

A NEW TECHNIQUE FOR THE EVALUATION OF SPACE SPRAYS AGAINST STORED PRODUCT INSECTS

Colin P. MORGAN, Graham BARSON, Estelle WATSON, David B. PINNIGER

Ministry of Agriculture, Fisheries and Food, Central Science Laboratory,
London Road, Slough, Berks, SL3 7HJ

Howard B. DAWSON
NCH (Europe), Codnor Gate Industrial Estate, Ripley, Derbyshire, DE5 3NW.

ABSTRACT

A frequent problem encountered in the use of persistent insecticides in laboratory tests using space sprays is the contamination of test chambers with insecticide deposits. A test technique using a portable and disposable polythene cubicle (tent) has been devised which eliminates all problems of carry-over of persistent deposits. Novel water based microemulsion space spray formulations of cypermethrin and bioresmethrin have been evaluated in a series of tests against the saw-toothed grain beetle (*O. surinamensis*), rust-red flour beetle (*T. castaneum*), cigarette beetle (*L. serricorne*), spider beetle (*P. tectus*) Mediterranean flour moth (*E. kuehniella*) and the stored product psocid (*L. bostrychophilus*). Dose response data obtained from these tests shows that 40ml/tent (3.95ml/m³) cypermethrin formulation gave complete knockdown (KD) of *O. surinamensis* and *P. tectus*. There was some survival of *T. castaneum* and *L. bostrychophilus* at this dose. At 20ml (1.97ml/m³) all *L. serricorne* and *E. kuehniella* were knocked down. Tests with bioresmethrin show that all *E. kuehniella* and 99.98% *L. bostrychophilus* were knocked down at 10ml and 40ml/tent respectively. However, at the doses tested bioresmethrin was ineffective against *P. tectus* and *L. serricorne*. Recovery from KD to cypermethrin and bioresmethrin was observed for *O. surinamensis*, *T. castaneum* and in particular by *L. bostrychophilus*, where a high percentage recovered from the two lowest doses. Recovery from KD to cypermethrin was also observed for *P. tectus* and to bioresmethrin by *L. serricorne*.

INTRODUCTION

The control of many species of stored product insect pests is achieved by the use of insecticides applied as space sprays. Such treatments can be effective in killing moths and other flying insects although this method may be less successful for the control of beetle species such as *Tribolium* sp. The success of the treatments are determined by the species of insect, environmental conditions, insecticide active ingredient and the way in which it is formulated. In order to test candidate space spray formulations it is necessary to be able to control the variables which

influence insecticide performance. A number of techniques have been devised over the years and these are usually based on the release of insects into controlled environment rooms and subsequent treatment of the room air space with insecticide. Some of these procedures have been adopted as standards within the industry (for example BS4172). To prevent cross-contamination of test rooms between treatments thorough cleaning of surfaces is essential. When insecticides which rapidly degrade, such as pyrethrins are being tested, normal cleaning practices will be sufficient to ensure that surfaces are satisfactorily decontaminated. However, the testing of more persistent insecticides such as permethrin and cypermethrin has given rise to some problems with retention of deposits which then influence subsequent tests. Even relatively inert materials such as glass and stainless steel appear to retain some of the persistent pyrethroids and elaborate cleaning or lining procedures have been necessary to prevent contamination. In response to a need to test space spray formulations of stable pyrethroids at The Central Science Laboratory (CSL) Slough, a test technique based on disposable polythene cubicles (tents) has been developed. The use of fresh cubicles for each test ensures that there is no carry-over of any toxic deposits between tests.

The tests reported in this paper were carried out to evaluate novel water-based formulations of cypermethrin and bioresmethrin. These microemulsion formulations have been developed by NCH (Europe) in response to a need for effective insecticides which are not based on oils or organic solvents. In addition microemulsions appear to offer several advantages when compared with conventional emulsion concentrate type systems:

1. Superior distribution of active ingredient in spray droplets.
2. Excellent stability characteristics, both as a concentrate and after dilution to field application rate.
3. Parity in biological activity, and in a number of cases enhanced activity.
4. Droplet size and hydrophilic-lipophilic balance can be readily controlled using a selection of salts and surfactants to give optimum efficacy. (Lankford, In Press; Scrimshaw *et al.*, In Press).

