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Research Article

Effect of Chemical Cues on the Foraging and Tunneling Behavior of Formosan Subterranean Termites (Isoptera: Rhinotermitidae)

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Abstract: Wood rot fungi can cause directional tunneling, aggregation behavior and increased wood consumption by subterranean termites. Because vanillin and guaiacol are byproducts of lignin degradation, these chemicals were tested as potential attractants to Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). In tunneling tests, termites tunneled faster in sand treated with vanillin, but not guaiacol, compared with control sand. Termite responses to vanillin were compared to two other chemicals that had been previously shown to affect bait discovery in field tests, Summon Preferred Food Source™ and a sports drink. When discovery of bait tubes was monitored in a foraging arena, only extracts of Summon Preferred Food Source™ significantly increased the number of bait tubes colonized by termites. When the tunneling behavior of termites was compared in foraging arenas with treated and untreated sand, the number of termites tunneling in treated foraging arenas was significantly greater when sand was treated with a sports drink compared with the other treatments. In no-choice tests, wood consumption was much lower in containers when sand was treated with a sports drink compared to other treatments, suggesting that termites were ingesting the carbohydrates from the moistened sand. Although termites responded to vanillin in tunneling tests, vanillin did not have a significant effect on termite foraging behavior in comparison to the two other chemicals.

Key Words: *Coptotermes formosanus***,** vanillin, guaiacol, wood consumption

Introduction

Baiting programs have been shown to be an effective method of control for Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) (Su 2003, Eger et al. 2012). Because subterranean termites do not detect the presence of sound wood in the soil at distances > 2.5 mm (Puche and Su 2001), attractants may provide a tool for improving the efficacy of bait discovery in the field. Chemical cues in the soil can cause directional tunneling behavior and may serve as cues to find food (Rust et al. 1996, Cornelius et al. 2002, Cornelius and Lax, 2005, Su 2005, Cornelius and Osbrink 2008). In addition, field studies have shown that the application of bait supplements, such as Summon Preferred Food Source™ (Cornelius and Lax 2005, Cornelius et al. 2009) or a sports drink (Cabrera and Thoms 2006, Getty et al. 2007), can increase the number of bait stations discovered by subterranean termites.

Wood rot fungi can cause directional tunneling, aggregation behavior and increased wood consumption (Esenther and Beal 1979, Rust et al.1996, Cornelius et al. 2002, Su 2005). Vanillin and guaiacol are byproducts of lignin degradation (Hartley 1971, Tien and Kirk 1984, Alvarez-Rodriquez et al. 2003). Therefore, these chemicals were tested as possible attractants for the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Cornelius and Osbrink (2008) showed that the rate of tunneling by Formosan subterranean termites increased significantly in sand treated with either an aqueous solution of Summon Preferred Food Source™ or a sports drink. This study evaluated the behavioral response of Formosan subterranean termites to vanillin compared with Summon Preferred Food Source™ and a sports drink.

Materials and Methods

Termite collections and maintenance. Termites were collected from field colonies in City Park, New Orleans, LA, using cylindrical irrigation valve boxes (lid: 17.8 cm diam; base: 21.6 cm; height: 22.9 cm) (NDS, Inc, Lindsay, CA) that are buried in the ground and filled with 100-150 blocks (7.5 by 3.8 by 0.8 cm) of wood (spruce, *Picea* sp,Pinaceae). Termites of at least the 3rd instar were used in experiments within two months of field collection. Termites were kept in the lab in 5.6-L covered plastic boxes containing moist sand and blocks of spruce *Picea* sp. until they were used in experiments.

Tunneling tests. Tunneling tests were conducted to determine if either vanillin or guaiacol affected termite tunneling behavior and to identify the most effective concentration. These tunneling tests were previously described in Cornelius and Osbrink (2008). Tests were conducted using glass t-tubes (stem: 5.5 cm; each arm: 5.5 cm; diameter 0.5 cm). The outer surface of the glass t-tube was marked with a permanent marker on the stem at a point 1 cm from the base of the stem and on each arm at a point 2 cm away from the base of the stem. The outer surface of one arm of the t-tube was marked to indicate that the sand in this arm would be treated. The t-tube was filled with dry sand (Ottawa sand, Frey Scientific, Mansfield, OH) until it reached the mark on the stem. Distilled water was slowly added to moisten the sand in the stem itself (approximately 40 µl). The t-tube was tilted to remove dry sand in the arms. The end of the treated arm of the t-tube was covered with tape. The t-tube was positioned so that the arm covered with tape was facing down and dry sand was added until it reached the mark on the control arm facing upwards. The tape prevented dry sand from escaping from the arm facing downward. Distilled water was slowly added until all the sand in the control arm of the t-tube was moistened (80 µl). The tape was removed, and the t-tube was tilted so that the dry sand was removed. The t-tube was then turned over so that the control arm was facing down and the treated arm was facing upward. The moist sand in the control arm stayed in place when the control arm was facing downward. Dry sand was added to the treatment arm until it reached the mark. Vanillin was dissolved in distilled water. The aqueous treatment solution was slowly added to the treated arm of t-tube until all of the sand in the treated arm was moistened (80 µl). An ethanol solution of guaiacol was applied to the sand in the treated arm of the t-tube and ethanol alone (80 µl) was applied to the sand in the control arm. After the ethanol evaporated, the sand in both arms was moistened with 80 µl distilled water.

