Effects of Four Chitin Synthesis Inhibitors on Feeding and Mortality of the Eastern Subterranean Termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Baiting for termite population management has shown great potential. Studies have shown successful use of diflubenzuron and hexaflumuron in commercially available baiting systems. Both are chitin synthesis inhibitors in the benzoyl phenyl urea chemical group. Other pesticides in this same chemical grouping have shown activity against termites, but comparative research has been lacking. This study measured changes in feeding and mortality of the Eastern subterranean termite *Reticulitermes flavipes* when exposed to paper discs treated with one of four chitin synthesis inhibitors including; diflubenzuron, hexaflumuron, lufenuron, and triflumuron in laboratory tests. All were benzoyl phenyl ureas, and tests included five different concentrations of each. Evidence of the “jackknifed” posture characteristic of chitin synthesis inhibition during eclosion when molting, overall mean survivorship, and survivorship through time were all observed. The results varied with chemical and concentration, but all treatments caused more mortality among termite populations than untreated controls. When presented with choices, *R. flavipes* did not show definitive preferences in a majority of the tests. Lufenuron was highly acceptable to *R. flavipes*, and caused high rates of mortality at all concentrations tested. Hexaflumuron and triflumuron showed similar acceptability and mortality at only two of the concentrations tested. Although diflubezuron was acceptable to the termites, mortality at six weeks post-exposure was significantly lower than with other chemicals tested.

Keywords: Subterranean termites, *Reticulitermes flavipes* (Kollar), chitin synthesis inhibitors, insect growth regulators, termite baiting systems.

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INTRODUCTION

Because of wide distribution, Reticulitermes flavipes Kollar, the Eastern subterranean termite, causes the most economic damage of any single termite species in North America (Austin et al. 2005; Austin et al. 2006; Cornelius and Osbrink 2001; Grace et al. 1989; Heitschel et al. 2006; Jones and LaFage 1980; Szalanski et al. 2003). These termites usually need contact with the soil in order to produce a successful colony, although some, including R. flavipes, are able to form 'aerial' colonies with no soil contact if sufficient moist conditions exist (Su et al. 1989). Subterranean termites are found in virtually every state in the United States with the exception of Alaska (Jones and LaFage 1980). Estimated costs associated with termite control and repair to urban structures range from $2 billion (Gold et al. 1996) to $4 billion (NPMA 2004) in the United States, with global estimates exceeding $22 billion (Su 2002). Subterranean termites account for approximately 80% of this damage (Su 1994). Reticulitermes flavipes present a challenge to pest management professionals (PMPs) and homeowners due to cryptic lifestyles. Homeowners are often only aware of the presence of these termites in their home when the damage that they cause is significant and the structural integrity of their home is compromised. Most frequently, it is only after the reproductive caste is encountered (the alates), and then only during the swarming season (Furman 2000). However, virtually all of the damage (to structural timbers) is done by the foraging castes (Su 1994; Su and Scheffrahn 2000), principally known as workers or pseudogynes.

Since the early 1940s, perimeter treatments using persistent soil-applied pesticides have been used to protect structures throughout the world. These perimeter treatments have been effective, but when inconsistent applications are made, even slight gaps in treatment areas may allow termites access to vulnerable structures, especially when repellant termiteicides are used (Kurichan and Gold 1998). A liquid chemical barrier to termites can be effective, but concerns over environmental contamination with pesticides have raised questions about the advisability of using this approach in the long term. For this reason, pest management professionals (PMPs) have employed several pre-construction options including the use of physical barriers, wood treatments, and bait delivery systems which exploit the biological demands of termites.

The use of termite baiting systems as a means of preventing and controlling subterranean termite populations was initiated in 1967, and these technologies have been extensively researched since that time (Esenther and Gray 1968; Pawson and Gold 1996; Su and Scheffrahn 1996; Haagsma and Bean 1998; Kistner and Sbragia 2001; Ring et al. 2001; Tsunoda et al. 2001). The theory behind using baits to control a termite population is that an entire colony can be affected when a slow-acting, non-repellent chemical is distributed to the colony by social interactions with the exposed foragers (Su et al. 1987). These social interactions include trophallaxis (stomodeal and proctodeal), allogrooming, and necrophagy.

Baits using Mirex were the first to show success in the field. Mirex baits were found to effectively control Reticulitermes in Southern Mississippi, but the United States Environmental Protection Agency (EPA) banned Mirex for use in the United States (Esenther and Beal 1974, 1978). This led entomologists to look at other chemicals that would have potential for use in baits, including chitin synthesis inhibitors (CSIs) (Esenther and Beal 1978; Doppeler et al. 1981; Pawson and Gold 1996; Jones 1984; Ahmad et al. 1986; Su and Scheffrahn 1991; Su and Scheffrahn 1993; Su et al., 1997; Haagsma and Bean 1998; Grace and Su 2001; Kistner and Sbragia 2001; Su et al. 2001; Tsunoda et al. 2001).