Microemulsions are a clear thermodynamically stable dispersion of two immiscible liquids. The droplet size of the dispersed phase is normally in the range 10-100 nm. They can be considered as two phase systems in which the stabilising monolayer consists of a primary surfactant and a cosurfactant (a mixed film) which is probably penetrated to some extent by molecules of the oil phase.

MATERIALS AND METHODS

Insects

Laboratory strains of the following insects were used; adults of *Oryzaephilus surinamensis*, *Tribolium castaneum* (FSS II strain), *Lasioderma serricorne*, *Ptinus tectus*, and *Ephestia kuehniella* and mixed stages of *Liposcelis bostrychophilus*. The Laboratory strains have been in culture at CSL for over ten years, and during this time have not been

exposed to insecticides. *O. surinamensis* were tested when 2-4 weeks old, *T. castaneum* 3-5 weeks, *L. serricorne* 1-3 weeks, *P. tectus* 1-3 weeks, and *E. kuehniella* 1h-4 days old.

Insecticides

Novel water external microemulsion space spray formulations of 3g bioresmethrin/litre and 1g cypermethrin/litre were assessed. Each formulation was sprayed at 5, 10, 20 and 40 ml/tent (equivalent to 0.49, 0.99, 1.97 and 3.95 ml/m³). Validation of the concentration of active ingredient in each formulation was carried out using HPLC analysis after the bioassays had been completed. Both formulations were found to be within $\pm 10\%$ of the stated concentration of active ingredient.

Experimental techniques

The test environment consisted of clear polythene (175 micron) cubicles (tents) measuring 2250 x 2000 x 2250 mm high. Access into the tent for spraying and for placement, removal and assessment of insect bioassays was through a vertical 1500 mm plastic zip located centrally in one face (width 2000 mm) of the tent. A clear acetate window 250 x 250 mm was used for test observations.

An Aerograph^R airbrush was used to spray the insecticide formulations or water controls into the tents through the zip sealer which was opened at the top just sufficiently to allow the operators hand and airbrush to pass through. To improve distribution the nozzle was traversed from side to side whilst the airbrush was inclined slightly upwards from horizontal thus ensuring treatment of the upper air space. The insecticides were applied undiluted.

Insect bioassay

E. kuehniella were removed from culture to 1lb glass jars (50/jar) just prior to testing. Fifty moths were released into each tent immediately before spraying.

O. surinamensis, *P. tectus*, *T. castaneum* and *L. serricorne*, were removed from culture to 75 mm x 25 mm dia glass tubes (25/tube) the day prior to testing and left overnight without food at 20°C 50% r.h. For the assay procedure beetles were confined on Whatman No 1 filter papers (placed on a glass plate for support) within 60 mm dia stainless steel rings coated with Fluon^R (PTFE) to prevent escapes. Four batches of 25 insects for each species were used in each tent, one placed in each corner approximately 30 cm from the tent wall.

Mixed stages of *L. bostrychophilus* were removed from cultures and counted into batches of approximately 300. Each batch was placed in a 50 mm x 30 mm glass crystallising dish (the inside rim previously coated with Fluon^R to prevent escapes) the day prior to testing, and left overnight without food at 20°C 70% r.h. Four dishes were used in each tent, one placed in each corner approximately 30 cm from the tent wall.

All tests were carried out at 20°C $\pm 1^\circ\text{C}$ and 50% $\pm 5\%$ r.h. For each dose of insecticide there were four replicate tests plus one control sprayed with water only at the appropriate volume. Insects were exposed to the insecticide

spray for 1 h (which included the spraying time), after which the air within each tent was extracted for a 10 min. period. All insects were assessed for knockdown (KD) 1.25 h after spraying had commenced (insects were considered knocked down if they were unable to co-ordinate their locomotory movements and regain a normal stance). After the 1.25 h KD assessment the insects were transferred to constant temperature and humidity rooms at 20°C, 50% r.h. Further assessment counts were then carried out at 24 and 48h.