The glass t-tube was then connected to a clear polystyrene, cylindrical screw top container (9 cm high x 7cm diameter) using a 5cm length piece of PVC tubing (I.D. 3/4", O.D. 5/16", Wall 1/32") (Nalgene, Rochester, NY) inserted through a hole in the bottom of the container and sealed in place with hot glue applied with a glue gun. The other end of the tubing was attached to the stem of the glass t-tube so that termites could move freely between the container and the t-tube. The ends of the arms of the t-tube were covered with plastic caps to prevent termites from escaping.

For each experiment, termites were collected from four different colonies, with three replicates from each colony. In each replicate, 100 workers were placed in the container. Tunneling behavior of termites was observed for a period lasting up to 6 h. These tests were conducted in the laboratory at ambient conditions. The time taken for termites to tunnel completely through the sand in each arm of the t-tube was recorded. Termites usually tunneled completely through the sand in both arms of the t-tube within 6 h. If tunneling was not completed in an arm of the t-tube within 6 h, a time of 360 min was recorded for that arm.

Bait tube tests. A 4-way choice test was conducted to compare the rate of discovery of sawdust-filled bait tubes treated with different chemicals in a foraging arena. Round plastic containers (9.5 cm height, 25.5 cm diam) (Pioneer Plastics, Dixon, KY) were filled with 4500 g sand (Play Sand, Quikrete, Atlanta GA), moistened with 900 ml distilled water. Plastic pipette tips were used as bait tubes by removing a 2 cm length section from the tip of each 5 ml plastic pipette tip (12 cm length x 1.4 cm diam). A 5 cm length section of the pipette tip was inserted into the sand on opposing sides of the container so that each tip extended 3 cm above the sand and was 12-13 cm apart from the next one and 2 cm from the edge of the container. The sand inside of the tip was leveled and then 1 ml of one of the following aqueous solutions was applied to the sand inside of each tip: control (no solution), sports drink (Gatorade), vanillin (1 mg/ml), or aqueous extracts of Summon Preferred Food Source (FMC, Philadelphia, PA) disks. Aqueous extracts of Summon disks were prepared by crushing up individual disks which weighed approximately 1.7 g each and placing disks in a beaker of distilled water (100 ml of water per disk), for 24 h. After 24 h, water was filtered through a glass funnel lined with Whatman #1 filter paper.

The top of each tip extending above the sand was filled with moist spruce sawdust. The order of the four treatments was rotated between replicates to take into account any positional bias. In the center of the container, a 5 cm diam lid of a plastic Petri dish (BD Falcon, Franklin Lakes, NJ) was embedded in the sand so that termites were able to easily climb out. Termites were released into the lid at an equal distance to the four bait tubes. The bait tubes were checked at Day 2, Day 5, Day 10 and Day 15 for the presence of termites in the sawdust or tunneling in the sand within the tube. After 15 d, surviving termites were counted. Termites were collected from four different colonies, with six replicates of each colony for a total of 24 replicates.

Foraging arena tests. The experiment was conducted in the laboratory at ambient conditions, using plastic ant farms (21.0 cm length by 1.0 cm width by 13.5 cm height) (Uncle Milton Industries, Corsica, CA) as test chambers. A thin piece of red oak (7.6 cm x 1.0 cm x 0.3 cm) was placed in the bottom of each test chamber. Each test chamber had a portal on each side, covered with a plastic cap. For each replicate, two test chambers were connected by removing the caps and attaching the ends of a 3-cm length piece of tygon tubing (0.8 cm diam.) to the portals of the two chambers. Both test chambers in each replicate were filled with sand (Play Sand, Quikrete, Atlanta GA), until the sand reached the same height as the portal. The first test chamber was moistened with 25 ml distilled water. The second test chamber in each replicate was moistened with 25 ml of one of the following solutions: distilled water, an aqueous solution of Summon disks (1 disk/100 ml), an aqueous solution of vanillin (1 mg/ml), or a sports drink (Gatorade). Termites (380 workers and 20 soldiers) were released into the first test chamber. After 30 d, the number of termites in each arena was counted. Termites were collected from three different colonies, three replicates of each solution.

