LONG TERM EFFICACY OF ACARICIDES AGAINST HOUSE DUST MITES (*DERMATOPHAGOIDES PTERONYSSINUS*) IN A SEMI-NATURAL TEST SYSTEM

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Abstract—House dust mites are together with pets the most important source of allergens in dwellings in western Europe. In a recently developed semi-natural test system, mites may be studied in an environment comparable to the dwelling situation. In this laboratory test, it should also be possible to determine the efficacy of intended measures against mites in dwellings. In the present study three acaricidal products were tested: Acarosan powder formula (Allergopharma J.Ganzer KG, Reinbek/Hamburg, Germany), Allersearch BT (Allersearch, Granville, New South Wales, Australia), and Tymasil (Gist-Brocades NV, Delft, The Netherlands). All three products have been employed in dwellings with more or less positive results. On soiled mattress models 50 house dust mites (*Dermatophagoides pteronyssinus*) were incubated and exposed to one of the three products. The surviving population after two different periods was determined by counting mite numbers following a heat-escape method.

In untreated blanks, mites developed from 0.5 mite per cm^2 to 1.6 mites per cm^2 in 8 weeks and to more than 30 mites per cm^2 in 24 weeks. After 8 weeks 99% of the mites were killed by Acarosan, while Allersearch and Tymasil proved less effective. The relative efficacy of each product after 8 week incubation correlates well with the results obtained in dwellings. However, when incubation is prolonged after treatment with Tymasil or Acarosan, the mite populations appeared to revive in considerable numbers, especially after treatment with Allersearch. From these results it appears that Tymasil is not an effective acaricide on soiled materials. While Acarosan and Allersearch BT decimate the populations in a one-hit treatment, neither is capable of keeping the number of house dust mites on mattresses low for 6 months. However, a first application of either of these products in late winter, when mite numbers are low in general, with a repeat after 2 or 3 months, should be sufficient to keep mite numbers low for the whole mite season.

INTRODUCTION

The most common mite in dwellings in Western Europe is the European house dust mite *Dermatophagoides pteronyssinus*. The favourite niches of this mite are the lower soil layers of textiles like furniture, mattresses, carpets and stuffed toys. The bed is one of the main niches. The number of mites in mattresses may amount to several thousands per m^2 [Abbott *et al.*, 1981; Carswell *et al.*, 1982].

The chemical extermination of mites in dwellings should not endanger the well-being of the dwellers. The use of acaricides in dwellings, and also of allergen removing or destructing agents, is therefore restricted to relatively inoffensive agents. The efficacy of these products may be tested in several ways. In direct-effect tests, a limited number of mites in a container is exposed directly to a specific concentration and formula of the agent; In an 'in-vivo' study, the number of living mites of one or more objects in a set of dwellings is determined before and after treatment with the agent to be tested. Both kinds of tests have draw-backs. The direct laboratory test has excellent reproducibility, but a low comparability with the 'in-vivo' circumstances (low validity). Efficacy tests in dwellings depend largely on the specific houses, on indoor and outdoor climate, on season, and on number and behaviour of inhabitants. In addition, they are time consuming and expensive.

In a third type of test that was developed by our task group, mites are installed in a reproducible laboratory simulation of a dwelling component [Schober *et al.*, 1992]. Development of mites may be determined after a selected period. It is expected that effects of acaricidal treatment or other measures within this semi-natural environment will relate more closely to the actual impact in situ. In the present study two acaricidal formulas where tested: Allersearch BT, containing a benzyl-tannate complex and alcohol, and Tymasil, containing 3.33% (w/v) natamycin and 0.02% (w/v) benzalkonium chloride. In all experiments untreated mattress models were used as a negative control. Acarosan powder, containing 5% (w/w) benzyl benzoate, is an acaricidal formula for carpet treatment which proved to be very successful in previous semi-natural tests on 4 different

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types of surfaces, including mattress models [Schober *et al.*, 1992]. It is used here as a positive control. All three acaricidal products have been employed in dwellings with more or less positive results [Elixmann *et al.*, 1988; Colloff *et al.*, 1989; Morrow Brown & Merrett, 1991; Tovey *et al.*, 1992].

