Buccal Manipulation of Sand Particles During Tunnel Excavation of the Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT  Serial tunneling behavior of Coptotermes formosanus Shiraki (Isoptera: Rhinotermitidae), the Formosan subterranean termite, includes excavation, loading, transportation, and deposition of sand particles. Functions of mouthparts, including mandibles, maxillae, labrum, and labium, for the tunneling behaviors were described. The four mouthparts form a buccal cavity for loading three to four sand particles (ranging 0.300–0.355 mm in diameter) during sand displacement. The maxillae, not the mandibles, are the major appendages for sand excavation and deposition. Previous studies speculated a “soil-compaction hypothesis” that subterranean termites might press the sand particles with their head to build the tunnel. Examination of video recordings of tunnel excavation through a microscope indicated sand was not compacted. When two photographs of tunnel tips taken before and after excavation were superimposed, sand particles surrounding tunnel tips remained in place, which demonstrated that termites did not press sand to either side. Observations and experiments indicate that particle displacement is the major mechanism for subterranean termites to build tunnels rather than particle compaction.

KEY WORDS  Coptotermes formosanus, tunnel architecture, excavating behavior, mouthparts

Underground foraging galleries of Coptotermes spp. have been unearthed by some researchers who found that these gallery systems ranged in depth from 48 to 304 cm and extended over a hundred meters from the nests at a depth from 5 to 300 cm (Oshima 1919, Ehrhorn 1934, Greaves 1962, Greaves and Florence 1966, King and Spink 1969). Despite their extensive underground gallery systems, Coptotermes spp. and other subterranean termites are not known to move soil to a great extent; thus, the speculation that subterranean termites compact soil to create gallery space was proposed by Ebeling and Pence (1957), and this “soil-compaction hypothesis” was generally accepted (Greaves 1962, Greaves and Florence 1966, King and Spink 1969, Lee and Wood 1971).

When termites were placed in two-dimensional arenas as described by Su et al. (2004), excavated sand was often moved to the empty space within the introduction chamber as the tunnel progressed. Transportation of excavated sand to an empty space does not in itself invalidate the soil-compaction hypothesis, because termites may move and compact soil simultaneously. The objective of this study is to describe the functions of termite mouthparts for tunnel excavation, and to validate the soil-compaction hypothesis.

Materials and Methods

Individuals of three field colonies of Formosan subterranean termites, Coptotermes formosanus Shiraki, were collected in Broward County, FL, by using underground bucket traps (Su and Scheffrahn 1986). Twenty workers (undifferentiated larvae third instar or older) from each colony were put in 80% ethanol for measuring their head width, and dissecting their mouthparts under a dissecting microscope (model SZX12, Olympus Optical Co., Ltd., Tokyo, Japan). The worker’s head was photographed with a stereomicroscope (model MZ 12.5, Leica Microsystems GmbH, Weltzlar, Germany) and enhanced with Auto-Montage program (version 5.02, Synoptics Ltd., Cambridge, United Kingdom). The photographs of each mouthpart were taken with a compound microscope with a digital camera (model BX51 and DP70, Olympus Optical Co., Ltd.).

Observations of workers’ soil manipulation behaviors were conducted by using a laboratory arena similar to that described by Li and Su (2008). The arena was constructed of two sheets of transparent Plexiglas (15 by 15 by 0.6 cm) separated from each other by four Plexiglas laminates (15 by 1.5 by 0.15 cm) placed between the outer margins to form a 0.15-cm gap of 12 by 12 cm for loading sand and was held together with screws (Fig. 1). To increase the color contrast between sand particles for identifying each sand particle

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under a microscope, 32 g of mixed colored sand (red, green, blue, purple, and gray) ranging from 0.300 to 0.355 mm in diameter was used for tunneling substrate. An empty square space (2.8 by 2.8 by 0.15 cm) was left at one corner for termites and a piece of moist wood (1.0 by 1.0 by 0.15 cm) was placed in the corner of this space. The top Plexiglas sheet had one access hole (0.6 cm in diameter) at each of two corners for injecting 8 ml water to moisten sand and to introduce termites (Fig. 1).