Statistical Analysis

Arcsine transformation was used on all knockdown data. Analysis of variance was then carried out on the data where appropriate to determine variations between doses, time and the dose-time interaction. Further comparisons were then made using two tailed t-tests on batched totals of 48 h counts only.

RESULTS

Analysis of variance showed that there was a significant difference in KD between doses and between count times for cypermethrin when tested against *E. kuehniella*, *O. surinamensis*, *P. tectus*, *L. bostrychophilus* and *L. serricornis* ($P < 0.01$ in all cases). There was a significant difference in KD between the count times for *T. castaneum* ($P < 0.01$). With bioresmethrin there was no difference in KD between the doses for *L. serricornis*, but there was a significant difference in KD between doses for *E. kuehniella*, *O. surinamensis* and *L. bostrychophilus* ($P < 0.01$). There was also a significant difference in KD between count times for all species tested ($P < 0.01$).

E. kuehniella

No control insects were knocked down during the tests. All insects exposed to 10 and 20 ml cypermethrin were knocked down after 24 h (Fig 1). At the lowest dose of 5 ml, 92% were knocked down after 48h (Fig 1). The results for bioresmethrin were similar to cypermethrin in that all insects exposed to 10 and 20 ml were knocked down after 24 h, and 89.5% were knocked down after 48 h at 5 ml (Fig 2). There was no observed recovery from KD with either insecticide formulation.

O. surinamensis

Control KD did not exceed 6.0%. In tents treated with 5 and 10 ml of cypermethrin <60% were knocked down after 48 h (Fig 1). However, cypermethrin was significantly more effective ($P < 0.05$) at 20 and 40 ml where 74.2% and 100% respectively were knocked down after 48 h. There was some evidence of recovery from knockdown between the 24 and 48 h assessments at 20 ml and between the 1.25h and 24 h assessments at 40 ml (Fig 1). Bioresmethrin was ineffective against adult *O. surinamensis* at 5, 10 and 20 ml, with only 21.5% knocked down after 48 h at 20 ml (Fig 2). At the highest dose of 40 ml 97.5% were knocked down after 48 h (Fig 2); a few insects having recovered from KD between the 24 and 48 h assessments.

P. tectus

Control KD did not exceed 6.0%. Knockdown (1.25 h) after exposure to cypermethrin was low ($\leq 5.0\%$) at 5, 10 and 20 ml. However, after 24 h KD had

