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EFFICACY OF (S)-METHOPRENE AGAINST *CIMEX LECTULARIUS* (HEMIPTERA: CIMICIDAE)

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Abstract Widespread resistance to conventional residual neurotoxic insecticides such as pyrethroids is likely to be a major factor in bed bug infestations. To identify an insecticide with an alternative mode of action that is effective against the bed bug, the juvenile hormone analogue (S)-methoprene, was evaluated. Adult and nymphal stages were exposed in the laboratory to deposits of technical (S)-methoprene at a range of doses. Results show that efficacy of the (S)-methoprene is expressed in a variety of ways including incomplete eclosion, formation of bedbugs with uneven cuticle, prolapses of the gut through the dorsal abdominal wall, and formation of supernumerary nymphs. The nymphs are most susceptible; there is least impact on adults. The overall impact of treatment is the failure of immature stages to develop to fertile adults. A dose of 30 mg/m^2 has been shown to interrupt development of susceptible bed bugs in laboratory experiments. The efficacy of (S)-methoprene was assessed against both a laboratory susceptible strain and a field strain resistant to pyrethroid and carbamate insecticides. The field strain is believed to be typical of wild bed bug populations in the United Kingdom. (S)-methoprene was as effective against the resistant strain, as it was against the susceptible strain, suggesting that there is currently little or no field resistance to this compound.
Key Words Bed bugs, juvenile hormone analogue, insect growth regulator.

INTRODUCTION

Bed bugs, *Cimex lectularius* (L.), are synanthropic blood-feeding insects. They are not recognised as disease vectors, although their biting behaviour causes distress to those bitten. Infestations cause significant problems for those managing residential premises. Since the late 1990's, the common bed bug has become much more abundant, and once again causes significant problems in both domestic and institutional accommodation (Boase, 2001). A wide range of explanations for the resurgence has been proposed, but there is increasing evidence of resistance to commonly used neurotoxic insecticides (Boase, 2008). The situation has prompted the search for alternative insecticides that may be used in conjunction with or instead of currently used compounds.

There is limited recent information available on the efficacy of insecticides against bed bugss, and very little on the efficacy of insect growth regulators. Against other insects, juvenile hormone analogues (JHA) are recognised as exerting a wide range of effects, including disruption of ecdysis, developmental anomalies in the reproductive organs, and increased melanisation (Edwards and Menn, 1981). However, whatever the mechanisms, the end result of a successful JHA treatment is the prevention of insect reproduction, resulting in a decline in the population (Retnakaran et al., 1985). Although methoprene has been successfully used for control of a range of holometabolous insects, there is limited data on hemimetabolous insects. Maiza et al. (2004) showed that methoprene is active against cockroaches, while Langley et al. (1990) reported the activity of methoprene against the hemipteran, *Rhodnius prolixus* (Stäl).

This study was intended to determine the relationship between dose and efficacy for (S)-methoprene and bed bugs, to assess how the efficacy was expressed, and to determine if resistance to conventional insecticides also conferred protection against (S)-methoprene.

MATERIALS AND METHODS

Insecticide and Insects

In these experiments 98.5 % (S)-methoprene technical (Babolna Bio) was evaluated against two strains of *Cimex lectularius*. The first, an insecticide susceptible laboratory strain, was originally sourced from Cambridge Entomology (Cambridge, United Kingdom) in 1999 and has been in laboratory culture for nearly 40 years without exposure to pesticides. The second, a field strain known to be highly resistant to both pyrethroid and carbamate insecticides, was collected from an infestation in London (United Kingdom) in 2006. Both strains are housed in the Department of Animal and Plant Science, University of Sheffield (United Kingdom) and are cultured at 26 ± 1ºC and 70 ± 5% RH, with a light cycle of L:D 12h:12h. All bugs are fed at weekly intervals on rabbit blood using the protocol of Davis (1956). All procedures involving rabbits adhered to the UK Animals (scientific procedures) Act 1986, and were covered by United Kingdom Home-Office licenses.

