Proceedings of the Ninth International Conference on Urban Pests
Matthew P. Davies, Carolin Pfeiffer, and William H Robinson (editors) 2017
Printed by Pureprint Group, Crowson House, Uckfield, East Sussex TN22 1PH UK

INTER- AND INTRASPECIFIC COLONY INTERACTIONS IN *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE)

MARK A. JANOWIECKI AND EDWARD L. VARGO
Department of Entomology, Texas A&M University, College Station, Texas, USA

Abstract Subterranean termites are the most economically damaging termites in the U.S. costing $11 billion annually for prevention, treatment, and repair of damage. However, termite colony spatial dynamics are an understudied topic. In previous laboratory studies, termite colonies of different species are aggressive while termite colonies of the same species are passive to each other. Conversely, in field studies, colonies of the same species have not been observed overlapping whereas congeneric colonies foraging ranges were observed overlapping. These seemingly conflicting findings require further research to clarify the inter- and intraspecific interactions of *Reticulitermes*. This project investigates these colony interaction dynamics in east Texas with three native species of *Reticulitermes: R. flavipes, R. virginicus,* and *R. hageni*. Two 14x14 grids of pine stakes spaced 2m apart were established in the Sam Houston State University Center for Biological Field Studies for a total of 392 stakes. These stakes were monitored monthly for active termite foraging and had a hit rate more than 10% in the first month alone. Collected termites will be genetically fingerprinted using microsatellites and colonies will be tracked for the duration of the study. Laboratory colonies will be established from termites at the field site and will be used for behavioral assays using both the petri plate and planar assay methods. Unique species and colonies will be paired in these assays to compare aggressive behavior. Finally, further sampling and investigation will occur at the areas of overlap to compare the behavior of different colonies of termites in the field to the results of the laboratory assays.

Key words Subterranean termites, genetic fingerprinting, behavior, planar assay.

INTRODUCTION

Subterranean termites, including *Reticulitermes*, primarily live in the soil and are the most structurally destructive family of termites in the United States (Suiter et al., 2002). While subterranean termites are urban pests, economically and environmentally, they are important because of their ability to consume cellulose materiel. Annually, Americans spend approximately $11 billion on prevention, treatment, and repair of subterranean termite damage (Su, 2002). Despite their pest status, termites recycle nutrients by decomposing wood and other nutrients back to the soil, thus providing a vital ecosystem service (Thorne and Forschler, 2001).

Although different termite species and colonies of the same species have often been observed in close proximity (Houseman et al., 2001; Deheer and Vargo, 2004), inter- and intraspecific termite colony dynamics and interactions have not been thoroughly studied in *Reticulitermes*. Houseman et al. (2001) investigated distributions of *R. flavipes* and *R. hageni*, but were unable to distinguish unique colonies. They attributed spatial distribution of these two species to temperature and soil moisture content with *R. hageni* preferring to forage in hot, dry periods and *R. flavipes* in cool, damp periods (Houseman et al., 2001). This confirmed an earlier laboratory study by Forschler and Henderson (1995) which found that *R. flavipes* is able to withstand water submersion the longest (LT_{50} of 19.6 h) of the several subterranean termites sampled. Laboratory aggression assays found a behavioral reaction between species of *Reticulitermes* (Thorne and Haverty, 1991), but no aggression between unique colonies of

R. flavipes (Bulmer and Traniello, 2002). In a field study, Deheer and Vargo (2004) found the opposite scenario, with overlapping colonies of different species, but no overlap in foraging ranges of colonies of the same species. This study tracked *Reticulitermes* colonies in Raleigh, North Carolina, U.S., for two years and determined colony identity using microsatellite DNA markers. Generally, they observed that low numbers of smaller colonies of *R. flavipes* never overlapped and fewer, larger colonies of *R. virginicus* overlap with colonies of *R. flavipes* ranges. In two cases, *R. virginicus* foragers were found in the same piece of wood that previously was inhabited by *R. flavipes* (Deheer and Vargo, 2004). *Reticulitermes* species and colony interactions are understudied and contain many discrepancies between laboratory and field trials investigating the same biological aspect. This study will examine these inter- and intraspecific colony interactions to determine what variables influence these boundaries and interactions. To investigate these colony interactions, I intend: 1) to characterize the species and colony boundaries of *Reticulitermes* in a given location, 2) to determine aggression between colonies of the same and different species in laboratory trials, and 3) to observe areas of actual or potential overlap of termite colonies in the field.

