Proceedings of the Fifth International Conference on Urban Pests
Chow-Yang Lee and William H. Robinson (editors), 2005.
Printed by Perniagaan Ph'ng @ P&Y Design Network, Malaysia.

EFFECT OF BAIT SUPPLEMENTS ON THE FEEDING AND TUNNELING BEHAVIOR OF THE FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

MARY L. CORNELIUS
United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd. New Orleans, LA 70124, U.S.A.

Abstract This study evaluated the effects of two commercially available products that have been used to supplement bait stations on feeding and tunneling behavior of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. A bioassay was conducted to examine the effects of water-soluble chemicals extracted from Summon™ disks on tunneling behavior. The average number of days taken for termites to discover pipette tips filled with material from ground-up disks was significantly less than the average number of days taken for termites to discover pipette tips filled with red oak sawdust. Feeding and tunneling tests were also conducted to determine how termites responded to the sports drink, Gatorade. Paired choice tests were conducted to compare the effect of Gatorade and water extracts of disks on feeding and tunneling behavior. There was no difference in the consumption of filter paper disks treated with Gatorade versus filter paper disks treated with water only and there was significantly greater consumption of filter paper disks treated with a water extract of disks versus filter paper disks treated with Gatorade. In paired choice tests, termites tunneled through sand moistened with water faster than through sand moistened with Gatorade and through sand moistened with a water extract of disks faster than through sand moistened with Gatorade. In this study, Summon™ disks showed more promise than Gatorade as an effective bait supplement for Formosan subterranean termites.
Key Words Feeding stimulant, termite tunneling, baits, termite control

INTRODUCTION

Commercial baits have been developed for the control of subterranean termite populations. The success of baiting programs depends on the ability of the termites to find and consume the bait, and to spread the slow-acting toxin to nestmates through trophallaxis. There are a variety of cellulose-based bait matrices on the market that termites will readily consume and pass on to their nestmates. However, in some cases, termites may not find the bait stations for several months or longer, causing homeowners to lose time and money using a pest control technique which is having no effect on the termite infestation in their homes. The development of methods to increase the probability that termites will discover bait stations will improve the efficacy of baiting programs for control of subterranean termites.

Subterranean termites use trail pheromones when foraging for food. The trail pheromone of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has been isolated and identified as (Z, Z, E) –3, 6, 8-dodecatrien-1-ol (Tokoro et al., 1992). There are also some non-pheromone chemicals that elicit trail following behavior in subterranean termites (Watanabe and Casida, 1963; Birch et al., 1970; Chen et al., 1998). For years, termite researchers have noted that ink from certain ballpoint pens would elicit trail following behavior in *C. formosanus* and *Reticulitermes* spp. Chen et al. (1998) isolated the compound, 2-phenoxyethanol, from Papermate ballpoint pens, and determined that this compound elicited trail following behavior by *C. formosanus*.

Workers of *Reticulitermes flavipes* (Kollar), orient shelter tubes towards wood blocks decayed by brown rot fungi, *G. trabeum* and *Poria incrassata* (Berkeley et Curtis) Burt (Amburgey and Smythe, 1977). Wood decayed by the brown-rot fungus, *Gloeophyllum trabeum* (Persoon: Fries) Murrill elicits trail following and aggregation behavior in *Reticulitermes* spp. (Esenther and Beal, 1979; Rust et al., 1996). Tunneling activity of *C. formosanus* was greater in sand treated with crude extracts of sawdust inoculated with the lignin-degrading fungus *Marasmiellus troyanus* (Murrill) Singer, than in sand treated with crude extracts of sawdust without fungus. These results clearly demonstrated that chemicals released from decayed sawdust influenced termite tunneling behavior (Cornelius et al., 2002).

This study examined the effects of water-soluble chemicals extracted from the commercially available product, Summon™ on the tunneling behavior of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Previous research determined that Summon™ disks acted as feeding stimulants, caused aggregation behavior and resulted in recruitment of termites to wood (Cornelius and Lax, in press). In addition, there was an increase in the rate of feeding on filter paper disks treated with a water extract of disks and an increase in the rate of tunneling in sand treated with a water extract of disks compared to untreated controls. These results demonstrate that water-soluble components of a disk influence termite foraging behavior. Furthermore, a field test determined that the presence of the disks increased the rate of discovery of monitoring stations by termites (Cornelius and Lax, in press). This increase in the rate of infestation in the field suggests that water-soluble components of the disks leach into the soil surrounding the station and cause termites to direct their foraging behavior towards the station. Hence, disks have the potential to improve the efficacy of commercial baits by increasing the rate of discovery of stations in the field. The current study examines how the presence of water soluble components of disks in the sand affect the direction of tunnel construction by termites.

