ENVIRONMENTAL AND BEHAVIORAL
CONTROL OF THE AMERICAN HOUSE DUST MITE,
DERMATOPHAGOIDES FARINAE HUGHES

A Thesis
Presented in Partial Fulfillment of the Requirements for
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ABSTRACT

The objective was to simulate the relative humidity and temperature that occurs in a mattress during human occupancy. Being able to reproduce these conditions enabled us to facilitate mite population growth. This artificial system was used to examine two control methods.

Mattress rotation was studied as a tactic to control house dust mites. The gradient difference in relative humidity that occurs between the top and bottom surfaces of a mattress is enough to discourage mite growth and survival. Rotation, in this context, is defined as the turning over of a mattress 180 degrees. By rotating a mattress, we found a forty-five percent decrease in mite number on rotated mattresses versus controls. Furthermore, a fifty-five percent reduction in allergen production was recorded. Regular mattress rotation could become part of a non-acaricidal strategy to reduce the dust mite population and allergen in a two-sided mattress.

The effectiveness of a high efficiency particulate air vacuum cleaner to remove dust mites and their allergenic debris was investigated. Vacuumed samples were taken from mite-infested mini-mattresses and the layers beneath were
removed. Vacuuming removes up to seventy-five percent of the dust mites and ranged from fifty-five to ninety percent. A proportion of the allergen present was also removed by vacuuming.

We monitored dust mite population growth on mini-mattresses under two different humidity conditions. In the laboratory setting, dust mites are cultured at a steady relative humidity. In the home, they survive the ambient humidity fluctuations. We compared how these two different humidity regimes would influence dust mite population in a mattress setting. Dust mites maintained at constant hydrating conditions for eight weeks totaled 55,109 mites. Dust mites grown at only eight hours per day of hydrating conditions for seventeen weeks totaled 26,332 mites. In only eight weeks at constant conditions, the dust mite doubled their population. Hence dust mites reproduced faster at a constant relative humidity (above 50%) than on varying conditions.

An excellent way of controlling house dust mites is a combination of these tactics. By rotating the mattress, many mites will die because of unsuitable living conditions. Vacuuming can be used to remove these mites and their allergen.
Dedicated to my grandfather:

Ramon Reyes Lopez
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CHAPTER 1

GENERAL INTRODUCTION

House dust mites is the common name given to the diverse group of mites that are commonly found in homes. They range from stored-products pests and their predators to nidicoles and parasites (Wharton, 1976). The two common species that are frequently encountered amongst the contents of domestic vacuum cleaners are *Dermatophagoides farinae* Hughes and *Dermatophagoides pteronyssinus* (Trouessart). Their common names are the American and European house dust mites, respectively. *Dermatophagoides farinae* is the most common dust mite in temperate climates while *D. pteronyssinus* is more abundant in higher humidity climates and coastal regions. In tropical and sub-tropical regions the predominant mite is *Blomia tropicalis* Bronwijk, Cock and Oshima. *Euroglyphus maynei* (Cooreman), like the American and European dust mites, is believed to have a worldwide distribution (Arlian, 1989). These mites belong to the family Pyroglyphidae, a family of parasitic or companion mites. Pyroglyphids are usually associated with birds or mammals but seldom both. They can be associated with the host itself (direct) or with its nest or stored products (indirect). Pyroglyphids
belong to a bigger group of mites called the Astigmata. Astigmatic mites are characterized by many features including reduction of the rutellar teeth, the lack of trichobothria and the desclerotized cuticle in adults (O'Connor, 1982). But one of their most important features, which separates them from other mite groups, is the lack of an internal respiratory system with external openings (stigmata). A pair of supracoxal glands, near the head, is involved in primary (active) uptake of water vapor from the air. Respiration occurs by diffusion of air across the integument.

Biology of house dust mites

House dust mites, like other acarines, have a life cycle that includes a larval and several nymphal stages. Their development and reproduction are very dependent on the ambient temperature and in the relative humidity. Embryonic development lasts for six to eight days. The larval stage takes five to eight days, followed by the two nymphal stages. The protonymphal stage lasts four to six days and is followed by the tritonymph lasting between four and five days. When the protonymph is ready to molt into a tritonymph it undergoes a resting period referred to as quiescence. Prior to molting it exhibits thigmotactic behavior and uses a sticky secretion to glue itself to a substrate. It then undergoes the internal changes necessary for molting. The mite then develops from protonymph to tritonymph but remains in a pharate stage inside the old protonymphal exoskeleton (Ellingsen, 1974). The tritonymphal stage will also undergo quiescence when molting into the adult. However these quiescent stages are moderately different.
The protonymphal quiescence has a half-life of water exchange of 159.7 days and consumes 28.5 times less oxygen/hour than an active protonymph (Ellingsen, 1974). The quiescent mite is therefore very resistant to desiccation, which increases its chances of survival during dehydrating conditions. The quiescent tritonymphal is not so resistant to desiccation. Ellingsen (1974) found that 94.6% of mites undergoing quiescence are protonymphs. Furthermore most of the adults that emerged from tritonymphal quiescence were males. They attributed the find to the fact that quiescence is a very energetic process and once undergone, with limited resources left, only a male could emerge because of the expense of molting into a female. During their life cycle, some cue not yet identified triggers the quiescence. It was believed to be the seasonal drop in relative humidity but dust mites in laboratory cultures will also undergo winter quiescence regardless of the constant relative humidity (Arlian et al., 1983). Reka et al. (1992), suggests that guanine induces quiescence but unfortunately quiescence has not been studied enough to learn its triggers and regulators.

Adult life expectancy varies between the sexes. Males have an average life span of forty-five days while females live up to ninety days. The life cycle takes about twenty-five days at 25°C and 75% RH, which are optimal conditions (Arlian, 1989). In temperatures extremes of 16°C and 35°C, the mites that were able to complete their life cycle did so in 122.8 and 15 days, respectively (Arlian and Platt-Mills, 2001). Both of these temperature extremes caused high mortality for all stages. Females control the mating process. Both sexes will mate more than once
(Larson, 1969). A mated female can lay as many as three eggs/day but these are
laid one at a time with an undefined resting period between them. During their
laying period females can produce 200 to 300 eggs (Wharton, 1976).

Environmental Requirements

House dust mites are very sensitive to changes in environmental conditions.
Their resistance to desiccation is well documented; however they are really quite
fragile. A small change in relative humidity and/or temperature can impact molting
or reproduction. Optimally, relative humidities of around 75 percent maintains
healthy and sizable dust mite populations. Humidity kept at 50% or less for more
than 11 days, will result in high mortality (Arlian, 1992). The effects of
temperature on their life cycle are also quite dramatic. However, dust mite
infestation in the home environment, where temperatures tend to be rather constant,
is driven primarily by relative humidity. Therefore dust mite populations in the
home vary with seasonal changes in the ambient humidity. The summer months
(June to August) are marked by the highest populations. As ambient humidity
decreases in the fall (September and October), the mite numbers start to decline,
reaching the lowest levels during winter (December to February). Spring (March to
May) is characterized by resurgence in dust mite numbers in the home (Arlian et
al., 1983).
Nutritional Needs

Dust mites are scavengers that commonly feed on dead human skin scales in the absence of other food items. An unanswered controversy in dust mite research is whether or not dust mites require molds in their diet. It is argued that molds facilitate them in digesting the harsh dead human skin scales. Molds as a nutritional requirement are more attributed to the European dust mite. Since *D. pteronyssinus* requires a higher relative humidity, and is more common on coastal areas, usually mold consumption is associated with it. However, *D. pteronyssinus* has been successfully cultured with a varying degree of skin scales and in the absence of mold. Although not recommended because of their own allergenicity, mites were grown in cat, cattle, dog, guinea pig and rabbit skin scales (Spieksma, 1976). In addition to that, researchers successfully grow European dust mite on a diet consisting of laboratory animal food, dried yeast and dried fish powder. Dust mites of both species are reared in laboratories with many different food sources.