Evaluations

(S)-methoprene was evaluated against bed bugs in four separate experiments: 1) efficacy against insecticide susceptible adults; 2) efficacy against insecticide susceptible nymphs exposed from the 1st instar; 3) efficacy against insecticide susceptible nymphs exposed from the 3rd, 4th and 5th instars; and 4) efficacy against insecticide resistant nymphs exposed from the 4th instar. All experimental animals were 1 week post eclosion and fed immediately prior to the start of the experiment. In all experiments the appropriate quantities of (S)-methoprene were dissolved in acetone and applied uniformly to filter papers (11 cm diameter) using a pipette. A total of 1.2 ml of solution was applied per paper, which was then allowed to dry horizontally for 24 hours. Bed bugs were confined to the treated surface using an upturned Petri dish (9 cm diameter).

Experiment 1. (S)-methoprene was evaluated against the pesticide susceptible adults at 0 (control), 8 and 16 mg/m^2. Three replicate batches of 10 adults (7 females plus 3 males) were used per treatment. Each batch of insects was confined continuously on the filter paper and fed at weekly intervals. At weekly intervals, the filter paper together with attached eggs was removed from each Petri dish and immediately replaced with a clean filter paper that had also been subjected to the same treatment at Day 0. The removed filter papers were individually incubated for 2 weeks to determine the viability of the attached eggs. The papers were then discarded. The following parameters were assessed weekly: mortality of adults; number of eggs laid; hatching success of eggs; and morphology and survival of emerging nymphs.

Experiment 2. (S)-methoprene was evaluated against pesticide susceptible nymphs exposed from the first instar to deposits of 0 (control), 8, 16 and 30 mg/m^2. Three replicate batches of 10 mixed sex nymphs were used in each treatment. At weekly intervals all nymphs were offered a feed, and the following parameters were assessed: mortality; feeding success; current nymphal instar; and morphological status. Upon imaginal eclosion the bugs were removed to an untreated surface and allowed to lay eggs over the following week. A total of 50 eggs were collected from across each of the 3 replicates within the 0, 8 and 16 mg/m^2 parental treatments. No adults were produced from the 30 mg/m^2 treatment, and thus no eggs could be collected. The eggs were split into 5 replicates of 10 eggs from each parental treatment group and incubated on untreated surfaces. The resulting nymphs were fed weekly and their survival assessed over the following 3 weeks.

Experiment 3. (S)-methoprene was evaluated against pesticide susceptible nymphs, which were exposed to 30 mg/m^2 deposits from the 3rd, 4th and 5th nymphal instars. Three replicate batches of 10 nymphs of each instar were confined to the treated surfaces, while 1 batch of 10 nymphs from each instar was confined to an untreated surface as a control. At weekly intervals all nymphs were offered a feed, and the following parameters were assessed: mortality; feeding success; current nymphal instar; and morphological status.

Experiment 4. (S)-methoprene was evaluated against the pyrethroid and carbamate resistant field strain, which was exposed from the 4th nymphal instar to 0 (the control), 30 and 50 mg/m^2 deposits. For each concentration, 3 replicate batches of 10 nymphs were confined to the treated surfaces. Three replicate batches of 10 4th instar nymphs from the insecticide susceptible laboratory strain were also exposed to each concentration as a positive control. At weekly intervals all nymphs were offered a feed, and the following parameters were assessed: mortality; feeding success; current nymphal instar; and morphological status.

Data Analysis

Data on the evaluations of susceptible adults (Experiment 1) were analysed with a mixed effects model using replicate ID as the random factor (Crawley, 2002). Proportion data were arcsin transformed (Crawley, 2002). The analysis was conducted using SPSS 14.0 for Windows. Differences in survival of the nymphs from the parental treatments (Experiment 2) were evaluated with a Kruskal-Wallis test (Rank Sums) (Crawley, 2002). The analysis was conducted using SPSS 14.0 for Windows. Data in Experiment 4 were analysed using a Cox regression survival analysis. Survival time was the dependent variable, and treatment, strain and their interaction term were the independent variables. Treatment and strain were coded as indicators with the highest treatment concentration as the reference (Crawley, 2002). Analysis was conducted using R 2.6.2 for Windows (Crawley, 2005).

