Proceedings of the Seventh International Conference on Urban Pests
William H Robinson and Ana Eugênia de Carvalho Campos (editors) 2011
Printed by Instituto Biológico, São Paulo, SP. Brazil.

S-METHOPRENE FORMULATIONS: LABORATORY TESTS FOR EFFICACY AGAINST BED BUGS

[1]DÁNIEL BAJOMI, [1]JÁNOS SZILÁGYI, [2]RICHARD NAYLOR AND [1]LÁSZLÓ TAKÁCS

[1]Babolna Bioenvironmental Centre Ltd., H-1107 Budapest X.,
Szállás u. 6, Hungary
[2]The University of Sheffield, Western Bank, Sheffield
S10 2TN, United Kingdom

Abstract New bed bug control products, methods and Codes of Practices have been introduced, but it is likely that until we have a novel insecticide molecule and their formulations to which the bed bugs are fully susceptible, we cannot achieve appropriate results. Following a series of individual laboratory trials, the authors have designed a novel semi-field laboratory trial method. The work introduces this new technique, and describes the benefits, results and conclusions. Presented are the short and long-term effects of combinations of S-methoprene insect growth regulator, natural pyrethrum and synergist. According to the tests performed, the combinations are active against pyrethroids- and carbamate-resistant bed bug strains, with the S-methoprene insect growth regulator interrupting the bed bug's development and reproduction.
Key Words IGR, bed bug, resistance, semi-field trial

INTRODUCTION

In Central Europe the number of bed bug occurrences has considerably increased in the last two decades. It is not only the number of bed bug infested locations that increased but also the level of infestation at these locations. The number of insect control treatments performed in Budapest by Bábolna Bio, a professional PCO company is an example. The number of cockroach and bed bug control treatments in 2000 were 720 and 10 respectively, by 2009 the number of cockroach control treatments decreased to 147 (by 79.6 %); while the number of bed bug control treatments increased to 95 per year which corresponds to a 950 % rise. Similar increase was reported in London where the number of treatments rose from 2-4 per year in 1996 to more than 140 per year in 2005 (Boase et al., 2006).

There are numerous explanations to provide for the increase of bed bug infestations: the lack of knowledge/experience; increased tourism and particularly traffic of guest workers; decrease of the number of insect control treatments by spraying as compared to gel treatments; the decrease of the types of insecticide actives used for spraying; ban of the use of organophosphates especially of the widely used DDVP; and last but not least, the worldwide resistance to pyrethroids and carbamates (Boase, 2008; Zhu et al., 2010; Romero et al., 2007a, b).

Which factor and to what extent is responsible for the settled situation, nobody knows. The lack of effective insecticides nowadays results in the necessity to perform treatments with synthetic pyrethroids at a location (flat) as many as 4-5 times before bed bugs are finally eliminated.

Objective. The authors have set up the goal to develop a new type of insecticide formulation which can be used for a long time in Europe even after the revision of biocides when the Biocidal Products Directive is completed. The Biocidal Products Directive already significantly reduced the number of active substances available for insect control. While in 2000 there were around 700 active substances used as indoor insect control products in Europe, this number – according to our current knowledge – will decrease to some 250 by 2014. Obviously, this is to further increase the risk of development of resistance among bed bugs.

It is evident that once resistance to a certain insecticide group (pyrethroids, carbamates) is developed, then it is advisable to use a group with alternative mode of action. Juvenile hormone analogues (JHAs) are such a group of actives. Before the start of the revision according to the Biocidal Products Directive, a number of active substances were used for indoor treatments in Europe; by the end of the revision in 2014, however, possibly only one or two JHA actives will remain (European Commission).

According to the above, it seemed quite obvious to investigate the efficacy and applicability of S-methoprene against bed bugs, with special regard to the fact that there had been hardly any relevant literature available.

The objective consisted in developing a formulation for use both by PCOs and – in ready to use form – amateurs which has a different mode of action: fast flushing out and killing effect, low toxicity, can be applied to various fabric (textile) surfaces and does not contain either pyrethroids or carbamates. In the theoretical formulation we contemplated to obtain the flushing out and the killing effects by using synergised natural pyrethrum while achieve the residual action with S-methoprene.