An examination of the literature reveals many food sources from varying types of mammalian foods to dust mites themselves. In the absence of a food source, dust mites will eat shed exoskeletons and dead mites. Dust mites produce an array of proteins that are allergenic to humans and animals. Several human deaths have occurred due to anaphylaxis because of dust mite allergens in food. On post-deaths examinations, mites (European, American and even *B. tropicalis*) were found living and consuming corn and wheat flours. They will also feed on dog food (Larson et al., 1969) and powdered biscuit meal (Griffiths and Cunnington, 1971). An
increased in production has been observed by the addition of animal fats to dust mite cultures (Waki and Matsumoto 1973).

The Health Connection

House dust mites produce allergens that escape the body in their faces and their shed exoskeletons (exuviae). These allergens are the most important indoor allergens present (Platts-Mills et al., 1987). Allergens are defined as antigenic substances, which are capable of provoking an allergic reaction in susceptible organisms. An allergic reaction is defined as an immune response in which the foreign substance stimulates IgE antibody production in the host body. This reaction can happen internally or externally. An external allergenic reaction can be something as simple as a small wheal or a rash and as extreme as atopic dermatitis. Internal reactions are as mild as sneezing, watery eyes and runny nose or as harsh as asthma or anaphylaxis. In fact, the presence of house dust mites have been linked to the development of asthma.

Asthma is a lung disease that is characterized by airway obstruction, airway inflammation, and an increased responsiveness to stimuli. Asthma has an array of symptoms, with coughing and wheezing being the most frequent of them. The American Lung Association estimates that 20.3 million Americans, including 6.3 million children, are currently suffering from asthma. Treatment expenses exceed 14 billion dollars annually. Recently, federal funding was increased for the research on and treatment of asthma. Currently more money is being spent on
treating asthma than on HIV. The number of asthma cases in developed countries has been steadily increasing since the early 1980s. Countries like the U.S. have had an increase of more than 30% in that time frame. Also in the last twenty years, asthma related hospitalizations have increased by 200% (Anderson, 1989). There have been documented deaths of people of all ages, ranging from newborns to the elderly, where an anaphylactic reaction to dust mites led to death. Since very early in the last century, it was found that one third of people tested were sensitive to dust mites (Kern, 1921, Cooke, 1922).

Allergens produced by house dust mites fall into two groups. Group 1 allergens include Der p1 and Der f1 and Group 2 allergens include Der p2 and Der f2. Group 1 allergens are digestive enzymes that are released into the environment via the excretory track (Tovey et al., 1981). Group 2 allergens are associated with the mite body and therefore found in the exuviae. Hence the dust mite allergy problem involves much more than exterminating the dust mites. There must be an effective way of removing or containing the allergen for control to be effective.

House Dust Mite Control

House dust mite control has included the usual armaments in the pest arsenal, from pesticides to temperature extremes. Several pesticides with miticide activity have been tried against dust mites. These are called acaricides and the ones tested include benzyl benzoate, tannic acid, pirimiphos methyl, permethrin and
disodium octaborate tetrahydrate (DOT). Fungicides have also been used because of the belief that dust mites require mold to digest human skin cells. The most common fungicide recommended for dust mite control because of its claims of disrupting reproduction is natamycin. Insect growth regulators like methoprene, telbuvizuron and flufenoxuron have also been tested on dust mites. Methoprene was found to be quite potent, while flufenoxuron was found to actually stimulate reproduction (Stepanova and Kostina, 1994).

Besides pesticides, various avoidance techniques are used. These include air filters, occlusive mattress encasements, pillow covers, vacuum cleaning, carpet removal and mattress replacement (Ferguson, 1995). Temperature extremes have also been used. Hot water laundering is one a way of reducing dust mite populations in the bedding materials. Steam cleaning of carpeted areas is recommended for use against dust mites. Liquid nitrogen was found to help remove dust mites from mattresses with the use of a vacuum cleaner (Co`loff, 1986). This treatment appear not to harm the mattress itself. Ten years later in a separate study, four houses were treated with liquid nitrogen. They found that post-treatment mite levels were around one fourth of pre-treatment levels but, resurgence occurred three to four weeks later, making this intervention short-lived.

It seems that control will not come from a single source but rather from a combination of efficient strategies. The first such tactic tested was the effectiveness of reducing the relative humidity in a mattress through rotation. Then the thoroughness of vacuum cleaning at removing the dust mites and their allergen from a mattress was tested.
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CHAPTER 2

The effects of mattress rotation on the population and allergen production of the American house dust mite, *Dermatophagoides farinae* Hughes.

INTRODUCTION

House dust mites are the primary source of antigens responsible for dust allergy in humans (Platts-Mills et al., 1987). Prolonged childhood exposure to amounts greater than or equal to 10 μg *Der* Group 1 allergen/gm of dust is said to cause allergy problems in adults (Platts-Mills et al., 1997). Furthermore, high exposure to mite allergens during infancy can lead to early sensitization and the development of asthma (Mahmic et al., 1998, Platts-Mills et al., 2000). Sensitization to house dust mites have been found in 45 to 85% of the asthmatics tested in the world (Platts-Mills et al., 1997). Consequently reducing exposure to dust mite populations and their allergens may greatly benefit asthmatics and allergy sufferers. Recommendations for dust mite environmental control include mattress encasement or replacement, hot water laundering of bedding materials, air filters, acaricide/fungicide treatments, and carpet removal.

