EVALUATION OF FEEDING STIMULANTS AND TOXIC SUBSTANCES FOR THE SUBTERRANEAN TERMITE *Reticulitermes flavipes* (Kollar) (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT

The objective of this study was to evaluate the use of glutamic acid and aspartame as feeding enhancers for the subterranean termite *Reticulitermes flavipes* (Kollar). For this purpose, these substances were added to filter paper at 0.005, 0.01, 0.05 M, plus distilled water as a control treatment, and evaluated in Petri dishes with 50 workers each of the termite. Mean filter paper consumption in mg per termite a day was compared at 30 d. No differences occurred in average paper consumption in mg per termite a day between treatments, so both substances at the concentrations evaluated did not stimulate feeding by *R. flavipes*. In addition, the insecticide effect of acetic and formic acids during 15 d was evaluated at 1, 5, 10, and 20%, plus distilled water as a control. The solutions were applied to pinewood blocks by soaking, and placed on separate Petri dishes with 30 termites each. The cumulative mortality curves indicated an effect proportional to the concentration of acetic acid, but not of formic acid. At day 15, the acetic acid caused over 50 and 80% mortality with the 10 and 20% concentrations, respectively; formic acid did not reach 50% control at any of the concentrations evaluated. Besides, acetic acid caused more homogeneous mortalities at the same concentrations than formic acid, which was reflected by the greater fit of the results to the regression curve.

Key words: Acetic acid, aspartame, formic acid, glutamic acid, termite bait.

RESUMEN

El objetivo de este estudio fue evaluar el uso de ácido glutámico y aspartamo como potenciadores de la alimentación para la termita subterránea *Reticulitermes flavipes* (Kollar). Para esto, estas sustancias se agregaron a papel de filtro a 0.005, 0.01, 0.05 M, más agua destilada como tratamiento de control y evaluaron en placas Petri con 50 termitas obreras cada una. El consumo promedio de papel filtro en mg por termita al día se comparó a los 30 d. No hubo diferencias entre los tratamientos, por lo que las sustancias a las concentraciones evaluadas no estimularon la alimentación de *R. flavipes*. También se evaluó el efecto insecticida de los ácidos acético y fórmico durante 15 d, al 1, 5, 10 y 20% y agua destilada como testigo. Las soluciones impregnaron bloques de madera de pino, y pusieron en placas Petri con 30 termitas. Las curvas de mortalidad acumulada indicaron un efecto proporcional a la concentración de ácido acético, pero no de ácido fórmico. Al día 15 el ácido acético causó mortalidades superiores al 50 y 80% con concentraciones de 10 y 20%, respectivamente, y el ácido fórmico no alcanzó el 50% a ninguna de las concentraciones evaluadas. Además, el ácido acético produjo mortalidades más homogéneas a una misma concentración que el ácido fórmico, lo que se reflejó en el mayor grado de ajuste de los resultados a la curva de regresión.

Palabras clave: Ácido acético, ácido fórmico, ácido glutámico, aspartamo, cebo para termitas.

INTRODUCTION

The subterranean termite *Reticulitermes flavipes* (Kollar) is native from North America, but it is widely distributed in the world. It was introduced in Chile in 1986 (Morales-Ramos and Rojas, 2003), and it can be currently found in the Metropolitan (MR), Valparaíso, and O'Higgins regions (Ripa and Luppichini, 2004). In 1998, the Chilean Institute of Technological Research (INTEC) and Forestry Institute (INFOR) found 80 infestation focuses in 29 counties in Santiago and in some areas of the 5th Region. Recent studies by the Chilean Institute of Agricultural Research (INIA) established 56 focuses in the MR, with a rapid growth since 1995, and damage reaching US\$15 million (Karsulovic and Bozo, 2006). As all subterranean termites, *R. flavipes* avoids exposure to light and needs to keep in contact with the ground or other humidity sources while searching for cellulose (Wiltz, 2012).

Subterranean termites affect undamaged wood, but they prefer partly deteriorated substrate, for example, by fungi (Cornelius et al., 2002). They have feeding preferences for some woods, and for a cellulose matrix with additives (Morales-Ramos and Rojas, 2001a; 2003). Certain organic compounds induce preference or increase the consumption of cellulose foods (Chen and Henderson, 1996; Cornelius and Lax, 2004; Swoboda et al., 2004).

Toxic baits, alone or combined with chemical barriers, are a good solution worldwide, but they are expensive and many of them are not sold in Chile. Therefore, efficient, inexpensive, and environmentally friendly alternatives need to be found (Munizaga, 2008). There are few attractants for termites, and most of them last a short-term. In general, commercial baits do not use attractants (Cornelius and Lax, 2004), and those available are based on termite food searching and foraging. They have feeding preferences and present consumption differences, while some substances added to cellulose feed increase preference significantly. To find preferred foods, feeding stimulants and toxic substances for *R. flavipes* added to a bait would improve monitoring and control of this pest.