This study also examined termite responses to sports drinks. Researchers at Dow AgroSciences have found that discovery and retention of subterranean termites at Sentricon bait stations can be increased by pouring the contents of a sports drink in the soil underneath the bait station (Claudia Riegel, personal communication). Feeding and tunneling tests were conducted to determine how termites responded to Gatorade. Tests were also conducted to compare the response of termites to Gatorade and to water extracts of disks.

MATERIALS AND METHODS

Termite Collections and Maintenance. Termites were collected from field colonies in New Orleans, LA, using underground bucket traps (Su and Scheffrahn, 1986) baited with spruce (*Picea* spp.) wood. Termites were kept in the lab at ambient conditions in 5.6 L covered plastic boxes containing moist sand and blocks of spruce wood until they were used in experiments.

Termite Bioassays Using Two Sand-Filled Foraging Chambers. This experiment was conducted using plastic ant farms as test chambers (Uncle Milton Industries, Corsica, CA). For each replicate, a small ant farm (21.0 cm length x 1.0 cm width x 13.5 cm height) was connected by a 4 cm length piece of tygon tubing to a large ant farm (37.5 cm length x 2 cm width x 23.5 cm height). Each small test chamber (ant farm) had a connector on the side. Two pieces of thin pine sheet wood (10.5 cm length by 4.5 cm width) were placed on the bottom of each small test chamber and each chamber was filled with sand so that the sand was level with the connector (approximately 100 g of sand). In this way, termites would enter the connector immediately after they were released into the test chamber. The sand was moistened with 20 ml of distilled water. In each replicate, termites (360 workers and 40 soldiers) were added to the small test chamber through an opening in the top of the chamber. Termites from two field colonies were used in this experiment. There were 6 replicates of each treatment, with 3 replicates from each colony.

The large test chamber (ant farm) was filled with sand to the center line (approximately 800 g of sand). In each test chamber, a hole had been made through the side of the chamber at a distance of 5 cm from the bottom of the test chamber and 6 cm from the surface of the sand. A piece of tygon tubing was inserted into this hole and sealed in place with a glue gun. Termites were able to enter the large test chamber through the tygon tubing connecting the two chambers.

A 2 ml disposable pipette tip (Sarstedt Ag & Co., Nümbrecht, Germany), with $1^1/_2$ cm cut off of the distal end of the tip, was filled with either red oak sawdust or material from a ground-up Summon™ disk. After the tip was filled with sawdust or Summon™, a 15 cm wooden Puritan Applicator stick (Harwood Products, Guilford, ME) was inserted into the tip. The applicator stick was inserted into the tip so that termites that entered the pipette tip from the bottom would tunnel up to the surface and be visible on the stick.

A mark was placed 2 cm from the edge of the large foraging chamber that was closest to the small chamber. The pipette tip was then inserted vertically into the sand at that mark so that the top of the pipette tip was level with the sand. The sand in the large foraging chamber was moistened with 150 ml of deionized water while also making sure that water was poured directly over the pipette tip. The two test chambers were then connected with tygon tubing.

The length of time (number of days) it took termites in each replicate to discover the pipette tip in the large test chamber was recorded. When the large test chamber was dismantled, it was determined whether or not a tunnel was constructed directly to the tip.

Bioassays Evaluating Feeding Behavior on Filter Paper Disks Treated with Bait Supplements. The effects of lemon-lime flavored sports drink, Gatorade (Gatorade Company, Chicago IL) on termite feeding behavior were evaluated using paired choice tests where termites were presented with a choice of a filter paper disk treated with either the sports drink or water alone. The response of termites to the sports drink versus water soluble components of the disks was compared using paired choice tests where termites were presented with a choice of a filter paper disk treated with either the sports drink or a water extract of disks. For the water extract of disks, two disks were crushed up and placed in a beaker with 100 ml of deionized water for 3 d. The water was then poured through a funnel lined with a Whatman #4 filter paper disk.

Bioassays were conducted to measure consumption after 48 h. For each test, Whatman #1 filter paper disks (2.5 cm diameter.) were weighed and numbered. Numbers were written in pencil in the center of each disk. For each filter paper disk, 60 microliters of the solution being tested was applied. Two filter paper disks with different treatments applied were placed on opposite sides of each other in a 14.0 cm diameter glass Petri dish containing 20 g of sand moistened with 5 ml of distilled water. The number and treatment of each disk was written adjacent to each disk on the outer surface of the dish. In this way, the disk could be identified even if termites had consumed most of the disk. For each bioassay, termites from four colonies were used, with 3 replicates from each colony. For each replicate, 200 termite workers were placed in each dish and the dishes were placed in an unlit incubator (29° C, 96% RH). After 48 h, filter paper disks were removed from the dishes, and left to air dry for 24 h. Weight loss of each disk was determined.