Mattress encasement has been studied many times and the outcome is controversial at best (Jooma et al., 1995, Thiam et al., 1999, Siebers et al., 2002,
Gotzsche et al., 2003, Terreehorst et al., 2003, van Strien et al., 2003, Woodcock et al., 2003). A study in South Africa found that covering mattress alone had no impact on patient health. As a consequence, regular encasement washing in hot water is required to statistically reduce mite allergens (Jooma et al., 1995). The same study found that house dust mites are able to live on cotton barriers and therefore allergen levels do not differ between encased and non-encased mattresses. In addition, researchers have proposed that synthetic bed covers might improve patient health by dramatically decreasing the amount of airborne house dust mite allergens in beds (Siebers et al., 2002). But then again, dust mite allergen’s ability to be propelled through the air has not been fully studied. A Swedish study observed a difference of 98% in dust mite numbers between the lower and upper surface of the encasement (Wickman et al., 1994). They demonstrated that the top of these encasements is as suitable for dust mite colonization as the top surface of the mattress. A recent study conducted by van Strien et al. (2003) in The Netherlands claims that mattress encasements have a significant reduction in the presence of mites and their allergen. However, they append that this reduction is modest and that only the initial mite allergen concentration was low. In response to this work Gotzsche’s group in the U.K. emphasized that the benefits of mattress encasements have not been shown beyond reasonable doubt. Mattress replacement may not be a good option because new mattresses can gain a significant amount of mite allergen in four months (Custovic et al., 1996). Since mite infestation is driven by humidity, it can be rapid in damp housing conditions (Placido et al., 2001).
The laundering of bedding materials and stuffed toys in hot water, usually above 50°C, is widely recommended for people with indoor allergies. This recommendation came to be by a study conducted by McDonald and Tovey (1992) in which the effectiveness of different temperatures for killing dust mites was assessed. They found that at 55°C or greater all dust mites were killed. They also noted that temperatures below that did not have an enhanced killing ability even after the introduction of pure detergents. An interesting find by them also was that at temperatures below 16°C, dust mites survived but 90% of the allergen present was denatured. A follow-up study by Tovey et al. (2001), almost ten years later found that the use of detergents at 25°C for five minutes was enough to extract the mite allergen from the bedding materials. So whether one uses very hot or cold water, eliminating dust mites or their allergen is viable. A negative effect is that surviving live mites may transfer from mite-infested to mite-free items (Arlian, 2003). Separating suspected infested items from other laundry, may prevent cross contamination.

Air filters are only effective in capturing trace amounts of airborne allergens in the bedroom, and therefore by themselves they cannot control the exposure issue. There have been several studies where air filters with a combination of regular vacuum cleaners filters and high efficiency particulate air (HEPA) filters have been tested. This work has only shown that there has been no health improvement to the patients in these studies (Warner et al., 2000, Thiam, 1999). Thus, so far there has been no work to represent air filters as efficient venues for
capturing airborne dust mite allergens. In addition, there is no work to date on the
dynamics of dust mite allergen when airborne. A novel allergen collecting method
that collects nasally inhaled dust into an adhesive surface may improve sampling
procedures. These allergens can then be analyzed in sensitive enzyme
immunoassays (Glass et al., 2003) or immunostaining can occur to observe under
the microscope (Renstrom, 2002).

Acaricides are ineffective at controlling house dust mite populations in
bedding. A common recommendation among allergy doctors is the use of benzyl
benzoate treatments on carpets and mattresses. New mattresses treated with benzyl
benzoate failed to reduce emerging mite populations and their allergens below
established sensitization thresholds (Rebmann et al., 1996). In addition,
researchers have shown that acaricidal application to mattresses provides no
additional benefits over mattress encasement alone (Weeks et al., 1995). Therefore
these acaricides not only fail to prevent colonization of the new mattress but they
are ineffective at eliminating established mite populations. Another acaricide that
recently has been studied in the mattress setting is permethrin. Although known of
its ability to kill mites, especially chiggers and fowl mites for more than twenty
years, recent work with permethrin treated mattress liners looks promising
found that permethrin-impregnated bedding reduced dust mite numbers in mattress
for up to twenty-seven months. They also observed a reduction in allergen
production for up to fifteen months. Although these researchers claim success with
this acaricide, permethrin is not tolerated by a small percent of the human population. For them it would be interchanging one allergen for another. Tannic acid has been explored as a control option but it does not kill the mites. Tannic acid denatures the protein and therefore reduces the allergen present (Ferguson, 1995). A new acaricide known as disodium octaborate tetrahydrate (DOT) was recently evaluated for dust mite control in a central Florida study, which found that DOT kills dust mites in carpet, and upholstered furniture. Furthermore the combination of its application with weekly vacuuming is recommended for better allergen control (Codina et al., 2003). DOT has good residual activity, and human toxicity is considered to be low.

It is believed by certain researchers that fungicidal treatments should be as effective at dust mite control as regular acaricides. The logic is that dust mites require molds to aid in the digestion of human skin scales. Although that has not been shown, what has been found is that the addition of yeast improves the diet but is not enough by itself (Spieksma, 1967). Seldom dust mites will consume mold surrounding their food source, although they are quite capable of surviving with mold free diets. The fungicide natamycin has been the popular choice of researchers to test against dust mites for twenty years. Some of the studies claim that the fungicide appears to hamper dust mites development but the effect was more pronounced by the used of a vacuum cleaner (Van Bronswijk et al., 1987, De Saint Georges-Gridelet, 1988, Colloff et al., 1989, Reiser et al., 1990). This makes sense because the vacuum cleaner will remove the dust mite allergen and therefore
the fungicide will seem more effective than it really was (Van Bronswijk, 1987). Another study found that natamycin was effective at controlling dust mite populations when the concentration used was twice the commercially available one (De Saint Georges-Gridelet, 1988). The researchers however did not comment on how this unorthodox concentration of natamycin might affect people or whether this treatment will even be approved for commercial use. It appears that fungicide treatments for dust mites hold little promise for mite control. Since the source of the problem is the mattress and perhaps the carpeted areas, acaricidal and fungicidal treatments are not likely possibilities for bedrooms.

Carpet removal may eliminate large surface areas of suitable mite habitat, but no data exists that supports the clinical benefits of this practice. Although the Carpet and Rug Institute will lead you to believe that dust mites in carpet is a minor problem, there is little evident either way. The mattress is a considerable higher source of dust mite allergens and therefore should be a priority (Sidenius et al., 2002). It would seem that the best strategy for mite control is to disrupt their living conditions in their home, the mattress.

While some of these approaches might be relatively inexpensive, others are not and they all share the mutual feature that they do not reduce mite allergens or improve patient symptoms dramatically by themselves (Woodcock et al., 2003, Terreehorst et al., 2003). Furthermore, none of these methods threaten dust mite populations by removing any key elements for their survival. Given that relative humidity is the main determining factor for dust mite survival in the home.
Reducing indoor humidity is a way of controlling mite populations (Korsgaard, 1983, Arlian, 1992). Therefore we decided to test how a simple procedure such as mattress rotation could affect the populations and allergen production of house dust mites. We attempted to mimic desirable conditions on a mattress for mite growth and reproduction. We did this in order to determine if mattress rotation affects dust mite survival through the decreased humidity and investigated whether or not the mites travel through the mattress to find suitable conditions.

