# Effect of Flooding on the Survival of Formosan Subterranean Termites (Isoptera: Rhinotermitidae) in Laboratory Tests*

by

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## ABSTRACT

Underground monitoring stations were active with Formosan subterranean termites, *Coptotermes formosanus* Shiraki, less than a month after City Park, an urban park in New Orleans, Louisiana, was inundated with 0.5 to 2.5 meters of flood water. This study examines whether *C. formosanus* are able to survive inundation by finding air pockets in either wood or their gallery system in the soil and whether termites move up from the substrate to higher ground in response to rising water. We found no evidence that termites are able to survive in their gallery system or within wood after submersion, and no evidence that termites attempt to move up from the substrate in order to escape rising water. However, significant numbers of termites located within the hollowed-out core of a wood block at the time of the flooding were able to escape slowly rising water. Formosan subterranean termite colonies most likely survived the flooding of City Park because these colonies were living within trees at the time of the flood. The construction of carton nests within the hollowed-out trunks of living trees may be a behavioral adaptation to survive flooding.

Key Words: *Coptotermes formosanus*, inundation, population, gallery system

## INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an economically important pest species that is estimated to cause $11 billion in damage annually in the United States (Su 2003). Formosan subterranean termites forage primarily within extensive underground gallery systems (King

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& Spink 1969). In a study characterizing 13 *C. formosanus* colonies in Louis Armstrong Park, New Orleans, LA, foraging territory sizes ranged from 83 to 1634 $m^2$ and the foraging territories of each colony remained relatively stable over a 4 yr period. In addition, 32% of the trees in the park were infested with *C. formosanus* (Messenger and Su 2005). Carton nests of *C. formosanus* are frequently found in living trees in New Orleans, LA (Osbrink *et al.* 1999, Osbrink *et al.* 2008). Formosan subterranean termites are able to travel above ground by constructing soil covered shelter tubes (Wood 1988, Chen & Henderson 1997) and are also capable of forming aerial colonies that have no connection to the ground (Su *et al.* 1989, Su *et al.* 1997).

When the levees broke in the aftermath of Hurricane Katrina, City Park was inundated with flood water for 2–3 weeks at depths ranging from 0.5 to 2.5 meters (NOAA 2008). Underground monitoring stations in the park were active with *C. formosanus* less than a month after the flood waters receded (Cornelius *et al.* 2007, Osbrink *et al.* 2008). In one study site, 59% of monitoring stations were occupied by *C. formosanus* before Hurricane Katrina, while 54% of stations were occupied by *C. formosanus* only 2–3 weeks after the water receded (Cornelius *et al.* 2007). However, in another study site, average numbers of termites collected in monitoring stations were significantly lower after the flood (Osbrink *et al.* 2008). These results suggest that sufficient numbers of termites survived the flood to rapidly re-infest monitoring stations, but that overall population numbers were suppressed. Because underground monitoring stations were located at the base of termite-infested trees, termites most likely survived in carton nests and gallery systems within the tree trunks (Cornelius *et al.* 2007, Osbrink *et al.* 2008). However, it is not known whether termites survived in submerged wood or gallery systems or whether they escaped drowning by moving up from the soil into trees.

Forschler and Henderson (1995) conducted an experiment to compare the survival of three subterranean termite species, *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks) and *C. formosanus,* when they were completely submerged in water for different lengths of time. *Reticulitermes flavipes* had an $LT_{50}$ of 19.6 h, compared to an $LT_{50}$ of 13.9 h for *R. virginicus*, and an $LT_{50}$ of 11.1 h for *C. formosanus.* Forschler and Henderson (1995) suggested that *C. formosanus* had the lowest lethal time of the three species because it is more likely to construct aboveground nests and live in trees than *Reticulitermes* spp. The reduced dependency on the construction of subterranean nests would decrease the necessity for adapting to periodic flooding.

