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ANALYSIS OF CO_2 AS BIOINDICATOR OF TERMITE DEGRADATION IN WOOD STRUCTURES

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ABSTRACT

The research performed exhaustive experiments to help better understand how subterranean termite colonies function in their biodegradation activity in wooden structural elements. Specifically, the research had as main objectives to analyse the usefulness of CO_2 as a bioindicator of the presence of termites and their wood biodegradation activity.

The obtained results have demonstrated that CO_2 emissions of termite colonies vary depending on their wood degradation activity. So, the amount of CO_2 emitted is closely linked to the population size and activity levels. The obtained relationship between the CO_2 concentration and time for different population sizes can predict termite biodegradation severity and help establish predictive models for pest monitoring in wood structures.

KEYWORDS: Termites, *Reticulitermes* spp., CO_2 emission, bioindicator, wood buildings.

INTRODUCTION

Subterranean termites are by far the most destructive species of wood-boring insect pests worldwide in different environments, especially in the tropical and temperate regions (Creffield 1996, Przewloka et al. 2007). They can attack any building containing wood without it necessarily showing external signs of deterioration (Ahmed and French 2008, Moreno et al. 2009).

Given that their total eradication is impossible, efforts must instead be directed towards controlling termite populations and limiting or reducing the risks to new wood constructions by implementing integrated pest management (IPM) strategies (Morris 1998). Preventive and remedial treatments are normally based on chemical products, mostly containing persistent organic pollutants (Ahmed et al. 2004). Monitoring of termites, as biodegradation agents, and their biological and environmental circumstances that influence them are key prerequisites for implementing IPM strategies (UNEP 2000). Thus, the emphasis on termite control changes

from a massive use of pesticides to IPM that involves knowledge of the ecology and behaviour of the insect and the use of safer biological or chemical methods of control (Nobre and Nunes 2007). The advantage of using IPM is sustainability by reducing dependence on (persistent) pesticides and damage to the environment, including natural enemies (Jacobs 2008). Furthermore, risks to human or animal health are also prevented, since termite pesticides can contaminate water systems and enter food chain (Lewis 2001).

Besides temperature and moisture content (Ahmed and French 2008), LED reflection (Oliver-Villanueva and Abián-Pérez 2013) or ultrasound (Taylor et al. 2011, Wilson 2013), carbon dioxide (CO_2) can be an adequate bioindicator for termite activity monitoring in wood constructions. CO_2 is produced by termites through methanogenesis, as a microbial process through symbiotic microorganisms (bacteria) in termite gut (Ohkuma 2008), in which organic matter is assimilated and metabolized with the production of biogas that contains methane (CH_4) and CO_2 in the approximate ratio of 2:1 (Gomathi et al. 2009). CO_2 gas emissions produced by termites in their biomass degradation activity have been studied in forest areas (Konemann et al. 2017, Ohashi et al. 2017, Sanderson 1996). Moreover, in closed places like wood buildings, the concentration of CO_2 produced by termites could be used to indicate their presence (Krishna and Weesner 1970) and to estimate their colony population size. Hence, in these buildings, CO_2 concentrations exceeding a certain threshold could indicate termite infestation of the wooden structures. Providing adequate clear, precise, and timely information to building managers and pest control professionals can facilitate the completion of termite control strategies based on IPM and may help to reduce the high cost of repairs and replacements required for proper reconstruction (Gutierrez Oliva et al. 1984, Moreno et al. 2009).

For this study we carried out bioassays to analyse the amount of CO_2 produced by the degradation activity of termites in wood samples in order to use it as a quantitative bioindicator for the presence of termites in wooden structures. Additionally, the research aimed to determine the nature of any relationship between CO_2 concentrations and different termite population sizes and biodegradation severity.

MATERIALS AND METHODS

To establish a relationship between the number of termites and the amount of CO_2 emitted during the wood degradation process, the bioassays have been designed and carried out following separate stages: (a) collection and breeding of termites; (b) medium preparation for termite breeding; (c) preparation of test colonies; (d) CO_2 measurements; (e) assay conditions and duration; and (f) evaluation of test colonies.