MATERIALS AND METHODS

We used fifteen mini-mattresses measuring 53cm x 55cm, which were identical in construction and materials to full-sized counter parts and were provided by the Sealy Corporation. To simulate the increased relative humidity in a mattress by human presence, a Miatech Inc. misting system was used. Figure 2.1 shows one of the three structures that was constructed to house five mini-mattresses each. Appendix A has several pictures on the entire experimental setup. These structures measured 325cm long, 104cm wide and 82cm tall. The surface where the mattresses were positioned was covered with the same fabric that is used to make box springs, (Sealy Corp.). Fifteen misters were placed on the wood frame above the mattresses at a height of 51cm. We used a three random block design to place the five mattresses in each block. Three mattress groups were chosen. Group I mattresses were rotated at nine weeks and had two impermeable barriers that may prevent mites from internally crossing from one side of the mattress to the other.
Figure 2.1: This is the wooden cage designed and built to house five mini-mattresses and their respective misters in an alternating pattern.
Group II were rotated equally but lacked the internal barriers and Group III were not rotated and served as a control. A hard plastic mesh was used to create a localized humidity gradient over each mattress. The mesh squares constructed measured 5cm in thickness and had the same area as the mini mattresses (Fig. A.3). They were placed on the top of the mattress sheet with a fabric over them that received the misted water. The fabric was a standard 50/50 polyester-cotton blend that is commonly used for bedding material construction. The fabric color was a dark blue-green to better visualize dust mites. HOBO (H8) relative humidity and temperature data loggers (Onset Corp) were used to record the conditions (Fig. A.8). Surfaces monitored continually included each mattress, top and bottom, the three blocks and the room. Water output by the misting system was calibrated to insure that the relative humidity was maintained between 65 and 75% during eight hours each day. The water output was checked weekly to insure that each nozzle was discharging the same amount of water throughout the experiment.

This system was constructed to simulate the humidity changes that occur when a person occupies the mattress. To monitor the relative humidity changes over a twenty-four hour period, we slept with several probes over the course of several days. The probes were placed in different regions adjacent to the body (Figs. 2.2 and 2.3). The room was maintained at 24-26°C. One mattress from each block was assigned randomly to ensure that the abiotic parameters desired were being maintained during the course of that day. The remaining probes were checked weekly.
Figure 2.2: Relative humidity and temperature fluctuations of a person sleeping eight hours/night over the course of three days. These readings were taken from the head area.
Figure 2.3: Relative humidity and temperature fluctuations of a person sleeping eight hours/night over the course of three days. These readings were taken from the area between the knees to the feet.
Mattresses were inoculated with American house dust mites, *Dermatophagoides farinae* Hughes. Five equally spaced inoculation circles measuring 100mm each were drawn on both the top and bottom surfaces of the mattresses (figure 2.4). Twenty mating pairs, picked from laboratory maintained bulk cultures, were placed on each top surface circle of all fifteen mattresses. Food weighing 600mg was added prior to the mites to each top circle, which consisted of a 3:1 combination of powdered egg and yeast, respectively. Nine weeks later a vacuum sample was taken (Filter Queen® HEPA vacuum cleaner, HMI Industries Inc.) from each mattress and the mattresses in groups I and II were rotated. The circles were vacuumed for one minute each. These samples were stored at 4°C until analyzed. The same amount of food (600 mg) was added to each circle on the new top surface of the rotated mattresses. Following nine weeks, the remaining circles were collected as above from the top, side and bottom of each mattress, as well as, the top of the substrate beneath each mattress (which simulated a box spring’s upper surface). All samples were stored at 4°C until analyzed. Then the mattress surfaces were slashed longitudinally across the circles (figure 2.4) and internal vacuum samples were taken. One circle from each mattress was used to check for *Der f-1* allergen using a standard ELISA technique (Luczynska, et al., 1989). ELISA results were reported as ng *Der f1/ml/cm²*. The remaining samples were counted for mites using a flotation technique (Thind, 1979). The data was then analyzed using descriptive statistics and ANOVA tests and compared.
Figure 2.4: These are the five circles that were drawn on the mattresses used to inoculate them and later sample from them. These circles are 100 mm in diameter.
RESULTS

A summary of dust mite counts per 58cm$^2$ circle(s) at the time of mattress rotation and at the end of the experiment, together with final allergen levels (ng/ml/cm$^2$) per circle(s) over the replicate mattresses is shown in Table 2.1. All sampling events in the experiment were marked by the presence of larvae, nymphs, males and females. Eggs were not counted because the nylon micron mesh used in the flotation technique did not allow for total egg recovery. Mite populations and allergen levels varied widely between mattresses. Mattresses were more variable the larger the dust mite populations and the higher the allergen levels were. Two control mattresses showed a decline (rather than growth) in dust mite population in the post-rotation portion of the experiment but the declined proportion in the rotated mattresses was much higher. Perhaps variable stochastic success at establishment by the dust mites initially might be at play. However, a randomized incomplete block design analysis of variance of the log (mite counts) showed that the initial mite establishment levels at the time of rotation were not significantly different over the three treatments ($F_{2,10} = 6.7$ p$= 0.532$) with the overall fitted mean being 132.7 mites per 100mm circle(s). The type of mattress and its handling does not affect initial dust mite build-up. There was a significant difference in final mite populations between the treatments ($F_{2,10} = 4.16$ p$= 0.048$). Fitted means were rotated (no barrier) : 48.8, Rotated (with barrier) : 77.6, Control : 92.7 mites per 100mm circle(s). Rotation reduces dust mite numbers by a third to a half.

Combining the two rotation treatments yields fitted values of 61.8 (flipped
<table>
<thead>
<tr>
<th></th>
<th>Mattress Treatment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rotated (no barrier)</td>
</tr>
<tr>
<td>Mite count at time of rotation</td>
<td></td>
</tr>
<tr>
<td>Mean (n)</td>
<td>156.2 (5)</td>
</tr>
<tr>
<td>SD</td>
<td>29.9</td>
</tr>
<tr>
<td>Range</td>
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<td></td>
</tr>
<tr>
<td>Final mite count</td>
<td></td>
</tr>
<tr>
<td>Mean (n)</td>
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</tr>
<tr>
<td>SD</td>
<td>27.2</td>
</tr>
<tr>
<td>Range</td>
<td>24 – 90</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Final allergen levels</td>
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<td>0.5367</td>
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<td>Range</td>
<td>0.184 – 1.450</td>
</tr>
</tbody>
</table>

Table 2.1: Mite counts for circle(s) at the time of mattress rotation (9 weeks), at the end of the experiment (18 weeks) and final allergen levels (ng/ml/cm²) are shown below. Randomized incomplete block design replicate treatments - rotation with and without a barrier versus control seeded with 200 mites at 24-26°C and 65-75% relative humidity.
mattresses) versus 91.8 (control mattresses) mites per 100mm circle(s). Whilst these were not quite significantly different due to small sample size ($t_{11} = 1.78$), nearly one and half times as many dust mites are found in control mattresses compared to those that are flipped. A randomized incomplete block design analysis of variance of log (allergen levels) showed that there was no significant difference in allergen levels between the treatments ($F_{2,10} = 0.04 \ p = 0.959$) with the overall fitted mean being 0.7244 (ng/ml/cm$^2$) per 58cm$^2$ circle(s). Although rotated mattresses had more than 50% lower allergen levels than the control; rotation did not have a dust mite allergen reduction that was statistically significant.

