CHARACTERIZATION of SUBTERREANEAN TERMITE POPULATIONS as PART of BAIT EVALUTION

Bart T. Foster, Anthony I. Cognato, and Roger E. Gold

Department of Entomology, Texas A&M University, College Station, Texas

Abstract The development of termite baits has demanded effective means of evaluating the success of commercial bait systems. Evaluation has been complicated by unknown factors concerning subterranean termite biology and foraging as well as a lack of definitive research methods. One important element of effective evaluation is the ability to characterize a termite population prior to treatment. In this study, monitoring, species identification based on soldiers, and DNA sequences from the mitochondrial A-T rich region were utilized to define termite populations in three field plots. Outpost TDS monitors (Bayer) produced no termite activity for a six-month period with continued activity on an independent monitoring system. Soldiers could be identified to species using a combination of labral shape and mandible curvature. Mitochodrial haplotypes were not definitive for each colony.

Key Words Identification monitoring

INTRODUCTION

Many changes have occurred over the past 20 years in the way subterranean termites are treated. The most significant departure from traditional efforts to establish a chemical barrier around a structure has been the development of commercial bait systems. Use of these systems has been both promising and challenging. A poor understanding of subterranean termite biology (Forschler and Jenkins, 1999) and the dynamic and cryptic nature of termite colonies (Su, 1991) complicate research methods aimed at assessment of bait efficacy (Thorne and Forschler, 2000).

Assessment of bait effectiveness includes: effective monitoring prior to baiting and after treatment (Thorne and Forschler, 2000); use of an independent monitoring system (Getty et al., 2000); and a method to distinguish the targeted termite colony and those termites that populate the area after treatment (Su and Scheffrahn, 1996). This project followed the above guidelines in an attempt to test bait efficacy and termite reinvasion. This paper is a summary of termite activity in the field sites through 18 months and provides an assessment of our experimental methodology.

MATERIALS and METHODS

The test site consisted of three 27.5 x 27.5 meter plots of similar habitat structures within an undeveloped park in Brazos County, Texas. Monitoring stations were placed in a 12 x 12 grid pattern at 2.5 m intervals for a total of 144 monitors per plot. Monitoring stations were a modification of a hollow wooden stake method (Ewart et al., 1992; Houseman, 1999) consisting of 15-cm lengths of 3.5 x 3.5 cm pine lumber. Each pine monitor had a 2 cm diameter hole drilled lengthwise though the middle of the stake and a single row of five or six 3mm diameter holes drilled from the outside to center on each of the four sides. The smaller holes on each side allow termite access to the larger center hole. Each station was placed into the soil with the large hole perpendicular to the soil surface and the top of each monitor left slightly above ground level. A rubber stopper was used to plug the center port while allowing access to view termite activity.

Foraging activities were recorded for each monitor starting in July of 2000 for two plots designated A and B, and in October of 2000 for plot C. Monitoring stations were inspected at

two-week intervals for termite activity, indicated by the presence of termites and mud crust, by removing the rubber stopper and examining the center hole with a flashlight. The period from establishment to July 2001 constituted a monitoring phase. During this time, the location for each active monitor was recorded and the number of termites present within the monitoring station was estimated using a number scale. Abundance estimates were ranked on a scale from 1 to 4; each number represented one of the following activity levels: 1 = 1-10 termites; 2 = 11-50 termites; 3 = 51- 150 termites; and 4 = 151 or more termites present. Similar ranking systems were used in previous foraging research (Haverty et al., 1999; Houseman, 1999). This method allowed a quick visual estimate of population with minimal disturbance and provided a means to rank activity before termites withdrew to their underground tunnels. Samples of the termites present within each monitor, particularly soldiers, were collected using an aspirator. Monitoring stations were replaced when termite feeding or rot reduced the structural integrity of the monitor to a point where it could be easily broken by hand. Environmental factors such as soil temperature, ambient temperature, and the amount of precipitation were recorded. Stainless steel pocket thermometers (Fisher Scientific Co.) were placed in the grids between every four monitors to record soil temperatures. Ambient temperature and precipitation data were obtained through weather readings recorded at Easterwood Airport in Brazos County, located approximately 11.25 km from the test sites. After one year, Outpost TDS monitors (Bayer), with wood but no active ingredients, were placed adjacent to any pine monitors attacked by termites during the first year.