*Reticulitermes flavipes* survived longer in submerged soil arenas than in submerged test tubes due to the presence of air pockets in the gallery system (Forschler & Henderson 1995). Although *R. flavipes* moved toward the soil surface to escape rising water within gallery systems, they never left the galleries and were eventually submerged by rising water (Forschler & Henderson 1995). The purpose of this study was to determine whether galleries in soil or wood increase survival of *C. formosanus* in flooded containers and if termites respond to rising water by moving up above the water line from selected substrates or wood blocks.

## MATERIALS AND METHODS

### Termite Collection and Maintenance

Termites were collected from field colonies in City Park, New Orleans, LA, using cylindrical irrigation valve boxes (22.5 cm by 14.8 cm) (NDS, Lindsay, CA) buried in the ground so that the lid was level with the surface of the soil and filled with blocks of wood (spruce, *Picea* sp.). The collected termites were maintained in the laboratory in 5.6- L covered plastic boxes containing moist sand and slats of spruce (8 cm by 4 cm by 0.5 cm) until they were used in experiments.

### Termite Survival After Inundation of Wood and Gallery Systems

In the first experiment, the test arena (34 cm by 23.5 cm by 1.5 cm) (Giant Ant Farm, Uncle Milton Industries, Corsica, CA) contained three blocks of spruce (11.5 cm by 3.7 cm by 1 cm) placed in bottom of the test arena. The arena was filled with topsoil (GardenPlus, Hope Agri Products, Powerly, TX) to a height of 11 cm, moistened with 50 ml of distilled water, and 5 g of termites (approximately 1500 individuals) with the same soldier proportion as in their field collection (5–15%) were added to each arena through openings in the top. There were eight replicates, with two replicates each from four colonies. Termites were allowed to build galleries in soil and wood for 60 d before flooding. After 60 d, test arenas were placed in large plastic containers filled with water to completely submerge soil and 72 h later, test arenas were removed and dismantled to count survivors.

In the second experiment, a test was conducted using wood bundles to determine if termites were able to create air pockets within the bundles. Two bundles of wood were placed on the bottom of a plastic 3.8 liter bucket (height 18.5 cm, diam 20 cm). Each bundle consisted of seven slats of spruce (7.7 cm by 3.5 cm by 0.5 cm) tied together with a plastic tie. Wood bundles were covered with topsoil to a height of 5 cm in each bucket and 200 ml of distilled water was added to moisten soil. Once a week, an additional 100 ml of distilled water was added to each bucket to maintain moisture. There were eight buckets, and 6 g of termites (approximately 1800 individuals) were added to each bucket, two each from four colonies, with the same soldier proportion as in their field collection (5–15%). Termites were allowed to build galleries in soil and wood for 60 d before flooding. After 60 d, water was poured into buckets until standing water reached a depth of 1 cm. After 72 h, water was drained off and soil and wood were examined for survivors.

## Termite Responses to Rising Water

*Sand-filled tubes.* Screw cap 50–ml Falcon tubes (28 by 114 mm) (Sarstedt, Newton, NC) were filled with 20 ml of sand and moistened with 7 ml of distilled water. A 7.5 cm length tongue depressor (Fisher Scientific, Worcester, MA) was inserted into the sand in each tube such that 4 cm remained above the sand. Termites (95 workers and 5 soldiers) were added to each tube. After termites were released into tubes, the tubes were placed in a dark environmental growth chamber (28° C, 97% RH) for 3 d to allow them to construct a gallery system in the sand before flooding. There were three treatments: non-flooded controls, rapid drip flooding and slow drip flooding. There were six replicates for each treatment. Termites were collected from three colonies with two replicates from each colony for each treatment. For rapid drip flooding, distilled water was added to each tube until standing water reached a height of 1 cm within approximately 60 s using a glass funnel covered with two Whatman #4 filter paper disks. For slow drip flooding, 20 µl of distilled water was added to each tube at 60 s intervals using a pipette until standing water reached a height of 1 cm within approximately 120 min. Termite behavior in the tubes was observed during the flooding process. After flooding, tubes were returned to the environmental growth chamber for 48 h, and then the number of survivors was counted.