Planning of the trial. Considering that only sporadic, but no systematic data was available on the efficacy of methoprene (RS; S) against bed bugs, first we investigated on fully developed (adult) individuals, and we also examined the effect of S-methoprene applied at 16 mg a.i./m^2 concentration on the eggs of treated females. Next we investigated the effect of S-methoprene applied at different concentrations (8 mg, 16 mg, 30 mg a.i./m^2) on 5^{th} instar larvae. In the next series of trials the effect of 30 mg ai/m^2 S-methoprene was investigated on different larval stages (3^{rd}, 4^{th}, 5^{th}). Considering the worldwide resistance problem, following we wanted to find out whether the same effect can be achieved with S-methoprene against pyrethroid and carbamate resistant bed bugs as against susceptible individuals.

Following assessment of the effect of S-methoprene on susceptible laboratory-bred and field-trapped resistant bedbug strains, we wanted to know what mortality can be achieved by direct spray treatment with the insecticide composition containing 1.8 g/kg pyrethrum, 4.2 g/kg PBO and 6 g/kg MGK 264 synergist, since experience shows that the insect growth regulators alone only exert their action slowly. People with bedbug infestations expect a rapid result following treatment, in order to prevent more biting and discomfort. The addition of a flush-out and fast-acting insecticide, such as natural pyrethrins, was therefore considered for a successful solution.

Finally, we wanted to model the practical treatment by the help of a special semi-realistic arena test elaborated by the University of Sheffield. The test was performed with the Biopren 6 EC concentrate containing 60.0 g/l S-methoprene, 42.8 g/l pyrethrum and 90.5 g/l piperonyl butoxide and 143.0 g/l MGK 264 synergist dissolved in water (50 ml Biopren 6 EC in 5 litre water). In the semi-realistic arena test, first we wanted to investigate the mortality of the resistant bed bugs in the harbourage by the direct spray method. Thereafter we intended to study the residual action of the direct spray treatment over a 6-week period. In the semi-realistic arena test (mimicking the field conditions), we wanted to establish the contact action of BIOPREN 6 EC treated surfaces.

MATERIALS AND METHODS

The first three tests were performed according to the following method: technical S-methoprene a.i. was evaluated at doses of 8, 16, 24 and 30 mg ai/m^2. The S-methoprene a.i. was dissolved in acetone at appropriate concentrations. Each filter paper was then evenly treated with 1.2 ml of S-methoprene a.i. solution, applied using a pipette. Each paper was air-dried for 24 hrs before being used for testing.

A long established insecticide susceptible strain of bed bugs (*Cimex lectularius*) was used. Tests were carried out on adult male and female bed bugs, and on 1^{st}, 3^{rd}, 4^{th} and 5^{th} instar nymphs.

Three replicate batches of 10 insects of each bed bug stage were exposed to each dose of S-methoprene a.i. In addition, three batches of each stage were also confined on untreated paper as a control in every test. Each batch of insects was confined continuously on the filter paper under a Petri dish, and offered a rabbit as a blood source at weekly intervals. Tests were carried out at 26°C, 70% R.H., and 12:12 light:dark.

The following parameters were assessed weekly: Mortality up to end of test; feeding success; rate of development through the nymphal instars; morphological status of insects; egg production from adults; viability of eggs; and mortality of nymphs emerging from eggs.

To assess the effect of S-methoprene on pyrethroid and carbamate resistant bed bugs was established from a sample collected in London in 2006. This strain showed to be highly resistant to pyrethroid and carbamate insecticides. The activity of S-methoprene a.i. against this strain was compared to the standard susceptible strain. The basic test technique used was identical to that described above, i.e. exposure of the insects to filter papers treated with technical S-methoprene.

When the goal was to assess the effect of synergised pyrethrum on the susceptible and the pyrethroid and carbamate resistant bed bugs was performed with the test product containing 1.8 g/kg pyrethrin, 4.2 g/kg PBO and 6 g/kg MGK 264. A groove was cut across the surface of wooden tiles, and batches of adult bed bugs were

encouraged to seek refuge in the groove (Picture 2). The tiles with bedbugs in the groove were then directly sprayed with the synergised pyrethrins so that the above formulation has a dose of 8 mg pyrethrins, 18.7 mg PBO and 26.7 mg MGK 264 / m^2, using a calibrated trigger sprayer. No S-methoprene a.i. was used in this test. Flushing, knockdown and mortality of the bedbugs were assessed at intervals after treatment.

