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Section 3 **Wood Protecting Chemicals**

Laboratory evaluation of four benzoylphenylureas against two species of *Reticulitermes* Holmgren, 1913 (Isoptera: Rhinotermitidae) from Southwest Europe

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Laboratory evaluation of four benzoylphenylureas against two species of *Reticulitermes* Holmgren, 1913 (Isoptera: Rhinotermitidae) from Southwest Europe*

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ABSTRACT
The use of benzoylphenylureas in baits for the control of termite pest is currently increasing. The chemical compounds used have been tested mainly with American termites. The effectivity against non-American termite species must be analyzed because the worldwide use of those compounds. In this paper four benzoylphenylureas (hexaflumuron, diflubenzuron, triflumuron and noviflumuron) have been tested with two European species (*Reticulitermes banyulensis* and *R. grassei*). The most effective concentrations not causing repellency were selected. The mortality rates obtained have been compared among all compounds tested. Our results show more than 95% mortality after a 6-week treatment in assays with noviflumuron, triflumuron and diflubenzurón, being more effective in the order given.

Keywords: *Reticulitermes banyulensis*, *Reticulitermes grassei*, Hexaflumuron, Diflubenzuron, Triflumuron, Noviflumuron, Baiting system.

1. INTRODUCTION

As it is known, termites represent a serious problem that has a huge economic impact resulting in the destruction of human beings (dwellings, documents, etc.) as well as agricultural cultures. In Europe, only three genera of termites have been referred: *Kalotermes* Hagen, 1853 and the introduced *Cryptotermes* Banks, 1906 (both drywood termites) and *Reticulitermes* Holmgren, 1913 a subterranean termite. In cities and villages, subterranean termites are the most destructive. The usual methods for termite control are chemical barriers, using less toxic products today; but, since the 90's, new methods with baiting systems have been developed (French J. 1994; Grace et al. 1996). The technical procedure in new baiting methods consists in offering palatable baits containing slow acting chemicals, which produce their effects after some days or weeks, resulting in most cases in the complete termite colonies elimination (Su 1994; Getty et al. 2005). Nevertheless, this method does not protect the affected area, so the reinfestations are possible (Husseneder et al. 2007). The most used commercial products as baits are the benzoylphenylureas (Tunaz 2004) which affect the moulting process of termites causing their death (they are chitin synthesis inhibitors -CSI-). Among the benzoylphenylureas, in Europe some products are used commercially as termicides: hexaflumuron (Sentritech® system) diflubenzuron (Exterra® or Termigard® systems); these products have been developed and tested with American termites and commercialized against the European *Reticulitermes* species. Noviflumuron is a new compound (developed to replace hexaflumuron in Sentricon® system in USA) which is not commercialized in Europe. Triflumuron is a benzoylphenylurea used as dust for the control of termites (Intrigue®) and tested as feeding-bait by Vahabzadeh et al. (2007).

Other benzoylphenylureas have been studied: lufenuron (Su & Scheffrahn 1996; Vahabzadeh et al. 2007), flufenoxuron (Muna 2002) or chlorfluazuron (Peters & Fitzgerald 2003), this last one approved for bait treatment in Australia (Exterra Requiem^{TM}).
In this paper the results of testing the most useful concentrations of four benzoylphenylureas against two European species of *Reticulitermes* are presented. The results will be compared with those of other authors obtained with non-European termite species.

2. MATERIALS AND METHODS

Termites

Due to the absence of significant morphological characteristics that allows to identify European species in genus *Reticulitermes* (Kutnik et al. 2004) and without a biochemical identification (cuticular hydrocarbons or DNA), the populations used for this study have been named as *Reticulitermes banyulensis* Clément, 1978 or *R. grassei* Clément, 1978, based on their geographical distribution, with separated populations and distant from distribution limits marked by Clément et al. (2001) and Kutnik et al. (op. cit) (Fig. 1).