*Wood block with hollowed-out core on cardboard.* This assay was conducted in order to determine how termites would respond to rising water in the absence of soil. This assay was conducted using plastic containers (22 cm by 21 cm by 17 cm) (Rubbermaid, Winchester, VA). Two pieces of corrugated cardboard (13 cm by 8.5 cm) were thoroughly moistened with distilled water and placed on the bottom of the container to provide moisture and to provide access by termites to the hollowed-out core in the bottom of the wood block. A block of spruce (15.7 cm by 8.5 cm by 3.7 cm) was placed in a vertical position on top of the cardboard. The block had a 1 cm diameter hole drilled all the way through the center of the block (15.7cm). In each container, 380 workers and 20 soldiers were added.

The rate of movement of termites from the bottom of the container up onto or into wood blocks was compared for flooded and non-flooded treatments at different time intervals. In flooded treatments, the container was slowly flooded with 1 liter of water at a rate of 12.5 ml/min. The cardboard was completely submerged and the water reached a height of 1 cm on the wood block. It took 80 min to flood each container. In all flooded treatments, survivors were counted 72 h after flooding. Flooded treatments included 1) flooded 0-hour; flooding immediately after termites were released; 2) flooded 1–hour; flooding 1 hour after termites were released; 3) flooded 24–hour; flooding 24 h after termites were released. Non-flooded controls included 1) non-flooded 0–hour; only termites that had moved up onto or within the wood block were counted 80 min after termites were released into container to account for time taken to flood treated containers; 2) non-flooded 1–hour; only termites that had moved up onto or within the wood block were counted 140 min after termites were released into container to account for time taken to flood treated containers; 3) non-flooded 24–hour; only termites that had moved up onto or within the wood block were counted 24 h after termites were released into container; 4) non-flooded 96–hour; all termites surviving in container were counted after 96 h to account for the 72 h period that containers flooded after 24 h were left submerged in water. There were six replicates, two from each of three colonies, except for the non-flooded 96–hour treatment where there was only one replicate each from three colonies.

*Stacked wood block with hollowed-out core.* A block of spruce (15.7 cm by 8.5 cm by 3.7 cm), with a 1 cm diameter hole drilled all the way through the center of the block, was cut in half to make an upper and lower block with a hollowed-out core. The hole at the bottom of the lower block was covered by taping a 2.5 cm diam filter paper disk over the hole to prevent termites from escaping. Termites (190 workers and 10 soldiers) were released into the hole at the top of the lower block using a plastic funnel and then the upper block was placed over the lower block and taped in place. At the top of the upper block, the hole was plugged with a rubber stopper to prevent termites from escaping. All termites remained inside the core in the stacked blocks for the duration of the experiment. The stacked blocks were placed in a 3.8 liter plastic bucket (height 18.5 cm, diam 20 cm). Termites were collected from three colonies, four replicates per colony, two treated and two control replicates.

Movement of termites from the lower to the upper block was compared for flooded and non-flooded treatments. In the treatment, buckets were flooded at a rate of 12.5 ml per minute for 200 min until the lower block was completely submerged. In non-flooded controls, the stacked blocks were left undisturbed in buckets for 200 min. After 200 min, the upper and lower blocks of the non-flooded controls were separated and numbers of termites in each block were counted. In flooded replicates, a solid wood block was placed on top of the submerged stacked blocks to prevent the blocks from floating, and the stacked blocks were left submerged for 24 h. After 24 h, the blocks were removed from the water and the number of survivors was counted.

