Sociobiology 61(1): 82-87 (March, 2014) DOI: 10.13102/sociobiology.v61i1.82-87

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - TERMITES

Comparative Evaluation of Three Chitin Synthesis Inhibitor Termite Baits Using Multiple Bioassay Designs

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Article History
Edited by
Evandro N Silva, UEFS, Brazil
Received 22 July 2013
Initial acceptance 26 August 2013
Final acceptance 08 October 2013

Keywords
Benzoylphenylurea, termite baits, *Coptotermes formosanus,* termite control

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Abstract

Use of chitin synthesis inhibitors has revolutionized the potential impact of termite baiting systems. Several chitin synthesis inhibitors have been used or tested against subterranean termites. We evaluated the effect of lufenuron on bait matrix consumption and mortality of *Coptotermes formosanus* and compared it with 2 other chitin synthesis inhibitors presently used for termite control: diflubenzuron and noviflumuron. Laboratory no-choice and multi-chamber bioassay designs were employed. At the end of 6 weeks, in both the no-choice and multi-chamber tests, mortality was significantly higher in all the chitin synthesis inhibitor treatments as compared to the controls; however, lufenuron treatment had significantly higher mortality than the other chitin synthesis inhibitors. Multi-chamber tests suggested no sign of feeding deterrence with any of the chitin synthesis inhibitors at the concentrations tested. Consumption of lufenuron cardboard or noviflumuron bait matrix was similar to that of control cardboard in the no-choice tests. We conclude that, based on the overall bait consumption and mortality data, lufenuron was at least as effective as noviflumuron and diflubenzuron.

Introduction

Present termite control methods mainly include application of liquid termiticides and baiting systems. Subterranean termite baiting systems exploit the termites' foraging behavior and their food transfer system (trophallaxis) to reduce or eliminate the colony population from an area (French, 1991; Su et al., 1995). For a typical bait to succeed, foraging termites must find the bait station, recruit more termites, consume sufficient amount of bait matrix containing a toxicant, carry the toxicant back to the colony and transfer a sufficient dose to the rest of the colony members by trophallaxis (Su et al., 1995; Grace et al., 1996; Su & Scheffrahn, 1996; Grace & Su, 2001).

It is suggested that bait treatments have advantages over alternative termite control strategies both by affecting a greater foraging area and by reducing the amount of toxicants placed in the environment (Su & Lees, 2009). The interconnected nests and galleries of a subterranean termite colony often spread over dozens of meters in the field. An excavation conducted by King and Spink (1969) revealed that underground tunneling system of *Coptotermes formosanus* Shiraki extended over 60 m and covered an area of over 0.58 ha. Su and Scheffrahn (1988) estimated 1.4 to 6.8 million foraging individuals in one colony of *C. formosanus*. Due to the huge population size and extensive foraging range of subterranean termite colony, a non-repellent and slow acting control agent has the best potential to impact the majority of individuals in a colony (Su, 1994; Su, 2005).

Chitin synthesis inhibitors (CSI) fall within the benzoylphenylurea group and are slow acting insect growth regulators (IGR) that induce malformation of cuticle and significant reduction of chitin synthesis (van Eck, 1979; Merzendorfer et al., 2012). Inhibition of chitin synthesis disrupts the molting process in insects leading to their death. CSI also prevents normal formation of peritrophic membrane (Zimmermann & Peters, 1987); as a result, the insects become more susceptible to infection by microorganisms such as nuclear polyhedrosis virus (Arakawa et al., 2002). Several chitin synthesis inhibitors such as diflubenzuron, hexaflumuron, noviflumuron, lufenuron, and novaluron have been used or tested with various degree of success in termite baiting systems (Su, 1994;

Cabrera and Thoms, 2006; Vahabzadeh et al., 2007; Lewis & Forschler, 2010; Osbrink et al., 2011).

There are quite a few studies, both laboratory and field, about the noviflumuron based termite bait (though not the durable bait we tested), however, very limited published studies have evaluated the lufenuron and diflubenzuron based termite bait. Lufenuron based termite bait was developed by Syngenta about a decade ago but is still not commercially available for termite control. This could possibly be because of the conflicting reports of the effect of lufenuron on subterranean termites (see for example, Su & Scheffrahn, 1996; Lovelady et al., 2008). The objective of this study was to evaluate the effect of lufenuron on bait matrix consumption and mortality of *C. formosanus* and compare it with 2 other CSIs: diflubenzuron and noviflumuron in the laboratory using two different bioassay designs. We believe that a comparative study in the lab using different bioassay designs would help to determine the efficacy of these chemicals against subterranean termites.