Bioassays Evaluating Tunneling Behavior in Sand Treated with Bait Supplements. The testing device was comprised of a 9 cm high x 7 cm diameter, clear polystyrene, cylindrical screwtop container (Consolidated Plastics, Twinsburg, Ohio) with a 5 cm length piece of Tygon tubing (0.8 cm I. D. diameter) inserted through a hole on the lower side of the container and sealed in place with a hot glue gun. A plastic Y-tube (stem: 3 cm; arms: 3 cm; diameter: 1 cm) was attached to the distal ends of the Tygon tube and another 5-cm length piece of Tygon tubing (0.8 cm I. D. diameter) was attached to each arm of the Y-tube. A 1 ml disposable pipette tip (length: 7 cm; diameter: 1 cm at wide end) was attached to the other end of the Tygon tubing. A thin layer of moist sand (Frey Scientific, Mansfield, OH) was placed on the bottom of the container. Termites were able to move freely from the container into the tubing. In each container, 200 *C. formosanus* workers were placed in the center of the container. Termites from four colonies were used, with 3 replicates from each colony.

Paired choice tests were conducted comparing either the sports drink to water or comparing it to a water extract of disks. For the water extract of disks, two disks were crushed up and placed in a beaker with 100 ml of deionized water for 3 d. The water was then poured through a funnel lined with a Whatman #4 filter paper disk.

In paired choice tests, one arm of the Y-tube was connected to a pipette tip filled with sand treated with the sports drink and the other arm of the Y-tube was connected to a pipette tip filled with sand treated with either deionized water or a water extract of disks, depending on the test. The position of the sports drink treated sand filled tips on the arms of the Y-tube was rotated between replicates to preclude any positional effects. Each pipette tip was marked on the outer surface with a permanent marker at a distance of 2 cm from the narrow end of the pipette tip. Tunneling activity was continuously observed until termites reached this mark. As soon as termites reached this mark in one of the tips attached to each Y-tube, both tips were removed and the number of termites in each tip was counted. These tests were conducted in ambient conditions in the laboratory.

Data Analysis. In the bioassay using two sand-filled foraging chambers, data on the number of days taken for termites in each replicate to discover the pipette tip were compared using one-way ANOVA. In feeding tests, consumption of the sports drink treated disks was compared with consumption of disks treated with either a water extract of disks or water alone using t-test for matched pairs, on a pooled data set from the four colonies. For tunneling tests, the number of tips of each treatment, which termites tunneled through first were compared using a binomial distribution (Sign Test, SPSS, 1996) and the number of termites in each tip were compared using t-test for matched pairs, on pooled data set from the four colonies.

RESULTS AND DISCUSSION

Termite Bioassays Using Two Sand-Filled Foraging Chambers. The average number of days taken for termites to discover pipette tips filled with material from ground-up Summon™ disks was significantly less (2 ± 0.63) than the average number of days taken for termites to discover pipette tips filled with red oak sawdust (11.3 ± 2.12) (ANOVA: F = 16.08; df = 1; P = 0.004). In 3 of the 6 replicates where the pipette tip was filled with material from a ground-up disk, termites constructed a tunnel directly upwards to the pipette tip at a perpendicular angle from the entrance point into the chamber. When the pipette tip was filled with red oak sawdust, there were no replicates where termites constructed a tunnel directly upwards to the pipette tip at a perpendicular angle from the entrance point into the chamber.

The tunneling behavior of termites is affected by the presence of disks in the substrate. When termites constructed a vertical tunnel at the point of entrance into the large test chamber, termites were able to discover the tips within a few hours. Hence, termites discovered tips much faster when tips were filled with material from disks than when tips were filled with sawdust. In a previous study, the rate of tunneling in sand was significantly greater when sand was treated with an extract of disks than in control sand (Cornelius and Lax, in press). These results demonstrate that water soluble components of a disk influence the construction of new tunnels and can potentially direct termite tunneling towards a bait station in the field.

Bioassays Evaluating Feeding Behavior on Filter Paper Disks Treated with Bait Supplements. There was no difference in the consumption of filter paper disks treated with the sports drink versus filter paper disks treated with water only (P = 0.712: t-test for matched pairs). There was significantly greater consumption of filter paper disks treated with a water extract of disks versus filter paper disks treated with the sports drink (P = 0.005: t-test for matched pairs) (Table 1). There was no evidence that the sports drink affected the feeding behavior of Formosan subterranean termites. These results and results from a previous study (Cornelius and Lax, in press) demonstrate that water-soluble components of disks act as phagostimulants for the Formosan subterranean termite.