CSIs are a category of insect growth regulators that interfere with the assembly of chitin after a molt (Pedigo 1996). The four chemicals that were used in this study belong to the chemical group called benzoylphenyl ureas (BPU) (Fig. 1). We use BPU and chitin synthesis inhibitors (CSIs) interchangeably throughout the paper, but their subtle differences should be noted, as described herein. The exact mode of action of BPU is not fully understood; however, it is known that BPU do not inhibit chitin synthetase, the enzyme controlling the last step of the process, but rather seem to interfere with the assembly of chitin chains and microfibrils. When immature stages of insects are exposed to BPU, they are incapable of completing ecdysis. As a consequence, termites die during the molting process (Graf 1999). BPU are highly lipophilic molecules, and when administered to the host, they tend to deposit in the body fat, where they are slowly released into the bloodstream. This mechanism is complemented by the fact that only a small amount of the molecule is metabolized. A high percentage of the excretion of these chemicals...
occurs in the form of the unchanged parent molecule. Because of this trait, a natural slow release occurs that prolongs the bioavailability of these compounds (Graf 1999). This slow action makes BPUs very good candidates for use in baits. Currently in the United States, hexafluron, novafluron and diflubenzuron are incorporated into commercially available baiting systems, while triflumuron is still being developed for use against termites. Lufenuron is approved for use, but efficacy testing for termite control is still underway.

Of the four benzoylphenyl ureas tested in this project (Fig 1), the first to be introduced was diflubenzuron [1-(4-cholorophenyl)-3-(2,6-diflubenzoyl) urea]. Diflubenzuron was first reported in the literature by J.J. van Daalen in 1972, and is now produced by Solvay Duphar B.V. (Tomlin 2000). It is currently the active ingredient in Exterra" Termite Interception and Baiting System marketed by Ensysyx Inc., and Advance Termite Bait System by Whitmire Micro-Gen. Doppelmeyer and Koriath (1981) reported that diflubenzuron caused mortality in R. flaveipes at between 10 and 1000 ppm with the concentration difference being insignificant. Ahmad et al. (1986) showed that diflubenzuron not only caused mortality in termites, but also reduced fecundity of female termites, and affected viability of eggs. Su and Scheffran (1993) reported that R. flaveipes showed high mortality (80%) when exposed to diflubenzuron in treated diet. While Ahmad et al. (1986) showed that termites would accept the diet at up to 1000 ppm diflubenzuron, Su and Scheffran (1993) stated that termites were deterred from feeding on diflubenzuron when concentrations were above 31.3 ppm.

Triflumuron [1-(2-cholorobenzoyl)-3-(4-trifluoromethoxyphenyl) urea] was first reported in the literature by G. Zoebelein et al. in 1979 and is produced by Bayer Environmental Science" (Tomlin 2000). Triflumuron has been used on a variety of different pests and has both larvicidal and ovicidal activity. It has shown effects on stored product beetles (Blumberg et al. 1985; Elek and Longstaff 1994), rice weevils (Smith and Grigarick 1989), flies (Broce and Gonzaga 1987; Knapp and Cilick 1988), spiny bollworm (Meisner 1987), cockroaches (Demark and Bennett 1989), scale insects (Eisa et al. 1991), and locusts (Wilps and Diop 1997). It is currently registered for use in crop protection, public health and animal health usage. At the present time, it is reportedly registered for termite control in Australia.

Hexafluron (1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy) phenyl]-3-(2,6-diflubenzoyl) urea) was first reported in the literature by R.J. Shrader et al. in 1983 and introduced by Dow Elanco (now DowAgroSciences) in 1987 (Tomlin 2000). It was the active ingredient in Sentricon® Baiting Systems.
Hexaflumuron has been shown to have effects on subterranean termites both in the laboratory and in the field (Pawson and Gold 1996; Haagsma and Bean 1998; Peters and Fitzgerald 1999; Prabhakaran 2001; Su 1994; Su et al. 1997, 2001). Hexaflumuron has also been shown to have potential to control fungus growing ants (Peppuy et al. 1998). Hexaflumuron has been reported to be a superior to lufenuron and diflubenzuron by Su and Scheffran (1993, 1996a) for use in termite baiting systems.

Lu fenuron [(RS)-1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl)phenyl)-3-(2,6-difluorobenzoyl)urea] was first reported in 1989 and introduced by Ciba-Geigy (now Syngenta Crop Protection, Inc) in 1990 (Tomlin 2000). Lufenuron has been shown to greatly reduce egg hatch in cat fleas by effecting the composition of the chorion of the egg (Meola et al. 1999).