Materials and Methods

Test Materials

Test materials included three chitin synthesis inhibitor bait materials and untreated cardboard as controls. Lufenuron treated cardboard (company code: NB 3401-91; 0.15% lufenuron) and control cardboard were received from FMC Corporation (Philadelphia, PA). They also provided samples of diflubenzuron bait (Advance® Compressed Termite Bait II, BASF Corporation, St. Louis, MO; 0.25% diflubenzuron) and noviflumuron bait (Recruit® HD Termite Bait, Dow AgroSciences LLC, Indianapolis, IN; 0.5% noviflumuron).

Termites

Formosan subterranean termites were collected in June 2012 from Brechtel Park, New Orleans, Louisiana using milkcrate trap methods as described in Gautam and Henderson (2011). In brief, the trap consists of a milkcrate filled with pine 44 wood (*Pinus* sp.) sticks arranged in a lattice structure. The crate is buried in the ground and checked after 1-2 months. When infested with ~10,000 termites or more, the crate is retrieved, brought back to the laboratory and maintained in a trash can by adding water whenever necessary. The termite groups used in the experiment were collected from such laboratory stocks. Termites from two colonies (separated by >500m) were used.

No-choice Tests

No-choice tests were conducted in individual Petri dishes (100 × 15 mm) each containing 50 g of autoclaved sand added with 6 ml of deionized water (12% moisture wt/wt). A pre-weighed cardboard piece or bait material (the lufenuron treated cardboard and untreated control cardboard were cut into pieces with separate scissors, whereas the diflubenzuron and noviflumuron baits were cut with a power operated band saw) was placed on moist sand surface in the dish. The cardboard or bait materials were pressed gently to the moist sand. One hundred termites (90 undifferentiated workers of at least the third instar and 10 soldiers) were released in each dish and the dishes were sealed with Parafilm® to retain moisture. There were 6 replications for each treatment (3/termite colony group) and control. The dishes were then placed in an incubator maintained at 27°C. Moisture content of the dishes was monitored regularly. Termite survival count was made at 6 weeks when the experiment was ended. The cardboard and bait materials were cleaned of debris, dried and weighed to determine the consumption. Consumption of diflubenzuron bait was not recorded as it was difficult to quantify due to its being spread throughout the dish by the termites and mixed with sand.

Multi-chamber Tests

To test how additional food sources may affect bait toxicity, an especially designed 16-chambered test arena with a slight modification from Gautam et al. (2013) was used. The arena consisted of one center chamber (8.5 cm diam × 3.4 cm, Pioneer Plastics©, North Dixon, KY) connected to 5 first peripheral chambers (5.08 cm diam × 3.63 cm, Pioneer Plastics©, North Dixon, KY) with Tygon tubing (5 cm long, 0.64 cm inside diameter, Watts Co., North Andover, MA). The first peripheral containers again connected to 10 second peripheral containers with the same length tubing making two peripheries of the chambers around the center chamber. The side chambers in the first periphery were connected to each other with 10 cm tubing and similarly the side chambers in the second periphery were also connected (Fig. 1). This test arena represented multiple feeding sites as might occur in a typical subterranean termite infested area.

The center chamber contained 60 g of autoclaved sand and the peripheral chambers contained 30 g each. Deionized water was added to each chamber to make the sand moisture content ~15% (wt/wt). The center chamber was the termite release chamber and contained nothing except the moist sand whereas the peripheral chambers contained 3 bait pieces of either lufenuron, diflubenzuron or noviflumuron in 3 randomly selected chambers and the remaining 12 chambers contained pinewood pieces pre-soaked in water for 1 h.

After 1 d of arena set up, 300 termites (90% undifferentiated workers of at least the 3rd instar and 10% soldiers) were introduced in the center chamber. The lids were put back and the arenas were placed on a laboratory bench at 27 ± 1.5ºC. There were 4 replications for each treatment and control. Control arenas contained no cardboard or bait, instead wood pieces were placed in all 15 peripheral chambers.

