

Use of liquid nitrogen in the control of house dust mite populations

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Summary

Laboratory and field studies on the use of liquid nitrogen as an acaricide, combined with vacuum cleaning, demonstrated this inert freezing agent to be extremely efficient in reducing the number of live mites in mattresses when compared to vacuum cleaning only. Preliminary observations suggest this treatment does no physical damage to mattresses. Any strategy in the control of house dust mites should incorporate procedures both for the reduction of the mite population and the removal of the allergen pool of faecal pellets and dead mites.

Introduction

It is well known that house dust mites are responsible for the production of potent allergens [1, 2] and that these allergens are a major cause of allergic asthma [3, 4]. Control of house dust mite populations has, in some studies, resulted in clinical improvement of asthma [5, 6]; whilst in others, no such improvement has been observed [7, 8].

This study examined the physical control of house dust mite populations in mattresses and mattress materials using liquid nitrogen as a freezing agent, followed by vacuum cleaning to remove dead mites and faecal pellets. The efficiency of this treatment was compared to vacuum cleaning only and I have attempted to assess, using scanning electron microscopy, any damage to mattress materials that may result from the application of liquid nitrogen.

Materials and methods

Laboratory study

Two blocks of open cell foam rubber (24 × 14 × 6 cm) were placed inside plastic boxes above a saturated solution of sodium chloride to maintain relative humidity at approximately 75%. Each block was infested with 1.0 g of culture medium (which consisted of a 1:1 mixture of baker's yeast granules) and dried *Daphnia* containing

Dermatophagoides pteronyssinus (Trouessart, 1897), the European house dust mite. The blocks were placed in an incubator at a temperature of 25°C for 24 hr to allow the mites to disperse. The upper surface of both blocks was then cleaned for 1 min using a portable industrial vacuum pump. Mites and culture medium were sucked direct into plastic sampling tubes, sealed at one end by 10 µm nylon mesh [9]. One of the blocks was then soaked with 500 ml of liquid nitrogen (temperature -195.8°C). The other block was left untreated. The treated block was allowed to thaw and then returned to the incubator. Sampling was repeated every 24 hr for 7 days followed by a final sample after 30 days. Samples were weighed and examined in glass Petri dishes. All live mites were counted and removed. Three ml of lactic acid and 0.1 ml of aqueous lignin pink solution were then added to the Petri dishes. Samples were heated at 50°C for up to 12 hr to clear the remaining mites which were counted under a binocular microscope.

Field trials

In order to compare the effects of liquid nitrogen treatment and vacuum cleaning with vacuum cleaning only the mattresses of ten non-atopic volunteers (age range 17–30, sex ratio m:f 3:2) were studied. Dust samples were taken using the same apparatus as was used in the laboratory study. The whole of the upper surface of each mattress was sampled using a zig-zag sweeping pattern of twenty 1 m sweeps in 1 min. This results in a sampling rate of 0.25 m²/min using a sampling tube nozzle with an internal diameter of 12.5 mm. This sampling technique was used in order to minimize the effects of repeat samples on the house dust mite population, to allow for even sampling of different densities of mites in different regions of the mattress and to allow for interconversion between time, surface area and sample weight.

After the first sample had been taken from all ten mattresses, five were completely soaked with liquid nitrogen (5 l for a single and 7 l for a double bed). Liquid nitrogen was transported to volunteers' homes by van in a liquid nitrogen refrigerator (Union Carbide U.K. Ltd.). An expanded polystyrene 'igloo' ice-bucket (Jencons Ltd.) was filled from the N₂ refrigerator and the contents poured onto the mattresses. Low-temperature gloves (Gallenkamp) and 'Pull Safe' visor were worn throughout this procedure. Doors and windows were left open during this process and for about 15 min to allow the mattresses to thaw. This precaution was to prevent the oxygen content of the air in the bedroom being replaced by nitrogen gas. The entire surface of the mattress was then cleaned using a Dejon heavy duty vacuum cleaner. The other five mattresses received vacuum cleaning only.