Termites collected from pine monitors were killed and stored in 70-95% ethanol for subsequent morphological identification and molecular analysis. Soldiers were identified to species using keys based upon labrum shape (Hostettler et al., 1995) and the curvature of the mandible (Scheffrahn and Su, 1994). Molecular characterization of termite population structure within plots utilized mitochodrial DNA sequences from the psuedergate caste. Mitochodrial DNA fragments from the AT-rich region were sequenced from individuals collected during the course of the study. DNA extractions of single crushed termite heads were conducted using a DNeasy tissue kit manufactured by Qiagen, Stanford Valencia, CA 91355). Two micro liters of individual extracted DNA samples were prepared for Polymerase Chain Reaction (PCR) by the addition of 35 ml PCR grade H_2O, 5 ml $MgCl_2$ free buffer, 4 ml $MgCl_2$, 1 ml dNTP's, 0.2 ml Taq polymerase, and 2 ml of a 5pmol solution of each PCR primer. PCR primers utilized were TM-N-193 (TGGGGTATGAACCAGTAGC [Taylor et al., 1993]) and AT-J-T1 (CACTAAGGATA-ATCAATTATACGTC [Jenkins et al., 1998]). PCR was carried out in a Peltier Thermal Cycler PTC-200 under the following conditions: 94°C for 150 seconds, 50°C for 30 seconds, 72°C for 60 seconds, for a total of 36 cycles, followed by 72°C for five minutes. PCR products were then electrophoresized in a 1X TBE Buffer at 100 volts for 30 minutes on 1.5% agarose gel stained with Ethedium Bromide (10 mg/ml solution) and visualized in UV light. Following electorphoresis, samples were cleaned using a Qiagen PCR cleanup kit and prepared for cycle sequencing. Cycle sequencing was performed with fluorescently dyed terminator nucleotides (Big Dye kit manufactured by Applied Biosystems, Foster City, Calif.) in a cocktail consisting of 8 ml of PCR grade H_2O, 2 ml of Big Dye, 3 ml of 5x sequencing buffer, 3 ml of primers, and 2 ml cleaned PCR product. Both strands of DNA for each PCR product were sequenced. Cycle sequencing products were then cleaned with sephadex columns and dried down using a speed vacuum to prepare each sample for sequencing. Cycle sequencing products were visualized on an ABI 377 automated sequencer (PE Applied Biosystems) operated by the Gene Technologies Lab (Department of Biology, Texas A&M University). The chromatographs of each strand of DNA were imported into the computer program Sequencer 3.1, and consensus sequences were constructed. The consensus sequences from all individuals were cut and pasted into a NEXUS file to enable phylogenetic analysis in PAUP (Swofford, 1998).

RESULTS

All monitors that were active in each plot are shown in Figures 1-3. During the 18-month time-period, 340 active (termites in monitor) pine monitors were recorded in plot A, 86 active monitors in plot B, and 31 active monitors in plot C. These numbers include repeated activity on the same monitors as recorded during examinations at two-week intervals. The number of termites per station and the duration of activity varied. Some monitors were active for only one examination while some monitors were active over several weeks.
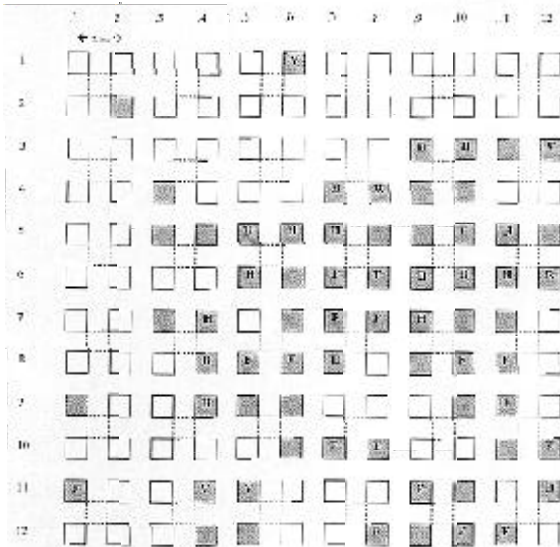