MATERIALS AND METHODS

Objective 1. A field site at the Sam Houston State University Center for Biological Field Studies was established in August 2016. Two 2-meter grids of pine stakes were created to provide similar clarity to previous studies that used a 2 meter and 2.5 meter grid, respectively (Houseman et al., 2001, Deheer and Vargo, 2004). Samples were collected monthly from any stake with active termite feeding, as done in DeHeer and Vargo (2004). Species will be determined genetically (Szalanski et al., 2003) for each sample. Colonies will then be characterized with previously established microsatellite DNA markers (Vargo, 2000; Dronnet et al., 2004) in a multiplex to differentiate and track colonies over time. The data obtained will be compared to previous studies (Houseman et al., 2001; Deheer and Vargo, 2004) and live termites collected will be kept in the laboratory for the next objective.

Objective 2. Although *R. flavipes* was previously shown to not be aggressive to other *R. flavipes* colonies (Bulmer and Traniello, 2002), this study assumed colonies were unique based on distance since it was before genetic markers were commonly used in termites. Now, with updated methods and in a different part of the species range (Texas rather than Massachusetts), I will examine interactions between termite colonies of the same and different species using the agonism assay outlined in Bulmer and Traniello (2002) as well as the planar areas from Chouvenc et al. (2011). Briefly, the Bulmer and Traniello (2002) method introduces 20 worker termites of each colony in an arena lined with moist filter paper and assesses mortality and injury at 24 hours after introduction. Termites are differentiated by feeding them dyed filter paper in advance of the experiment. This temporarily dyes the termite gut and is visible through its exoskeleton. Controls will consist of both 40 workers collected from the same wooden stake and combinations of workers collected from different stakes that were genetically determined to be the same colony. The second method in Chouvenc et al. (2011) has a modified arena consisting of soil sandwiched between two sheets of Plexiglas. This method has shown to have generally higher survival rates and provides a more realistic setup and allows the termites to tunnel (Chouvenc et al., 2011). These methods will be replicated with as many samples as possible obtained from the first objective.

Objective 3. After the termite colonies have been mapped out for each field site, the areas of overlap or near overlap will be investigated in greater detail. Additional wooden stakes will be added in these areas to make a 0.5 m grid that will provide more clarity of the overlap and ranges of each colony. Any additional wood debris present in these areas will be examined for potential foragers of either colony. Observations will be made at these overlapping areas as to how termites avoid or conflict with other colonies of the same or different species.

RESULTS

In the first five months at the field site (September 2016 to January 2017) there was an average of 48.4 active termite monitors per month, greater than 10% of the total stations. Currently, the genetic analysis is ongoing, but it appears that the abundance in the field station is directly correlated with the moisture in each site. The total number of active stations is approximately stable over the first five months, but the abundance in the two plots vary over time (Figure 1).

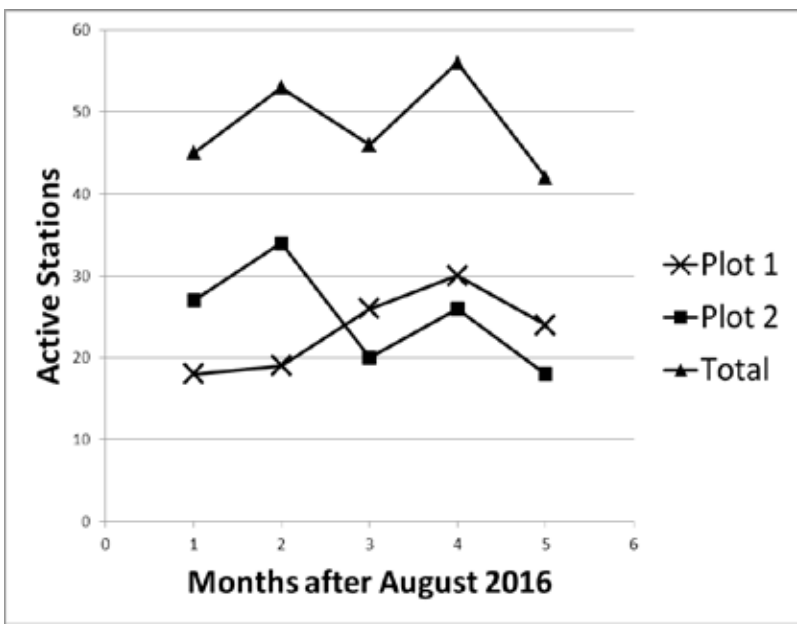

Figure 1. Number of active stations over the first five months for Plot 1 and Plot 2.