Termite collection

Subterranean termites were collected on a private property in the town of Bétera (Spain) in April 2015 using soil baits stations, which were self-made using black polyvinyl chloride pipes (75 mm diameter by 250 mm long). 5 mm-wide slots into the pipe surface allow termites' access to the inside. The pipe interiors were filled with a matrix of wetted cardboard (three layers corrugated cardboard: recycled fibre test liners with grammage of 115 $g \cdot m^{-2}$ and recycled fluting medium with grammage of 110 $g \cdot m^{-2}$, produced by CARTONAJES LEVANTE in Picanya, Spain) impregnated with cellulose powder (ARBOCEL®, produced by J. RETTENMEIER & SÖHNE GmbH & Co. in Rosenberg, Germany) and the ends were closed with cork stoppers and wrapped with adhesive tape for greater support. A total of thirty bait stations were buried in the infested area identified. After installation, the area next to each bait station was irrigated with

water. The baits were periodically inspected for termite activity. Three weeks after installation they were digged up and transported in opaque plastic containers to the laboratory.

Termite breeding

Following Austin et al. (2002), Clément et al. (2001) and Vargo and Husseneder (2009), the collected subterranean termites were identified as *Reticulitermes banyulensis* in the accredited entomology laboratory of AIDIMME Technological Institute. At this laboratory, two breeding colonies were set up with two plastic containers with a substrate containing a 3:1:1 mixture of peat, sand and substrate collected from the area the termites were sampled. The containers lids were not watertight to facilitate termite extraction for later trials. To make the new environment more familiar to the termites and to buffer their changing conditions, 250 mm × 200 mm ×10 mm poplar wood slats were added, bundled together with elastic bands (Fig. 1).

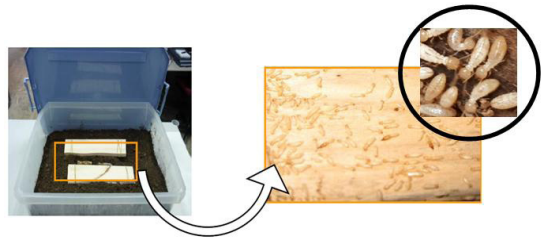

Fig. 1: Example of testing colony: detail of wooden slats infested with termites.

Termites were collected from the bait stations and individually deposited into the breeding containers. Following Shelton and Appel (2000), temperature influences CO_2 emissions in termite wood degradation activity. So, the containers were then kept in the climate chamber at a constant temperature of 26 ± 2°C, following Smith and Rust (1994). The moisture content was also controlled at 70 ± 5%, following the standard EN 117. A thermo-hygrometer (TESTO 625) has been used to check the optimal environmental conditions for termites, and if necessary, added small amounts of water to maintain optimum substrate moisture levels (Przewloka et al. 2007).

Preparation of test colonies

A total of 16 test colonies were populated with a total of 8,800 termites: specifically, four groups of 200, 400, 600 and 1,000 termites each. A brush was used to gently extract and manually transfer the termites, discarding any individuals that were injured, motionless, mite-infested, or in the larval or moulting phase. Worker termites were the predominant caste because these are the most active individuals and they therefore have a higher metabolism. Around 5% soldier and nymph termites were added to the worker groups to replicate the proportions of their originating colonies.

The testing containers were prepared by adding sterilized vermiculite as substrate and 15 x 25 x 50 mm samples of pinewood (*Pinus sylvestris* L.) as representative timber species for structural uses and unique termite food source inside the testing containers. Termites were then individually introduced and the containers sealed with their corresponding watertight caps and labelled with the date and number of transferred individuals. Finally, the test colonies were immediately moved to the climate chamber. Correct test colony set up was verified 24 hours later by checking for active termite circulation and their distribution over the entire substrate volume; any containers not displaying these characteristics were discarded and replaced by others.