No-choice wood consumption test. Groups of 200 termites (190 workers; 10 soldiers) were placed in a single clear polystyrene, cylindrical screw top container (9 cm high x 7cm diameter). Each container was filled with 50 g sand (Play Sand, Quikrete, Atlanta GA), moistened with 12 ml of either distilled water, a sports drink (Gatorade) , an aqueous extract of Summon Preferred Food Source™, or an aqueous solution of vanillin (1 mg/ml).

Blocks (4.2 cm x 3.8 cm x 1 cm) of spruce, *Picea* sp., were oven-dried at 90° C for 24 h and then weighed. A wood block was rinsed in distilled water and placed on top of the sand. Containers were placed in a dark incubator (28º C, 97% RH). After 30 d, all wood blocks were removed, cleaned, oven-dried at 90° C for 24 h, and weighed. Surviving termites were counted. Termites were collected from three different colonies, with two replicates of each treatment per colony for a total of six replicates per treatment.

Statistical analysis. For all data, tests for normality and equal variance were performed prior to statistical analysis. If either of these assumptions were violated, nonparametric tests were used.

In the tunneling tests, the number of arms which termites tunneled through first were compared using a binomial distribution (Sign Test) and the number of minutes taken to tunnel through each arm was compared using a paired choice t-test. In the bait tube test, number of bait tubes discovered on Day 2, Day 5, Day 10, and Day 15 for each treatment were compared using a Kruskal-Wallis one-way ANOVA on Ranks. Means were separated using Tukey's test with ranked sums (Systat 2008). In the foraging arena test, termite survival and the number of termites in the treated arena were compared for each treatment using a one-way ANOVA and means were separated using a Tukey test. In the no-choice wood consumption test, survival of termites and weight loss of blocks were compared using a Kruskal-Wallis one-way ANOVA on Ranks. Means were separated using Tukey's test with ranked sums (Systat 2008).

Results and Discussion

Termites tunneled faster through sand treated with 1 mg/ml of vanillin than through sand moistened with distilled water (Table 1). These results were similar to previous studies evaluating tunneling behavior in sand treated with aqueous extracts of Summon Preferred Food Source™ and a sports drink (Cornelius and Osbrink 2008). Termites did not respond to sand treated with guaiacol.

In bait tube tests, termites discovered significantly more bait tubes treated with an aqueous solution of Summon disks than controls on Day 5 (H = 13.7; P = 0.003) , Day 10 (H = 14.2; P = 0.003), and Day 15 (H = 15.4; P = 0.002). On Day 2, significantly fewer bait tubes treated with vanillin were discovered compared to tubes treated with Summon (H = 16.1; P = 0.001). There was no difference in the discovery of bait tubes treated with vanillin compared to the other treatments after 5, 10, and 15 days (Fig. 1). The Summon extract was the only treatment that increased bait discovery compared with controls. In field tests, Summon Preferred Food Source also significantly increased the rate of bait discovery (Cornelius and Lax 2005, Cornelius et al. 2009).

In foraging arena tests, different treatments had no effect on termite survival (F = 0.99; df = 3, 11; P = 0.44). There were significant differences in the number of termites in treated arenas (F = 7.9; df = 3, 11; P = 0.009). Significantly more termites were located in arenas treated with a sports drink than in arenas treated with either vanillin or the Summon extract (Table 2). Sugars act as phagostimulants for subterranean termites (Waller and Curtis 2003, Swoboda et al. 2004, Saran and Rust 2005, Castillo et al. 2013). Field studies found an increase in bait discovery with the addition of a sports drink (Cabrera and Thoms 2006, Getty et al. 2007).