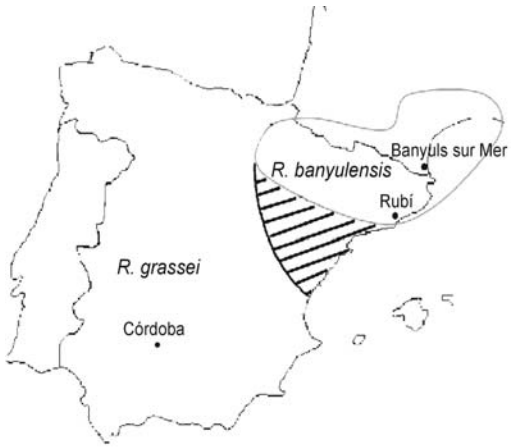

Figure 1: Sites of termites collection. See text. Map modified from Kutnik et al. 2004. Dashed area: additional territory assigned to *R. banyulensis* in Clément et al. 2001.

R. banyulensis was collected near type locality (Banyuls sur Mer, France); additional colonies were sampled in Rubí (Barcelona). Populations of studied *R. grassei* were field-collected in El Brillante (Córdoba). Termites were sampled with corrugated cardboard baits or dead wood (Banyuls sur Mer). After being removed in laboratory, termites were maintained in Petri dishes (9 cm) into a rearing room at 26 $\pm$ 1º C and 80 $\pm$ 5% RH in dark conditions. This temperature showed more efficiency in consumption and effect of tested chemicals (Van den Meiracker et al. 2005). Termites were maintained with corrugated cardboard or Whatman filter paper no. 5 until the beginning of the experiments (maximum two months).

Chemical Compounds

The benzoylphenylureas used in this study have been diflubenzuron (PS-1208; Chemical Service 99% purity), hexaflumuron (PS-2074; Chemical Service, 99% purity), triflumuron (PESTANAL Riedel-de Haën; #35029; 99’9% purity) and noviflumuron commercial recruit IV 0’5% a.i. (Dow AgroScience). All these chemicals have demonstrated effectiveness against termites of genus *Reticulitermes*.
Feeding-baits are the most common application form of these compounds (Su et al. 2004) but in a chemical concentration not causing repellency or consumption deterrence. Thus, each

compound has been prepared following similar concentrations given by other authors or by commercial products. In the case of triflumuron, the concentrations used have resulted from a previous repellency study.
Tested concentrations were prepared in acetone to obtain the following relations (weight:weight) between the active ingredient and cellulose (filter paper): hexaflumuron at 1000 ppm and 2000 ppm; diflubenzuron at 750 ppm, 1000 ppm and 2000 ppm and triflumuron at 100 ppm, 500 ppm and 1000 ppm. Noviflumuron was prepared in form of 50 $\pm$ 0.5 mg of commercial recruit IV.
Tested chemical compounds were offered to termites by impregnation of 100 µl in 50 $\pm$ 0.5 mg Whatman filter paper no. 5, uniformly distributed over each piece of paper. 100 µl of pure acetone was applied on 50 $\pm$ 0.5 mg Whatman filter paper for control. In all cases papers have been air dried (at room temperature) before being offered to termites.

Bioassays
Bioassays were made in Petri dishes (9 cm). Groups of 50 workers (middle to late instars) and one soldier, like proportions in field colonies, were placed in each dish. Moistened vermiculite was added for humidity control. Six replicas of each test were made. All assays had two papers in order to evaluate repellency, one marked and impregnated with a CSI and other for control. After this procedure was made, the termites were introduced into the Petri dishes and placed into the chamber. Two days a week all experiments were observed and termites counted individually so as to know the relationship between compound concentration and mortality. All compounds were tested in *R. banyulensis*, but a final test was made for comparing the efectiveness of noviflumuron between the former species and *R. grassei*.

Statistical analysis
Mortality results of all assays were analyzed using an analysis of variance (ANOVA) followed by Fisher's LSD separation of means. An alpha level of 0.05 was used to determine significance. All calculations were performed using the analytical software SPSS.

RESULTS

Consumption preferences
None of the compounds caused repellency at any of the concentrations tested in this study.