CO_2 measurements

In the climate chamber, CO_2 was measured inside each testing container with the sensors of the measuring device CARBOCAP® GMP343 produced by VAISALA, so as not to disturb the termites and to preserve the optimal temperature and humidity conditions. The CO_2 readings had to be taken when the termites were active and, preferably, feeding, because CO_2 production is highest during cellulose digestion. Thus, the measurements were automatically recorded each minute during two days. First, the wood sample was disinfected with 96° ethanol, then an O-ring was attached to avoid possible gas leaks; the wood sample was then introduced through the opening on the container lid. Once inside the colony, the device was turned on for at least 15 minutes to stabilize the read values, and data were recorded at one-minute sampling intervals. After the two days, the probe was then removed, the data downloaded to a computer, and the sensor humidity and temperature stabilized outside the climatic chamber for 15 minutes. The process was then repeated for each container and test colony.

Testing conditions and duration

To establish the nature of the relationship between the number of termites and the amount of CO_2 gas emitted, the trial lasted around 10 weeks, testing the individual containers for two days. Each test colony was maintained inside the climatic chamber in absolute darkness to prevent the nymphs from swarming because they are attracted to light. While in the climatic chamber, the test colonies were reviewed every 12 hours to check for the presence, location, and activity levels of the termites, as well as any possible anomalies such as the growth of fungi and moulds or cannibalistic behaviour upon encountering dead nestmates (Neoh et al. 2012).

Evaluation of test colonies

At the end of the assay, the number of living individuals was counted for each test colony, according to their social caste (worker, soldier or nymph), and the percentage survival rate relative to the starting number was determined following standard EN 117 (2012).

RESULTS AND DISCUSSION

Test validity by evaluation of termite mortality

After the trials, the overall survival rate of the test colonies was calculated by counting the total number of surviving individuals, classified them according to their social caste. The within-castes survival rate ranged from 99% to 100%. The global average for each test colony (200, 400, 600 and 1,000 termites) was 99.3%, 99.5%, 99.1% and 100%, respectively. This mortality rate below 1% validates the bioassays. The main reason to achieve such a low mortality after the trials was the optimized termite selection strategy in field and lab as well as the working sterility conditions in the lab.

Evaluation of the CO_2 produced by each test colony

The initial concentration of CO_2 was very similar among all testing colonies. So, without termite activity, the concentration has been between 400 and 600 ppm. During the trials the CO_2 concentration increases in all test colonies, maintaining a rising trend in all cases.

The CO_2 emission increase begins directly after breeding with the wood samples. Only after one hour all colonies show significant CO_2 emissions. While 200, 400 and 600 termites' colonies remain around 1,000 ppm, the 1,000 termites colony presents an emission of approximately 2,500 ppm already in these first moments of the test, directly after attacking the wood. After

12 hours, the CO_2 increases significantly, with clear differences between the test colonies. So, the average concentration of the 200 colony is 1,004 ppm. The 400 and 600 colonies present very similar values, 1,296 ppm and 1,351 ppm, respectively. The 1,000 colony achieves a very high concentration of 2,980 ppm. Consequently, only 12 hours after wood degradation activity, all termite colonies show a significant difference in CO_2 concentration in comparison to the initial conditions.

After 24 hours, the obtained results are more consistent. The average CO_2 concentration of the 200 colony is 1,150 ppm. The 400 and 600 colonies show again similar values, 1,603 and 1,689, respectively. The 1,000 colony present again a record value with an average of 3,291 ppm.

After 36 hours, the general increase of all colonies begins to decline, as can be observed in Fig. 2. So, the average CO_2 concentration of the 200 colony is 1,243 ppm. Again, 400 and 600 colonies show similar values, 1,823 and 1,848, respectively. The 1,000 colony present the highest value with an average of 3,412 ppm.

Finally, after 48 hours total duration of the measurements, the observed trends and differences become more consistent and definitive. So, an ANOVA test demonstrates the significant differences between the observed values after 12, 24 and 36 hours, but not more between 36 and 48 hours. This means that the increased CO_2 concentration begins to stabilise only two days after termite activity, independently of the population size or the severity of the wood degradation.