Lufenuron is also used to control Lepidoptera and Coleoptera larvae on cotton, maize, and vegetables, as well as citrus whiteflies and rust mites on citrus fruit (Tomlin 2000). It has been registered as Zyrox for termite control.

MATERIALS AND METHODS

**Termite Collection**

All Reticulitermes flavipes used for these experiments were collected from colonies in College Station, Texas. They were collected using trays of moistened corrugated cardboard housed in polyvinylchloride (PVC) pipes that were 12.5 cm tall with a 10 cm diameter. After being sorted in the laboratory, the termites were maintained in Falcon ISO x 25mm sterile plastic petri dishes and supplied with Fisher brand tongue depressors for food and shelter. Termites used in the experiments were held in the laboratory for less than one month.

**Bait Preparation**

The chemical solutions containing the benzoylphenyl ureas were all prepared with high performance liquid chromatograph grade acetone from EM Science. A solution of each of the four BPUs at 1000 ppm were prepared, and then serial (1:10) dilutions were made to yield 100, 10, 1, and 0.1 ppm solutions, respectively. Hexaflumuron, diflubenzuron and triflumuron were all obtained from Chem Services (West Chester, PA) and were 99% pure. Lufenuron was obtained from Sigma Chemical (St. Louis, MO) and was 99.7% pure.

**Termite Feeding Substrates**

A 4 x 6 cm piece of one-ply BayWest 1002 paper was soaked in the treated solution in a 100 x 20mm Kimax dish. Non-treated controls were prepared with the same paper stock which was either soaked in pure acetone, to test for acetone effects (acetone controls), or put into the dish without any treatment (untreated controls). The treated papers were then held horizontally and sequentially rotated for 24 h at 100% RH to minimize a differential concentration gradient of the chemical from one end of the paper. The paper was then placed in the lid portion of a Fisher brand 100 x 15mm sterile plastic petri dish and placed in a 'humidity dish'. Humidity dishes were constructed to keep the environment at 100% RH to optimize feeding by the termites. The humidity chambers were made of a Falcon 150 x 25mm sterile plastic petri dish in which approximately 40 g of sand was spread evenly on the bottom and moistened thoroughly. The dishes containing the paper were then put on the sand and the lid of the larger dish was put in place. This procedure was repeated three times for each concentration of the four chemicals in evaluation.

**Termite Feeding Trials**

To initiate a feeding trial, 300 worker termites were aspirated and placed in a 100 x 15 mm sterile plastic petri dish and held in the humidity dish. A dish of 300 termites was prepared for each of the concentrations of the BPUs to be tested. Both of the dishes containing the paper and the termites were allowed to sit for 24 h prior to testing. This allowed the treated paper to moisten in the humid environment without direct application of water, making it more acceptable to feeding termites, and to prevent differential concentration gradients of the chemical to the edges of the paper. During this period, the termites were also starved. After 24 h, the termites were split into groups of 100 and placed into the dishes with the treated papers or controls. Each concentration-chemical group had termites from the same colony. The termites were allowed to feed on the paper for 5 d, after which time they were removed and placed into clean petri dishes for evaluation of survivorship. The treated paper was left in the dish, and the bottom portion of the dish was placed on it to keep the paper in place. The area of the papers remaining were then determined. Area loss measurements were done by analyzing digital images taken of each paper before and after testing, similar to the procedures
Photographs were taken with a digital camera (Nikon Coolpix 4300, Tokyo,
Japan). All images were incorporated into Adobe Photoshop (Adobe Systems
Inc. 2001) where they were converted to a black-and-white color scheme to
increase measurement accuracy. This required translating the images' color
to grayscale and setting the contrast to a maximum level. An electronic paint
tool was used to correct small blemishes that remained inside the perimeter
of the paper after the color conversion with Adobe Photoshop software
(Adobe Systems, San Jose, California). The amended images were analyzed
using SigmaScan Pro 5 (SPSS Inc. 1999) to determine the area remaining
following feeding was then subtracted
from the original known area of the paper to estimate the amount of paper
consumed by the termites.

Survivorship Experiments

After being removed from the treatment dishes, 100 termites were placed
in a 100 x 15 mm petri dish with a smaller version of the tongue depressor
structure used to house freshly collected termites in our laboratory. Su and
Scheffran (1993) reported the "symptoms of ecdysis inhibition" as cannibal-
ization of appendages and antennae by nest mates, as well as the "jackknifed"
position. In this study, both the number of surviving termites, and the number
that showed the jackknifed pose were recorded as evidence of chitin synthesis
inhibition. The numbers of surviving termites were recorded on a weekly
basis for six wk beginning at 1 wk after placement on non-treated food (12
d post-exposure). Experimental units were maintained in an Elliot-Williams
Environmental chamber held at 24 ± 2°C and 24h D:D. A Hobo Pro Series
monitor was placed in the chamber to record temperature and humidity.