Sampling, followed by vacuum cleaning, was repeated every 2 weeks for 2 months. Samples were stored at 4°C to inhibit mite population increase, and 80% relative humidity to prevent desiccation for up to 24 h before examination.

After weighing, the dust was transferred to glass Petri dishes, and lactic acid and lignin pink were added, as in the laboratory study. Mites were removed, mounted in gum chloral, and identified and counted under a compound microscope. Mites were recorded as 'damaged' or 'intact' as an indirect estimate of whether the mites were alive or dead at the time of sampling. The major criterion used in this assessment was the integrity of the exoskeleton and associated structures. When a house dust mite dies it immediately starts to dehydrate since the water-balance system is no longer operating. The cuticle becomes shrunken and susceptible to damage. It is hypothesized that much of this damage results from the movements of the occupier of the bed.

'Intact' mites possessed an intact exoskeleton with all leg segments setae and

ambulacra ('feet') present. In addition, the oesophagus was intact and food particles were visible in it. 'Damaged' mites lacked some portion of their anatomy, and/or the exoskeleton and oesophagus was ruptured. Exuviae (moulted exoskeletons) were detected by the dorsal split through which the new instar emerges and by their characteristically crumpled appearance.

This indirect technique was used because live and dead mites in dust samples become coated in fine particles and are extremely difficult to find. Heating the sample in lactic acid and ligin pink dissolves much of the non-chitinous material present, and stains and clears the mites making them easier to find.

The accuracy of this technique was tested in the laboratory using a single-blind trial. The author was given ten coded microscope slides for examination on which a colleague had mounted known numbers of live and dead mites (total mites: 100 per slide). The accuracy in determining live and dead mites in this way was found to average 94.1% (range: 98–89%).

Assessment of damage to mattress materials following liquid nitrogen treatment

Pieces of the cloth cover and foam rubber filling of a discarded second hand mattress were soaked in liquid nitrogen and allowed to thaw three times. Five × 5 mm squares were cut and mounted on aluminium stubs with double sided sellotape. The specimens were sputter coated with gold in a Polaron E 5000 s.e.m. coating unit at 0.8 torr, 750 V, and 25 mA for 4 min and examined in a Phillips 500 scanning electron microscope at 3 Kv.

Statistics

Analysis of covariance was used to compare the reduction in numbers of intact and damaged mites between liquid N₂-treated and untreated mattresses. This was done after using a transformation of $\log n + 1$ on all the raw data to eliminate the large variances due to differences in abundance of mites between mattresses.

Results

Laboratory study

Figure 1 shows that no live mites were extracted from the treated block after liquid nitrogen had been applied. By comparison it took from day 1 to day 6 to reduce the numbers of live mites to below 50/100 mg of culture medium in the untreated block. By day 30 the numbers of live mites in the untreated block had risen to 61/100 mg. The mean percentage reduction in numbers of dead mites over 6 days as calculated from lines of best fit derived from regression analysis were 89.8% for the treated block and 87.2% for the untreated block, suggesting that liquid nitrogen treatment has little or no effect in making dead mites more available for removal by vacuum cleaning.

Field trials

Comparison of the reduction in numbers of damaged mites from liquid N₂ treated and untreated mattresses showed no significant difference (variance of slopes of regression lines = 0.022 with 1 and 46 degrees of freedom) even though the mean percentage reduction in numbers of damaged mites, as calculated from lines of best fit, were 67.2% for the treated mattresses and 50.98% for the untreated mattresses. This lack of any significant difference was largely due to the large amount of variance, despite