*Wood block with hollowed-out core in soil.* For all treatments, a block of spruce (15.7 cm by 8.5 cm by 3.7 cm) was placed in a vertical position in the center of a 3.8 liter plastic bucket (height 18.5 cm, diam 20 cm). Topsoil was added to a height of 8 cm such that half of the block was covered by soil and half extended above the soil. Soil was moistened with 200 ml of distilled water, and 100 ml weekly thereafter to maintain soil moisture. Treatments included 1) non flooded hollowed-out block where termites were released onto the soil; 2) flooded hollowed-out block where termites were released onto the soil; 3) flooded hollowed-out block where termites were released directly into the hollowed-out core using a plastic funnel; 4) flooded solid block where termites were released onto the soil. For treatments where the block had a hollowed-out core, the block had a 1 cm diameter hole drilled all the way through the center of the block. At the top of the block, the hole was plugged with a rubber stopper. Termites were collected from five colonies, one replicate of each colony for each treatment. In each replicate, 1200 termites were released in each bucket with the same soldier proportion as in their field collection (5-15%). Termites were allowed to build galleries in soil and wood for 30 d before flooding. After 30 d, buckets were slowly flooded until the standing water on top of the soil was 2 cm deep. Buckets were flooded by placing a plastic container with a small hole in the bottom filled with water over each bucket. The water dripped through the hole in the bottom at a rate of 12.5 ml/min. Water was continuously added to the container to keep the rate of flow constant. It took approximately 90 min to flood each bucket. Buckets were left with standing water for 72 h. After 72 h, the wood block was removed and examined for survivors. The location of feeding damage was also noted. After water was drained from each bucket, the soil was examined for survivors.

### Statistical Analyses

Differences in survival were compared using an ANOVA or a one-way Kruskal-Wallis if tests for normality or equal variance failed. Means were separated using Tukey's Honestly Significant Difference (HSD) test. For the experiment with stacked wood blocks, numbers of termites located in wood blocks in flooded and non-flooded containers were compared using a Mann-Whitney U Rank Sum Test and the number of termites located in the upper and lower blocks in non-flooded controls was compared using a *t*-test. All analyses were conducted using SigmaPlot 11.0 (Systat Software, 2008).

## RESULTS

### Termite Survival After Inundation of Wood and Gallery Systems

With the exception of a single soldier who survived in an air pocket inside of a wood block in one of the replicates in the test arena with wood blocks on bottom, there were no other survivors in any of the replicates in either experiment where termite galleries were submerged in water.

### Termite Responses to Rising Water

*Sand-filled tubes.* Survival was significantly greater in non-flooded controls than in either the rapid drip or slow drip flooded treatments ($H = 23.6$; $df = 2$; $P < 0.0001$), but there was no significant difference in survival of termites in treatments flooded with a rapid or a slow drip (Table 1). Only individuals that were located above the water line survived the flooding. Most survivors in the flooded replicates were observed on the wood extending above the sand when flooding was initiated. Only a few termites near the surface were observed moving up from the substrate to avoid rising water. Most of the termites within the gallery system stopped moving when they contacted rising water and became submerged. A few termites were observed floating on the water, but they were all dead after 48 h.

*Wood block with hollowed-out core on cardboard.* There was a significant effect of treatment on the number of termites surviving and those located within wood blocks ($F = 10.6$; $df = 6, 18$; $P < 0.0001$) (Table 2). The number of termites surviving in non-flooded containers after 96 h was not significantly different than the number of termites surviving in containers flooded after 24 h, or from the number of termites that had moved up into wood blocks in non-flooded controls after 24 h. However, survivorship in non-flooded controls was significantly greater than the number of termites surviving in containers flooded after 0 and 1 h and the number of termites that had moved up into wood blocks in non-flooded controls after 0 and 1 h (Table 2).