**DISCUSSION**

The difference in relative humidity between the top and bottom mattress surfaces can range between 20 to 50%. We learned that the relative humidity produced by a person in bed naturally fluctuates and there are differences between the head, torso and leg areas (Figs. 2.2 and 2.3). These fluctuations diffuse through the mattress and can be observed at the bottom where there humidity is decreased (Fig. 2.5). The top surface had an average relative humidity in the high 60s during the misting interval, while the bottom surface had an average in the 30s. Because house dust mites require the ambient humidity to be above 50% to absorb water from the air, this type of interruption was effective. We found that the average reduction in mite numbers was close to 45%. The difference in allergen produced between the controls and the rotated mattress was in fact closer to 55% less allergen
Figure 2.5: Relative humidity and temperature fluctuations on top and bottom surface of mattress number 12. You can see the delay in the humidity transfer from top to bottom. Also the bottom curve peaks at lower percentages.
detected in rotated mattresses. The range in mite number reduction varied from 35% to as high as 80%. We believe that mattress rotation can be used to drastically decrease the dust mite population in any mattress. We did encounter variability with three of the fifteen mattresses in the study, where we saw the direct opposite of what we had expected.

Mattress 2.9 was a group 1 mattress that was rotated and had the impermeable barriers present. This mattress had a slight increase in mite numbers from 141 mites on December to 210 on February. This was the only group 1 mattress that had an increase in its dust mite population in the second half of the study. According to the humidity and temperature probe data, the relative humidity for the first week post-rotation was too high. On several days the ambient humidity on the surface of the mattress reached and remained at 100%. This in turn caused the bottom side of the mattress, the one housing the mites at this point, to also have an increased humidity modestly over 50%. Once the ambient humidity crossed the 50% threshold, the dust mites were able to actively take water from air and hence prolong their survival. Since de Boer and Kuller (1997) showed that dust mites will reproduce with as little as 1.5 hours of high humidity (90%), even if the remaining 22.5 hours is as low as 10%, the population growth was expected. In addition to that, the following week had conditions that were almost identical. This proved to be quite the damaging occurrence when the bottom probe recorded a record high of 71% relative humidity. As a result, the bottom surface was just as suitable for mite colonization as the top. Until this point the highest bottom
measurement had been 55% RH. Among the other 14 mattresses the highest recorded relative humidity on the bottom surface was 43%. On the third week, the relative humidity situation corrected itself. The overall relative humidity average was 65% with a range in the low 50s to upper 80s. Unfortunately this aberration initiated weeks of optimal conditions that accounted for the increase in mite numbers. We cannot explain the irregular relative humidity in this one mattress. The two adjacent mattresses (2.8 and 2.10) had normal readings and their ambient humidity never reached saturation.

Mattress 1.2 (control) decreased from 284 mites to 75 instead of continuing the expected increase for a control. Relative humidity for this mattress was close to average through two weeks (post-rotation). During week three after rotation the humidity on the top of this mattress was extremely low, peaking at 43% and remaining below this for the entire week. The following week (December 28th to the 1st of January) the relative humidity reached only 30%. We suspect that these 14 days of dehydrating conditions had a substantial impact on survival and maturation. On January 1st, the reverse happened and the humidity reached 64%. During these three weeks the temperature was a few degrees lower than on all of the mattresses. The mite populations in that mattress were severely affected by this. Unexpectedly the same problem arose ten days later with relative humidity remaining under 36% for 7 days. On the 8th day, humidity was re-established at 70% where it remained for the duration of the experiment. I believe that some of the nymphal mites became quiescent and survived until the conditions became
favorable again. Final sampling occurred just 12 days after the conditions became favorable, so there was insufficient time for the mites to recover and reproduce.

Mattress 1.5 (control) also decreased from 522 mites (which was the highest count for the experiment) to 73 at post-rotation sampling. This 522 count was the highest observed for the experiment and it clearly marks this data point as an outlier (calculated using the 1.5xIQR rule). Clearly on December 7th that circle had a thriving population compared to the other circles on the mattress. That could have affected the rest of the mattress, because the remaining populations were smaller. For three weeks post-rotation the relative humidity and temperature readings were optimal. However, on January 1st the relative humidity dropped to the mid 20s and fluctuated between 25 and 35% for most of January, until the 27th. The factor that influenced the dust mite populations in this mattress the most was destructive sampling. These two factors (destructive sampling and outlier effect) made the changes that occur in this mattress appear more drastic than they actually were.

Except for these three mattresses, the other twelve had dust mite counts in the expected range of increased numbers for the controls and decreased counts for the rotated ones. On average, mattress rotation reduced dust mite numbers and allergen production. From Table 1 we see the difference between mattresses rotated in groups 1 and 2. At both points, pre and post-rotation, the barrier mattresses had more mites. But the overall reduction was around 63% for group 2 and only 43% for group 1. There appears to be some association between the
barrier material and environmental conditions favorable for dust mites. A close examination of the probe data shows that relative humidity at the bottom of the mattresses with the barrier material was higher than the non-barrier ones. Hence the barrier material likely exaggerated the problem by retaining water.

Mattress rotation may not be the absolute answer to the question on dust mite control but it is a very good start. This is a very simple mechanism that gives advantageous results when put to the test. Because of this we believe that we may have found a very easy way of controlling house dust mite populations. We can disrupt their living conditions and therefore the life cycle of this mite by a simple rotation schedule. Since patient compliance seems to be one of the biggest issues in treating asthma and allergies, we have tested a technique that can affect dust mite populations without a lot of work. We recommend mattress rotation every three months and that when the already rotated mattress is rotated a second time, the bottom surface of the mattress and the top surface of the box spring or adjacent substrate should be vacuumed. This will capture and remove allergens and mites. We believe that the rotation of the mattress and the vacuuming of the surfaces prior to rotation, can lead to a lower dust mite population and hence a lower allergen level. Therefore the minimizing of allergen exposure should lead to improvement among allergy sufferers and asthmatics.
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CHAPTER 3

An evaluation of a high efficiency particulate air vacuum cleaner at removing American house dust mites, *Dermatophagoides farinae* Hughes, from mattresses.

INTRODUCTION

The main challenge faced when attempting to control house dust mite populations is the allergen exposure issue. Even when acaricides and other invasive treatments kill the mites, they leave the feces and exuviae in the environment. Residual antigens can still cause allergic reactions in susceptible individuals. The strategy for dealing with dust mite control should be two-fold. There should be a primary treatment with an acaricide or, preferably some other tactic, to kill the source of the allergen followed by vacuuming to extract as much allergen as possible. Much of the dust mite literature deals with one or the other but not both in combination. This study focuses on the former tactic, removal of mites from a primary exposure site, the mattress.

Numerous studies have been conducted in the last twenty years to assess the efficiency of different types of vacuum cleaners at removing dust mites and their allergens (Munir et al., 1993, Colloff et al., 1995, Hegarty et al., 1995, de Boer et al., 1996, Adilah et al., 1997, Endo et al., 1997, Wickman et al., 1997, Glass, 2002,
Vichyanond et al., 2002). Vacuum cleaning has positive short term effects that can be maintained by frequent vacuuming, however long term studies are lacking (Adilah, 1997). Since short term vacuuming is beneficial, there is no reason to think that long term vacuuming is not equally beneficial. It was found that commercial wet carpet cleaning has short term effectiveness (de Boer, 1996). At one month post cleaning, the carpet was not entirely suitable for dust mite colonization although some of the inoculated mites survived. However, at three months post cleaning the infestation continued at a greater scale.