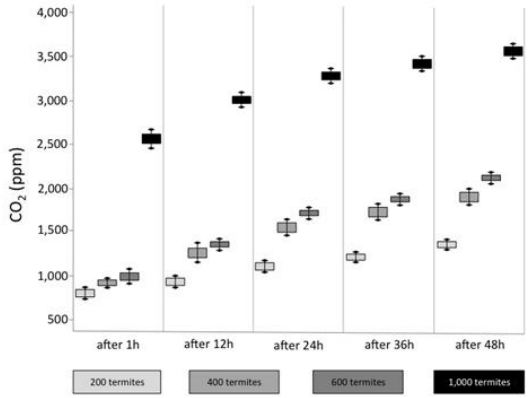

Fig. 2: Total comparison of CO_2 concentration (ppm) between test colonies after two days.

Following a second ANOVA among population sizes, the 400 and 600 colonies present a significant higher value (1,957 ppm and 2,079 ppm, respectively) in comparison to the 200 colony, but without significant differences between these two populations. The 1,000 colony is with an average of 3,477 ppm significantly higher as all other colonies. So, the population size and, consequently the biodegradation severity, influence significantly the CO_2 emission, tending to a maximum value only few days after beginning of the termite activity. Gomathi et al. (2009) found similar trends for the associated CH_4 emissions with termites of genus *Macrotermes*.

CO_2 emission as bioindicator of termite degradation severity in wood structures

In order to verify the consistency of the obtained data to establish prediction models, we performed regression analyses for the different test colonies. We also explored and quantified the relationship between the dependent criterion (*y*) representing the accumulated CO_2 concentration

and the independent, or predictive, variable (*x*), time, to develop an equation to predict the amount of CO_2 produced in a given time by a given starting population. We tested linear, quadratic, and cubic regressions to find the equation that best fitted the data points obtained in relation to the accumulated CO_2 over time. A regression line coefficient of determination (R^2) was used to determine how closely each regression line describes the relationship between the two variables. The R^2 values demonstrate the high determination level of the obtained regressions. Tab. 1 present the different regression models obtained for the colonies tested.

Tab. 1: Prediction models of termite activity in dependence on CO_2 emission following regression analysis.

Test colonies	Regression analysis	R^2
200	$y = 879 + 0.202x + 6.591e^{-0,009}x^2 - 4.799e^{-0,009}x^3$	0.870***
400	$y = 1{,}060 + 0.391x + 8.533e^{-0,006}x^2 - 4.203e^{-0,009}x^3$	0.878***
600	$y = 982 + 0.454x + 4.582e^{-0,006}x^2 - 7.609e^{-0,010}x^3$	0.952***
1,000	$y = 2{,}155 + 0.649x + 1.930e^{-0,004}x^2 - 9.800e^{-0,008}x^3$	0.964***

CONCLUSIONS

First, the methodological development and the research results allow us to conclude that successful bioassays with termites validated by low mortality rates after trials depend highly on an optimized termite selection strategy in field and lab based on soil baits, on the one side, and on controlled working sterility conditions in the lab, on the other side.

Termite colony CO_2 emissions vary depending on their diet and so this factor must also be considered. Wood samples without bark containing high proportion of cellulose are recommended, e.g. pine or poplar.

The amount of CO_2 emitted by termites is closely linked to their population size and activity levels. So, CO_2 concentration increases after beginning of the biodegradation activity and can be significantly documented only after few hours.

With independence of the termite population and the biodegradation activity, the CO_2 concentration maintains a rising trend achieving a maximum value after few days, which in all cases exceeds the value of 1,000 ppm, i.e. more than double as in normal conditions without termite infestation degrading wood samples.

Highly significant statistical models based on quadratic adjustments to the relationship between the CO_2 concentration and time for different population sizes allow the prediction of termite populations and consequently degradation severity in dependence on CO_2 concentration.

From the point of view of a practical application of the research results obtained, we can conclude that CO_2 emission by termite activity can be accepted as a quantitative bioindicator for biodegradation in wooden buildings, e.g. for inclusion in sensors to monitor termite activity.

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