Ultrasonic waves as a physical barrier for damage of the subterranean termite *Reticulitermes flavipes* Kollar (*Isoptera: Rhinotermitidae*) in wood of *Pinus radiata* (D. Don)

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Abstract

The behavior of the subterranean termite *Reticulitermes flavipes* Kollar (*Isoptera: Rhinotermitidae*) when applying ultrasonic fields under three single frequencies (100, 500, and 1000 kHz), in order to establish the conditions to create a physical barrier to their action, using 1 by 4 by 10 cm wood stick probes adapted from the European norm EN118. The three frequencies inhibited feeding while the acoustic field was kept active, with a lesser feeding rate at 500 kHz, and a greater mortality at 1000 kHz. These results demonstrate that ultrasound affects the physiology and behavior of *R. flavipes*.

As they feed on cellulose, termites are important in nature, but some species, particularly *Reticulitermes*, cause severe damage to wood structures in buildings. Just in the United States, termites cause more than 3 billion U.S. dollars in damage to wood structures, 80 percent of which is by the subterranean termite *Reticulitermes flavipes* Kollar (Lewis 1997). This species, identified initially as *R. hesperus* Bank, was introduced from the United States into central Chile in the 1970s (Camousseight 1999), where it has increased in distribution in recent years. Despite its particular effect on low income people, no government policies have been established to diminish its impact. The subterranean habits make it difficult to detect this termite, allowing it to disperse throughout large underground. They also reach wood materials on the surface even when separated from the soil, by building tunnels on walls. To maintain the stable humidity and temperature that they require (Smith and Rust 1993a, 1993b, 1994), termites build communication tunnels mixing saliva, feces, and substrate, which also provide them the obscure environment they prefer and protection against natural enemies. Studying the behavior of *R. flavipes* and *R. santonensis* De Feytaud, Grace et al. (1995) found that they orient themselves through their tunnels using chemo-tactile and chemo-kinetic mechanisms.

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Forest Prod. J. 57(9):114-117.

Eradication of termites is not possible, and efforts must be directed to control populations and to limit or diminish risks to buildings by implementing integrated pest management strategies (Morris 2000). To manage termites adequately, it is necessary to initiate prevention, that is, to establish physical or chemical barriers, and control methods. In general, physical barriers are conceived for preconstruction treatments (use of sand, steel nets, impregnated plastics, etc.). Chemical barriers are both preventative and curative treatments in pre- and post-construction, with the objective to exclude the subterranean termites from the structures. Curative methods rely on the use of chemicals and other substances in enclosed spaces, such as CO_2 (Delate et al. 1995), hot air (Woodrow and Grace 1998), liquid nitrogen (Lewis 1997), microwaves and high-voltage electricity (Lewis and Haverty 1996). Insecticides are applied by drilling and injection in localized areas. To apply curative methods it is necessary to have systems for termite inspection or detection in wood structures. Several techniques have been developed for this, such as acoustic emission (Lemaster et al. 1997, Yanase et al. 1998, Mankin et al. 2002), tension waves (Ross et al. 1997, De Groot et al. 1998), ultrasound (Wilcox 1998), detection of termite metabolic gasses (methane and CO_2), infrared imaging, microwaves, X rays, etc.

Ultrasound affects biological systems in animals including insects. Thus, termites may be susceptible to their action at a certain frequency and intensity affecting their communication, feeding, or other biophysical and/or biochemical changes that have immediate effects on their behavior and survival. Ultrasound comprises elastic vibration waves above human hearing, that is, above 20 kHz. Propagation of ultrasonic vibrations in diverse media is analogous to that of sound, although their absorption or degree of attenuation is much greater. The practical interest in the uses of ultrasound resides either in the application of the energy emitted (dispersions, chemical reactions, generation of heat, etc.), or in the analysis of the beam once it is made to pass through a medium (physical inspections of materials, absorbance studies, etc.). The ultrasonic bundle has diverse effects and behaviors that depend on the energy, wavelength, and interfaces in its path. When an ultrasonic beam is emitted through a nonuniform medium such as wood or soft tissues, its intensity is reduced or attenuated by several mechanisms, including scattering, absorbance, reflection, diffraction, and refraction. Ultrasound absorbance occurs when the energy of the wave is dissipated at a molecular level, and this produces heat (Kenneth 1988).