Mortality
After six weeks all chemicals have probed their effectiveness in relation to their concentration. Hexaflumuron achieves more than 85% mortality at 1000 and 2000 ppm (Table 1 and Fig. 2). It seems that the mortality follows the same line at both concentrations. Hexaflumuron has been the phenylurea included in Sentricon® baits; but it was recently replaced by noviflumuron. That is the reason why both compounds are compared in the same figure. Noviflumuron is clearly more effective, showing the same mortality as hexaflumuron in four weeks and achieving 100% mortality in the fifth week (Fig. 2).
Diflubenzuron (Fig. 3) showed a clear relationship between concentration and mortality; the higher the concentration, the higher the efficacy. The achieved mortality at 750 ppm was 56% , at 1000 ppm 85% and at 2000 ppm 94% (Table 1).
Triflumuron has shown very good results at 500 ppm and 1000 ppm (Table 1). This shows that future assays at higher concentrations could improve the mortality rate achieved in this study (Fig. 4). The more effective solutions produced almost 100% mortality in six weeks, showing a higher effect after the fourth week.

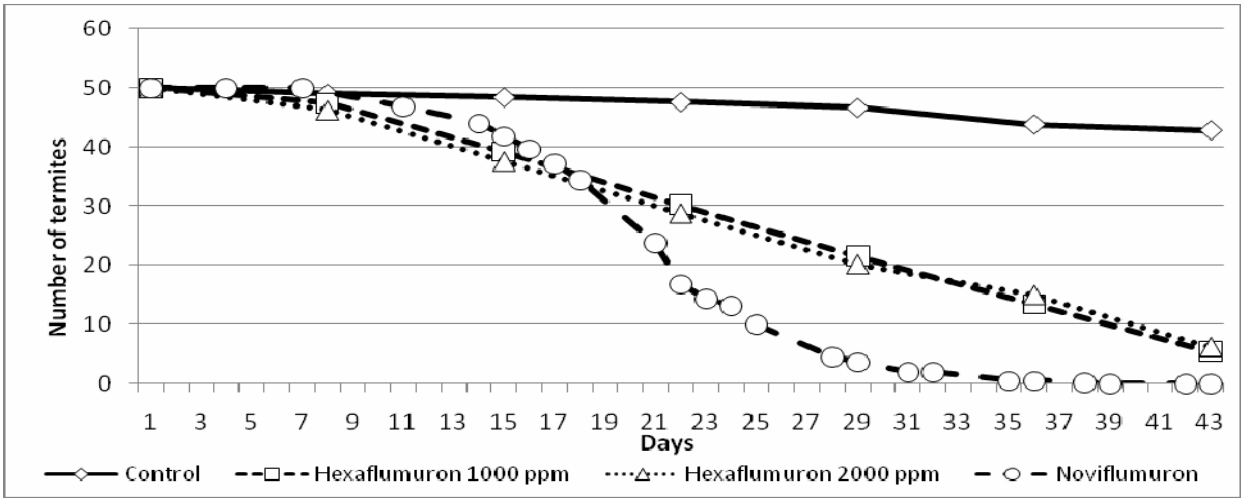

Figure 2: Mortality caused by hexaflumuron and noviflumuron against *R. banyulensis*, compared with the control group.