Choice Tests

Choice tests were carried out in Falcon 150 x 25 mm sterile plastic petri
dishes. The cellulose sources used were discs (7 mm dia.) of Fisherbrand
Qualitative PS Filter paper cut with a standard hole punch out. Past projects
done in our laboratory showed that R. flaveipes would feed on this paper. The
disks were soaked in either 0.1, 1, 10, 100, or 1000 ppm solutions of diflubenz-
zuron, hexaflumuron, lufenuron or triflumuron, respectively. The control
disks were not treated, but were handled just as the treated discs. For each
test, two discs, each treated with a different BPU of the same concentration,
or a non-treated control were place at equal distance from the center of the
dish. The termites were given only two choices based on preliminary experi-
ments that found that termites seemed to be 'overwhelmed' when presented
more than two choices, and hovering in the center of the dish, not visiting
any of the choices. The clear dishes were labeled on the bottom so that the
circles could be identified through the translucent dish without marking on
the actual paper disc. The discs were moistened with 25 l of water and 50 R.
flaveipes workers were aspirated and placed in the center of the dish. After
giving the termites at least one hour to 'acclimate', the number of termites
at each disc were counted every hour for 6 h on the first day (24 h). On the
second day, the termites were counted once in the morning and once in
the late afternoon. The termites were counted once in the afternoon of the
third day. After the initial day, the paper circles were moistened twice a day.
Termites on the edge of the disc were counted as feeding on the paper. Every
possible pair-wise combination of the four CSs and the non-treated control
were tested. Between observations, the dishes were kept in large sealed plastic
containers with moist sand in the bottom so as to maintain 100% RH.

Voucher Information

Vouchers of the termites used in these studies were placed in the Department
of Entomology, Texas A&M University Insect Collection, in the Minnie
Belle Heep Building, College Station, TX.

RESULTS

Subterranean Termite Feeding

Tests for significant differences and interaction effects were conducted
applying an analysis of variance (ANOVA) (SPSS 2001). Significant differ-
ences among survivors were detected for day (F = 129.44, df = 5, p < 0.001),
Treatment (F = 48.907, df = 4, p < 0.001), Concentration (F = 31.815, df =
4, p < 0.001), Treatment x Concentration (F = 20.565, df = 12, p < 0.001),
and Day x Treatment (F = 3.014, df = 25, p < 0.001). Post Hoc tests for means
comparisons were performed applying Tukey's highly significant difference
(HSD) at the α = 0.05 level. Reticulitermes flaveipes fed on all of the treated
and control papers, and the amount of feeding varied depending on chemical
Table 1. Mean percent of test papers (2 cm²) treated with diflubenzuron, triflubenzuron, hexaflurazon, and lufenuron consumed by *Reticulitermes flavipes* at the concentrations indicated using digital analysis of the mean area remaining following feeding for 5 days.

<table>
<thead>
<tr>
<th>Concentration, ppm</th>
<th>Diflubenzuron</th>
<th>Triflubenzuron</th>
<th>Hexaflurazon</th>
<th>Lufenuron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Area Remaining (cm²)</td>
<td>% of paper consumed</td>
<td>Mean Area Remaining (cm²)</td>
<td>% of paper consumed</td>
</tr>
<tr>
<td>Acetone control</td>
<td>15.99±0.68 a</td>
<td>33.38</td>
<td>15.99±0.68 a</td>
<td>33.38</td>
</tr>
<tr>
<td>Paper control</td>
<td>14.52±1.49 ab</td>
<td>39.50</td>
<td>14.52±1.49 ab</td>
<td>39.50</td>
</tr>
<tr>
<td>0.1</td>
<td>11.13±2.80 c</td>
<td>52.38</td>
<td>15.34±4.00 bc</td>
<td>44.54</td>
</tr>
<tr>
<td>1</td>
<td>10.55±2.36 c</td>
<td>56.04</td>
<td>12.50±0.50 bc</td>
<td>55.94</td>
</tr>
<tr>
<td>10</td>
<td>12.29±1.11 bc</td>
<td>48.79</td>
<td>11.06±0.72 de</td>
<td>53.92</td>
</tr>
<tr>
<td>100</td>
<td>14.2±2.26 ab</td>
<td>59.92</td>
<td>10.00±0.72 de</td>
<td>53.92</td>
</tr>
<tr>
<td>1000</td>
<td>16.9±0.13 c</td>
<td>29.38</td>
<td>14.13±0.61 abc</td>
<td>41.12</td>
</tr>
</tbody>
</table>

*Means within column followed by the same letter were not significantly different from each other applying Tukey's HSD at the α = 0.05 level (SPSS, 2001)."
line of the means of survivors using linear regression and comparing means by ANOVA (SPSS 2001).

**Diflubenzuron** - The slopes of the percentage of survivors for the termites fed on diflubenzuron-treated paper were variable. The termites fed on paper treated with 10 ppm diflubenzuron solution died significantly ($p<0.001$) slower than the termites fed on the other treated papers (Fig. 2).