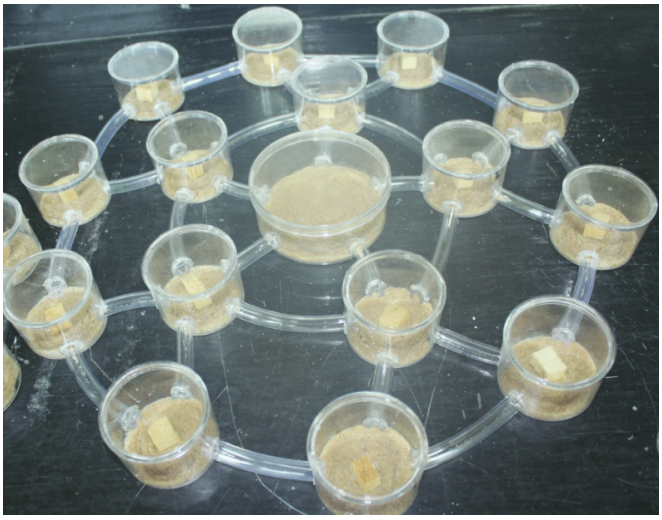

Fig. 1. Image of a laboratory test arena consisting of multiple feeding chambers connected with tubings.

The test arenas were monitored regularly for moisture and water was added when necessary. Live termites were counted at 6 weeks when the experiment ended, and wood and bait material consumption was recorded as in the no-choice tests. In each test arena, consumption of all 12 wood blocks or all 3 bait materials was added to calculate the total consumption. In controls, consumption of all 15 wood blocks was determined. Unlike no-choice tests where consumption of diflubenzuron bait was not quantified, a best effort attempt of consumption was quantified in the multi-chamber tests. Unconsumed diflubenzuron bait was carefully separated from sand and scrapped off the container, then dried and weighed to determine consumption.

Statistical Analysis

Data analysis was done using proc mixed model in SAS 9.3. Data were transformed using either arcsine of the square root method (mortality data) or log transformation method (consumption data) when necessary, especially to improve the normality. Post ANOVA comparisons were made using Tukey's HSD and significance level was determined when $\alpha < 0.05$.

Results and Discussion

No-choice Tests

There was no significant difference in consumption among lufenuron, noviflumuron and control baits ($F = 1.18$; df = 2, 15; $P = 0.334$) (Fig. 2). CSIs had a significant impact on termite mortality ($F = 17.53$; df = 3, 20; $P < 0.0001$). All three CSIs caused significantly higher mortality as compared to the control. When compared among the three CSIs, lufenuron caused significantly higher mortality (87%) than either diflubenzuron (55%) or noviflumuron (65%) at 6 weeks (Fig. 3).

Multi-chamber Tests

Among the 3 CSIs, bait matrix consumption was significantly different ($F = 44.54$; df = 2, 9; $P < 0.0001$). Consumption of diflubenzuron bait was significantly higher than noviflumuron bait or lufenuron bait and the consumption of noviflumuron bait was significantly higher than the lufenuron bait (Fig. 4). Similarly, wood consumption was significantly impacted by the CSI baits ($F = 247.19$; df = 3, 10; $P < 0.0001$). Wood consumption was significantly lower in all the CSI tests at 6 weeks (range: 120-333 mg) compared to the controls (4190 mg). Consumption of wood was not significantly different among the CSI baits (Fig. 5).

As in no-choice tests, termite mortality was significantly impacted by the CSI baits in multi-chamber tests ($F = 21.25$; df = 3, 10; $P < 0.0001$) and the mortality varied depending on the type of CSI. Approximately 100% mortality was observed in lufenuron tests at 6 weeks, which was significantly higher than the mortality in diflubenzuron or noviflumuron tests. There was no significant difference in mortality between diflubenzuron and noviflumuron (Fig. 6).

The results showed that the 3 CSIs tested induced significant mortality of *C. formosanus* foragers with lufenuron causing the highest percentage mortality in 6-week test. It is interesting to note that the relatively low concentration of

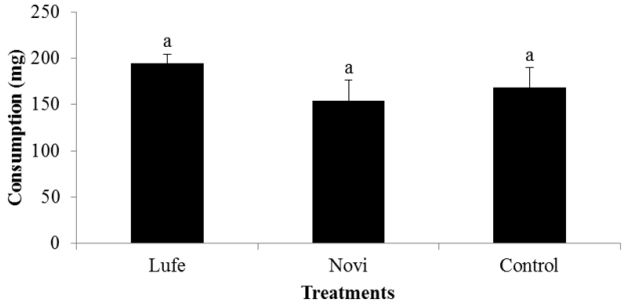

Fig. 2. Consumption (mean ± SEM) of 2 chitin synthesis inhibitor baits and control cardboard in no-choice tests at 6 weeks after exposure. Same letters above the bar indicate not significantly different from each other. Lufe = lufenuron, Novi = noviflumuron.