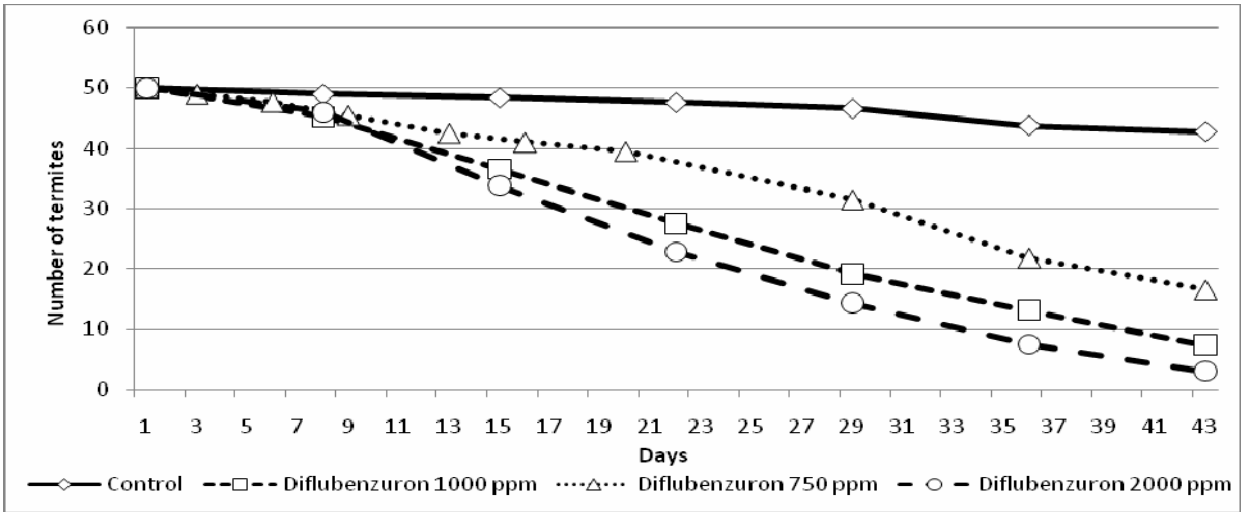

Figure 3: Mortality caused by diflubenzuron against *R. banyulensis*, compared with the control group.

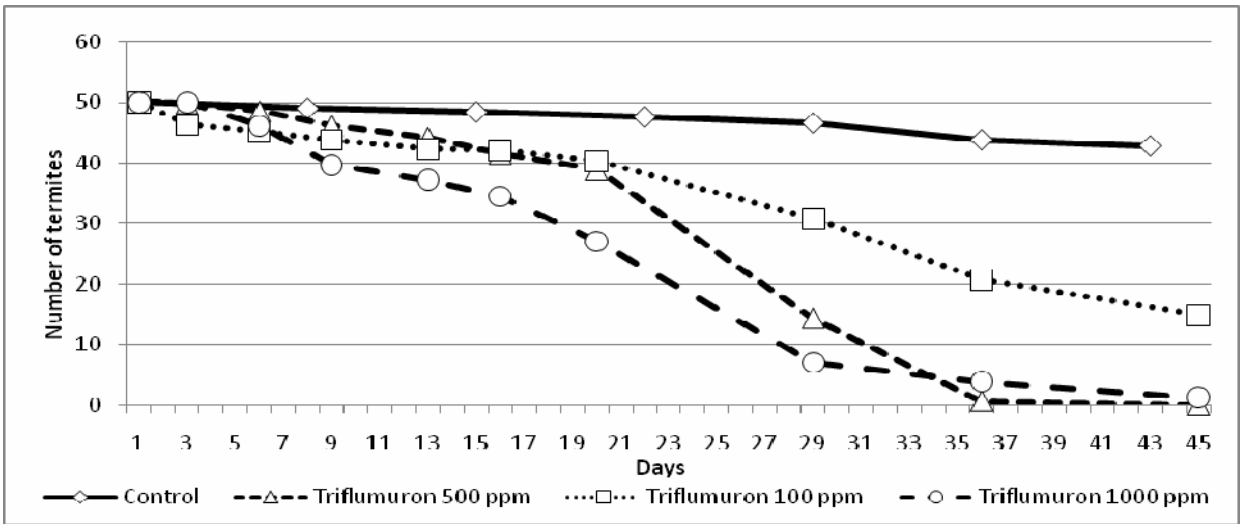

Figure 4: Mortality caused by triflumuron against *R. banyulensis*, compared with the control group.

Due to the efficacy shown by noviflumuron, this assay was repeated with the other species of *Reticulitermes* and mortality rate reached in six weeks with *R. grassei* was quite similar to that from *R. banyulensis* (Fig. 5 and Table 1). The effect of this benzoylphenylurea seems to be stronger against *R. banyulensis*, increasing the mortality rate after the second week, but with similar results in the fifth week.