From conventional to steam vacuum cleaners and the newest ones with high efficiency particulate air (HEPA) filters, the use of vacuum cleaners for removing the dust mites and their allergen is recommended. Dust mite levels in the dust bag of a vacuum cleaner are a good indicative of the dust mite infestation in the home (Twiggs, 1991). This removal strategy works with varying degrees of effectiveness, in spite of which it is not the most popular approach. The problem encountered here has to do with the capture of the dust mite allergen by the machine itself. Conventional vacuum cleaners (non-HEPA) remove the allergen from the carpet but re-circulate it into the air and eventually resettling in the carpet. Thus vacuuming potentially becomes an activity that is both harmful and dangerous for asthmatics and those with indoor allergies. In the past it was believed that dust mite allergens were large enough that a conventional vacuum cleaner could capture them and prevent re-entry into the air. But it was discovered that Group 2
allergens, and perhaps Group 1s, exists in small particles that escape the vacuum cleaner bag and can then be inhaled deep into our respiratory tracks (Custovic et al., 1999).

A HEPA filter in a vacuum cleaner captures allergens and prevents their re-circulation into the air. These filters effectively capture small particles from the air including pollen, dust mite allergens and even smoke. The re-circulated air carries much less allergen. The acronym HEPA actually refers to the filters themselves, however vacuum manufacturers have different terms (HEPA vacuum, Allergy Vac, etc.) for models featuring HEPA filters. Findings show these filters can remove 50 to 85% of the dust mite allergen present in test areas, which included carpet, upholstered furniture and mattresses (Munir et al., 1993). In another study, HEPA/Allergy vacuum cleaners outperformed conventional ones (Hegarty et al., 1995). They also added that there was little difference between the different brand name machines featuring HEPA technology. The use of these vacuum cleaners has been found to improve patient health. A reduction in atopic dermatitis was recorded in a study where vacuum cleaning became a rigorous part of everyday life (Endo et al., 1997).

The efficiency of conventional vacuum cleaning versus water trap and central vacuum cleaners has been studied. In patient mattresses, they found a considerable reduction in mite allergen (up to 78%) with all three vacuum cleaners (Wickman, 1997). The negative aspect of this comparative study was that intense vacuuming was claimed to be required to achieve this level of control on the
mattress. Other work has demonstrated that intense vacuuming does not improve allergen capture. And a recent study found that short term vacuuming does not reduce allergen levels in the mattress significantly over the long term (Vichyanond et al., 2002). They vacuumed the mattresses at one month, then at four months and found that the allergen content was higher at four months and therefore the cleaning protocol was ineffective. They failed to consider that the surviving mites at the one month cleaning continued to reproduce and produce more allergen.

Steam cleaning is somewhat effective at controlling dust mites. A study on the effectiveness of this procedure found no mites alive after vacuuming and 86.7% reduction in allergen present (Collof, 1995). Reductions of 100% of the allergen present have been recorded, in addition to 100% mortality in mini-mattresses (Glass, 2002). Steam cleaning does leave the carpeted area warm and humid; two key elements that promote dust mite growth. That is why it does not seem to be an efficient approach for mattresses. Since previous studies only looked at re-infestation or allergen removal, we decided to test the efficiency of a HEPA vacuuming cleaner at removing dust mites from mattresses.

MATERIALS AND METHODS

Fifteen mini-mattresses measuring 53cm x 55cm and 29 cm in height were inoculated with dust mites and followed for twenty five weeks. These mattresses were similar in dimensions to the ones used in the mattress rotation study (Chapter 2), but the stitching pattern on the top was changed. A single, centrally located
stitching circle was used to facilitate sampling (Fig. A.6). The mattresses were placed in three wooden frames measuring 325cm in length, 104cm in width and 82cm in height. The surface on which the mattresses were placed was covered with the same fabric that is used to make box springs. To simulate the relative humidity conditions of a person occupying a mattress, a (Miatech Inc.) misting system was used. One mister was positioned over each mini-mattress at a height of 50cm. The misting system was programmed to dispense the appropriate water output. The output was checked on a weekly basis to ensure consistency. The misting system setup is shown on figure 3.1 and Appendix A.

Based on the challenges in the prior study, we made some adjustments to the design. The wooden frames were covered with a clear plastic sheet on the top and three quarters of the way down on the front, back and sides (Fig A.1). This aided in maintaining the relative humidity and minimized the impact of the overhead air ducts. The plastic mesh squares, measuring 5cm in thickness, that were used to receive the misted water had the fabric glued to them to ensure humidity preservation (Fig. A.4). This white fabric was a 50/50 cotton and polyester blend. Onset corporation HOBO (H8) temperature and relative humidity probes were used to monitor the conditions on the top and bottom surfaces of each mattress, on each block and in the room.

All fifteen mattresses were inoculated with 120 mating pairs of Dermatophagoides farinae Hughes from bulk laboratory cultures. Six 100 mm circles were drawn on the mattress surface (Fig. 3.2). Food (600 mg) and the mites
Figure 3.1: This is the wooden frame designed and built to house five mini-mattresses and their respective misters in an alternating pattern (mattresses are not shown).
Figure 3.2: This is the six circle pattern that was drawn on the mattresses so that we could inoculate them and later sample from them. These circles are 100cm in diameter. Here we can see where two circles were removed for counting.
were placed on each circle. The food consisted of a 3:1 mixture of powdered egg and yeast. One of the fifteen mattresses was used as a sentinel mattress to assess population levels so sampling could be optimized. The room was maintained at 24 - 26°C. During the winter months, three space heaters (The Holmes Group Inc.) were positioned to help maintain the temperature. One mattress from each block was chosen randomly on a daily basis to check temperature and humidity, data on all others was collected weekly. For the first seventeen weeks, the humidity on the mattress surface was maintained at about 75% for eight hours and under 50% for the remaining sixteen hours. For the last eight weeks the humidity was maintain at 75% for twenty-four hours per day. Seventeen weeks after inoculation, three randomly chosen circles from each of the fourteen mattresses were sampled, using a Filter Queen® HEPA vacuum cleaner (HMI Industries Inc.). These circles were vacuumed for one minute each and then cut out from the mattress. The top ticking, polyester and two foam layers were removed and placed at 4°C until they could be counted, along with the vacuumed samples. At this point, an additional 200 mg of food media was placed on each on the 42 remaining circles. Eight weeks later those circles were vacuumed, removed as previously described and stored at 4°C. All samples were eventually counted and the data was analyzed and compared overtime and by layer.
<table>
<thead>
<tr>
<th>Mattress #</th>
<th>sub-surface</th>
<th>vacuumed</th>
<th>total mites</th>
<th>%removed by vacuuming</th>
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<tbody>
<tr>
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Table 3.1. The total number of dust mites found in the vacuumed samples and the cut out portions of the mattresses are shown. The percent of dust mites removed by vacuuming from the total present mites at seventeen weeks is also shown. The subsurface counts refer to the cut out portions of the mattresses.