There was no significant difference in the number of termites located in wood blocks in flooded and non-flooded containers after 0, 1, and 24 h (Table 2). In both non-flooded and flooded containers, < 20% of termites had moved up into the wood block after 80 and 140 min. After 24 h, > 60% of termites in non-flooded controls and 83.9% of termites in flooded containers had moved into wood blocks. Although only termites located within the hollowed-out core or on the outer surface of wood blocks survived the flooding, there was no evidence of increased movement of termites from the cardboard into the hollowed-out core or onto the surface of the wood block in containers that were slowly flooded.

*Stacked wood block with hollowed-out core.* There were equal numbers of termites located in the upper and lower blocks in the non-flooded control blocks after 200 min (*t*-test: $P = 0.99$). There was no significant difference in termite survival between flooded and non-flooded blocks (Table 3). Although a few dead termites were found in the flooded lower blocks, most of the termites moved up within the hollowed-out core of the lower block to avoid drowning. There were significantly more termites in the lower blocks in non-flooded replicates than in flooded replicates (Table 3).

Table 1. Mean (±SE) number of termites surviving in Falcon tubes 48 h after being flooded at a rapid rate (60 s), a slow rate (120 min) and in non-flooded controls.

| Treatment | Percent Survivorship |
|---|---|
| Non-flooded control | 97.8 ± 4.2a |
| Slow drip | 25.5 ± 7.5b |
| Rapid drip | 12.5 ± 4.2b |

Means followed by the same letter were not significantly different (P > 0.05; Kruskal-Wallis, Tukey's HSD).

Table 2. Mean (±SE) percent termites surviving in containers 72 h after being flooded with a liter of water and in non-flooded control containers

| Treatment | Percentage |
|---|---|
| **Non-flooded controls** | |
| 96 h (percent survival of termites in container) | 97.6 ± 0.7a |
| 24 h (percent termites that moved onto/ into wood block) | 61.5 ± 8.3a |
| 1 h (percent termites that moved onto/ into wood block) | 16.9 ± 11.4b |
| 0 h (percent termites that moved onto/ into wood block) | 9.3 ± 5.2b |
| **Flooded** | |
| 24 h (percent survival of termites in container) | 83.9 ± 4.7a |
| 1 h (percent survival of termites in container) | 16.0 ± 14.7b |
| 0 h (percent survival of termites in container) | 15.2 ± 10.4b |

Means followed by the same letter were not significantly different (P > 0.05; ANOVA: Tukey's HSD).

Table 3. Mean (±SE) percent of termites surviving in stacked upper and lower blocks where the lower block was completely submerged in water and in stacked upper and lower blocks of non-flooded control blocks after 200 min.

| Location Of Termites | Percent Individuals in each Location | | |
|---|---|---|---|
| | Non-flooded blocks | Flooded blocks | P Value |
| Upper block | 47.8 ± 15.2 | 87.0 ± 3.2 | 0.07 |
| Lower block | 47.9 ± 15.0 | 0.0 ± 0.0 | 0.002 |
| Total survival | 95.7 ± 1.7 | 87.0 ± 3.2 | 0.07 |

Location of termites in flooded and non-flooded blocks was compared using a Mann Whitney U Rank Sum Test.

*Wood block with hollowed-out core in soil.* Survival in non-flooded controls was significantly greater than in the flooded treatment with a solid wood block (F = 4.4; df = 3; P = 0.024). However, there were no significant differences in survival between flooded treatments or between the control and flooded treatments where the block had a hollowed-out core (Table 4). Termites survived

Table 4. Mean (±SE) percent of termites surviving in containers 72 h after being flooded with a liter of water and in non-flooded control containers

| Treatment | Location of Termite Release | Percent Survivorship |
|---|---|---|
| Non-flooded control | Soil | 76.0 ± 3.8a |
| Flooded-hollowed-out core | Soil | 40.0 ± 12.6a |
| Flooded-hollowed-out core | Core | 33.2 ± 17.2a |
| Flooded-solid block | Soil | 0.0 ± 0.0b |

Means followed by the same letter were not significantly different ($P > 0.05$; ANOVA, Tukey HSD test).

the flooding within the hollowed-out core of the wood block in most of the replicates. Survival of termites in flooded treatments depended on whether or not termites had been feeding inside of the hollowed-out core within the blocks before flooding. In replicates where carton and feeding damage were observed exclusively inside of the core, survival of termites ranged from 57 to 78%. In two replicates where feeding damage was located only on the outer surface of the block, there were no survivors. In replicates where feeding damage was located both inside the core and on the outer surface of the block, survival ranged from 7% to 40%.