METHODS AND MATERIALS

In this study the efficacy of the three products is compared in a defined semi-natural environment. 10 × x10 cm pieces of mattress models, 10 mm thick, were manufactured from two layers of polyurethane foam, 4 mm thick, between two layers of an acetate/viscose mattress tick, stitched with zigzag stitches along the edges and straight stitches across the surface. The models were soiled with 1 g artificial dust, consisting of 50% mineral powders, as used in carpet industry for cleaning tests, and 50% organic material, i.e. 1 part milled yeast and 3 parts dander. Thus, the artificial dust has a texture and chemical composition as might be found in floor or mattress dust [Schober, 1991]. The test pieces are laid in Petri dishes, that are subsequently covered with a poplin (batiste) fabric. Mites are kept inside by applying a sticky substance (Tangle Trap, Tanglefoot Cy., Michigan) to the upper edges of the dish. The test environment is placed into airtight polythene bags (Polypax, Baarn, the Netherlands, air exchange 1 g/m²/24hrs). Relative humidity inside is constant, due to a saturated salt solution in water, and a moisture buffering cardboard. The bags are placed in a thermostated room. Temperature and humidity are checked by a hair hygrometer and a hygroscope (Rotronic AG, Zurich, Switzerland) placed in one of the bags. Prior to incubation of organisms, the mattress pieces are acclimated for 10 to 14 days to 75 ± 2 % relative humidity at 20 ± 1 °C, using a saturated ammonium sulphate solution. For each piece of mattress, 50 house dust mites (*Dermatophagoides pteronyssinus*) were randomly picked one by one from a developing culture, raised on the artificial dust. After 3 days, the mattress pieces were treated with either of the products. 0.2 ± 0.03 ml of Allersearch or 3 × 87 ± 11 mg of Tymasil (with two-week intervals) were sprayed on the pieces. 0.63 ± 0.01 g of Acarosan was applied with a spatula. The population development was continued for 8 weeks after first application, which is approximately two life cycles for these mites. Dishes were ventilated every two weeks to avoid carbon dioxide accumulation. All tests were run in duplicate and in two series, separated in time and space. Blanks were added for the determination of undisturbed population development. Additional incubations of mites on mattresses treated with Allersearch and Acarosan were allowed to develop for 24 weeks. The surviving populations after the two different periods were determined by counting mite numbers following a heat-escape treatment [Bischoff *et al.*, 1986]. The significance of results was determined with the Mann-Whitney U test. Limit of confidence was set at 0.05.

RESULTS

In untreated blanks, populations developed up to 600 mites per model in 8 weeks. The geometric mean of 160 mites per model equals 1.6 mites per cm². Distribution of mite numbers was not gaussian, as expected. The number of mites recovered from the treated pieces indicate that after 8 weeks most mites are killed in presence of Acarosan, while Allersearch and especially Tymasil appear less effective (figure 1).

Reduction of growth was calculated for each test as:

$$R_G = \left(1 - \frac{N_{treated}}{N_{no\ treatment}}\right) . 100\%$$

with $N_{treated}$ = number of surviving mites on treated mattresses, and $N_{no\ treatment}$ = geometric mean of untreated duplicates in the same test series. Mean reductions were 99% due to Acarosan (range 97% to 100%, n = 12), 85% due to Allersearch (range −24% to 97%, n = 4), and Tymasil scored 40% reduction compared to the untreated blanks (range 29% to 49%, n = 4). The rate of development of mites treated with Tymasil did not differ significantly from the undisturbed development (Mann-Whitney, $p > 0.05$). All other comparisons between treatments showed a significant difference.