In the no-choice wood consumption test, termite survival was not affected by the different treatments (F = 1.5; df = 3, 23; P = 0.24); however, wood consumption was significantly different (F = 55.8; df = 3; 23, P < 0.001). Wood consumption was significantly lower in replicates treated with the sports drink than in the other treatments (Table 3). These results were consistent with a previous study where termites fed less on wood blocks when sand was treated with Gatorade. Cornelius and Osbrink (2008) found that a significantly higher number of termites turned blue in containers with Gatorade-treated sand (100.3 ± 21.4) when Nile Blue A dye was dissolved in Gatorade than in containers where Nile Blue A dye was dissolved in water (4.7 ± 1.7) and then applied to sand. The uptake of Nile Blue A from the Gatorade-treated sand, but not the control sand, indicated that termites were ingesting carbohydrates from the moistened sand. Therefore, the reduced wood consumption in arenas treated with a sports drink is most likely due to the increased consumption of carbohydrates from the sports drink.

Conclusions

Because vanillin and guaiacol are byproducts of lignin degradation, they were evaluated as potential attractants for subterranean termites. At a concentration of 1 mg/ml water, vanillin significantly increased the rate of tunneling in treated sand compared with control sand. However, an aqueous extract of vanillin did not cause an increase in either the number of bait tubes discovered or the number of termites foraging in treated arenas. Guaiacol had no effect on termite tunneling behavior. Since both of these chemicals are byproducts of lignin degradation, it is possible that termites might respond more strongly to a combination of these two chemicals than the two chemicals alone. Further research could explore how combinations of chemicals released from decaying wood affect termite tunneling behavior and whether termites are more responsive to a specific ratio of chemicals than to single compounds. This study provides evidence that chemicals released from decaying wood could influence termite tunneling behavior and that chemical cues could potentially be used to direct the tunneling behavior of termites towards bait stations.

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Table 1. Tunneling behavior of termites in glass T-tube tests where sand in one arm of the t-tube was treated with either vanillin or guaiacol and sand in the control arm was moistened with distilled water.

	Number of Arms Tunneled Through First[1]		Mean (±SEM) Minutes Taken To Tunnel Through Sand[2]		t-test
Experiment[3]	Treated	Control	Treated	Control	P Value
Vanillin (2mg/ml)	7	5	204.5 ± 26.0	204.2 ± 26.4	0.99
Vanillin (1mg/ml)	10	2*	149.2 ± 21.3	208.4 ± 21.1	0.001
Vanillin (0.1mg/ml)	7	4	165.2 ± 16.9	212.1 ± 26.2	0.15
Guaiacol (1 mg/ml)	6	4	261.1 ± 27.1	270.3 ± 28.5	0.84
Guaiacol (0.1 mg/ml)	7	5	231.7 ± 20.4	255.4 ± 27.5	0.46
Guaiacol (0.01 mg/ml)	6	3	204.6 ± 31.9	269.7 ± 23.2	0.22
Guaiacol (0.001 mg/ml)	8	4	226.7 ± 28.1	231.5 ± 21.0	0.87

* $P \leq 0.05$

[1] numbers tunneled through first compared using a binomial distribution (Sign Test)

[2] minutes taken to tunnel through sand compared using a Paired choice t-test

[3] There were 12 replicates for each experiment. If termites did not tunnel through either arm of the t-tube within 6 h, that replicate was not included in the analysis.

Table 2. Mean (±SEM) number of termites in each location and mean (±SEM) percent survival.

Treatment	Mean (±SEM) Number Termites Located in Treated Arena	Mean(±SEM) Percent Survival
Sports drink	187.7 ± 9.0a	88.7 ± 3.5a
Vanillin	103.3 ± 18.3b	88.0 ± 2.6a
Summon	109.7 ± 12.2b	82.5 ± 5.2a
Water	156.0 ± 15.6ab	81.4 ± 3.2a

Means followed by the same letters were not significantly different (P> 0.05; Tukey's Test).

Table 3. Mean (±SEM) percent survival of termites and wood consumption in sand-filled containers.

Treatment	Mean (±SEM) Percent Survival	Mean (±SEM) Wood Consumption (mg)
Sports drink	84.3 ± 2.2a	18.3 ± 13.3a
Vanillin	89.3 ± 2.1a	795.0 ± 58.2b
Summon	88.4 ± 2.1a	735.0 ± 60.8b
Water	90.0 ± 1.9a	703.3 ± 47.7b

Means followed by the same letters were not significantly different (P> 0.05; Tukey's Test).

Figure 1. A 4-way choice test with sawdust-filled bait tubes in a foraging arena with the following aqueous solutions: control (water), vanillin, sports drink, Summon Preferred Food Source. Mean (±SEM) number of bait tubes discovered after Day 2, Day 5, Day 10 and Day 15. Means followed by the same letters were not significantly different (P> 0.05; Tukey's Test).