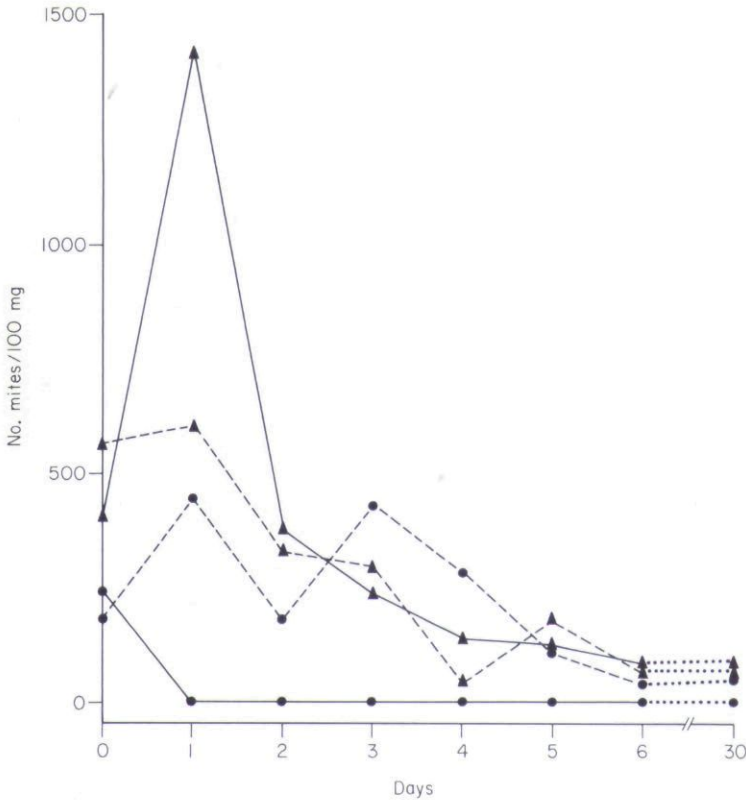


Fig. 1. Numbers of live (●) and dead mites (▲) extracted from liquid N₂-treated (—) and untreated (----) blocks of foam rubber.

Table 1. Mean numbers of mites per 100 mg dust taken from mattresses on five biweekly sampling occasions

	1	2	3	4	5
<i>N₂-treated mattresses (n = 5)</i>					
Damaged mites	150.0	234.9	144.9	85.1	55.3
Intact mites	31.2	20.9	11.5	4.3	0.3
<i>Untreated mattresses (n = 5)</i>					
Damaged mites	69.8	56.5	57.6	40.3	33.7
Intact mites	50.8	35.8	29.4	27.3	21.1

logarithmic transformation, in numbers of dead mites between mattresses within the treated and untreated groups (Figs 2 and 3). However, for intact mites the difference was significant with a value of $P = < 0.05$ (variance of slopes of regression lines = 7.50 with 1 and 46 degrees of freedom). These data indicate that vacuum cleaning alone is as efficient as vacuum cleaning plus liquid N₂ treatment in removing dead mites but the latter treatment is significantly more efficient in reducing live mite populations. The presence of intact mites in mattresses some time after application of liquid N₂ suggests

either re-infestation (e.g., from carpets) or incomplete eradication of entire mite populations.

Assessment of damage to mattress materials following liquid nitrogen treatment

No observable structural damage to mattress materials could be detected by scanning electron microscopy. None of the volunteers reported any damage to their mattresses following application of liquid nitrogen. These observations do not establish however that repeated applications over a longer period do not cause structural damage or alteration of the tensile strength of the mattress materials.

Discussion

The results of the laboratory study and field trial clearly illustrate two aims in the control of house dust mites; the eradication of live mites and the reduction in the non-living component of the allergen pool which consists of faecal pellets and dead mites.

Compared with dead mites, live mites are far less susceptible to removal by vacuum cleaning. Sucker-like ambulacra at the tips of each leg help attach them firmly to the substrate and only a relatively small proportion of the population will be removed by each vacuuming treatment. The application of liquid nitrogen kills the mites, effectively converting the large residual live population into the non-living component of the allergen pool. The resulting improvement in extractability accounts for the large increase in numbers of dead mites found in the first dust sample taken after liquid nitrogen treatment (Figs 1 and 2). Differences in the degree of improvement probably account for the increased variance in numbers of dead mites between mattresses within the treated group.

House dust mite infestations are mainly confined to the surfaces of mattresses [10, 11] probably because human skin-scales on which they feed are concentrated here.