Ultrasound may modify matter by two mechanisms, generating heat, negligible at low power applications as in ultrasonography (mainly for medical uses), and cavitation, that is, the formation and collapse of cavities in a liquid in which there either exists high vacuum or that is saturated with dissolved gases. Continuity of the medium is destroyed in cavitation, a nonlinear effect phenomenon (Shutilov 1998). Extensive studies have been done on the effects of ultrasound on chemical systems and on biological molecules (Kenneth 1988).

All insects contain stable microscopic gaseous bodies, which oscillate under the influx of ultrasound, generating micro streams in the adjacent tissues that are responsible for the effects observed in many studies with eggs, larval, and prepupal stages (Kenneth 1988).

Termites are 70 percent liquid, and although polar liquids have a low to moderate absorbance, it is important to consider that in their metabolic process, termites liberate acetic acid, which is absorbed by and undergoes oxidation in the insect, and which benefits the protozoarian symbionts which allow them to digest cellulose, but when accumulated, damages the insect (Hungate 1975). Maximum absorbance of ultrasound in acetic acid occurs at 500 kHz (Almagro 1967), making this frequency interesting for termite irradiation.

Some insects and other inferior animals die quickly from the effect of ultrasound, and embryos develop abnormally. If the target organs are submerged, the effects are more intense, largely because of the greater surface exposed. Cavitation destroys cell membranes depending on irradiation frequency, among other factors. Insect and larval responses to ultrasound are significantly influenced by the presence of microscopic air pores in their tissues (Child et al. 1989).

The objective of this research was to study the application of ultrasonic waves as physical barriers for termites, and represents a new contribution to the existing techniques for control, an area with no previous works published.

Materials and methods

Collecting and maintaining termites

Termites for the experiment were obtained from two colonies in Santiago, Chile, using 7.5-cm diameter by 25-cm-long PVC tubes filled with corrugated carton bait and 5-mm-wide slits to allow for termite entry, buried superficially for 20 days in adequate places.

Five colonies were set with the termites captured, installed in a chamber with controlled temperature (25 to 27 °C) and relative humidity (70% to 80%), to provide them the best environmental conditions for optimal maintenance and development. Colonies were set on 60 by 45 by 30 cm glass cages with a lid and ventilation window covered with a metallic screen, containing a substrate formed by a mixture of coarse sand, soil, quartz sand, and feeding material (small radiata pine wood chips). Distilled water was added daily to maintain the humidity of the substrate. Six radiata pine wood stick probes tied with wire were placed inside these cages, which were taken out according to requirements for the experiments, and returned to the colonies.

Ultrasound experiments

The European norm EN188 was used with modifications, that is, instead of the specified protective layer, an ultrasonic field was used as a barrier, using frequencies of 100, 500, and 1000 kHz. The ultrasound system consisted of a 5058PR (pulser/receiver) Panametrics Ultrasonic pulse generator, and Videoscan P/N V194-RB Panametrics contact piezoelectric transductors producing longitudinal waves, with 0.024 W/cm^2 maximum irradiation intensity.

Twelve 10-cm-long radial and 1 by 4-cm transverse section pine sticks were used, with three as controls, having a mean density of 613 kg/m^3 at a 12 percent MC, with their sides covered with aluminum paint to avoid humidity losses. Glass tubes 2.5-cm diameter and 1.1-cm long open in the edges were attached to the sticks with epoxy adhesive. Quartz sand was introduced into the tubes up to 7 cm from the tube base. The sand was kept permanently wet adding distilled water (1:4 = water:sand). Rearing wood (0.2 g) was added at 1 cm from the bases, to feed the termites during adaptation, previous to the experiments. Then 250 worker termites were introduced into each of the 12 tubes. The weight of the assemblages was kept constant during the experiment by adding distilled water

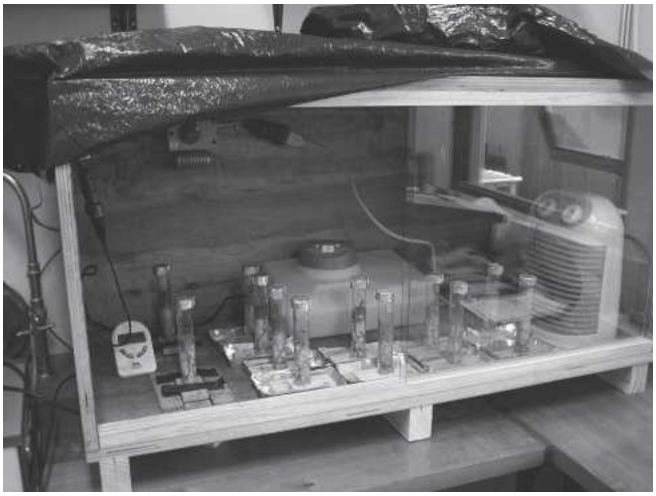

Figure 1. — Temperature and humidity controlled chamber used in the study. The black plastic cover provided darkness during the experiment.

daily. The assemblages were placed in a specially designed chamber to maintain controlled temperature and humidity during irradiations (**Fig. 1**).