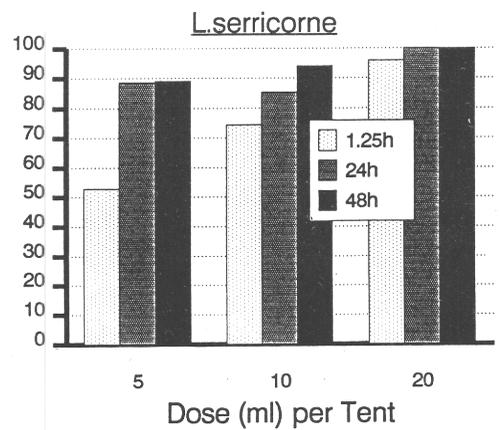
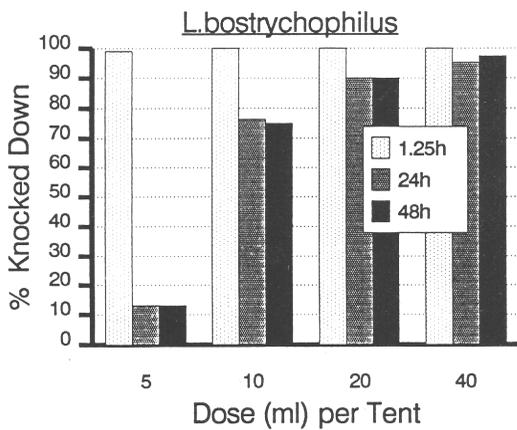
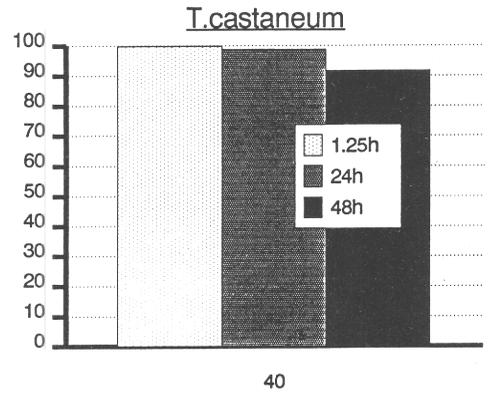
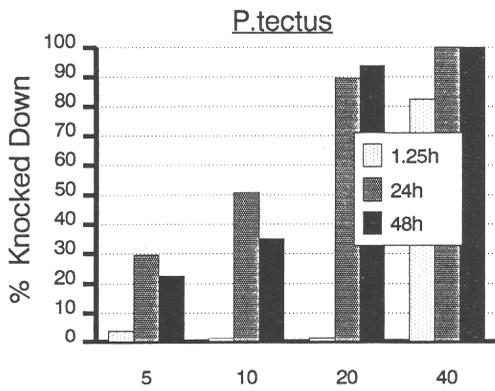
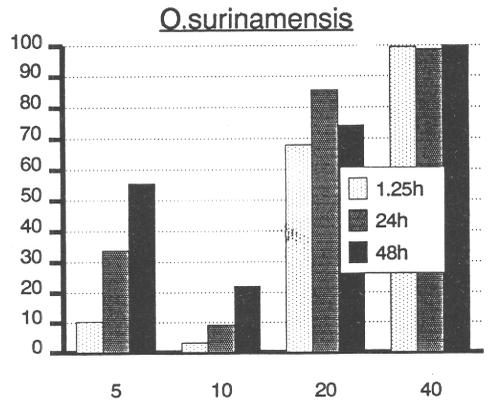
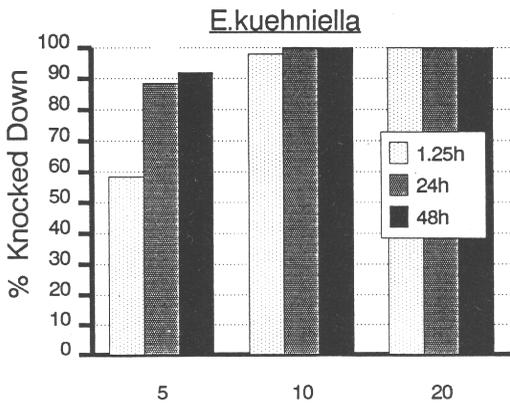


Figure 1. Knockdown (%) of six species of insect pests after exposure to cypermethrin space spray.

increased to 29.8, 46.1 and 89.3% respectively (Fig 1). After 48 h, a small proportion of insects had recovered from KD to 5 and 10 ml (Fig 1). No recovery was observed at 20 or 40 ml where 93.8% and 100% were knocked down after 48 h; the difference in KD between these two doses was significant at the $P < 0.01$ level. Bioresmethrin was only tested at 20 ml and was ineffective, giving $< 10\%$ KD after 48 h (Fig 2).

T. castaneum

No control insects were knocked down during the tests. Only one dose (40 ml) of each formulation was tested. After 1.25 h, 99.75% of insects exposed to cypermethrin were knocked down, but after 48 h, some insects had recovered giving a KD of 91.75% (Fig 1). A similar pattern was observed with bioresmethrin, where all insects were knocked down after 1.25 h, but after 48 h, 16.5% had recovered giving a KD of 83.5% (Fig 2).