**Trifluriluron** - All but one concentration had a significantly different slope than the acetone control (Table 2); however, none were significantly different from the untreated control group (Fig. 2).

**Hexaflumuron** - There was no significant difference between the treated groups and those fed on untreated paper (Fig. 2). There was a significant difference ($p<0.001$) among two of the groups and the acetone control. Of the treated groups, the termites fed on paper treated with 0.1 ppm solution died the slowest.

**Table 2.** Mean steps of the percentage of survivors over a six-week period challenged to various active ingredients.

![Fig. 2](image)

**DISCUSSION**

The first objective of this study was to determine the concentration of each of the four BPs that was the most acceptable to *Reticulitermes flavipes*. Although a specific 'threshold' concentration was not observed, it is apparent that *R. flavipes* will adjust to the amount of feeding on a treated food source depending on the concentration of the treatments. There were significant differences in amount of feeding not only among the four BPs, but also among different concentrations of the same BP. Lufenuron was highly acceptable to *R. flavipes* at four of the five concentrations tested. This BPU treated disc was the most consumed by *R. flavipes* in this portion of the experiment. Su and Schefran (1996a) report lufenuron to be less acceptable to *R. flavipes* than hexaflumuron. In their study, lufenuron was found deterrent at 0.4% of the concentration at which hexaflumuron was found deterrent. In the present study, hexaflumuron at 1 and 100 ppm had similar acceptability to lufenuron, while the other four concentrations tested were generally less acceptable (to *R. flavipes*).

**Diflubenzuron** at 0.1 and 1 ppm and
triflumuron at 10 and 100 ppm also had similar acceptability to those of hexafluuron at 100 ppm for R. flavipes. These differences may be attributed to subtle differences among colony preference for either a cellulose food source or the active ingredients applied to them in the present study or in the study by Su and Schettfran (1996a). Acceptance of diflubenzuron and hexafluoron bait products from field studies have been comparable for both rhinotermiid groups in Texas (JWA, personal observation).

Feeding: Amount of Paper Consumed

The amount of treated material that R. flavipes will consume is an indication of how acceptable that treated material is to the test termite population. The results of this test indicate that R. flavipes will feed on BPU-treated material depending on the CSI and the concentration at which it was treated.

Survivorship: Mean percentage of survivors

The goal of any termite baiting treatment is to ultimately reduce the colony to a point where it is not causing significant damage to a structure or to eliminate it completely. Observing the mean percentage of survivors of R. flavipes 45 days after being exposed to four CSIs at five different concentrations allowed us to conclude that these chemicals have an adverse effect on termite survival. These results were concentration dependent and quite variable (Fig. 2).

The optimal CSI concentration incorporated into a bait would kill slowly enough for the foragers to transfer the treated material to its colony mates. It is not only important to look at overall mean percentage of survivors, but also the rate at which the termites died. A chemical that kills too quickly would not be effective as a termite bait tool; however, in these tests even low concentrations produced effects in one to two weeks. Although this should be sufficient time for termites to move the CSI into the colony, the rate of transfer can depend largely on the size, age, and nesting structure of a colony. While a slow-acting chemical is optimal, it must cause mortality within the population within a reasonable period of time in order to prevent extensive damage to the structure.

The second object was to determine if feeding on diet treated with these four BPUs would have an effect on R. flavipes. Termites exposed to diets treated with all four BPUs exhibited characteristic jackknifed postures associated with death from chitin synthesis inhibition. Many also showed different survivorship and rates of mortality from untreated control groups. Once again, there was variation among the four BPUs, and within different concentrations of the same BPUs; however, lufenuron caused the most mortality in the test period under the constraints of this experiment. Four of the five lufenuron concentrations tested caused at least 90% mortality in approximately 7 weeks of this experiment (Fig. 2). Similarly, hexafluoron elicited at least 90% mortality with three of its concentrations, followed by triflumuron with two of the concentrations tested. These results differ from Su and Schettfran’s 1996 findings that non-deterrent concentrations of lufenuron caused less than 80% mortality even after 9 weeks. The reason for these differences remains unknown. One can only speculate that subtle differences among various natural populations of termites collected from the field would likely influence their respective susceptibilities to these BPUs.

Effects of Benzoylphenyl Ureas: Jackknifing

Although a specific mode of action for chitin synthesis inhibitors is unknown, their effects can be observed. Termites fed on all four of the BPUs used in this experiment showed the jackknifed posture, which indicated that death would occur due to BPU exposure. The frequency of jackknifing, like the other observations in these experiments, was dependent on the chemical and concentration.