Figure 1. Subterranean termites detected in plot A. Wooden monitors arranged in a grid pattern in a 27.5 x 27.5 meter plot are represented by each box. Rows and columns are numbered respectively as 1-12 and .1-.12 to give each monitor a unique number such as 1.1. Spacing between monitors was 2.5 meters. Shaded boxes indicate termite activity during an 18-month period. Species identifications based on the soldier caste are indicated by the letters V, F, H, and R. V represents Reticulitermes virginicus, *F represents* R. flavipes, *H represents* R. hageni, *and R represents the presence of more than one of these species at the indicated monitor over the 18-month period.*
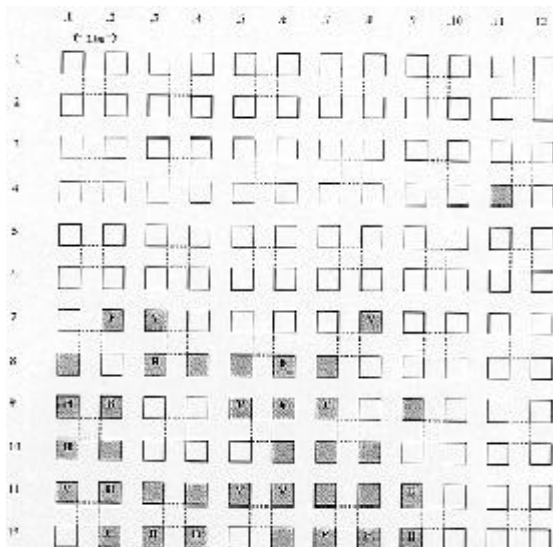

Figure 2. Subterranean termites detected in plot B. Wooden monitors arranged in a grid pattern in a 27.5 x 27.5 meter plot are represented by each box. Rows and columns are numbered respectively as 1-12 and .1-.12 to give each monitor a unique number such as 1.1. Spacing between monitors was 2.5 meters. Shaded boxes indicate termite activity during an 18-month period. Species identifications based on the soldier caste are indicated by the letters V, F, H, and R. V represents Reticulitermes virginicus, *F represents* R. flavipes, *H represents* R. hageni, *and R represents the presence of more than one of these species at the indicated monitor over the 18-month time period.*
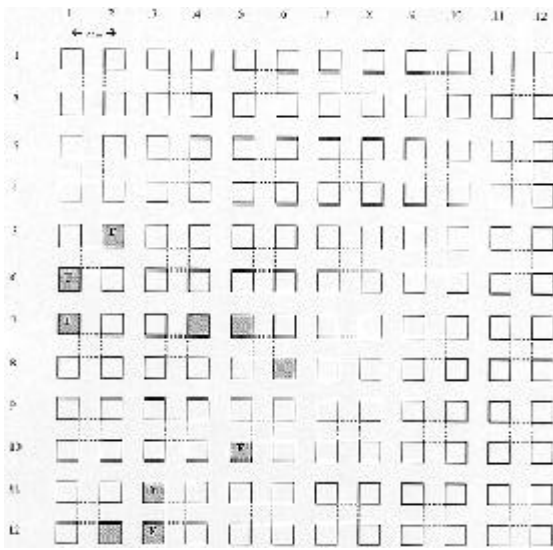
Figure 3. Subterranean termites detected in plot C. Wooden monitors arranged in a grid pattern in a 27.5 x 27.5 meter plot are represented by each box. Rows and columns are numbered respectively as 1-12 and .1-.12 to give each monitor a unique number such as 1.1. Spacing between monitors was 2.5 meters. Shaded boxes indicate termite activity during a 14-month period. Species identifications based on the soldier caste are indicated by the letters V, F, H, and R. V represents Reticulitermes virginicus, F represents R. flavipes, H represents R. hageni, and R represents the presence of more than one of these species at the indicated monitor over the 14-month time period.

The most striking result from this experiment is the lack of termite feeding on the commercial monitors. A total of 98 commercial monitors were placed in the field plots, next to independent monitors that had been attacked by termites during the first year. During six months of commercial baiting, 128 active infestations were recorded on the 432 independent monitors, while 0 infestations were found in any commercial monitor. After discounting repeated hits on the same monitors, a total of 61, or 14.1%, of the independent monitors were active at some point during this six-month time period.