L. bostrychophilus

No control insects were knocked down during the tests. A high level of KD was achieved with cypermethrin after 1.25 h at all doses tested (Fig 1). However, a proportion recovered from KD at each dose. The proportion which recovered decreased as the dose increased, from 30% recovery at 10 ml to 2.5% recovery at 40 ml (Fig 1). After 48 h, KD was high at 20 and 40 ml being 90.8 and 97.5% respectively (Fig 1). KD was significantly lower at 5 and 10 ml ($P < 0.05$) being 13.0 and 74% respectively. The KD results for bioresmethrin were similar to those for cypermethrin, with a high level of KD after 1.25 h at all doses followed by recovery of 61% at 10 ml to 20.8% recovery at 20 ml. At 40 ml recovery was minimal at 0.02% (Fig 2). Final KD assessments after 48 h were $< 30\%$ at 5 and 10 ml (Fig 2), but increased to 79% and 99.98% at 20 and 40 ml respectively.

L. serricorne

Control KD did not exceed 1.0%. Cypermethrin was effective against adult *L. serricorne* at the three doses tested (Fig 1), where 89%, 94.2% and 100% were knocked down after 48 h at 5, 10 and 20 ml respectively. There was a significant difference in KD after 48 h between the 10 and 20 ml doses ($P < 0.01$) but there was no difference between the two lowest doses of 5 ml and 10 ml. No recovery from KD was observed. Bioresmethrin was ineffective at all doses tested; only 7.2% were knocked down at the highest dose of 20 ml (Fig 2).

DISCUSSION

The microemulsion spray formulation of cypermethrin was effective in knocking down all adult *E. kuehniella* at 10ml/tent, *L. serricorne* at 20ml and *O. surinamensis* and *P. tectus* at 40ml/tent within the 48h test period. However, there were some survivors of *T. castaneum* (8.25%) and *L. bostrychophilus* (2.5%) after 48h at 40ml/tent. Bioresmethrin also knocked down all *E. kuehniella* at 10ml/tent and was more effective than cypermethrin having knocked down 99.98% *L. bostrychophilus* at the highest dose of 40ml/tent. But, bioresmethrin was ineffective against *P. tectus* and *L. serricorne* at 20 ml. There were some survivors of *O. surinamensis* (2.5%) and *T. castaneum* (16.5%) after 48 h at 40ml/tent.

Recovery from knock-down to cypermethrin and bioresmethrin was observed for *O. surinamensis*, *T. castaneum*, and in particular

