Charring does not affect wood infestation by subterranean termites

C.J. Peterson1*, P.D. Gerard2 & T.L. Wagner1
1USDA Forest Service, Southern Research Station, Insects, Diseases and Invasive Plants Unit, 201 Lincoln Green, Starkville, MS 39759, USA, 2Experimental Statistics Unit, Mississippi State University, Mississippi, MS 39760, USA
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Abstract
Fire is an important part of forest ecosystems, as is the insect fauna. Changes in wood brought about by fire may alter the ability of termites to use the wood, interrupting the decay cycle of woody debris. The ability of termites to find, infest, and feed upon wood after it had been charred was evaluated in the laboratory and field. Eastern subterranean termites, Reticulitermes flavipes (Kollar) (Isoptera: Rhinotermitidae), fed on char from burned wood had significantly reduced numbers of protozoa compared to termites fed on pine shavings, but significantly more than starved termites. The ability of termites to find and infest wood was not affected by surface charring. In a laboratory choice test, there were no significant differences in the onset of feeding by termites between charred and non-charred wood boards. Likewise in the field, no differences were observed in the time to initial attack by termites on charred and non-charred wood boards or bolts. Because termites will likely survive fires of low to moderate intensity, in most cases, there should be no disruption of the termite contribution to forest nutrient and carbon cycles.

Introduction
Termites play an important role as decomposers of organic material in forest ecosystems. Following ingestion by termites, woody material is converted by symbiotic microorganisms in the hindgut into acetate and butyrate that is used by the termites as an energy source (O’Brien & Slaytor, 1982). Termites concentrate nitrogen (and probably other nutrients as well) in their bodies (Matsumoto, 1976), and predation upon termites by other organisms results in movement of nitrogen and nutrients into local food chains (Schaefer & Whitford, 1981). Soil chemistry is also affected by termite activities, resulting in increased levels of carbon, nitrogen, phosphorous, potassium, magnesium, and calcium (for a review, see Lobry de Bruyn & Conacher, 1990). It is probable therefore that any interruption of termite activity could result in a disruption of the flow of material and energy through forested ecosystems. The future health and productivity of a forested site depend partly on active and healthy subterranean termite populations.

*Correspondence: C.J. Peterson, US Department of Agriculture (USDA) Forest Service, Southern Research Station, Insects, Diseases and Invasive Plants Unit, 201 Lincoln Green, Starkville, MS 39759, USA. E-mail: cjpeterson@fs.fed.us

Fire is an important disturbance agent in healthy and productive forests, consuming underbrush, and breaking down woody material. Fire severity depends upon two components: intensity and duration (Certini, 2005), which are in turn affected by site fuel loading and moisture content of the fuel (Massman et al., 2003). In a series of low-intensity experimental fires, the maximum soil temperature decreased exponentially with increased depth; 100 °C at 0.5 cm, 65 °C at 1 cm, 45 °C at 2 cm, 35 °C at 3 cm, and 30 °C at 4 cm (Bradstock & Auld, 1995). The upper lethal limit (the temperature above which termites did not recover from heat stress within 1 h) was about 47–48 °C for Reticulitermes flavipes (Kollar) (Isoptera: Rhinotermitidae) and about 49–50 °C for Coptotermes formosanus Shiraki (Hu & Appel, 2004). Therefore, termites in the soil at 2 cm and below should survive a fire, provided that the fire was of short duration and/or low intensity. In the tropics, where direct measurements have been made, fires of low to medium intensity did not directly affect termite abundance, and any observed suppression of termite populations was usually attributed to reduction of the food supply (Prabhoo et al., 1983; Abbott, 1984; Majer, 1984; Benzie, 1986).

When wood burns, it undergoes chemical and structural changes. Char, sometimes referred to as black carbon,
results from incomplete combustion of organic matter (Baldock & Smernik, 2002), and represents a continuum of chemical and physical properties (Czimczik et al., 2002) rather than a substance of discrete identity. Although individual components were not identified, in response to burning O-alkyl and di-O-alkyl carbon compounds (such as cellulose and hemicellulose) were converted to aryl (aromatic and phenolic) and O-furan-like compounds (Baldock & Smernik, 2002; Czimczik et al., 2002). If termites do not recognize charred wood as a potential food source, or if they are not able to utilize ingested char, then further decomposition of burned woody material must rely on other means. Indeed, char is largely biologically inert, and chemical changes induced by charring reduced the ability of micro-organisms to mineralize charred carbon to carbon dioxide, and mineralization decreased with increasing combustion temperature (Baldock & Smernik, 2002). Delay in the decomposition process increases the amount of time it takes for nutrients to return to the soil and the time required for coarse woody debris to disappear from an area.