DISCUSSION

While much is known regarding termite biology, there are still many knowledge gaps that require further investigation. Although studies have scratched the surface of *Reticulitermes* colony interactions in the lab and field (Thorne and Haverty, 1991; Houseman et al., 2001; Thorne and Forschler, 2001; Bulmer and Traniello, 2002; Deheer and Vargo, 2004; Chouvenc et al., 2011), a consensus and understanding of the interactions is lacking. This project seeks to investigate these basic interactions between colonies of *Reticulitermes* of the same and different species to produce the foundation required to further study the mechanisms that mediate these interactions.

Termites are very significant economic pests, costing billions of dollars annually in the U.S. (Su, 2002). Colony interactions, as investigated in this project, have the potential to be applied to future pest management strategies. It has been suggested by Thorne and Haverty (1991) that the ability to understand and breakdown cues that termites use to distinguish colony members from outsiders could be manipulated to cause a "civil war" in the colony, and thus destroy the colony. Interfering with the cues that determine whether a colony fights or avoids another colony could also be used as a tool to control pest species.

ACKNOWLEDGEMENTS

We are grateful for the help and support of the members of the Texas A&M Urban and Structural Entomology Laboratory, particularly Carlos Aguero. Additional funding support came from the William L. and Ruth D. Nutting Research Grant through the North American Section of the International Union for the Study of Social Insects (IUSSI-NAS) as well as the Texas A&M Urban Entomology Endowment.

REFERENCES CITED

Bulmer, M.S. and J.F.A. Traniello. 2002. Lack of aggression and spatial association of colony members in *Reticulitermes flavipes*. J. Insect Behav. 15: 121-126.

Chouvenc, T., P. Bardunias, H.-F. Li, M.L. Elliott, and N.-Y. Su. 2011. Planar arenas for use in laboratory bioassay studies of subterranean termites (Rhinotermitidae). Fla. Entomol. 94: 817-826.

Deheer, C.J. and E.L. Vargo. 2004. Colony genetic organization and colony fusion in the termite *Reticulitermes flavipes* as revealed by foraging patterns over time and space. Mol. Ecol. 13: 431-441.

Dronnet, S., A.G. Bagnères, T.R. Juba, and E.L. Vargo. 2004. Polymorphic microsatellite loci in the European subterranean termite, *Reticulitermes santonensis* Feytaud. Mol. Ecol. Notes 4: 127-129.

Forschler, B.T. and G. Henderson. 1995. Subterranean termite behavioral reaction to water and survival of inundation: implications for field populations. Environ. Entomol. 24: 1592-1597.

Houseman, R.M., R.E. Gold, and B.M. Pawson. 2001. Resource partitioning in two sympatric species of subterranean termites, *Reticulitermes flavipes* and *Reticulitermes hageni* (Isoptera: Rhinotermitidae). Environ. Entomol. 30: 673-685.

Su, N.-Y. 2002. Novel technologies for subterranean termite control. Sociobiology 40: 95-101.

Suiter, D.R., S.C. Jones, and B.T. Forschler. 2002. Biology of subterranean termites in the eastern United States. University of Georgia Cooperative Extension Service Bulletin 1225. 16pp.

Szalanski, A.L., J.W. Austin, and C.B. Owens. 2003. Identification of *Reticulitermes* spp. (Isoptera: Reticulitermatidae [sic] Rhinotermatidae) from south central United States by PCR-RFLP. J. Econ. Entomol. 96: 1514-1519.

Thorne, B.L. and M.I. Haverty. 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. Sociobiology 19: 115-145.

Thorne, B.L. and B.T. Forschler. 2001. Biology of subterranean termites of the genus *Reticulitermes*; Subterranean termite biology in relation to prevention and removal of structural infestation, National Pest Control Association, Fairfax, VA.

Vargo, E.L. 2000. Polymorphism at trinucleotide microsatellite loci in the subterranean termite *Reticulitermes flavipes*. Mol. Ecol. 9: 817-829.