The tunneling behaviors in the arena were observed and recorded through a video-equipped dissecting microscope. The speed of mouthpart movement was quantified by counting the video frames (60 frames per s). The number of sand particles moved over 5 cm per trip by 20 workers was counted separately. The observations were made only of tunnels not touching the arena edges (Fig. 2A, tunnel b). Tunnels excavated along the arena edge (e.g., Figure 2A, tunnels a and c) were not used because termites were able to press sand particles in only one direction. At the tip of the nonedge tunnel, a transparent film with printed Xs (tunnel b, pointed by arrow tips, Fig. 2), at each corner of a 0.6-cm square was placed on the top Plexiglas sheet of the arena for photographic orientation (Fig. 2). Back-lit digital images were taken, before and after tunnel excavation beneath the four marks, respectively (Fig. 2, A and B). At the midpoint of the marks, the width of the tunnel (Wt) was measured (Fig. 2B). Digital images were also taken with an overhead light source through the microscope, before and after termites excavated the tunnel beneath the four marks, respectively (Fig. 2, C and D). By superimposing the two images (Fig. 2, C and D), the sand particles that remained stationary were identified along with particles that were moved; thus, the interface between the stationary and moved sand particles could be determined. The distance between the two interfaces on two sides of the tunnel, termed as the width of the moved sand (Wm), was measured (Fig. 2D). Wt and Wm were measured at the same point. Five subsamples of Wt and Wm were measured along tunnel b in each replication. The significant difference between Wm and Wt was tested by paired t-test at \( \alpha = 0.05 \) (SAS Institute 1985). If termites only removed sand particles, then the width of a tunnel must be the same as the width of the moved sand or Wm = Wt. However, if termites removed and pushed sand particle simultaneously, then Wm > Wt.

**Results and Discussion**

**Tunneling Behaviors.** The worker buccal cavity is enclosed by the dorsal labrum, the lateral mandibles and maxillae, and the ventral labium (Fig. 3A). Each mouthpart is synchronized to perform a four-step tunneling behavior that includes excavating sand, loading sand in buccal cavities, holding sand for transportation, and depositing sand. The video clips of Formosan subterranean termite’s tunneling behaviors can be viewed at http://flrec.ifas.ufl.edu/ent_nem/structural_entomology_su_videos.shtml.

Excavating sand is defined as moving sand particles from its original location into the termites’ buccal cavity. The maxillae are the major appendages for excavating sand (Fig. 3D). The basal plates, the stipes (S), support the laciniae (La) and the galeae (Ga) distally and maxillary palps (Mp) laterally. The lacinia terminates in a heavily sclerotized double tooth. The galeae cover the laciniae above and laterally. Before excavating, the maxillary palps in front of the laciniae and galea first touched the sand particles. The excavating behavior was initiated by a lateral extension of the laciniae, and the distance between the two laciniae can be as wide as the width of the head. The mean \((\pm SE)\) worker head width was 1.179 \(\pm\) 0.008 mm \((n = 20)\). The extended maxillae moved forward and the distal laciniae were inserted into gaps between particles followed by grasping and moving sand particles from original location to the buccal cavity. This process may take place as fast as 0.5 s. Termites often angled their heads so that the maxillae can easily grasp and remove sand particles. When the pair of maxillae moved backward while holding a sand particle, the mandibles which bear heavily sclerotized teeth (Fig. 3C) simultaneously opened and received the particles.

Loading is defined as packing sand particles into the buccal cavity. After placing the first sand particle into buccal cavity, the maxillae repeatedly moved forward and backward to stuff the first sand particle deeper into the cavity, or continued excavating and loading a second particle that simultaneously pushed the first particle deeper inward. Termites also rotated sand particles in the buccal cavity with the maxillae. During loading, the labrum and labium were pushed upward and downward, respectively, and some of the sand particles may slip out of the cavity. The labrum, the dorsal appendage, is a transparent, convex, and semi-

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**Fig. 1.** Experimental arena to observe workers’ tunneling behaviors and to test the soil compaction hypothesis. (Online figure in color.)
circular flap (Fig. 3B) covering the mandibles and prevents sand particles from slipping off the mandibles. The labrum can bend over a 60° angle when sand particles projected above the dorsal surface of the mandibles. As shown in Fig. 3E, the labium, the ventral appendage, consists of the postmentum (Po) and the prementum (Pm). The postmentum is a shield like plate, and the prementum consists of a pair of labial palps (Lp) and the ligula. The ligula is a four-lobed structure with two inner lobes (the glossae [G]) and two outer lobes (the paraglossae [Pg]). The labium can angle downward to 50° or greater, and the four lobes of the ligula spread laterally to support the sand particles. During loading, termites sometimes partially extruded sand particles out of the buccal cavity by using their hypopharynx (H) (Fig. 3A), to reposition the particles. Termites may excavate and load sand alternately or simultaneously several times at the excavation sites before moving sand to deposition sites.

During the step of transporting sand, termites moved it from the excavation site to the deposition site. The mandibles, maxillae, labrum, and labium held the sand particles together until termites arrived at the deposition site, and no movement of buccal appendages was observed during the transportation. The average number of sand particles carried by one worker per trip was 3.5 ± 0.15 (n = 20).