The acetic (CH_3COOH) and formic (HCOOH) acids could be used as toxic compounds for termite control with low environmental impact. Acetate derived from acetic acid is found in large amounts in the digestive tract of *R. flavipes* and other termites (Odelson and Breznak, 1983). Increasing the acetic acid in their guts may produce an imbalance that could kill them. The use of formic acid could be useful, as this compound is used for defense by ants, which are recognized enemies of termites.

This study evaluated feeding stimulants and the use of potential toxic compounds to develop an efficient and inexpensive bait to control *R. flavipes*.

MATERIALS AND METHODS

This study was conducted with termites from a colony maintained at 23 to 27°C, in a Laboratory at the Department of Wood Engineering, College of Forestry and Nature Conservation Sciences, University of Chile, Santiago, Chile.

1. Bioassay of potential feeding stimulants. Based on a study by Chen and Henderson (1996), three concentrations of glutamic acid and aspartame at 0.05, 0.01, and 0.005 M, plus distilled water for the control, were evaluated. These bioassays were conducted separately and not simultaneously, with four replicates each (Munizaga, 2008).

Filter paper discs (10 cm diameter) were dried at 70°C during 24 h, dehydrated at a 30 mm Hg vacuum for 30 min, and then weighed to obtain the initial dry weight. The dried papers were placed in Petri dishes, and a volume and 1.5 mL of the corresponding solution was added to each; this added volumen was sufficient to wet the filter papers and allow termite mobility. Then 50 termite workers were added per dish. Each dish was weighed immediately for humidity control. During the bioassay, the dishes were weighed twice a week and water was added to restore their initial humidity.

The termites were removed from the dishes and counted at 30 d. The paper disks were brushed and then dried at 70°C during 24 h. Subsequently, they were dehydrated 30 min at a 30 mm Hg vacuum, and weighed. Weight value of the solute present in 1.5 mL of the corresponding treatment was deducted from the final weight of each paper disk, and this corrected weight was deducted from the initial weight to obtain the total consumption mg/termite/day. A completely randomized design was used. The data from each bioassay were subjected to a simple ANOVA, while the means of the treatments were compared using a Tukey test at 95%.

2. Bioassay of toxic substances. Solutions of acetic and formic acids were prepared at four concentrations, plus distilled water as a control (Table 1).

Pinewood blocks (4 x 2 x 0.5 cm) were immersed completely in a vase with a solution, and treated by sorption at 60 and 20 mm Hg vacuum in an impregnation cylinder for 20 and

Table 1. Acids and concentrations evaluated in the bioassay for *R. flavipes* feeding.

Treatments	Concentrations (v/v %)
Acetic acid	1
Formic acid	1
Acetic acid	5
Formic acid	5
Acetic acid	10
Formic acid	10
Acetic acid	20
Formic acid	20
Control (water)	100

5 min, respectively. Subsequently, they were dried out in tissue paper, and weighed.

Petri dishes with a 4:1 mixture of sand and water ~3 mm deep and an impregnated wood block on top were used for a bioassay, with three replicates. Termites (30) were placed on the border of each plate, preventing them from falling on the wood blocks, and their initial reactions were observed. The dishes were covered with cotton cloth to avoid the evaporating gases to create a fumigation chamber, prevent moisture losses from occurring quickly, and imitate a natural environment. The termites (alive and dead) in each dish were counted the next 3 d, and every other day thereafter, to determine the variation in mortality in all treatments until the end of the test on day 15, when the wood blocks were taken out, separating and counting the surviving termites. Relative humidity in the dishes during the test varied from 43 to 47%. Mortality data were corrected with Abbott's (1925) formula. Mortality percentages on day 15 were adjusted with the Minitab software to quadratic regression curves for each acid. These curves were used to estimate and compare the effect of the acids and their concentrations on termite mortality.

RESULTS AND DISCUSSION

1. Bioassay of possible feeding stimulants.

Neither the aspartame nor the glutamic acid treatments caused significant differences in filter paper consumption (Table 2). Thus, these compounds did not stimulate feeding by *R. flavipes* at the concentrations evaluated.

Aspartame was evaluated because it contains aspartic acid. Same as in a study by Chen and Henderson (1996), both glutamic and aspartic acids, evaluated at 0.01 M together with other amino acids, caused a greater consumption in choice tests. However, the glutamic acid and aspartame did not caused a significant consumption increase in our study with *R. flavipes* at several concentrations in no-choice tests.