Table 1. Mean (± SEM) weight loss of filter paper disks in paired choice tests.

	Weight Loss of Disks (mg)
Experiment 1: Gatorade versus Water	
Gatorade	16.0 ± 2 mg
Water	14.0 ± 3 mg
Experiment 2: Gatorade versus water extract of Summon™ disks	
Gatorade	17.0 ± 2 mg
Summon™	27.0 ± 3 mg**

** P < 0.01; t-test for matched pairs

Bioassays Evaluating Tunneling Behavior in Sand Treated with Bait Supplements. Termites tunneled through sand moistened with water faster than through sand moistened with the sports drink (9 to 1; P = 0.021: Sign Test). However, the number of termites in tips with the sports drink (30.67 ± 3.11) was significantly greater than the number of termites in tips with water (22.58 ± 2.77) (P = 0.05: t-test for matched pairs). Termites tend to cluster at the base of the tunnel in the sports drink treated sand, indicating that it does affect termite behavior. In this bioassay, termites generally constructed only a single tunnel in the pipette tips. Hence, the rate of tunnel construction was slower when termites clustered at the base of a new tunnel because they blocked termites carrying grains of sand from backing out of the tunnel to deposit the grains.

In paired choice tests, termites tunneled through sand moistened with a water extract of disks faster than through sand moistened with the sports drink (10 to 1; P = 0.012: Sign Test). Also, the number of termites in tips where sand was moistened with water extract of disks (28.0 ± 1.9) was significantly greater than the number of termites in tips where sand was moistened with the sports drink (19.0 ± 1.8) (P = 0.006: t-test for matched pairs). Termites constructed tunnels faster through sand treated with a water extract of disks. Termites were able to efficiently construct a single tunnel into extract-treated sand without blocking the tunnel entrance, even though there were greater numbers of termites in tips filled with sand treated with an extract of disks than in tips filled with the sports drink treated sand. In this study, disks showed more promise than the sports drink as an effective bait supplement for Formosan subterranean termites.

ACKNOWLEDGMENTS

I would like to thank Leanne Duplessis and Silvia Cosenza for their invaluable technical assistance in conducting the bioassays.

This article presents the results of research only. Mention of a commercial or proprietary product does not constitute endorsement or recommendation by the USDA.

REFERENCES CITED

Amburgey, T. L. and R. V. Smythe. 1977. Shelter tube construction and orientation by *Reticulitermes flavipes* in response to stimuli produced by brown-rotted wood. Sociobiology 3**:** 27- 34.

Birch, A. J., K. B. Chamberlain, B. P. Moore and V. H. Powell. 1970. Termite attractants in *Santalum spicatu.* Australian Journal of Chemistry 23: 2337-2341

Chen, J., G. Henderson and R. A. Laine. 1998. Isolation and identification of a 2-phenoxyethanol from a ballpoint pen ink as a trail-following substance of *Coptotermes formosanus* Shiraki and *Reticulitermes* sp. J. Entomol. Sci. 33**:** 97-105.

Cornelius, M. L. and A. R. Lax. 2005. Effect of Summon™ Preferred Food Source on feeding, tunneling and bait station discovery by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. In press.

Cornelius, M. L., D. J. Daigle, W. J. Connick Jr., A. Parker and K. Wunch. 2002. Responses of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) to three types of wood rot fungi cultured on different substrates. J. Econ. Entomol. 95: 121-128.

Esenther, G. R. and R. H. Beal. 1979. Termite control: decayed wood bait. Sociobiol. 4**:** 215-222.

Matsumura, F., A. Tai and H. C. Coppel. 1969. Termite trail-following substance, isolation and purification from *Reticulitermes virginicus* and fungus-infected wood. J. Econ. Entomol. 62**:** 599-603.

Rust, M. K., K. Haagsma and J. Nyugen. 1996. Enhancing foraging of western subterranean termites (Isoptera: Rhinotermitidae) in arid environments. Sociobiol. 28**:** 275-286.

SPSS. 1996. SYSTAT statistical package, version 8.0. SPSS, Chicago, IL.

Su, N.-Y. and R. H. Scheffrahn. 1986. A method to access, trap, and monitor field populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the urban environment. Sociobiology 12: 299-304.

Tokoro, M., M. Takahashi and R. Yamaoka. 1992. Identification of the trail pheromone of the subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Mokuzai Gakkaiski 38**:** 284-300.

Watanabe, T. and J.E. Casida. 1963. Response of *Reticulitermes flavipes* to fractions from fungus infected wood and synthetic chemicals. J. Econ. Entomol. 56: 300-307.