<table>
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<th>%removed by vacuuming</th>
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<tr>
<td>14</td>
<td>277</td>
<td>419</td>
<td>696</td>
<td>60</td>
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<tr>
<td>15</td>
<td>376</td>
<td>1372</td>
<td>1748</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 3.2 The total number of dust mites found in the vacuumed and cut out samples is displayed here along with the percentage corresponding to the vacuumed mites at twenty five weeks. The subsurface counts refer to the cut out portions of the mattresses.
RESULTS

The total number of dust mites recovered in the vacuum samples and the cut-out circles for both time points are shown in tables 3.1 and 3.2. These tables show the total mites for the vacuum samples, the cut out portion of the mattress and the percent of the total that were removed via vacuuming. The mites from the vacuum samples were counted using a flotation technique (Thind, 1979) and the cut out portions of the mattresses were examined under dissecting microscopy. During both sampling events all mite life stages were observed. Therefore these counts reflect all stages, including eggs. Mating pairs were observed as well as mite clusters (Glass et. al, 1998). The presence of mite clusters was linked with the observation of live mites after being stored at 4°C for two weeks. The top layer, the ticking, was examined under microscopy on both sides. The next layer: in the mattress is a fibrous polyester layer. All the mites present in this layer were counted and the number presented is for the entire area of the layer. The foam layer measures around 2.5cm in thickness. Even though the mites in this last layer could have been separated into top and bottom part of the layer, they were not. The reasoning was that this foam layer is semi-porous and therefore many dust mites could go into the foam itself. Those were counted and recorded but no possible advantage was seen in separating this count into three different numbers. The variation in the total number of mites present was considerable and it ranged from an average per mattress of 43 to 907 on the sub-surface cut-out portions. The distribution of the remaining mites in the mattress is represented in figures 3.3 and
Figure 3.3 and 3.4: The total number of dust mites per layer in the cut out portions of the mattress. The layers are the top ticking (TT), the bottom ticking (BT), the polyester layer (PL) and the foam layer (FL). For the last two layers, these counts include the total mites found on that layer.
Figure 3.5: The beginning of the experiment was marked by the inoculation of 240 mites. At seventeen weeks the total of mites remaining in the mattresses was 8725 and at twenty-five weeks the number was 17031.

3.4. The vacuumed samples also varied widely from an average per mattress of 121 to 7627. Vacuuming the mattress surface for a few minutes with a HEPA vacuum cleaner captured 75 percent of the dust mites. This type of intervention removes dust mites in the range of 55% to almost 90% of all mites, dead or alive. An ELISA technique was used to analyze the allergen (Der f 1) concentrations at seventeen weeks. Those results ranged from 3.493 to over 100 ng Der F1/ml/cm², with over half of them exceeding 10ng/ml/cm².
DISCUSSION

Based on prior HEPA filter work, we hypothesized that vacuuming would be very efficient in removing mites in the mattress setting. Unlike previous work, our goal was to quantify the number of dust mites removed rather than expect to remove all of them. This practice is rarely ever recommended probably because most people would not think to vacuum the mattress. However a removal of 75 percent of the dust mites present with a few minutes of vacuuming is advantageous.

The distribution of the remaining mites in the mattress was also recorded (Figs 3.3 and 3.4). Three mattress layers were cut out and analyzed for dust mites (Figs. A.7 and A.8). The mites that did remain in the mattress apparently did so because they were either trapped or the vacuum suction was not enough to remove them. Microscopic examination revealed many remaining mites in between strands of fabric or deep within the mattress itself. The under surface of the ticking had a very high number of eggs present and mite clusters. The variation in mite numbers in the different layers was far less than expected. Most of the dust mites were found in the thick and stringy polyester layer. Under the pressure of the mattress stitching, this layer is extremely thin probably a millimeter or two. But once the stitching is released the layer has a width of 2-3 centimeters. At seventeen weeks around 60 percent of the mites remain in the polyester layer (Fig 3.3). This layer seems to be a very suitable environment for these mites. It is very similar to the carpet environment, with fibers everywhere pressed together tightly. This layer retains humidity better because of its construction, according to our own data. At
twenty-five weeks the number of dust mites in the polyester layer was an impressive 75 percent. This suggests that over time the dust mite populations in this layer will increase. The graph on figure 3.5 shows where the initial 240 mites were placed and where the mites that avoided the vacuum cleaning went at seventeen and twenty-five weeks. This graph shows that the dust mites at the ticking and foam layers, barely increased in numbers throughout the experiment but the high numbers are consistent in the polyester layer. Besides dust mites showing an apparent preference for this layer, vacuuming removed most of the mites in the ticking layer as well as some from the polyester and foam layers. Therefore it cannot be assumed that the dust mites are present in the polyester layer merely by preference but rather by a combination of factors, one of which might be suitable habitat.

A phenomenon that was observed during the course of this experiment was unexpected. In the period of time post vacuuming, the samples were placed at 4°C until they could be analyzed. Samples were chosen randomly on a daily basis for a little over two weeks and counted. However two full weeks passed from vacuuming until the first sample was counted. After these two weeks in the fridge, live dust mites were encountered during counting. This was observed in the cut-out portions of the mattresses only and not in the vacuum samples. Initially all dust mites present looked dead. Counting particular circles varied in the time it took as some had more than 5000 mites. During this time it was observed that some of the dust mites slowly became active. We had expected that all mites would have been
killed by the prolonged cold but apparently some survived. The mites that were observed coming out of the temporary stupor were in clusters of more than 30 mites. Whether this represents a type of cold hardiness mechanism or just a benefit of the clustering behavior, more work is necessary.

A reasonable conclusion is that vacuuming of the mattress surface can remove about three-fourths of the dust mites and a significant portion of the allergen. Vacuuming prior to mattress rotation (Ch. 2) is an effective and inexpensive tactic for removing dust mites and their allergen. This tactic has the flaw that it relies 100 percent on patient compliance, which is less than desirable. Future work should test whether this intervention is as useful in the long term as it is in the short term.
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CHAPTER 4

Population growth of the American house dust mite, 

*Dermatophagoides farinae* Hughes in mattresses, under 
constant and varying relative humidity.

INTRODUCTION

House dust mites are encountered in a variety of climates. Presence and abundance of dust mites is influenced by those climates. *Blomia tropicalis* Bronwijk, Cock and Oshima is a tropical/sub-tropical species, while *Dermatophagoides pteronyssinus* (Trouessart) is encountered in humid and coastal areas. *Dermatophagoides farinae* Hughes and *Dermatophagoides microceras* Griffiths and Cunnington seem to be more predominant in temperate climates. 

Ambient relative humidity is the most important factor in determining the occurrence and abundance of house dust mites in an area (Arlian, 1992, Placido et al., 2001). High humidities are critical because the mites have a mechanism to actively uptake ambient water vapor. Given that they lack an internal respiratory system with external openings, these humidities help retain water.

Relative humidity is the limiting factor that affects their survival in the home environment. By lowering the ambient relative humidity in a house, the mite
populations and their allergen production can be reduced (Arlian, 1977). As the humidity drops below 50%; the mites desiccate and eventually die. Females are the most resilient active stage when it comes to desiccation. Active immatures are the most susceptible stages to low ambient humidity (Arlian, 1975, Brandt and Arlian 1976). Arlian (1999) found that lowering humidity from 85% to 75% caused mites to feed 75% less and consequently produce much less feces. Since the Group 1 allergen is associated with mite feces, less allergen will be released into the environment. A reduction in humidity causes a feeding reduction and decreased reproduction, which leads to diminished dust mite allergen.