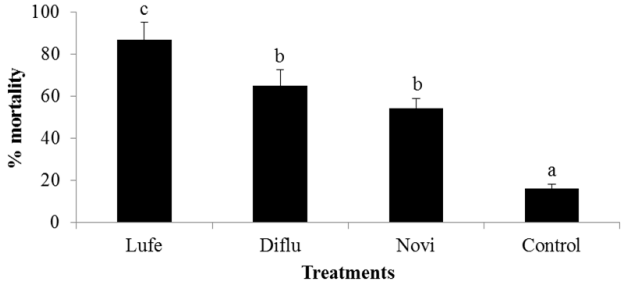

Fig. 3. Percentage mortality (mean ± SEM) of *C. formosanus* exposed to 3 different baits and control in no-choice tests at 6 weeks after exposure. Different letters above the bars indicate significantly different from each other. Lufe = lufenuron, Diflu = diflubenzuron, Novi = noviflumuron.

lufenuron (1500 ppm) inflicted a significantly higher mortality than the higher concentrations of either diflubenzuron (2500 ppm) or noviflumuron (5000 ppm) in 6 weeks. These results are consistent with the findings by Vahabzadeh et al. (2007) who reported that lufenuron caused significantly higher mortality than diflubenzuron in 6 weeks for *Reticulitermes flavipes* (Kollar). Rojas and Ramos (2004) found significantly lower queen fecundity in the lufenuron treatments but not in the diflubenzuron or hexaflumuron treatments when compared with the control. In 6 weeks, only lufenuron treatment caused ~100% mortality of *C. formosanus* individuals in multi-chamber tests suggesting a faster knock-down of lufenuron. The multi-chamber test, where only 3 of 16 chambers were provisioned with bait matrix, was particularly designed to dilute the baiting effect which may occur in the field. We observed that there was no noticeable impact on termite mortality due to the presence of other food sources. There are successful field results of lufenuron termite bait developed by Syngenta (Greensboro, NC), using a cardboard matrix containing 1500 ppm lufenuron (Lovelady et al. 2008, Haverty et al. 2010). Lovelady et al. (2008), in their review paper, reported that all of the 4 baited *R. flavipes* colony sites in Columbus, OH were eliminated of termites within 3.5-10.5 months of lufenuron bait placement. The authors further reported that the lufenuron bait successfully eliminated subterranean termites from 78 out of ~100 infested structures in a large multi-site study covering all of the major termite regions across the US (22 States). Likewise, Haverty et al. (2010) reported the cessation of termite activity within 70 days of the lufenuron bait placement in all the 6 baited colony sites containing 21 colonies of *R. hesperus* Banks in Placerville, CA. Successful elimination of an aerial colony of *R. flavipes* from a six-story apartment building has also been reported using 1500 ppm lufenuron bait (Bowen & Kard 2012). Wang et al. (2013) reported that a combination of a lower concentration (1000 ppm) of lufenuron and opportunistic pathogens such as *Pseudomonas aeruginosa* (Schroeter) Migula caused higher percentage mortality of *C. formosanus* in a relatively short period. Su and Scheffrahn (1996), however, reported only ~60% mortality of *C. formosanus* individuals in the laboratory with 4000 ppm or 8000 ppm lufenuron in 6 weeks after exposure.

Contrary to the reports that lufenuron treatment elicited feeding deterrence at >1000 ppm for *C. formosanus* and >50 ppm for *R. flavipes* (Su & Scheffrahn, 1996), the present multi-chamber tests indicate that lufenuron does not cause noticeable feeding deterrence to *C. formosanus* at 1500 ppm. Feeding deterrence was not reported by Vahabzadeh et al. (2007) at any concentration (0.1-1000 ppm) tested. In fact, the authors mentioned that lufenuron was highly acceptable to *R. flavipes* and was the most palatable of the 4 chemicals evaluated: lufenuron, diflubenzuron, hexaflumuron and triflumuron. Similarly, Lovelady et al. (2008) and Haverty et al. (2010) stated that *R. flavipes* and *R. hesperus* readily consumed the cardboard bait matrix loaded at a rate of 1500 ppm lufenuron.

Noviflumuron, a CSI which replaced hexaflumuron used in Recruit III termite bait (Sentricon System, Dow AgroSciences), is a more extensively studied chemical. Dow AgroScience scientists reported that this chemical is non-deterrent at up to 10,000 ppm on filter paper to *R. flavipes* (Karr et al., 2004) and successful results in suppressing the populations of subterranean termites by others has also been reported (Su 2005; Cabrera & Thoms, 2006; Thoms et al., 2009).