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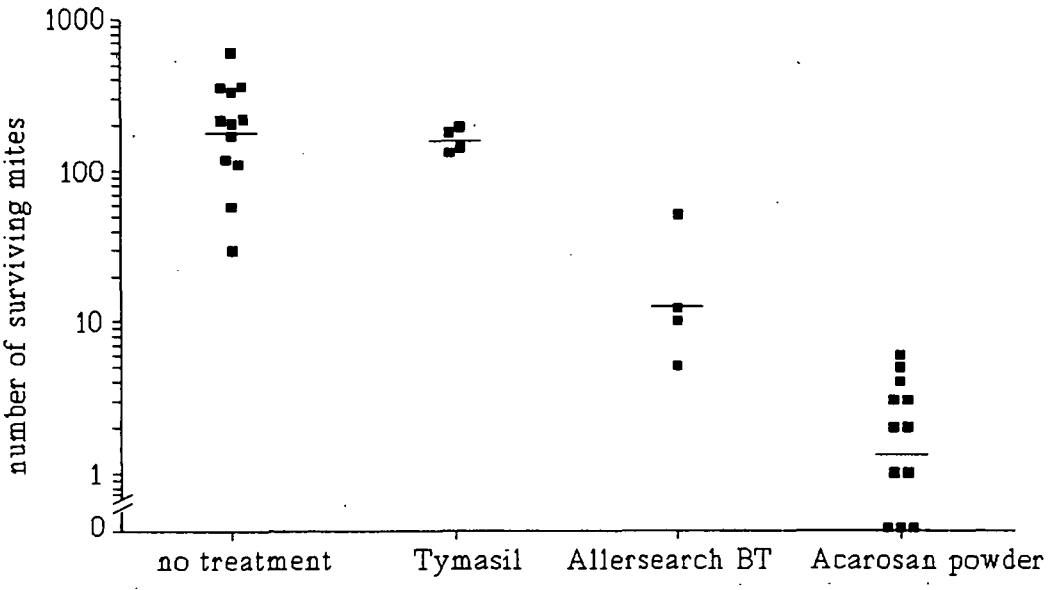

Fig. 1. Numbers of living mites *D.pteronyssinus* per test, 8 weeks after acaricidal treatment. Temperature was 20± 1°C, relative humidity was 75± 2 %, starting number of mites was 50. Horizontal bars indicate geometric mean.

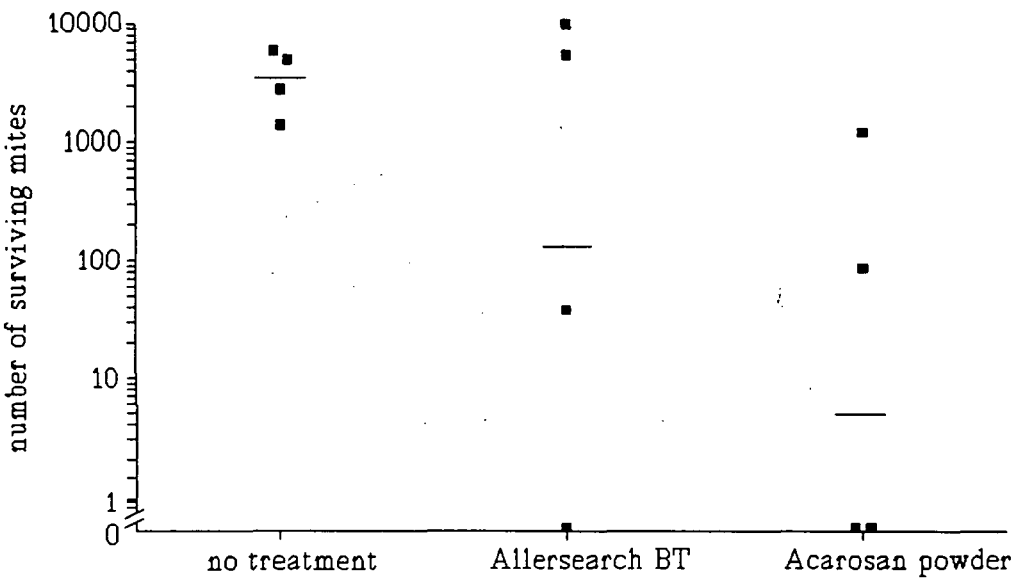

Fig. 2. Numbers of living mites *D.pteronyssinus* per test, 24 weeks after acaricidal treatment. Temperature was 20± 1°C, relative humidity was 75± 2 %, starting number of mites was 50. Horizontal bars indicate geometric mean.

