Suisse Zool. 94: 545-551.

SAS Institute. 1999. SAS user's guide: version 8. SAS Institute, Cary, N. C.

Su, N-Y. 1982. An ethological approach to the remedial control of the Formosan subterranean termite *Coptotermes formosanus* Shiraki. Doctoral Dissertation, University of Hawaii, pp 124.

Su, N-Y., R. H. Scheffrahn, and P. M. Ban. 1995. Effects of sulfuramid treated bait blocks on field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology 88: 1343-1348.

Su, N-Y., M Tamashiro, J. R. Yates, and M. I. Haverty. 1982. Effect of behavior on the evaluation of insecticides for the prevention of or remedial control of the Formosan subterranean termite. Journal of Economic Entomology 75: 188-193.

Tamashiro, M., J. K. Fujii and P.-Y. Lai. 1973. A simple method to trap and prepare large numbers of subterranean termites for laboratory and field experiments. Environmental Entomology 2: 721-722.

Thorne, B. L. and N. L. Breisch. 2001. Effects of sublethal exposure to imidacloprid on subsequent behavior of subterranean termite *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). Journal of Economic Entomology 94: 492-498.

Tschinkel, W. R., and P. G. Close. 1973. The trail-pheromone of the termite *Trinivitermes trinervoides*. J. Insect Physiol. 19: 707-721.

Traniello, J. F. A., and R. H. Leuthold. 2000. Behavior and ecology of foraging in termites. Pp 141-168 *in* Abe, T., Bignell, D. E., & Higashi, M. (Eds). Termites: evolution, sociality, symbioses, ecology. Dordrecht, Netherlands, Kluwer Academic Publishers.