The third objective of this study was to determine if R. flavipes would exhibit a preference for different treatments of BPUs or untreated controls. There were some preferences evident. The majority of the choice tests showed no preference between the four BPUs represented. When compared to controls, diflubenzuron was the only BPU chosen more often than an untreated control, and that was in only one concentration tested. This would indicate that in a setting in which BPUs are incorporated into termite baits and placed around a structure, there is an equal chance that R. flavipes would visit an untreated food source as there is a chance that it would visit the bait. Even though colonies of R. flavipes can be highly localized in confined urban settings where the frequency of interaction between adjacent foraging populations would be expected high, the directed foraging of termites to bait stations can be relatively low (Vargo 2003). Many variables, such as seasonal weather patterns, predation, competition between nearby colonies, size or age of a
colony, and the number of alternative food sources available can affect the viability of a termite colony and confound the interpretation of the action of the bait (Forschler and Ryder 1996a, 1996b).

**Choice Tests**

Both choice and no-choice tests were conducted with *R. flavipes* to treated paper. When the treated materials were presented to groups of termites, the termites often showed no preference, when observing the number of termites visiting discs at set intervals. But also observed were the number of discs that were completely consumed at the end of the experiment. In only seven of the 30 choice tests between treated discs did *R. flavipes* show a preference for one treatment over another.

Around a structure *R. flavipes* will probably not be given a choice between two different commercial baits. They will however be presented with the choice of feeding on a treated bait and untreated materials, namely the structure that is supposedly being protected by the bait. In the choice tests in which the termites had a choice between a treated disc and an untreated control *R. flavipes* showed a preference in eight of 20, respectively. A breakdown of their feeding preferences follows:

**Difluubenzuron**

*Reticulitermes flavipes* consumed more of the paper treated with lower concentrations (0.1 - 10 ppm), with the 1 ppm solution being the most acceptable. At the higher concentrations, 100 and 1000 ppm, feeding was inhibited. It is apparent that there might be slight attractiveness at concentrations between 1 and 10 ppm. There was a significant difference in percentage of survivors depending on concentration. This is contrary to the findings of Doppler and Korith (1981) who observed no significant concentration related differences in production of mortality in *R. flavipes*.

*R. flavipes* fed on difluubenzuron-treated paper had some of the highest percentages of survivors in the portion of the experiment. Four of the five concentrations had similar survivorship percentages as the untreated controls. Termites feeding on 10 ppm treated discs with difluubenzuron had the highest mean percentage of survivors in the entire experiment (≈ 61%), even outnumbering the survivors in the controls. 44 and 26% for acetone and untreated paper controls, respectively. *R. flavipes* fed on difluubenzuron-treated paper had some of the slowest mortality, similar to the untreated controls; however, the effects of difluubenzuron may take longer to observe than 6 weeks as suggested by Doppler and Korith (1981). They observed 100% after 14 weeks of observation. Although jackknifed termites were observed, difluubenzuron had fewer jackknifed termites when compared to lufenuron and triflumuron at the same concentrations and time. The majority of this jackknifing occurred at the higher concentrations; however, feeding decreased above 10 ppm. This suggests a threshold of preference which is concentration-dependent.

Difluubenzuron was preferred over triflumuron at 10 ppm while the opposite was true at 100 ppm. Interestingly enough, in the 10 ppm choice test, more triflumuron discs were consumed and the opposite was true in the 100 ppm choice test. Although there was no significant difference in the number of termites visiting the discs treated with 1000 ppm dilutions, there were two discs of difluubenzuron completely consumed while no visible consumption of triflumuron discs were observed. This would imply that *R. flavipes*, given a choice, prefers difluubenzuron to triflumuron at 10, 100 and 1000 ppm. There was no difference in preference for difluubenzuron when compared to lufenuron; with about the same number of discs completely consumed of each (Figure 2).

When the termites had a choice between hexaflumuron and difluubenzuron, they showed a preference in two of the tests. At 1 ppm, the termites visited the hexaflumuron discs more often, which was supported by the fact that two of the hexaflumuron discs were completely consumed while only one difluubenzuron disc was completely consumed. At 10 ppm the difluubenzuron-treated discs were visited more often and both chemicals had one disc completely gone. Although at 100 ppm, the number of termites visiting discs of each BPU were exactly the same, at two hexaflumuron discs.

Difluubenzuron-treated discs were visited more often than the control at both 1 and 10 ppm. When the disc was treated with 1000 ppm, the termites visited the control disc more often. This was the only test in which the number of disc consumed were noticeably different, with seven difluubenzuron discs completely consumed as compared to four of the controls were completely consumed.

Difluubenzuron had a moderately positive correlation between concentration and amount of feeding ($R^2 = 0.764$). This implies a general trend for
R. flaviipes to be less attracted to treated discs as concentration or diflubenzuron increased. This could be a disadvantage to this CSI when attempting to formulate an active concentration range that would be amenable to most target termite species.