The control to be performed under practical conditions was modelled in the semi-realistic laboratory arena test as follows. Batches of bed bugs, of a pyrethroid resistant field strain, were encouraged to rest within artificial harbourages. Each harbourage contained 10 adults, 10 4th instar nymphs and 10 1st instar nymphs. The entrance to the harbourage was sprayed with Biopren 6 EC, resulting in the spray directly contacting some of the bugs. The harbourage together with the bed bugs it contained was then immediately placed on the floor at one end of an arena measuring c. 80 x 60 cm. At the other end of the arena (under the floor of the arena) was an electric heat source, which was turned on every night to create a warm patch (c. 37^{o}C) on the floor of the arena, to which the bedbugs were attracted. However between the harbourage and the heat source was an area of wood and textile partially treated with Biopren 6 EC. Bed bugs seeking the heat source, or returning to the harbourage, crossed the wood and textile surface, but were able to choose between treated or untreated routes. The harbourage and the wood and textile surfaces within the arena, were treated with Biopren 6 EC using a calibrated trigger sprayer. A 10 cm wide corridor across the wood and textile was left untreated, to provide bedbugs with a choice of routes (Figure 1).

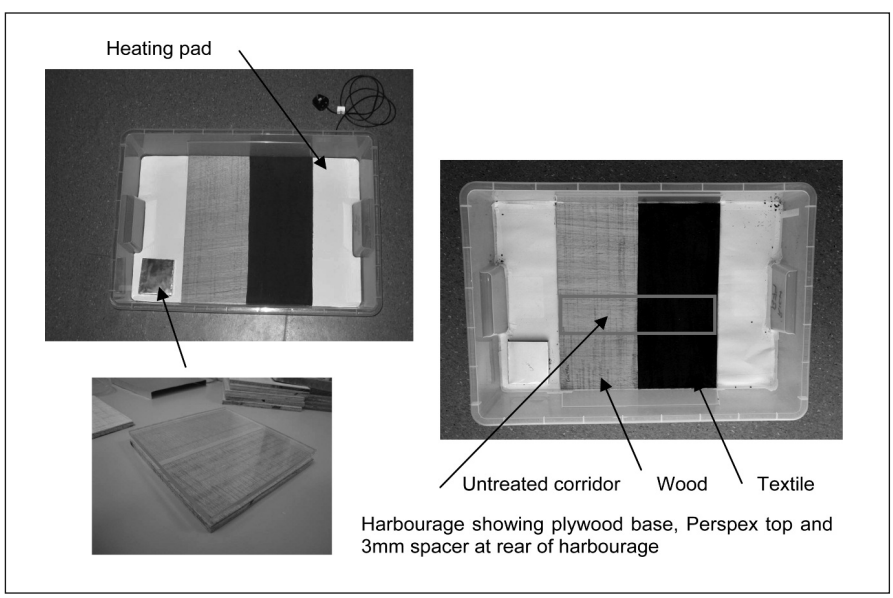

Figure 1. Semi-field test arena

Biopren 6 EC containing 60.0 g/l S-methoprene, 42.8 g/l pyrethrins and synergists was diluted in water (50 ml in 5 litre water), and the resulting emulsion applied at 50 ml/m^2, to give a deposit of 30.0 mg/m^2 S-methoprene and 21.4 mg/m^2 pyrethrins. Untreated batches of bed bugs, in untreated arenas, were also assessed to provide a control. There were three replicates of each treatment.

In addition to the main protocol outlined above, where complete mortality of the directly sprayed bed bugs was obtained within 24 hrs (i.e. in 2 of the 3 arenas), and additional test was carried out as follows. The dead bed bugs from the original test were removed, and a fresh batch of ten adult, ten 4th and ten 1st instar bed bugs were then introduced to each arena. These new bed bugs could also enter and rest in the treated harbourage, and crawl across the partially treated wood and textile, or rest on untreated surface. However they were not directly sprayed. The bedbugs were removed from the arena at weekly intervals, offered a rabbit as a blood source, and then replaced in the arena. Flushing, knockdown and mortality of the bed bugs treated in the harbourages, were initially assessed at frequent intervals. The bed bugs subsequently introduced into the treated arenas, were assessed for development, mortality, oviposition and location, over the 8 weeks following introduction.

RESULTS AND DISCUSSION

Activity of S-Methoprene on Adult Bed Bugs

Table 1. Activity of S-methoprene a.i. against adult bedbugs

Assessment		Untreated	16 mg ai/m²
% mortality of treated adults over weeks 1-5	Males	0 %	22 %
	Females	0 %	14 %
Eggs laid / live female / day		1.9	1.3 (29 % redn)
% hatch of eggs produced by treated females over weeks 1-5		99 %	94 %
Mortality up to 3rd instar of nymphs hatched from eggs laid by treated adults		14	14

Overall, treatment of the adults showed that although, there were modest impacts of S-methoprene a.i., at a dose of 16 mg ai/m² the levels of activity were unlikely to contribute much to the control of an infestation.