Most studies of the effects of fire on termites have been conducted in the tropics with mound-building termites, which are typically found in a central nest. No studies exist on the effects of fire on North American subterranean termites, located more diffusely in the soil. The goal of this study was to determine if wood charring from fires would have any negative impacts on termite infestation of charred wood. Specifically, we examined the length of time required for termites to infest charred wood in laboratory and field tests. If termites are unable to utilize char as a food source, then termites forced to feed on char should display symptoms of starvation. Therefore, this study also reports the effects of consumption of char on symbiotic protozoa populations.

Materials and methods

**Termite protozoa populations in no-choice tests**

Char was obtained by burning 9 × 0.5 × 0.5 cm sticks of commercially purchased yellow pine (*Pinus* spec.) in a large crucible and grinding it to a powder by using a mortar and pestle. Pine shavings for the control group were obtained by using a tabletop planer. No-choice feeding tests were conducted over 14 days from 8 to 22 December 2005 in 60 × 15 mm Petri dishes containing 5 g sand moistened with 1.5 ml distilled water (23% moisture). Control groups consisted of dishes with pine shavings (pine-fed group), and treatment groups consisted of dishes with char (char-fed group) or sand only (starved group). Twenty eastern subterranean termite workers (*R. flavipes*) from a colony collected in March 2005 from naturally infested pine logs in Oktibbeha County, MS, USA, were added to each dish. On days 5, 8, 11, and 14, two termites were removed from each Petri dish and dissected to count protozoa. Protozoa were counted by removing the gut from a living termite, and pulling it apart in 40 µl Ringer’s solution (0.75 g NaCl, 0.04 g KCl, and 0.02 g CaCl₂ in 100 ml water). Ten µl of the solution was taken up with a micropipette and placed on a bright line hemacytometer. Gut protozoa were counted, identified, and then placed in the following groups: *Trichonympha*; small *Dinenympha* (probably *Dinenympha gracilis*); large *Dinenympha* (probably *Dinenympha fimbriata*); *Pyrononympha*; *Spirotrichonympha* (Koidzumi, 1921); and unidentified protozoa [containing but not necessarily limited to Holomastigotoidea, Microjoenia, Monocercomonas, and *Spironympha* (Lewis & Forschler, 2004)]. Counts were taken in each of the four hemacytometer quadrants and the total number of protozoa per termite was calculated. This test was replicated five times. All statistical analyses for the protozoa counts were conducted by using mixed model analysis of variance in SAS (SAS Institute, 2001) with repeated measures.

**Infestation of charred wood boards in the laboratory**

Boards of commercially purchased pine sapwood (8.75 × 6.25 × 3.75 cm) were charred over their entire surface by using a propane torch. Plastic boxes (13.5 × 19 × 7.5 cm deep) were filled with 500 ± 10 g sand and moistened with 75 ± 5 ml distilled water. Two non-charred boards (monitor groups) or one charred and one non-charred board (test groups) were placed in each box. In the monitor groups, note was made of board position in the box, either to the right or to the left to provide a dummy ‘charred’ and a dummy ‘non-charred’ board for statistical considerations. Termite (*R. flavipes*) workers and soldiers (250 total) were added to each box. Lids were placed on the boxes and the boxes were placed in an incubator at 25 °C in the dark at 50% r.h. The test was conducted over 47 days from 10 November 2005 to 27 December 2005. Boards were examined at regular intervals for evidence of termite feeding and the number of days until feeding commenced was recorded. Wilcoxon signed-rank tests (SAS Institute, 2001) and paired Prentice–Wilcoxon tests for censored paired data (O’Brien & Fleming, 1987) were each conducted separately on the monitor and test groups. The latter test was required due to the fact that some boards were not attacked during the course of the study, resulting in censored attack times.