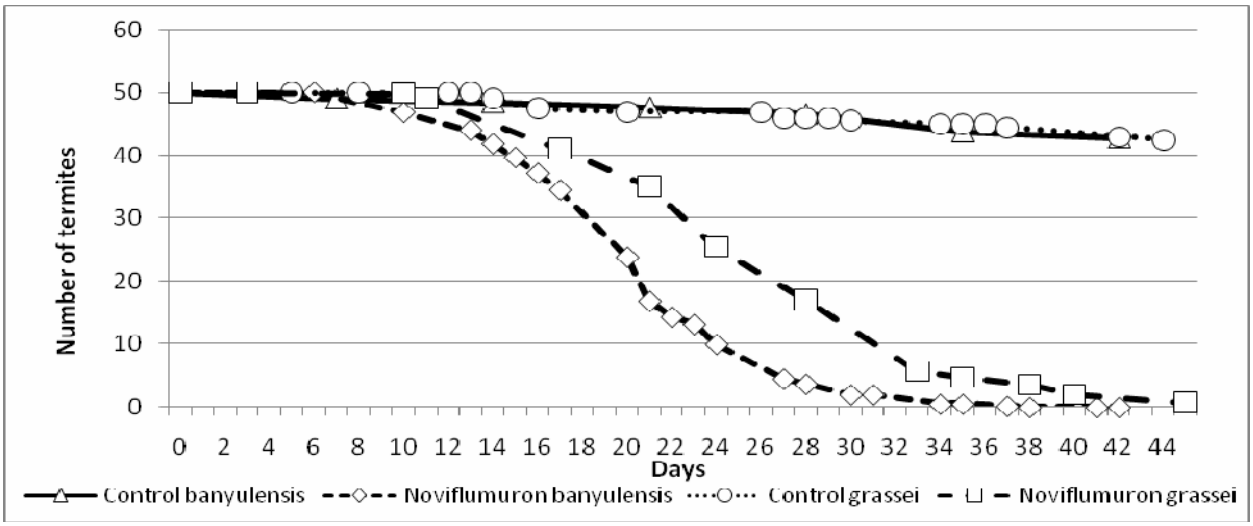

Figure 5: Mortality caused by noviflumuron against *R. banyulensis* and *R. grassei* compared with both control groups.

Taking the best results of each compound (Fig. 6), noviflumuron seems to be the best termicide in this study, which reached 100% mortality sooner than hexaflumuron and diflubenzuron. New assays with triflumuron must be made to find the repellency limit in its concentration.

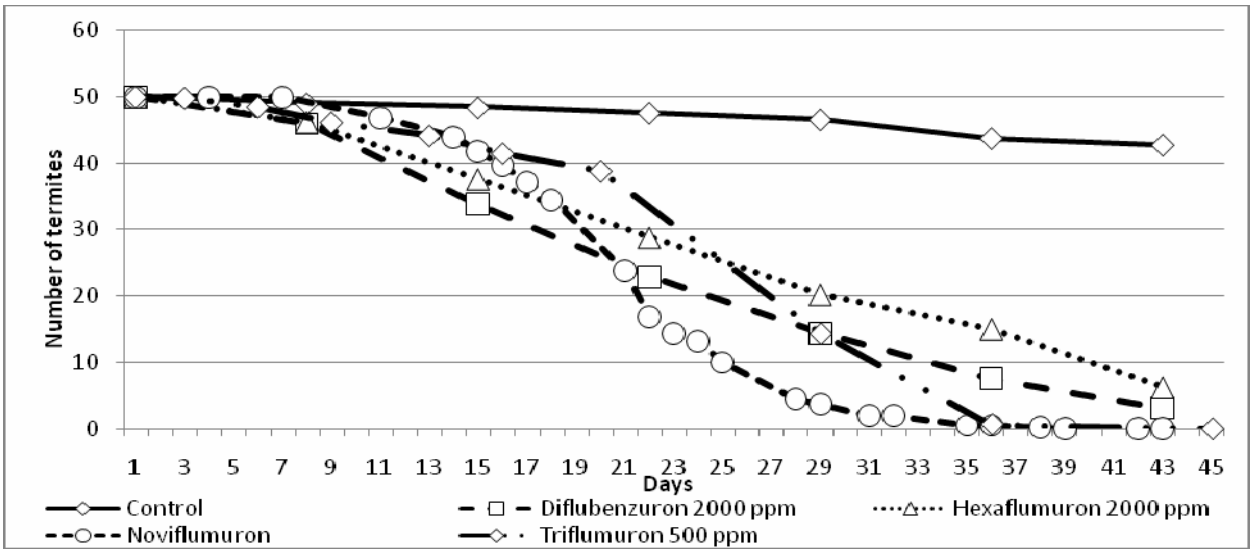

Figure 6: Comparison of the best results of each tested compound against *R. banyulensis*, related to the control group.