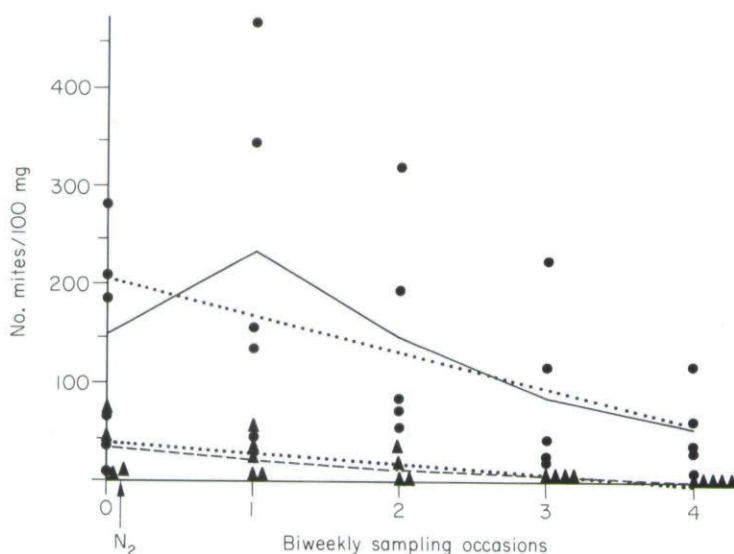


Fig. 2. Numbers of damaged (●) and intact (▲) mites extracted from liquid N_2 -treated mattresses. Solid line represents mean of damaged mites; dashed line represents mean of intact mites; dotted lines represent regression lines of best fit.

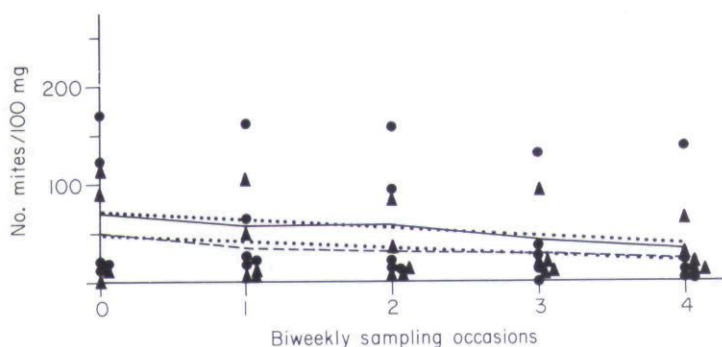


Fig. 3. Numbers of damaged and intact mites extracted from untreated mattresses. Solid line represents mean of damaged mites; dashed line represents mean of intact mites; dotted lines represent regression lines of best fit.

Since an application of liquid nitrogen soaks right through the mattress, there seems little possibility that any residual population, even if it was capable of existing in the food-deficient depths, could survive treatment to migrate out and re-colonize the surface layers. In any case, seven out of ten mattresses used in this study were internally sprung. The centre of this type of mattress consists of a large air space.

The major difference between liquid nitrogen treatment with vacuum cleaning and vacuum cleaning only is that the former treatment achieves a considerable reduction in live and dead mites whilst the latter, although achieving a comparable reduction in dead mites has only a small impact on the numbers of live mites. This situation can be improved if vacuum cleaning is continued at regular intervals for long periods of time, but if treatment ceases, residual populations build up again as do the amounts of house dust mite allergens.

Liquid nitrogen treatment has several distinct advantages over other acaricidal techniques. Chemically, the substance is inert. Thus the possibility of sensitization by atopic patients is eliminated. Use of chemical acaricides such as paragerm A.K. [12], natamycin [13, 14] or lindane [15] in the personal environment of atopic patients should be discouraged because of this potential risk, despite the fact that no apparent toxicity to humans may have been reported for the substance concerned.

Liquid nitrogen is fast-acting. An application to a mattress takes only a few minutes and the effects are immediate. Control of house dust mites by reducing the absolute humidity of the air as advocated by Korsgaard [16], presumably by increasing indoor heating, takes a long time, is expensive, and must be continuous to be effective [17].

Stringent measures of household cleanliness as detailed by Murray & Ferguson [18] are probably the cheapest and most effective means of reducing the allergen pool in the environment of a patient suffering from allergic asthma, but they must be strictly adhered to and carried out by someone other than the patient. Liquid nitrogen treatment can provide a valuable additional effect to such basic measures.

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