RESULTS

Three main types of morphological impact of (S)-methoprene on the bed bugs were observed. Some nymphs failed to ecdyse successfully and the old exuvium remained attached to either the head/thoracic region (Figure 1), or to the distal end of the abdomen. Such events were observed in all instars. When this incomplete ecdysis occurred, in some cases the nymph did not survive beyond that instar, while in others the nymph completed the next moult. In some nymphs, the gut prolapsed through the dorsal surface of the abdomen, during ecdysis (Figure 2). This event was only observed after the 3rd instar. In other individuals, the gut contents appeared to have leaked into the haemolymph, suggesting internal damage to the gut wall. This event was typically shortly followed by the death of the insect. The most significant morphological impact observed was the supernumerary nymphs that occurred at the final nymphal moult (Figure 3). These supernumerary nymphs were identified as such by the absence of external genitalia, and the continued presence of the ecdysial line. Although no measurements of length or weight were taken, these nymphs were similar in size to slightly larger than the normal adults. They also exhibited other signs of (S)-methoprene action, such as incomplete ecdysis, malfunctioning limbs, and an uneven or corrugated cuticle. Some of the supernumerary 6th instar nymphs were able to feed successfully, and moulted into 7th or even 8th instars. No supernumerary nymphs subsequently moulted into adults.

The rate of development of untreated nymphs and those exposed to (S)-methoprene were identical, up to the end of the 5th instar. At that point there was a divergence; untreated nymphs then moulted to normal adults, while those treated with the higher doses of (S)-methoprene either died or moulted into supernumerary nymphs.

Figure 1. Nymph trapped within partially ecdysed exuvium.

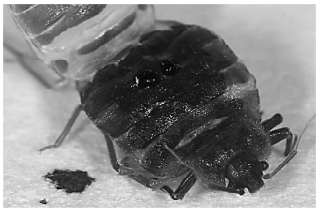

Figure 2. Nymph with mid-gut prolapsed through abdominal wall.

Figure 3. Normal adult (right), supernumerary 6th instar (left); ecdysial line just visible.

Experiment 1 — Efficacy Against Adults

The results of Experiment 1 are summarised in Tables 1-3. There was a significant effect of the 16 mg/m^2 (S)-methoprene treatment on the number of eggs laid (Mixed Effects Model: $t_6 = -2.44$, $p = 0.0499$) and the hatching success (Mixed Effects Model: $t_6 = -3.04$, $p = 0.023$) relative to the control. However, there was no significant effect of the 8 mg/m^2 treatment on the number of eggs laid (Mixed Effects Model: $t_6 = -2.18$, $p = 0.07$) or the hatching success (Mixed Effects Model: $t_6 = 1.42$, $p = 0.21$) relative to the control. There was also a significant effect of time on number of eggs laid (Mixed Effects Model: $t_6 = 4.67$, $p = 0.0001$) but not on the hatching success ($p > 0.05$), suggesting that egg laying rate but not hatching success increases

with maturity. The interaction of time by concentration did not significantly affect either number of eggs or hatching success ($p > 0.05$), indicating that the effects of the three (S)-methoprene concentrations were consistent over time. Table 3 shows that the nymphs that hatched from eggs produced by adults exposed to the (S)-methoprene, showed no treatment related mortality.

Table 1. Mortality of (S)-methoprene treated adults over weeks 1-5.

Sex	Untreated	8 mg	16 mg
Males	0%	0%	22%
Females	0%	19%	14%

Table 2. Daily egg production and viability by (S)-methoprene treated females over weeks 1-5.