Activity of S-Methoprene A.I. on Bed Bug Nymphs

Results show that the rate of nymphal development was unaffected by S-methoprene a.i. Mortality of early instar nymphs did occur, but this was variable and did not show a clear dose response. However there was a very clear impact of S-methoprene on the nymphs that reach the 5th instar: Exposure to the 8 mg rate allowed the majority of 5th instar nymphs to develop to adults. Exposure to the 16 mg rate allowed a few insects to reach the adult stage, while some formed supernumerary stages. Exposure to the 30 mg rate resulted in the death of all nymphs before reaching the adult stage. As a result of these tests, 30 mg S-methoprene a.i./m² was selected as the most appropriate dose for further evaluation.

Table 2. Impact of S-methoprene a.i. at a range of doses, on 1st instar nymphs

Assessment	S-methoprene dose (mg ai/m²)			
	Untreated	8 mg	16 mg	30 mg
Weeks to reach 5th instar	5	5	5	5
% mortality up to 3rd instar	7	20	47	7
% of 5th instar nymphs moulting to supernumerary stages	0	0	50	88
% of 1st instar nymphs reaching adult stage	90	80	27	0

Activity of S-Methoprene A.I., When Exposure Commences at 3-5th Instar

Where nymphs are exposed at the 3rd or 4th instar stages, development to the adult is completely blocked. When the start of exposure is delayed until the 5th instar, some nymphs moult successfully to the adult, although these adults die prematurely. The typical impacts of S-methoprene are: reaching supernumerary stages, cuticle thinning, irregularities in cuticle deposition thus trapped in exuviae, or having feeding problems due to deformations of gut. IGRs interfere with the formation of the new cuticle or disturb metamorphosis. All these effects cause death (Figure 2).

Table 3. Impact of S-methoprene at 30 mg ai/m^2 on instars 3 to 5

Instar at start of test	% mortality by end of 5th instar	% moulting to supernumerary stage	% mortality of supernumeraries within 2wk of reaching the supernumerary stage	% of original nymphs reaching adult stage	% mortality of adults within 2 wk of reaching adult stage
3	7	93	50	0	-
4	20	80	30	0	-
5	80	7	100	13	100
Untreated (instars 3 + 4 + 5)	0	0	-	100	0

Figure 2. The exuviae remaining partially attached to the insect after moult

Activity of S-Methoprene A.I. Against Resistant Field Strain Bed Bugs

Table 4 shows that the 30 mg dose of S-methoprene a.i. completely blocked the development of nymphs to the adult stage, in both laboratory (insecticide susceptible) and field (resistant to synthetic pyrethroids and carbamates) strains. The susceptible strain showed slightly higher mortality than the field strain. This susceptible strain is more active and mobile than the field strain, and the difference in mortality may be a result of this increased activity, resulting in greater pick-up of insecticide, rather than any fundamental difference in susceptibility.

Table 4. Activity of 30 mg/m² S-methoprene a.i. on laboratory and field strain bed bugs

Treatment	Strain	% mortality over 4 weeks from start of test	% moulting to supernumerary nymphs	% reaching adult stage
Untreated	Susceptible	0	0	100
	Susceptible	0	0	100
30 mg/m^2 S-methoprene a.i.	Susceptible	70	100% of survivors	0
	Resistant	63	100% of survivors	0

Table 5. Effect of spray of susceptible and resistant bed bugs with synergised pyrethrins

Strain	% knockdown + mortality, at times shown after treatment			
	1 hr	6 hr	24 hr	96 hr
Susceptible	96	96	98	100
Resistant	14	32	44	56

Activity of Synergised Pyrethrins Against Susceptible, Pyrethroid- and Carbamate-Resistant Bed Bugs
Results show that the pyrethrin treatment is, as expected, less effective against the resistant field strain than the susceptible strain, nonetheless, significant mortality was still achieved. It was anticipated that in practice, any initial survivors of a pyrethrin + S-methoprene direct spray treatment, would still be exposed and succumb to, the S-methoprene component of the product.