**Infestation of charred wood boards in the field**

Field tests of charred wood boards were conducted at the John W. Starr Memorial Forest in Oktibbeha County, MS,
USA, from 29 April 2005 to 27 January 2006. The ground over a 30 × 60 cm area was cleared of litter and roots to a depth of 5 cm for each plot. Cement cinder blocks, with two 15 × 15 cm cavities, were placed on the cleared ground. Test groups consisted of cinder blocks with a 5 × 5 × 10 cm board of yellow pine sapwood (monitor board) in one cavity and a board of yellow pine that had been charred over its entire surface (charred board) in the other cavity. Monitor groups consisted of cinder blocks with a non-charred board in each cavity, one board being a dummy ‘charred’ and the other being a dummy ‘monitor’ for statistical considerations, and note was made of board position, either to the right or to the left. Each cinder block was covered with a 0.75-cm thick piece of opaque plastic sheeting and a brick to hold the sheeting in place. The wood boards were checked at 1-month intervals for the presence of termites for 9 months. The test was a randomized complete block design with 10 replications. Note was made of the length of time (in months) to termite penetration of each board. Paired Prentice–Wilcoxon tests for censored paired data (O’Brien & Fleming, 1987) were conducted separately on the monitor and test groups.

Infestation of charred bolts in the field

Field tests of charred bolts were conducted at the John W. Starr Memorial Forest in Oktibbeha County, MS, USA from 29 April 2005 to 27 January 2006. Bolts of yellow pine (greater than 30, but less than 45 cm in diameter, cut to 60-cm lengths, and bark removed), which had weathered for about 1 year and were free of termites, were charred over either one-half of their surface (half-charred, 10 bolts) or their entire surface (fully charred, 10 bolts) by using a propane torch. The half-charred bolts simulate a situation where the wood was burned after it was already on the ground, and the fully charred bolts simulate a situation where the wood was burned while still standing and fell later. Two bolts were placed within 15 cm of each other with their long axes in contact with the soil. Each bolt was buried to a depth approximately equal to its radius so that one-half of the bolt was exposed. Monitor groups contained two non-charred bolts (one a dummy charred and the other a dummy monitor). The first test group contained one monitor bolt and one half-charred bolt, while the other test group contained one monitor bolt and one fully charred bolt. In the case of the half-charred bolts, the charred portion was faced upward. Bolts were examined at 1-month intervals for 9 months. Note was made of the length of time (in months) to termite penetration of the monitor bolt and the charred bolt in each pairing. This test was a randomized complete block design with 10 replications. Paired Prentice–Wilcoxon tests for censored paired data (O’Brien & Fleming, 1987) were each conducted on the monitor and test groups, separately.

Results

Protozoa

Termites force fed char had black abdomens within a few days of test initiation, indicating that they were consuming the char. In the char-fed and starved groups, the total number of protozoa per termite declined with time (Figure 1). Counting the total number of protozoa present, termites in the pine-fed group had the highest number of protozoa at each time-point, followed by the char-fed group, and starved termites had the fewest. When all food groups (pine-fed, char-fed, and starved) were analyzed together, the effect of food on total protozoa number became more significant with an increase in time (i.e., a significant interaction between food and time; F_{6,18} = 3.30, P = 0.0227) (Table 1). This is expected because more protozoa will die as nutrition reserves are depleted.

When statistical analysis was conducted comparing the char-fed and pine-fed groups, the effect of food on total protozoa number depended upon time (F_{1,6} = 4.98, P = 0.0136). As can be seen from Figure 1, there was little difference at day 5, but the number of protozoa declined over time in termites fed on char. When analysis was conducted comparing the char-fed and starved groups, however, the only significant factor was food (F_{1,6} = 7.48, P = 0.0340). There were significantly less protozoa in termites that had been starved compared to those fed char, and the number of protozoa in the two groups declined similarly over time.

Infestation of charred wood boards in the laboratory

When the food sources are equivalent, that is, two non-charred boards are used, the termites showed no preference between boards placed on the right or the left, which is indicated by the Prentis–Wilcoxon test (P = 0.784) (Table 2).

The termites fed readily on charred boards in the test boxes, and termites with black abdomens were observed within a few days of test initiation. Portions of the charred boards were stripped by the termites to reveal the non-charred wood below. Termites were first observed feeding on the charred boards on average 7.7 days into the test and on monitor boards within 19.2 days (Table 2). For the charred boards, 80% were attacked by 8 days, and the last board was attacked by 18 days. For the monitor boards, half were attacked within 11 days, 80% were attacked by 39 days, and the last board was attacked by 47 days. The charred board was attacked first six times, the monitor was attacked first three times, and in one box both boards were
attacked at the same time (in one replication, all the termites had died before the monitor board was attacked).

Although on average termites attacked charred boards 11.5 days sooner than monitor boards, and the charred boards reached 50, 80, and 100% attack sooner than the monitor boards (Table 2), there was no significant difference between charred and monitor boards based on the paired Prentice–Wilcoxon test ($P = 0.319$). This lack of significance is likely due to the large variability in the relative difference between days to attack on each board, even when the boards are equivalent. In the monitor groups, the relative difference between the time when one board was attacked and the other board was attacked varied from 0.16 to 6.25 (where if both were attacked at the same time, the relative difference would equal 1.0).