Viability	Untreated	8 mg	16 mg
Eggs/live female/day	1.9	1.5	1.3
% hatch of eggs	99%	98%	94%

Table 3. Mortality up to week 3 of nymphs hatched from eggs laid by (S)-methoprene treated adults

Nymphs	Untreated	8 mg	16 mg
% mortality	4	2	14

Experiment 2 — Effect on Nymphs Exposed From 1st Instar

The bed bug mortality that occurred over the duration of this experiment did not show an entirely consistent dose response (Figure 4). The untreated bed bugs and those exposed to the 8 mg/m^2 (S)-methoprene deposits showed < 20% mortality. Increasing the dose to 16 mg/m^2 resulted in 47% mortality; however, increasing the dose to 30 mg/m^2 produced mortality of only 23%.

The formation of supernumerary nymphs showed a more consistent dose response. No supernumerary nymphs were produced at the 0 and 8 mg/m^2 rates, and all surviving nymphs moulted to normal adults. At the 16 mg/m^2 rate 50% of the surviving nymphs moulted to normal adults, while the other 50% (27% of the total batch) moulted to supernumeraries. At the 30 mg rate, no normal adults were produced, and all surviving nymphs (88%) developed into supernumeraries.

Of the eggs that were laid by the females exposed to the 0, 8 and 16 mg rates, the percentage hatch was 99, 98 and 94% respectively. Of the nymphs that hatched successfully from the 3 treatments and which were then kept on an untreated substrate, mortality over the following 3 weeks was in the range of 0 — 4%.

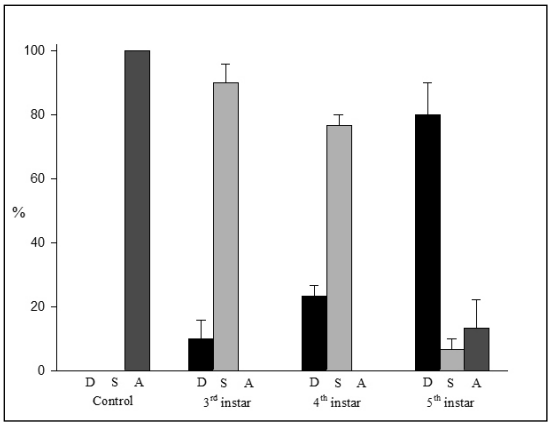

Figure 4. Bed bugs exposed from 1st instar to (S)-methoprene. Died during one of the nymphal instars (D); became supernumerary nymph (S), became adult (A). Error bars = 1 SE.

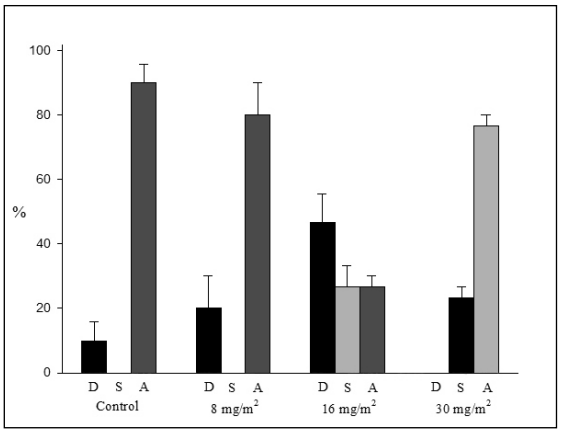

Figure 5. Bed bugs exposed to 30 mg/m^2 (S)-methoprene from 3rd, 4th and 5th instars. Control exposed to untreated surface from 3rd instar. Died during one of the nymphal instars (D), became supernumerary nymph (S), became adult (A). Error = 1 SE.

Experiment 3 — Efficacy Against Nymphs Exposed From 3rd, 4th and 5th Instars

The results of Experiment 3 are shown in Figure 5. The impact on the 3rd and 4th instar nymphs was similar, with mortality up to the end of the 5th instar of 7% and 20% respectively (similar to that seen in Experiment 2 on 1st instar nymphs), and all surviving nymphs moulting to supernumeraries. However, when 5th instar nymphs were exposed, mortality prior to the end of the 5th instar was much higher (80%), and the survivors then either moulted to supernumeraries (7% of the batch), or to adults (13% of the batch). All the adults were clearly affected by the treatment; none survived longer than 2 weeks and no eggs were produced.