The stick probes were irradiated after 7 days adaptation of the termites, treating them for 2 hours daily with each of the three frequencies, until reaching 20 hours of cumulative irradiation. Data obtained were subjected to a qualitative analysis, according to EU norm EN118, which is presented in **Table 1**.

Table 1. — Classification of infestation by R. flavipes *according to damage intensity (EU norm EN 118, 1992).*

Levels of infestation	Description
0	Without infestation.
1	Attempted damage: scratches or superficial markings of nonmeasurable depth.
2	Attempted damage: scratches < 1 mm, limited in extension maximum to 1/4 of area exposed, or unique perforation < 3 mm deep, with no other damage symptom.
3	Medium damage: superficial < 1 mm, extending to > 1/4 of area exposed, or 1 to 3 mm erosion of an area ≤ 1/4 of that exposed, or point perforations > than 3 mm, but without extending in caverns or not reaching through.
4	Severe damage: erosion > 1/4 of area exposed, or damage penetrating > than 3 mm, extending into caverns in the stick o without extending in caverns, bur reaching through.

Results and discussion

Ultrasound experiments

Mean survival (%) for the frequencies evaluated appear in **Table 2**. The EN118 European norm considers tests valid where two of three controls reach a survival > 50 percent, a level obtained in this study, where control termites had 54 percent mean survival.

Table 2. — Mean survival of R. flavipes *under each frequency.*

Treatments (kHz)	Individuals (No.)	Survival (%)
1000	86	34.4
500	154	61.6
100	142	56.8
0[a]	135	54.0

[a]Control

Survival for the 100 and 500 kHz frequencies were very similar and greater than 50 percent, in comparison with the 1000 kHz treatment which had the least survival 34 percent. However, damage levels under 0, 100, and 1000 kHz were severe (level 4), and medium (3) at 500 kHz, as presented in **Table 3**. Even so, this last treatment had the highest level of survival (61.6%). Before analyzing this apparent contradiction, it is necessary to notice some particularities during the experiment. As indicated, the glass tubes were filled with substrate up to 7 cm high, which increased 2 cm in the controls and only 1 cm in the treated tubes due to substrate displacement produced by termite activity during their construction of galleries through it to reach the sticks for feeding. This indicates that there existed an effect of ultrasound on the particles of the substrate due to the vibrations, which compacted the material. Also, after 1 week of the experiment, galleries began to form on the walls of the tubes with the treated sticks, a development that did not occur in the controls. This may have led to an effect simulating the negative geotropism of termites. Another important effect noticed was that when the ultrasonic field was being applied, termites stopped feeding and went up the substrate until a minimum height of 5 cm on the stick, resuming feeding when irradiation ceased, showing clearly the barrier effect produced by ultrasound.

Table 3. — Mean damage by R. flavipes *suffered by the sticks (EU norm EN 118, 1992).*

Frequencies (kHz)	Level of infestation[a]
1000	4
500	3
100	4
0[b]	4

[a]3 = medium; 4 = severe.
[b]Control

The 100 kHz frequency did not have a significant effect on termite survival and damage during the period of time that they were irradiated. At 500 kHz, where there existed a greater survival and lesser damage, termites may have been more affected when reaching the food, or there may have been some influence in their metabolic process, which influenced their feeding rate, but without causing lethal effects. With respect to this, the metabolic effects may have been produced by the acetic acid, which participates in the digestion process, absorbing the ultrasound.

At 1000 kHz there was the least survival but severe damage to the sticks. Termites here may have kept themselves very close to the substrate-wood interface during irradiation, given the attenuation which occurs at this high frequency, without producing major alterations. However, this may have meant that termites were subjected to a greater irradiation, which caused the lethal effect because of a greater cumulative exposure time. It is important to remember that the effect of ultrasound in biological tissues depends not only on the intensity and frequency of irradiation, but also on the time of exposure (Kenneth 1988).

Conclusion

The results show clearly that irradiation at the ultrasound frequencies evaluated were a barrier for termites to reach their feeding source, despite the low intensity of the ultrasonic field.

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