### Infestation of charred wood boards in the field

This portion of the test was terminated after 9 months, as continuation would not have had an effect on the conclusions beyond what had already been observed. When the two wood sources were equivalent, that is, both boards were non-charred, there was no effect due to board placement (to the right or the left) by the Prentice–Wilcoxon test ($P = 0.404$).

In the test cinder blocks, the monitor board was attacked first once, the charred board was attacked first twice, and both boards were attacked in the same month

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**Table 1** Effect of food (char, pine shavings, and starvation) and time on protozoa counts in *Reticulitermes flavipes* by mixed analysis of variance with repeated measures by total protozoa count and by group

<table>
<thead>
<tr>
<th>Protozoa group</th>
<th>Food F$_{2,8}$</th>
<th>P</th>
<th>Food*time F$_{3,15}$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protozoa</td>
<td>13.38</td>
<td>0.0028</td>
<td>3.30</td>
<td>0.0227</td>
</tr>
<tr>
<td><em>Trichonympha</em></td>
<td>9.73</td>
<td>0.0072</td>
<td>1.76</td>
<td>0.1641</td>
</tr>
<tr>
<td>Small <em>Dinenympha</em></td>
<td>10.36</td>
<td>0.0060</td>
<td>2.85</td>
<td>0.0393</td>
</tr>
<tr>
<td>Large <em>Dinenympha</em></td>
<td>10.88</td>
<td>0.0052</td>
<td>2.11</td>
<td>0.1025</td>
</tr>
<tr>
<td><em>Pyrsonympha</em></td>
<td>4.36</td>
<td>0.0524</td>
<td>1.03</td>
<td>0.4369</td>
</tr>
<tr>
<td><em>Spirotrichonympha</em></td>
<td>21.95</td>
<td>0.0006</td>
<td>1.55</td>
<td>0.2196</td>
</tr>
</tbody>
</table>

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**Figure 1** Counts (mean ± SE) of protozoa numbers per *Reticulitermes flavipes* termite for the three treatment groups (char-fed, starved, and pine-fed) on day 5 (solid bars), day 8 (open bars), day 11 (dark gray bars), and day 14 (light gray bars).
In four cinder blocks, neither board had been attacked 9 months into the test. In four cases where both boards were attacked within a cinder block, in three cases both boards were attacked in the same month, and in the fourth case the two boards were attacked within 1 month of each other. The paired Prentice–Wilcoxon test found no difference in time to first attack between the monitor and charred boards ($P = 0.585$).

**Infestation of charred bolts in the field**

By the end of 9 months, five bolts had not yet been attacked: three in the monitor group and two in the half-charred group (Table 3). In the monitor group, employing pairs of non-charred bolts, there was no difference in attack between the dummy monitor and the dummy charred bolts by the paired Prentice–Wilcoxon test ($P = 0.839$).

Among the 10 half-charred bolts, half were attacked by the 1st month, eight were attacked by the 4th month, but two were not attacked by the 9th month (Table 3). Of the monitor bolts in these plots, five were attacked within 2 months, eight were attacked by the 4th month, and all 10 were attacked by the 7th month. On average, the half-charred bolts were first attacked by 1.5 months, and monitor bolts by 3.1 months. There was no difference in the length of time to first attack between charred and monitor bolts based on the Prentice–Wilcoxon test ($P = 0.383$). The monitor bolt was attacked first twice, the charred bolt was attacked first five times, and both were attacked in the same month three times.

Of the 10 fully charred bolts, six were attacked by the 1st month, eight were attacked by the 2nd month, and were all attacked within 4 months. Of the monitor bolts in the same plots, seven were attacked in the 1st month, nine by the 4th month, and all were attacked by the 7th month. The first attack averaged 2.2 months for the monitor bolts and 1.7 months for the charred bolts. There was no significant difference in the time to first attack between the monitor and charred bolts by the paired Prentice–Wilcoxon test ($P = 0.456$). The monitor bolt was first attacked twice, the charred bolt was first attacked twice, and both bolts were attacked in the same month six times.