In another study on *Reticulitermes* spp. with sugars and amino acids, Swoboda et al. (2004) found significant consumption differences in choice tests. Uric acid was preferred at 0.001 M, and some amino acids stopped feeding at 0.1 M. However, no compound stimulated feeding in no-choice tests, which agrees with the results herein. In another test with two alternatives (Cornelius and Lax, 2004), filter papers treated with aspartic acid at several concentrations were not more consumed by *C. formosanus* than the control.

In Huixin et al. (2003), 2-fenoxiethanol at very low concentrations increased feeding by *C. formosanus*. In addition, hydroquinone (1,4-dihydroxybenzene) and some related compounds have stimulated termite feeding (Reinhard et al., 2002).

In laboratory choice tests, *Mastotermes darwiniensis* Froggatt and *Coptotermes acinaciformis* Froggatt consumed significantly more wood with hydroquinone, but adding glucose or amino acids did not increase the feeding stimulant effect of hydroquinone (Reinhard et al., 2002). However, other choice tests with synthetic chemicals presented clear preferences of *Reticulitermes santoniensis* Feytaud for sugar compounds at 10 and 100 mmol. And the amino acids induced only slight preferences (Reinhard and Kaib, 2001).

Table 2. Mean daily consumption (mg) of *R. flavipes* in treatments with aspartame and glutamic acid.

Treatments	Aspartame	Glutamic acid
Distilled water	0.0617 a	0.0459 a
0.005 M	0.0561 a	0.0427 a
0.01 M	0.0613 a	0.0437 a
0.05 M	0.0555 a	0.0401 a

Means with the same letter are statistically similar ($P \leq 0.05$), according to a Tukey test.

According to Rojas et al. (2004), certain nitrogenous compounds of the urea group and uric acid are effective as feeding stimulants and/or "subterranean termite grouping substances" at concentrations $\leq$ to 1000 ppm (0.1% of weight).

In this study, the aspartame or glutamic acid solutions did not caused attraction or aggregation effects on *R. flavipes*.

Several patents refer the use of chemicals to increase the consumption of cellulosic baits. One of them indicates that several termite species, *R. flavipes* among them, have increased feeding of cellulose baits containing a plant lipid called sitosterol (James et al., 2003). Patent US No. 5.637.298 describes the use of 2-naftalenomethanol to attract and stimulate feeding (Stowell, 1997); US Pat. No. 6.203.811 describes a fungus extract as feeding stimulant (McPherson and Wood, 2001); and US Pat. No. 3.858.346 the mixture of a carbohydrate with wood sawdust bait for increasing consumption (Bailey, 1975).

However, many chemicals that are preferred in choice tests, have not stimulated feeding in no-choice tests, as uric acid and sugars like fructose, galactose, glucose, raffinose, sucrose, and trehalose (Swoboda et al., 2004). Saran and Rust (2005) found that several sugars acted as feeding stimulants for *Reticulitermes hesperus* (Banks) at concentrations much greater than those found naturally in wood. The greatest consumption occurred in paper discs treated with ribose at 5%, followed by xylose at 3%, and maltose, fructose, arabinose, and ribose at 2%. However, termites did not discriminate between ribose, fructose, xylose, and maltose at 2% in multiple choice tests conducted by Saran and Rust (2005).

Multiple choice tests do not differentiate the feeding preferences caused by feeding stimulants or inhibitors (Swoboda et al., 2004). The greater the alternatives, the effect of any particular substance is less identifiable, and it is convenient to perform no-choice tests with the substances that have been preferred in multiple choice tests (e.g., Chen and Henderson, 1996; Morales-Ramos and Rojas, 2001b; Swoboda et al., 2004).

Considering the evidence obtained in feeding studies, few compounds really stimulate feeding.

Many papers wrongly refer to feeding stimulants, as they confuse the preference in choice tests with a feeding stimulant effect (Munizaga, 2008).

2. Effect of acetic and formic acids in mortality of *R. flavipes*. At the beginning of the tests, acetic acid at 1% had an immediate effect on termite attraction and aggregation in the wood blocks, apparently influenced by temperature; this was observed at 18-20°C but not in the colony at 23-25°C. Formic acid at 1, 5, 10, and 20% caused a slight repellent effect. A similar effect was observed with acetic acid at 20% as the termites did not approach the blocks, which differed from the control. However, these effects were not quantified and most of them did not persisted longer than 2 d.

The concentrations of acetic and formic acids used, and the mortality means on day 15 are presented in Table 3. The water control had 10% natural mortality, which was deducted from the other means applying Abbott's (1925) correction. The least concentration of acetic acid presented a corrected mortality of 1.23%, and that of formic acid of 16.05%. Mortalities were similar with both acids (lower than 17%) at concentrations of 5%. The 10% concentration caused 51.85% and 41.98% mortality with acetic acid and formic acid, respectively. The greatest concentration of acetic acid caused a $> 80\%$ mortality, and $< 50\%$ with formic acid.