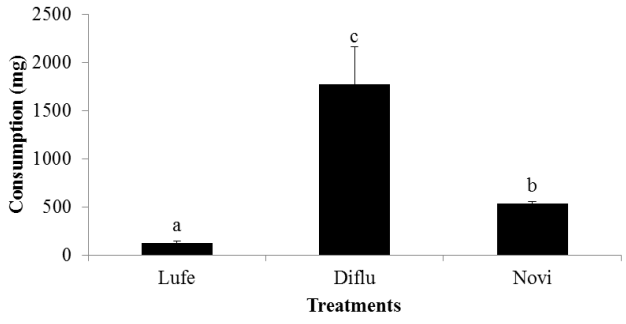

Fig. 4. Consumption (mean ± SEM) of 3 chitin synthesis inhibitor baits in multi-chamber tests at 6 weeks after exposure. Different letters above bars indicate significantly different from each other. Lufe = lufenuron, Diflu = diflubenzuron, Novi = noviflumuron.

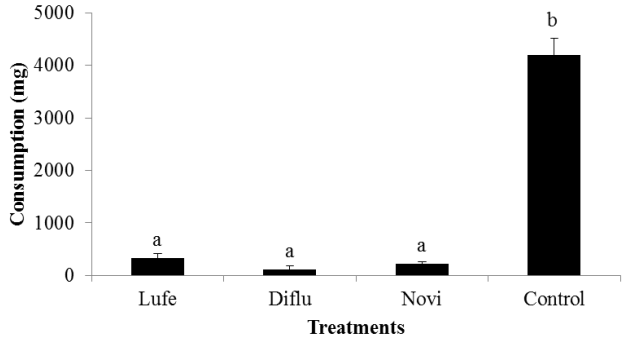

Fig. 5. Consumption (mean ± SEM) of wood in treated and control arenas in multi-chamber tests at 6 weeks after exposure. Different letters above the bars indicate significantly different from each other. Lufe = lufenuron, Diflu = diflubenzuron, Novi = noviflumuron.

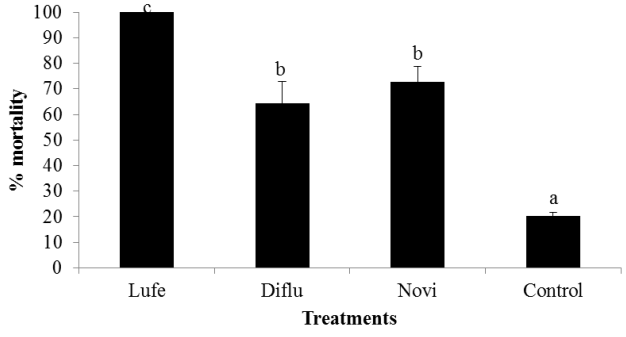

Fig. 6. Percentage mortality (mean ± SEM) of *C. formosanus* exposed to 3 different baits and control in multi-chamber tests at 6 weeks after exposure. Different letters above the bars indicate significantly different from each other. Lufe = lufenuron, Diflu = diflubenzuron, Novi = noviflumuron.

Relatively low mortality caused by diflubenzuron in both the no-choice and multi-chamber tests in 6 weeks indicated a slow action of this chemical similar to that of noviflumuron. Osbrink et al. (2011) reported that field tests of bait matrix containing 1000 ppm diflubenzuron had no noticeable impact on *C. formosanus* or *R. flavipes* populations when checked regularly for 3 years after bait placement. We are not sure, however, if the lower concentration of diflubenzuron used in their study was the reason for not achieving a significant mortality. However, other studies have also reported not so promising results with diflubenzuron (Green et al., 2008; Su & Scheffrahn; 1993). Although Rojas and Ramos (2001) reported that diflubenzuron caused 100% mortality of *C. formosanus* in 9 weeks after exposure in the laboratory tests, their field testing was less impressive (Rojas et al., 2008). Nevertheless, the present results demonstrated that lufenuron is an effective CSI against *C. formosanus* and is relatively fast acting.

Acknowledgements

We thank Louisiana Department of Agriculture and Forestry and FMC Corporation for partial funding and material support in this study. We thank two other anonymous referees for their comments and criticisms on the manuscript. This manuscript is approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 2013-234-9630.

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