We defined sand deposition as the releasing of sand particles from the buccal cavity at deposition sites. Termites deposited sand in a given empty space, including the tunnel that they were digging, and they did not always deposit all particles in one trip at the
same site. Termites usually deposited one sand particle at a time. Termites rotated their heads alternately from side to side against the walls of deposition sites and pushed the sand particle from several directions with maxillae to fit the edge of the walls. Termites minutely adjusted the position of each sand particle with the tip of maxillae (lacinia) rapidly at 0.25 s per round and rounds repeated for up to 2–5 s. After depositing all sand particles, termites returned to the previous excavation site and started another cycle of excavation, loading, transportation, and deposition.

In this study, the tunneling material used was colored sand of uniform size, which facilitated observations and quantifications of the behavior and function of each mouthpart. However, it should be noted that soil properties such as moisture (Su and Puche 2003), density (Tucker et al. 2004), and particle size (Cornelius 2005) can alter termite-tunneling behavior. Moreover, the behaviors of excavating soil and cellulose involved different steps, because it probably takes less effort to separate soil particles than to cut and tear off cellulose fibers. Indrayani et al. (2007) reported that C. formosanus separated wood fragments by cutting and pulling activities. The maxillae grasped the wood and then the mandibles cut wood fragments. Termites pulled

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Fig. 3. The mouthpart of the worker of C. formosanus. (A) Frontal view of the head. (B) Labrum. (C) Mandibles. (D) Maxillae. (E) Labium. B, bristle; G, glossa; Ga, galea; H, hypopharynx; L, labrum; La, lacinia; Lp, labial palp; M, mandible; Mp, maxillary palp; Pa, primary articulation point; Pg, paraglossa; Pm, prementum; Po, postmentum; S, stipes; Sa, secondary articulation point. (Online figure in color.)
wood fragments with both their maxillae and mandibles while rocking their bodies. In this study, termites could easily separate sand particles by using their maxillae with no rocking motion.

The function of the termite’s mandibles for excavating several substrates has been reported previously. These functions include pushing sand particles to create the tunnel (Reticulitermes hesperus Banks; Ebeling and Pence 1957), grasping and cutting kaolin clay to form a pill [Reticulitermes flacipes (Koller); Whitman and Forschler 2007], and cutting and pulling wood fragments (Incisitermes minor Hagen, C. formosanus, and Reticulitermes speratus Kolbe; Indrayani et al. 2007). Because the maxillae are covered beneath the mandibles, and their movement is rather fast, their function has not been well described. In this study, by examining the video frames recorded through the microscope, we found that the maxillae were the major appendage to excavate and deposit sand, and the function of mandibles was only for holding the sand particles during the serial tunneling behaviors.

The head capsule movement during excavation and deposition of soil or other material has been previously described. Ebeling and Pence (1957) noted that R. hesperus pushed their heads forward through the sand and pushed sand to either side, which is similar to the head alternating rotations of R. flacipes during excavation and deposition (Whitman and Forschler 2007) and to the side-to-side motion of the head of Zootermopsis spp. during placement of the fecal pellets on fecal cement (Stuart 1967). However, the interpretations of the function of the head movement differed among observers. Ebeling and Pence (1957) thought that the head movement was used to press sand particles to create tunnel space. Whitman and Forschler (2007) indicated that the head movement functioned to pull soil held by the mandibles during soil excavation, but they offered no explanation for the same behavior during soil deposition. Stuart (1967) posited that the termites moved heads to push the fecal pellets held by mandibles onto the wall with fecal cement. Our video recording showed that the rotating movement of the head capsule during sand deposition mainly allowed the maxillae to push sand particles into wall edges from several directions. C. formosanus did not use the mandibles for pressing or pushing sand particles.

Rejecting the Soil Compaction Hypothesis. Wm and Wt were 3.16 ± 0.18 and 2.90 ± 0.15 mm, respectively. There is no significant difference between Wm and Wt (n = 15, P = 0.12), which indicates that sand was not pushed to any significant degree. We conclude that C. formosanus removed the sand particles to make tunnels without pushing the sand particles to either side during excavation.

During sand deposition, the maxillae minutely adjusted and pushed every sand particle to fit the edge of the wall one by one, which led to the proposal of the modified soil-compaction hypothesis, in which termites deposit sand in a greater density than before it was removed to create space (Li and Su, 2008). However, the modified hypothesis also has been rejected. Based on our behavioral observations and experiments, we speculate that soil displacement is the main mechanism for subterranean termites to build underground galleries in sandy soil.

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