The regression curves of the concentrations versus termite mortality at the end of the test are presented in Figs. 1 and 2. The results with acetic acid had a better fit to the curve, and a narrow relationship between concentration and increased mortality. In this case, concentrations $\geq 10\%$ eliminated over 50% of the termites, and the 20% concentration eliminated over 80% in 15 d. The curve equation allowed calculating an LC_{50} for acetic acid of 10.73 mL in 100 mL solution. A concentration of 22.55 mL acetic acid in 100 mL would be required to kill 90% (LC_{90}) of the termites.

The curve adjusted for the formic acid treatments did not have a good data fit, because mortality was very variable at a given concentration. Besides, not even the greater

Table 3. Corrected termite mortality (%) on day 15 with the concentrations evaluated of acetic and formic acids.

Concentration (mL acid/100 mL solution)	Mortality, %	
	Acetic acid	Formic acid
1	1.23	16.05
5	13.58	16.05
10	51.85	41.98
20	82.72	46.91

Fig. 1. Quadratic regression of *R. flavipes* mortality (%) vs acetic acid concentration.

concentration evaluated eliminated 50% of the termites at 15 d.

Figs. 1 and 2 indicate that acetic acid was much more effective for termite control than formic acid. The regression curves were done with the cumulative mortality at day 15, because a notorious decrease in mortality occurred in most treatments around that day, which caused a decrease in the slope of the cumulative mortality curves. However, mortality did not stop in all treatments at the end of the test. The cumulative mortality curves during the test are presented in Figs. 3 and 4 for acetic and formic acids, respectively.

Fig. 3 indicates a mortality response proportional to the concentration used of acetic acid. On the contrary, the 1 and 5% concentrations of formic acid caused almost the same effect according to the mortality curves, while the 10 and 20% concentrations were also similar.

Both acetic and formic acids are far less toxic to *R. flavipes* than insecticides like thiamethoxam or fipronil. Remmen and Su (2005) evaluated the effect of several concentrations of these insecticides on *R. flavipes* for 24 h, reporting mortalities over 80% in 2-4 and 5 d with 50 mg kg⁻¹ thiamethoxam and 1 mg kg⁻¹ fipronil, respectively.

Fig. 2. Quadratic regression of *R. flavipes* mortality (%) vs formic acid concentration.

Fig. 3. Cumulative mortality of *R. flavipes* workers exposed to pinewood impregnated with acetic acid at four concentrations during 15 d.

Fig. 4. Cumulative mortality of *R. flavipes* workers exposed to pinewood impregnated with formic acid at four concentrations during 15 d.

No references were found on the use of formic acid to control termites, but this substance is used against parasite acari of honey bees (*Apis mellifera* L. and *Apis cerana* F.). Espinosa-Montaño and Guzmán-Novoa (2007) evaluated 65% formic acid to control the parasitic mite *Varroa destructor* Oudemán, applying it 4 times at 4 d intervals, and obtained 66.4% mortality in 5 wk. However, the methodology used by these authors differs from that used in this study. Another study aimed at determining the best concentration-time (CT) combination to control *V. destructor* without damaging bees reported that the survival of mites and bees was affected by the concentration of formic acid, with an interaction for the CT_{50} between temperature, concentration and species (Underwood and Currie, 2003).

Patent US 6586470 (Lojek and Lojek, 2003) describes an aqueous composition of acetic acid

as insecticide, consisting essentially of acetic and citric, malic or ascorbic acids, in 10:1 to 1:1 weight ratios, and with a concentration of acetic acid less than 8 g L⁻¹. However, no scientific studies were found to verify its insecticide action.

Rahman Saljoqi et al. (2014) conducted a study to characterize insecticides for the termite *Heterotermes indicola* L., and indicated that those with slow action at low concentrations require a longer time to reach the mortality achieved at high concentrations. At the end of their study, the low concentrations presented cumulative mortality curves close to those with high concentrations. However, those with rapid action resulted in lower mortality rate at low concentrations. Based on this, the cumulative mortality curves observed herein do not indicate a slow action of the acids. A toxic substance to be applied in baits needs to be of slow action to allow its transmission to other termites that have not

fed yet from them, and avoid the accumulation of dead or sick termites near the feeding station, which would prompt other termites to leave the area (Beyond Pesticides, 2007).

CONCLUSIONS

Glutamic acid and aspartame did not result in a greater consumption of filter paper at any of the concentrations evaluated, indicating that these substances would not stimulate feeding by *R. flavipes*.

Mortality of *R. flavipes* was greater (over 50 and 80% at 10 and 20% concentrations, respectively), and more homogeneous with acetic than formic acid.

The mortality results with toxic substances suggest that further studies are required to evaluate the effect of acetic acid at 10 and 20%, and formic acid at 20%, using a larger number of termites over a larger period of time, and under conditions similar to their natural habitat.

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