Surprisingly, when incubation is prolonged, in some tests the mite population seems to revive in considerable numbers. This prolonged test was only executed with the two more effective products on new mattress models. On blank mattress models, populations increased to a geometric mean of 33 mites per cm^2 in 24 weeks (figure 2). Reduction of mite populations on treated models compared to the non-treated blank was now 24% and 78% for Allersearch and Acarosan respectively. Statistical comparison of groups showed however no significant differences, except for the comparison of undisturbed mite populations with Acarosan ($0.01 < p < 0.05$).

DISCUSSION

Acarosan powder is a benzyl benzoate formula, including allergen removing components, for carpet treatment. In a semi-natural test it was found to be the most effective acaricide, in addition to liquid nitrogen [Schober, 1992]. It was used in present study as a positive control. Indeed, 8 weeks after treatment a median value of 1.1 live mites was found, where controls scored a median of 160 mites.

Natamycin is used in drugs and alimentary products as a broad spectrum fungicide, usually effective in a 0.1–1% concentration [Kucers & MckBennett, 1987]. In agriculture it is used against plant mites. In addition to fungicidal activity, natamycin is also a mite ovicide [St.Georges-Gridelet, 1987]. Schober [Schober *et al.*, 1987] found 3% natamycin to induce no significant acaricidal effects on 10 mites, soaked in this solution. However, a 10% solution added to nutrients reduced the number of mites to less than 10% of the undisturbed population after 8 weeks. This seems to indicates that indeed the acaricidal mechanism of Natamycin is a nutrient defect: A suppression of fungi that predigest essential lipid components for mites. In the present study no significant reduction by Tymasil was discovered. Tymasil is apparently not an effective acaricide on soiled materials. Bronswijk [*et al.*, 1987] detected some hampering of mite population growth after Tymasil treatment. Colloff [*et al.*, 1989] found in a controlled clinical trial less effect of natamycin than of regular vacuum cleaning, which corroborates present findings.

It appeared difficult to obtain the precise names and concentrations of the active ingredients of Allersearch BT. It contains according to the manufacturer a 'benzyltannate complex' in an alcohol base; Allersearch DMS, which has been recently used with success in a clinical trial by Tovey *et al.*, [1992], contains a 'benzyl derivative' and tannic acid. These may well be the same ingredients. Short-term exposure tests showed a strong acaricidal potency of DMS: within 3–4 minutes, 95–100% of the exposed mites were killed [Green *et al.*, 1989]. In the present study, Allersearch BT reduced mite populations with 85% after 8 weeks, compared to undisturbed populations.

After prolonged incubation on some mattress models, mite populations of treated mattress models appeared to revive, compared to the 8-week incubation. The mite numbers of treated mattresses have a much wider range than was determined after 8 weeks incubation, even on a logarithmic scale. The exponential growth of the individual populations will account for some of the width. Furthermore, when all mites die in an early stage, no revival of the population can be expected, but one surviving (female) mite in the first incubation week may have an offspring of several thousands in 24 weeks.

These results indicate that in a one-hit treatment neither Allersearch nor Acarosan is capable of keeping the number of house dust mites low on mattresses for 6 months. Since the incubation environment is limited, it is not very likely that some of the mites have been able to avoid contact with the benzyl benzoate during their lifetime. The soiling layer may have reduced toxicity by its diluting and absorbing effect. Furthermore we must consider that not all mites may be equally sensitive to benzyl benzoate. Nevertheless, resistance to benzyl benzoate has not been recognized as yet.

In dwellings, a first application of the benzyl benzoate products in late winter, when mite numbers are low, with a repeat after 2 or 3 months, that is before the opening of the 'mite season', should be sufficient to keep mite numbers low the whole mite season.

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