## DISCUSSION

The results of these experiments indicate that Formosan subterranean termites are not able to survive in their gallery system or within wood once submerged. In addition, *C. formosanus* appears to lack any behavioral adaptation to flooding because, similar to *R. flavipes* (Forschler & Henderson 1995), termites failed to move up from their galleries to higher ground to avoid becoming submerged. However, *C. formosanus* appears to move up within wood to escape rising water. In the test using stacked wood blocks where termites were released directly into the core of the lower block, 87% of termites were located in the upper block when the lower block was slowly flooded, compared to only 48% in non-flooded controls.

Also, results indicate that survival of termites was greater when they were located within the core of a wood block at the time of the flooding. Termite survival in flooded replicates appeared to be related to the location of feeding damage. No survivors were found in two flooded replicates where all of the feeding damage on the wood block was located on the outer surface of the block, but the majority of termites survived in replicates where all the feeding damage was located inside of the core. These results suggest that termites did not move out of the soil and into the core in response to slowly rising water, but that only termites located within the core at the time of the flooding were able to survive.

The distribution of Formosan subterranean termites has been largely unaffected by the heavy flooding in the aftermath of Hurricane Katrina (Cornelius 2008, Osbrink *et al.* 2008). In addition, termite numbers rebounded more quickly in monitoring stations located at the base of oak (*Quercus* spp.) trees than in monitoring stations located at the base of pine (*Pinus* spp.) trees, indicating that populations with carton nests within the hollowed-out trunks of oak trees had better rates of survival than populations infesting pine trees (Osbrink *et al.* 2008).

Formosan subterranean termites frequently build carton nests within the hollowed-out trunks of living trees in New Orleans, LA, (Osbrink *et al.* 1999, Mankin *et al.* 2002, Osbrink & Lax 2002, 2003). The construction of carton nests within living trees could be an adaptation to survive flooding. In the Amazonian rainforest, healthy, living trees commonly have rotten cores infested with termites and termites from the genus *Coptotermes* were most frequently found nesting in trees (Apolinário & Martius 2004). In City Park, most of the monitoring stations were located at the base of trees on golf courses where the trees themselves were the only food sources available (Cornelius *et al.* 2007). Therefore, it is likely that a substantial portion of the population visiting these stations were from nests located within the tree trunks, and survived the flooding of City Park because they were located in trees at the time of the flood. The distribution of termites remained largely unchanged after the flood, but numbers of termites collected in monitoring stations were reduced due to the lack of survival of any individual termites that were foraging in the soil or feeding in stations at the time of the flood (Cornelius *et al.* 2007, Osbrink *et al.* 2008).

These results have implications for termite control programs. The lack of behavioral adaptations to slowly rising water suggests that Formosan subterranean termite foragers within the underground network of tunnels or monitoring stations at the time of the flood drowned. However, the seemingly high mortality of foragers during the flood did not cause a significant reduction in termite activity in monitoring stations in heavily flooded areas of City Park (Cornelius *et al.* 2007). Average numbers of termites collected in stations associated with oak trees were initially reduced, but began rebounding after a year (Osbrink *et al.* 2008). Although termite treatments may kill large portions of the foraging populations, colonies may be able to recover and re-infest structures within a short period of time. The capacity of individual colonies to recover even after suffering high levels of mortality to foragers may contribute to the difficulties of controlling *C. formosanus* colonies infesting structures.

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