Triflumuron

When fed to R. flaviipes, triflumuron treated disc preference were different to diflubenzuron treatments. Ten and 100 ppm solutions of triflumuron were the most acceptable to R. flaviipes, while the other three concentrations of triflumuron were less attractive. Termites fed on triflumuron-treated paper exhibited variability in mean percentage of survivors (ranging from 0.50 – 37.33%). Termites fed on paper treated with 0.1 and 1000 ppm solutions of triflumuron had very low mean percentage of survivors (5.0 and 0.50%, respectively), while termites fed on 100 ppm solution had a high percentage of survivors (37.33%). It is unclear if these results simply demonstrate the lack of preference to this active ingredient-concentration in terms of feeding or possible aversion, which subsequently resulted with the higher percentage of survivors.

In the triflumuron trials, most of the concentrations showed a slope for mortality that was significantly different from controls (Table 2), indicating some level of effectiveness. Triflumuron at 0.1 ppm showed mortality, but the mean percentage of survivors at the beginning of the experiment was ~40% while the three remaining concentrations had initial survivorship ~60%. A higher initial survivorship could allow more foragers to return to the colony and potentially transfer the treated material to other termites. Conversely, a higher survivorship may reflect greater aversion to the various treatment combinations. Reticulitermes flavipes that were fed on paper treated with triflumuron exhibited the most jackknifing compared to the other three BPs in this experiment. The majority of this jackknifing was observed in termites feeding on paper treated with 1 and 10 ppm solutions. This implies that this chemical may be an effective bait active ingredient at low to moderate concentrations. R. flaviipes showed a preference for hexaflumuron paper discs at 1000 ppm when given a choice between hexaflumuron and triflumuron. It was observed that overall eight hexaflumuron discs were consumed while only one triflumuron disc was completely consumed for the same concentration (1000 ppm).

Only one choice test between triflumuron and lufenuron showed a difference in the number of termites visiting discs. At 100 ppm, the termites visited the lufenuron-treated disc more often. Seven discs of triflumuron were completely consumed at the end of the experiment while only three of lufenuron were completely consumed. Three tests were significantly different (p < 0.0001) in the choice between triflumuron and the untreated control. Triflumuron was preferred at 1 and 100 ppm while the control was more readily frequented at 10 ppm. Although inconclusive, these results suggest that triflumuron at 1 and 100 ppm could be good candidates for use in termite baiting situations around structures. Other than the strong positive correlations between mean percentage of survivors and the slope (R^2=0.97%), triflumuron showed no specific trends among the measures factors in this experiment.

Hexaflumuron

The results of the hexaflumuron trials were variable and did not show a definite pattern with relation to the amount of feeding. The most acceptable concentrations of hexaflumuron were at 1 and 100 ppm. Overall hexaflumuron caused mortality that was significantly different (p < 0.0001) than the untreated control groups at 10, 100 and 1000 ppm. Termites fed on hexaflumuron-treated paper had high initial percentages of survivors (~80%), and all but one concentration (0.1 ppm) showed a slope significantly more negative than that of the controls (Table 2). Similar to our findings Sheets et al. (2000) demonstrated that the rate of uptake, level of maximum uptake, and amount of insect-to-insect transfer were concentration dependent for hexaflumuron, and that even after 40 d exposure it was not further metabolized, thus demonstrating the ability to spread throughout a termite colony in an efficacious manner.

R. flaviipes challenged with hexaflumuron treated paper were observed to exhibit jackknife trends similar to diflubenzuron; however, there was less jackknifing than in the termites fed on triflumuron or lufenuron at the same concentrations over the same period of time (Fig. 2). The majority of the jackknifing observed in hexaflumuron baited termites were observed among the higher concentrations. There was only one choice test in which the termites showed a preference between hexaflumuron and lufenuron. A
1000 ppm, more termites were observed at the hexaAumuron-treated discs. This suggests that even at high concentrations of hexaAumuron, termites will continue to feed and consume treated cellulose materials, one possible explanation as to why it was selected as the BPU of choice among the earliest bait systems on the market. Further evaluation of this BPU with other active ingredients resulted with an equal number of discs being consumed. In only one test between hexaAumuron and the untreated control did the termites exhibit any preference. At 10 ppm, the control was preferred to the hexaAumuron-treated disc. As with triAumuron, the only strong correlation ($R^2 = 0.962$) in hexaAumuron was between the mean percent of survivors and its slope (Table 2).