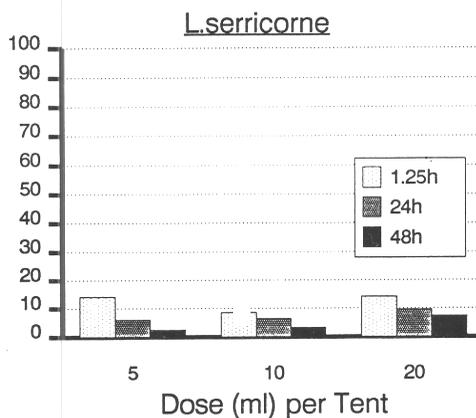
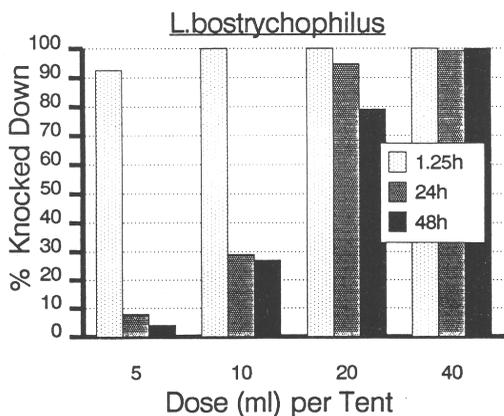
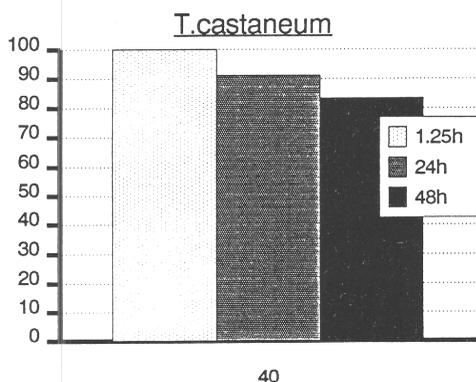
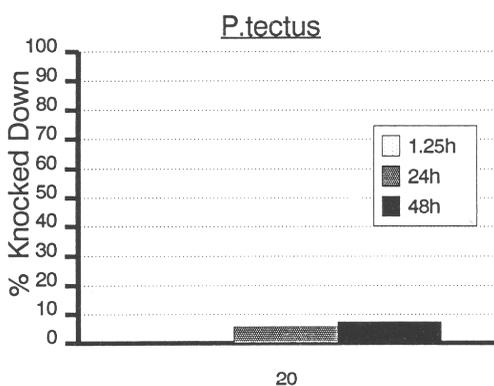
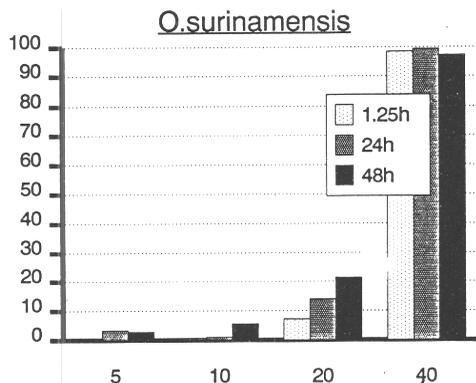
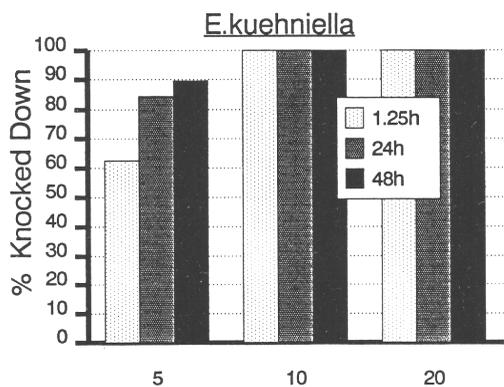


Figure 2. Knockdown (%) of six species of insect pests after exposure to bioresmethrin space spray.

L. bostrychophilus. A proportion of *L. bostrychophilus* recovered from all doses of both formulations and this was particularly evident at 5 and 10 ml/tent. Recovery from KD to cypermethrin was also observed for *P. tectus* and to bioresmethrin by *L. serricornis*. Recovery from KD by stored product insect pests exposed to synthetic pyrethroids has also been observed by Watters *et al.*, (1983) and Barson (Unpublished results).

The reliability of the results provided by this test method should enable future work to include the evaluation of insecticide efficacy and selection for insecticide resistance. Dose-response data achieved using this technique may also enable determination of resistance levels to be more representative of field application methods.

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UNE NOUVELLE TECHNIQUE D'EVALUATION DES APPLICATIONS
INSECTICIDES PAR AEROSOL DANS LES VOLUMES

C.P. MORGAN, H.B. DAWSON, G. BARSON (1),
E. WATSON ET D.B. PINNIGER

Ministry of Agriculture,
Fisheries and Food
ADAS Central Science Laboratory,
London Road Slough SL3 7HT
(1) NCH Mohawk Laboratories,
Codnor Gate Industrial Estate,
Ripley, Derbyshire, DE5 3NW

RESUME

Un problème fréquent rencontré dans l'utilisation d'insecticides persistants, dans les essais de laboratoire pour le traitement de volumes par des aérosols est la contamination des chambres d'essai par les dépôts d'insecticides. On a conçu une technique utilisant une cabine en polyéthylène jetable ce qui élimine tous les problèmes de contamination par les dépôts persistants. Une série de tests visant à évaluer l'efficacité d'aérosols de cyperméthrine et de bioresméthrine montre que des données sur l'effet dose peuvent s'obtenir pour des espèces telles *O. surinamensis*, *Lasioderma serricorne*, *Stegobium paniceum*, *Ephestia kuehniella* et *Liposcelis bostrychophilus*.