Results of Direct Spray of Bed Bugs in Harbourages

The treatment had a rapid initial impact on the bedbugs, with 90 % mortality across all 3 replicates by 6hrs after treatment. The survivors were all 1st instar nymphs, in one arena, and appear to have been shielded from the spray by larger bedbugs at the moment of the treatment.

Table 6. Percentage of mortality following direct spray of pyrethroid resistant bedbugs in harbourages; mean of all 3 replicates.

Treatment	Assessment	Hours after treatment			
		0.5	**1**	**6**	**24**
Biopren 6 EC	Mortality	34	71	90	93
Untreated	Mortality	0	0	0	0

At the dose used in this study, Biopren 6 EC has direct spray activity on pyrethroid resistant bedbugs. This initial activity is likely to be due to the activity of the synergized pyrethrins, which are used in this formulation at a dose 2.6 x greater than that in the direct spray treatment with synergized pyrethrum described above. Observations on the one arena containing survivors continued up to 6 weeks after treatment.

Table 7. Longer term % response to direct spray treatment; mean of all 3 replicates

Treatment	Assessment	Weeks after treatment					
		1	**2**	**3**	**4**	**5**	**6**
Biopren 6 EC	Mortality	93	95	95	95	98	99
Untreated	Mortality	0	0	0	0	0	0

The survivors were in one arena only. Therefore counts were discontinued in the two arenas with complete mortality, but continued in the arena with survivors. Nonetheless the percentage mortality figures in Table 7 above are the mean of all three replicate arenas. These results show that even for those bedbugs that survive the initial direct spray treatment, exposure to dry deposits of Biopren 6 EC results in high mortality.

Results of Contact Action of Biopren 6 EC Treated Surfaces
In the two arenas in which complete mortality of the bed bugs was reached within 24 hrs, all the dead bed bugs were then removed from the arenas, which were then repopulated with new bed bugs. The effects of the exposure of these new bed bugs to the dry deposits of Biopren 6 EC in the arenas are summarised in Table 8.

The impact on insects that escape or survive the initial treatment, but which are subsequently exposed to a dry deposit of the product, varies according to stage. Table 8 confirms that exposure of bedbugs to the dry deposits of Biopren 6 EC causes mortality. The mortality is highest in the small nymphs (80% by 6 weeks), intermediate in the large nymphs (45% by 6 weeks) and low in the adult group (5% by 6 weeks). This delayed impact is likely to be due largely to the S-methoprene content. Interestingly, the number of eggs laid by the surviving adults

exposed to Biopren 6 EC appeared normal, with hatching success of these eggs also largely normal. The fertility of the adult bedbugs that developed from treated nymphs was checked. The sample size was small, but the majority appeared fully fertile.

Table 8. Mortality % of bedbugs subsequently introduced to arenas

Treatment		StageWeeks after introduction							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Untreated	Small nymph	0	0	0	0	0	0	-	-
	Large nymph	0	0	0	0	0	0	-	-
	All nymphs	0	0	0	0	0	0	5	5
	Adult	0	0	0	5	5	5	5	5
Biopren 6 EC	Small nymph	0	0	10	35	75	80	-	-
	Large nymph	5	25	25	25	30	45	-	-
	All nymphs	2.5	12.5	17.5	30	52.5	62.5	67.5	-
	Adult	0	0	5	5	5	5	5	-

CONCLUSIONS

The current difficulties in control of bedbug strains resistant to conventional insecticides (synthetic pyrethroids and carbamates), adds weight to the development of products with an alternative mode of action. The series of tests described here show that at a dose of 30 mg ai/m^2, S-methoprene is active against both susceptible bedbugs, and strains resistant to synthetic pyrethroids and carbamates. The activity is expressed primarily as a block to the development of nymphs into adults. Mortality arises partly from unsuccessful moults, and from ruptures of the gut and abdomen wall.

Semi-realistic tests designed to simulate practical usage, have been designed and carried out. Results show that the combination of synergised pyrethrins and S-methoprene in Biopren 6 EC provides a high initial mortality of directly treated bedbugs, and good gradual mortality of any that escape or survive the direct treatment.

This activity profile places emphasis on careful direct treatment of bed bugs in practice, with subsequent retreatment to intercept any surviving adults, and young bed bugs as they emerge from newly hatched eggs, thus Biopren 6 EC concentrate is able to suppress and control bed bug infestations. Accordingly, this insecticide combination may be a new weapon in the fight against resistant bedbugs.

ACKNOWLEDGMENTS

The authors appreciate and thank Clive Boase for his valuable suggestions and his coordination of the tests.

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