Statistical analysis

Using the analysis of variance (ANOVA), results of all assays can be included in different groups due to its effectiveness, and results inside the same group are followed by the same letter and do not present significant differences (Table 1). Group (a) has only control assays, with very

high survival rate. Group (b) includes diflubenzuron 750 ppm due to its relative effect as termicide with poor mortality. The little differences between mortality in some assays make difficult to include Group (c) in one group or another, as shown on Table 1; triflumuron at 100 ppm is closer to group (b) and diflubenzuron at 1000 ppm and both hexaflumuron assays are closer to group (d). The most important effect and almost 100% mortality are reached in compounds inside group (d): diflubenzuron 2000 ppm, triflumuron 500 and 1000 ppm and noviflumuron.

Table 1: Mean ± SEM mortality of *R. banyulensis* (except when indicated) after six weeks of exposure with treated filter paper.

Treatment concentration	No. Dead termites/50 workers after 6 weeks exposure
Control	8.40 ± 1.50 a
Diflubenzuron 750 ppm	28.16 ± 2.35 b
Diflubenzuron 1000 ppm	42.66 ± 1.60 cd
Diflubenzuron 2000 ppm	47.00 ± 1.50 d
Hexaflumuron 1000 ppm	44.60 ± 1.74 cd
Hexaflumuron 2000 ppm	43.80 ± 2.33 cd
Triflumuron 100 ppm	35.16 ± 3.88 bc
Triflumuron 500 ppm	50.00 ± 0.00 d
Triflumuron 1000 ppm	48.83 ± 0.83 d
Noviflumuron	50.00 ± 0.00 d
Noviflumuron (*R. grassei*)	48.71 ± 0.71 d

Means followed by the same letter were not significantly different

DISCUSSION

We will compare our results with those provided by other authors, but we must take into account the different species tested and the lack of knowledge of the moulting state of the individuals, that can provide some differences.

Hexaflumuron was the first compound widely commercialized as bait and used against American and European *Reticulitermes* termites. The a.i. concentration in recrute II is 0.5% and seems to be an effective termicide. Our mortality results at 1000 and 2000 ppm are similar to those by Su & Scheffrahn (1993) at 1000 ppm with *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar). Moreover, Rojas & Morales–Ramos (2001) achieved 100% mortality in *C. formosanus* at 250 to 1000 ppm.

In relation to diflubenzuron, Su & Scheffrahn (1993) obtained 100% mortality in Eastern subterranean termite in 9 weeks at more than 500 ppm, but the authors indicate a significant consumption deterrence at higher than 31 ppm levels. Our results are quite similar in 6 weeks and same concentrations (750 and 1000 ppm). Recently King et *al*. (2005) using also *R. flavipes*, found quite less mortality at higher concentrations (2500 and 5000 ppm weight:weight), that can be explained by a certain repellency at these compound levels.

Laboratory assays with noviflumuron were made by Karr et al. (2004), obtaining 100% mortality in 6 weeks at 10.000 ppm and the same rate in 8 weeks at 1000 ppm. Results achieved by King et *al.* (op. cit) are quite similar to ours in the same 6-week period. Field studies were carried out by Getty et *al.* (2005) and Cabrera et *al.* (2006) demonstrated the effectiveness of the compound, eliminating colonies between three months and one year.

Triflumuron has been used as dust termicide (Madden 1999) and the compound is commercialized in this way (Intrigue®). Triflumuron has recently been tested as bait

(Vahabzadeh et al. 2007) and the authors found high rate mortality, with similar results to hexaflumuron but better than diflubenzuron.
Results given in this study have shown that these compounds are really effective against both tested European termites. Among benzoylphenylureas analyzed, noviflumuron seems to be the most suitable, followed by triflumuron which needs new assays to find the repellency limit in its concentration.
Our laboratory studies suggest that the two termite species found in Spain can be suppressed with the four tested compounds (see Table 1), but the most promising termicide is noviflumuron that without repellency or distastefulness, has shown the efficiency with the two tested species of *Reticulitermes*.
Indeed, our studies are promising. However, a more precise evaluation of the concentrations is needed for further researches on (1) More laboratory studies about the concentrations studied in order to ratify our results in both species; (2) The same technique applied to a greater number of individuals and (3) field assays.

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