Experiment 4 - Efficacy Against Field Strain Compared to Lab Strain

The laboratory strain consistently experienced slightly higher mortality than the field strain, although the differences were not significant (Cox regression survival analysis: Wald = 0.09, d.f. = 2, p = 0.95, Figure 6). In both strains, the development of normal adults was completely prevented on both the 30 and 50 mg/m^2 deposits of (S)-methoprene.

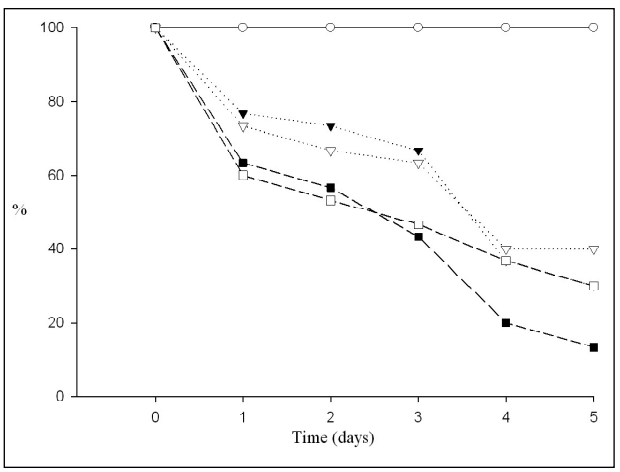

Figure 6. Percentage mortality/days of laboratory strain (open shapes) and field strain (closed shapes) when exposed to 0 mg/m^2 (circles), 30 mg/m^2 (triangles) and 50 mg/m^2 (squares) deposits of (S)-methoprene. Plots for the field strain 0 mg/m^2 treatment are obscured behind the plots for the laboratory 0 mg/m^2 treatment.

DISCUSSION

The results presented above clearly show that (S)-methoprene exhibits classical JHA activity against bed bugs. The overall impacts on mortality, morphology, ecdysis and fecundity are comparable with those seen with other hemimetabolous insects such as cockroaches (Das and Gupta, 1974; King and Bennett, 1988). In particular, at the doses evaluated here, (S)-methoprene has relatively little impact when applied to adult bugs, but has very significant impact on the development of nymphal bed bugs.

In term of practical pest control, those experiencing a bed bug infestation need both immediate relief from the biting activity, and eradication of the infestation. Results show that (S)-methoprene alone is unlikely

to deliver the former, as nymphal mortality is of slow onset, some supernumerary nymphs will continue to take blood-feeds, and adult insects are relatively unaffected. It is likely therefore that in practice, JHAs such as S-methoprene would be used in conjunction with an effective fast-acting insecticide. Such a combination would ensure rapid kill of the majority of the bed bugs, and prevent any survivors developing to fertile adults and continuing the infestation. However such an approach needs to be carefully evaluated under practical conditions. Combinations of JHAs and conventional insecticides are already used for control of other urban insects (Bennett et al., 1986).

The activity of (S)-methoprene on both susceptible insects and the strain resistant to neurotoxic insecticides is unsurprising. JHAs have a very different mode of action from sodium channel modulators such as pyrethroids, and cholinesterase inhibitors such as organophosphates and carbamates. Kramer et al. (1990) showed that if insects resistant to conventional insecticides were treated with JHAs, then their susceptibility to the conventional insecticide increased. No such effect was looked for here.

CONCLUSIONS

The data presented here indicate that exposure of bed bug nymphs to appropriate doses of (S)-methoprene will completely prevent the development of fertile adult insects. The compound is active against both the susceptible strain, and a field strain resistant to neurotoxic insecticides. Given the current increase in bed bug infestations in many countries, compounds with alternative modes of action such as (S)-methoprene, may have a potentially useful role, particularly when used in conjunction with effective fast-acting insecticides.

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