Three species of subterranean termites — *Reticulitermes flavipes* (Kollar), *Reticulitermes virginicus* (Banks), and *Reticulitermes hageni* (Banks) — were found in plots A and B; two species, *R. flavipes and R. virginicus,* were found in plot C. Locations of species within each plot are shown in Figures 1-3. *R. virginicus* soldiers were readily identified using the labrum shape (Hostettler et al., 1995) as shown in Figure 4. Subtle differences were seen between the labrum shapes of the other two species (Hostettler et al., 1995), as shown in Figures 5 and 6. The degree of mandible curvature (Scheffrahn and Su, 1994) was also useful in separating *R. flavipes* from *R. hageni*. Pronotal width (Scheffrahn and Su, 1994) was not definitive for each species, but this event may have been a consequence of small sample sizes from each collection (Hostettler et al., 1995). A characteristic not mentioned in the labrum key (Hostettler et al., 1995), which was consistent for all *R. virginicus* soldiers, was the rectangular shape of the dark area on the forward part of the labrum (Figure 4). This dark labral region was always triangular shape for *R. flavipes* and *R. hageni* soldiers (Figures 5, 6). Although there were periods of activity and inactivity, the location of species in each plot was relatively stable during the 18 months of observation.

Mitochodrial DNA haplotypes were not unique for each colony. Seven unique mtDNA haplotypes were found for 23 samples processed from seven different collections. Termites collected from the same station, at the same date and time, had different haplotypes, a fact that suggests they were descendants of different maternal lineages. This conclusion supports work that suggests a possible exchange of individuals between colonies (Jenkins et al., 1999).
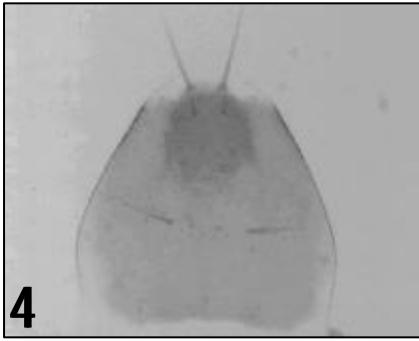
Figure 4. Slide mounted Reticulitermes virginicus *soldier labrum at 100x.*
Figure 5. Slide mounted Reticulitermes flavipes *soldier labrum at 100x.*
Figure 6. Slide mounted Reticulitermes hageni *soldier labrum at 100x.*

DISCUSSION

The poor acceptance of the commercial monitoring system by the termites could be due to several factors. Unlike the pine monitors, the wooden pieces used in the commercial monitors were not weathered prior to installation. From experience in termite collecting, we have observed weathered or aged wood appears more effective in attracting termites than is newly processed lumber. All of the commercial bait systems available utilize un-aged wood. Weathering this wood may be a simple method to increase the attractiveness of bait monitors. It is also possible that the styrofoam, which surrounds the wood in the commercial bait monitors, physically hinders termites foraging. Further explanations of these factors need to be evaluated.

Identification keys were useful tools in characterizing the termite populations. Within each plot, termite populations could be partially segregated under the assumption that different species constitute different colonies.

At the onset of this project, our intention was to use the frequency of the haplotypes to define the termite populations pre- and post-bait treatment. The population prior to treatment was characterized by seven haplotypes. Failure to attract termites to the bait monitors precluded the remainder of this study. However, haplotype frequency changes and/or the presence of new haplotypes could indicate that the bait treatments reduce the pre-treatment population and would suggest that the post-treatment population immigrated from outside the immediate study site. No change in haplotype frequency would suggest that the bait treatment did not effectively reduce the original population. Changes in haplotype frequency may occur independent of bait treatment. Thus temporal changes of haplotype frequency will be assessed for the samples collected during 18 consecutive months (July 2000-January 2002). This revised focus will allow us to gauge the use of genetic characterization of populations in future assessment of bait treatments.

Termite baiting is a challenging, but promising, new approach to termite control. Assessment of baiting systems is important, but the methodology for these assessments is certainly in the developmental stage. Even with the challenges, field experiments such as this study are necessary not only to test the bait systems, but also to develop and perfect evaluation techniques.

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