**Lufenuron**

Lufenuron was the most palatable of the chemicals evaluated. Four of the six most highly consumed disc areas remaining were lufenuron treated papers. Results suggested by the quantity of paper consumed by *R. flavipes* in this portion of the experiment imply that lufenuron was a highly acceptable BPU at most concentrations tested. Termites that fed on paper treated with 1000 ppm dilutions of lufenuron had no survivors within the first two weeks of the six week observation period, while most of the other CSs and controls had surviving termites well into the six week observation period. It is unclear if rapid death was due to direct toxicity or if chitin inhibition played a more significant role, particularly in instances where internal gut morphology may have been affected. The other three concentrations of lufenuron had similar mean percentages of survivors; these being fairly low relative to other CSs. The slopes of survivors for termites that fed on lufenuron-treated paper exhibited some variability. Termites fed on 0.1, 1 and 10 ppm dilutions of lufenuron had initial survivorship ~ 90%, and had consistently negative slopes for survivorship (Table 2), indicating that most termites would likely die between 55 and 60 days, just a few days after the hexaAumuron treated termites. Termites that were fed on lufenuron-treated discs at 100 ppm had a lower initial survivorship (~ 60%), and were all dead after four weeks of observations. As stated before, most termites exposed to lufenuron at 1000 ppm were dead upon the initial observation period. *R. flavipes* that fed on paper treated with lufenuron exhibited jackknife postures, although not as frequently as with triflumuron. Like triflumuron, their jackknife postures were observed in termites which fed more readily at lower concentration which are more, and died more slowly than the two highest concentrators applied. Some death without observable jackknife postures may be due to internal damage elicited by BPUs.

Chitin synthesis inhibitors have been demonstrated to affect the production of the peritrophic membrane of locust *Locusta migratoria* (L.) (Clark et al. 1977), blow fly *Calliphora erythrocephala* Meigen (Becker 1978), and both the pupal integument of yellow mealworm, *Tenebrio molitor* L. (Soltani 1984) and its developing peritrophic membrane (Soltani 1984). It may also be that *R. flavipes* possess a less sensitive target site for lufenuron's mode of action (Bogwitz et al. 2005), resulting in more rapid toxicity and death. It is well established that different organisms possess variable proportions of cholinergic receptors, which invariably influence insects differently when challenged to different insecticides (Liu & Casida 1993) and likely with most BPUs. Even when overt jacknifing is not observed, internal damage to various sites is occurring. Morales-Ramos et al. (2006) support this notion and have demonstrated that BPUs consumed by termites damage peritrophic matrices, sometimes preventing their full development.

In only one test between lufenuron and the untreated controls did termites demonstrate a preference. At 100 ppm, the control was preferred to lufenuron-treated discs, indicating that lufenuron may not elicit attractant properties at this concentration. Lufenuron expressed a strong correlation between concentration and slope of the mean % of survivors ($R^2 = 0.899$). As treatment concentrations increased, their slopes increased (or became less negative). Lufenuron also exhibited a strong negative correlation between mean area remaining and mean % of survivors ($R^2 = 0.713$). A strong linear relationship between the amount of treated material consumed and the number of termites that died was observed, and lufenuron was the only BPU that showed the relationship between the amount of treated material consumed and mortality so clearly (see Fig. 2). Previously, lack of mortality has been attributed to the deterrence of feeding at higher concentrations for other chemicals. Interestingly, lufenuron was the only BPU that did not show a strong correlation ($R^2 = 0.195$) between the mean percent of survivors and the slope. This was due to the rapid mortality observed at higher concentra-
Whereby a greater number of termites may die before they are capable of disseminating the active ingredient throughout the colony (or before deleterious consequences should be considered.

When evaluating the impact of CSIs on the survival and egg viability of another rhinotermitid termite, Coptotermes formosanus, Rojas & Morales-Ramos (2004) observed no statistical differences in egg viability as all CSIs prevented larva from hatching, and with first instars failing to eclose: no differences were observed in the mortality of queens and kings exposed to diflubenzuron and hexaflumuron treatments and controls. Only lufenuron treatments showed significantly increased mortality rates within a month of exposure to the active ingredient. Furthermore, Rojas and Morales-Ramos (2004) also observed that the estimated number of eggs oviposited during the first 100 d was significantly lower in the lufenuron treatment group compared to hexaflumuron, diflubenzuron, and controls. They suggest that lufenuron appeared to be the most potent of the CSIs tested against primary queens and kings of C. formosanus and that hexaflumuron was the least potent, showing no difference in adult mortality between treatments and controls after 6 mo of exposure.

Based on this preliminary investigation, lufenuron, particularly at low concentrations, has great promise for termite control if incorporated into a termite bait matrix. In general, most of the benzoylphenyl azoles exhibit a lot of variability in their palatability and acceptance by R. flaveolus, but show potential for use in remedial termite baiting systems at some concentrations. Diflubenzuron, although acceptable to feeding by these termites, lagged behind the other three BPs in the level of mortality exacted to R. flaveolus under these experimental constraints. With the demonstrated success of several CSIs for remedial termite control, results of this study suggest that lufenuron should be given careful future consideration for potential evaluation as another active ingredient which may reliably control unwanted termite populations.

REFERENCES