### Table 2

Summary of *Reticulitermes flavipes* infestation of boards in the laboratory study (n = 10)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Control Left</th>
<th>Control Right</th>
<th>Charred Monitor</th>
<th>Charred Charred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of times attacked first</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Number of times both boards attacked at the same time</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average difference in time (days) to attack between boards in the same box</td>
<td>11.2</td>
<td>16.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average time (days) to first attack</td>
<td>10.2</td>
<td>11.4</td>
<td>19.2</td>
<td>7.7</td>
</tr>
<tr>
<td>Time (days) to 50% attack</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Time (days) to 80% attack</td>
<td>12</td>
<td>18</td>
<td>39</td>
<td>8</td>
</tr>
<tr>
<td>Time (days) to 100% attack</td>
<td>32</td>
<td>25</td>
<td>47</td>
<td>18</td>
</tr>
</tbody>
</table>

### Table 3

Summary of *Reticulitermes flavipes* attacks on bolts in the field study (n = 10)

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Control Top</th>
<th>Control Bottom</th>
<th>Half-charred Monitor</th>
<th>Half-charred Charred</th>
<th>Fully charred Monitor</th>
<th>Fully charred Charred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of times attacked first</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of times both logs attacked at the same time</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average difference in time (months) to attack between bolts in the same plot</td>
<td>0.2</td>
<td>1.5</td>
<td></td>
<td></td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Average time (months) to first attack</td>
<td>1.4</td>
<td>1.6</td>
<td>3.1</td>
<td>1.5</td>
<td>2.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Time (months) to 50% attack</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Time (months) to 80% attack</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Time (months) to 100% attack</td>
<td></td>
<td>–1</td>
<td>7</td>
<td>–1</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

1Two bolts not attacked by the 9th month.
2One bolt not attacked by the 9th month.
Discussion
A differential loss of gut protozoa in response to starvation and diet has been demonstrated previously (Cleveland, 1925; Veivers et al., 1983), but identifying protozoa to group was not necessary in this case to determine effects due to food. When each protozoa group was analyzed separately, the food factor (pine-fed, char-fed, or starved) had a significant effect on the count of *Trichonympha*, large *Dinonymphia*, and *Spirotrichonympha* without a significant food*time interaction (Table 1). Although counting individuals of a particular group may be used and this may avoid a significant interaction term, it was not necessary to do so to determine effects due to char consumption.

Although force-feeding char to termites significantly reduced protozoa populations, the effect was different than that of starvation. Termites may derive some nutritional value from char, as evidenced by partial survival of gut protozoa vs. starved termites, but it could not be fed indefinitely as a sole food source. Declining protozoa numbers would eventually cause starvation of the termite. Baldock & Smernik (2002) noted that the biological inertness of char depends upon the degree of thermal alteration of the wood. Had the wood used here been more extensively burned, we expect that it would have been less nutritious to the termites. In nature, it is unrealistic to expect a situation where char is the only food source available to termites. As seen in the board and bolt experiments, termites quickly penetrated the char layer to feed on the non-charred wood below. Presumably, there would be little effect on the protozoa in nature, and therefore little interruption of the termite’s ability to digest the wood.

In the board laboratory test, it seemed that termites attacked the charred boards more readily than the non-charred boards, but several statistical methods were unable to detect significance, despite our use of 10 replications. Bernklau et al. (2005), whose work was not yet published when the current study was conducted and who do not report any statistical analysis, noted that charred stakes were attacked more readily than non-charred stakes. If charred wood is indeed more readily attacked than non-charred wood, a potential reason could be the thermal decomposition or volatile loss of repellent compounds in the wood, as noted for the increased attraction of termites to fungal-decayed wood (Cornelius et al., 2004).

Experimental evidence in the tropics indicate that termites usually survive low-intensity fires (Prabhuoo et al., 1983; Abbott, 1984; Majer, 1984; Benzie, 1986), and in informal observations, on separate occasions, two of the authors (CJ Peterson and TL Wagner) found living termites in wood burned a few hours before. Together with our experimental results presented here showing that termites attack surface-charred wood as readily as non-charred wood, there should be no interruption of termites’ contribution to the nutrient and carbon cycles of a forest following a burn.

Fire in forests, however, has several characteristics that differ from those simulated here. Trees killed by larger fires increase unburned dead biomass below ground, increasing the amount of food available to the termites. The effect of fire on wood-decaying fungi, some of which may be attractive or repellent to termites, has not been examined. Forest fires can also produce a large amount of smoke (submicron particles and gaseous volatiles) and ash, which would not be produced by our use of a propane torch. As well, the effects of fire on termites already in the wood being burned have not been quantitatively examined, nor has the long-term fitness of termites fed on char been evaluated.

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References