In 1998, it was found that by exposing dust mite females to eight hours of 75 percent relative humidity instead of the constant 24 hours they receive in the laboratory cultures, 84% of them survive through twenty-eight days. Egg production was reduced to a third of what it would be at constant RH (Arlian, 1998). The impact of small reductions in relative humidity on dust mite populations is well documented (de Boer, 1997, Hart, 1998, Arlian, 2001). Exposing dust mites to hydrating (>50%) and dehydrating (<50%) conditions during the same twenty-four hour period, results in a greater percentage that emerges as males rather than females (Arlian, 1998). Probably operating the same way that quiescent tritonymphs emergence does (Ch. 1). Most likely there are not enough resources to molt into a female.

Knowing their vulnerability to relative humidity, we decided to study population growth in mattress. Being able to mimic the presence of a person on a
mattress (Ch. 2), we wanted to compare two different environmental conditions. In laboratory cultures, dust mites are usually reared under constant hydrating (~75%) relative humidity. In the home in the North Temperate Zone, they only have hydrating (>50%) humidity conditions when a person is occupying the mattress. Therefore we wanted to monitor population growth of dust mites under these two conditions in a mattress. First we ran the experiment with eight hours of hydrating relative humidity followed by 16 of desiccating conditions. The growth of the dust mite populations was compared with that of dust mites under twenty-four hours of hydrating conditions.

MATERIALS AND METHODS

The experimental setup and design was identical to that in chapter 3. This was part of the previous study. We used fifteen mini-mattresses measuring 53cm x 55cm and 29cm in height. These mattresses were similar in dimensions to the ones used in chapter 2, but the stitching pattern on the top and bottom was changed. The mattresses were placed in three structures measuring 325cm in length, 104cm in width and 82cm in height. The surface where the mattresses were placed was covered with the same fabric that is used to make box springs. To mimic the ambient relative humidity needed for the experiment, a (Miatech Inc.) misting system was used. One mister was positioned over each mini-mattress at a height of 51cm. The misting system was calibrated to ensure the appropriate water output, which was checked on a weekly basis to assure consistency.
To better maintain the ambient humidity, the three structures were covered with a plastic liner on the top and three quarters of the way down on the front, back and sides (Fig A.1). The plastic mesh squares, that received the misted water, were covered and glued to a fabric. This white-colored fabric was composed of a 50/50 cotton and polyester blend. HOBO (H8) temperature and relative humidity probes (Onset Corporation) were used to monitor the conditions on and around the fifteen mattresses. Inoculation was done over six spaced 100mm circles on the mattress top surface. We used 120 mating pairs of Dermatophagoides farinae Hughes from bulk laboratory cultures and placed them along with 600mg of food media, 20 pairs/circle. The mite food consisted of a 3:1 combination of powdered egg and yeast. Mattress fifteen was intermittently sampled to assess the population growth. The conditions in the room were maintained around 75% RH and at 24-26°C. Three thermostat controlled ceramic space heaters (The Holmes Group Inc.) helped maintain the room temperature during the winter months. One mattress from each block was randomly chosen on a daily basis to monitor its temperature and humidity. Data for the remaining fourteen were collected weekly. For the first seventeen weeks, the relative humidity on the mattress upper surface was maintained at about 75% relative humidity for eight hours/day. With the misting system off for sixteen hours, dehydrating conditions range from 30 to 40%. During the last eight weeks, the misting system maintained 75% relative humidity for twenty-four hours/day.
At seventeen weeks three circles from each mattress were chosen randomly and vacuumed (Filter Queen® HEPA vacuum cleaner, HMI Industries Inc) to remove the mites. The circles were then cut away from the mattress and inspected under dissecting microscopy for mite content. The procedure was repeated at twenty-five weeks with the remaining circles. All samples were stored at 4°C until they could be counted and analyzed. The data was analyzed and compared.

RESULTS

We found that the difference in dust mite numbers between the two periods was evident. For the first seventeen weeks when hydrating conditions were only maintained for eight hours a day, we recorded a total of 26,332 mites. This count includes eggs, larvae, both nymphal stages and adults. However, these stages were not counted separately since our objective was total numbers in the populations. Figure 4.1 shows the range of counts amongst the mattresses, which was significant. When the final sampling was done, we recorded 55,109 mites. This number also includes all life stages. Figure 4.2 shows the distribution of the counts per mattress at the end of the experiment, which also varied widely. Both sampling events were marked by the presence of mating pairs and mite clusters.
Figure 4.1: Total dust mites found in the mattress circles added together to get the total mite count per mattress. This growth represents seventeen weeks under eight hours of hydrating (75%) and 16 hours of dehydrating conditions (30→40%) and temperature between 24 and 26°C.

Figure 4.2: Total dust mites found in the mattress circles added together to get the total mite count per mattress. This growth represents twenty-five under twenty-four hours of hydrating conditions (75%) and temperature between 24 and 26°C.
Figure 4.3: Total dust mites found in the mattress circles. The 25th week sampling is super imposed on the 17th as to see what the additional growth was and overall totals.

DISCUSSION

At first examination the difference in the mite number is not striking. At seventeen weeks we had 26,332 mites and at twenty-five weeks we had 55,109. The difference between the counts is 28,777, which is very close to the seventeen week number. However, this growth happened in only eight weeks. Therefore the mites were able to reproduce in eight weeks under constant hydrating conditions faster than at eight hours of hydrating conditions during seventeen weeks. Faster reproduction rates at high humidity are expected. It has been observed that with four hours of hydrating conditions, the mites reduce their reproduction to a third of
that under constant hydration (Arlian, 1998). In spite of this, we wanted to see how this system operated on a mattress setting. As shown in figure 2.5, the ambient humidity diffuses through the mattress creating a humidity gradient and microhabitats. Data (Figs. 4.1 and 4.2) represents mattresses where the mites were able to take advantage of these suitable micro habitats and some where they did not. Individual circle counts in a mattress vary widely. It also appears that the mattresses with more dust mites stayed consistently so through the experiment (Fig. 4.3), except for number eight.

Several factors may be at work here including microhabitats, genetics and mating. The microhabitats created by the humidity gradient may be affecting reproduction in the different circles. Each mattress, top and bottom, was monitored but individual circles were not. Hence, there may have been humidity differences amongst the circles on a mattress, leading to the different mite counts.

Yet another factor could be mating status. Since we randomly inoculated the mattresses with twenty mating pairs, these could have been in their first or second mating. It was recently shown that egg production can decrease with a second mating (Alexander, 2002). Thus it is possible that second mated females were used. For high population circles continuous mating may have also occurred, leading to higher egg production (Alexander, 2002). There could also be unknown factors operating in this system, skewing the populations to one extreme or another.

One unexplored factor is migration rates. For example, we believe that mite movement in the mattress could explain a population number of 7,627 while the
next circle had only 348 mites. This phenomenon was observed on mattress number four. The mites seem to move toward one circle in the mattress and aggregate in larger numbers. Usually circles adjacent to high populations have very low population by comparison. This movement is likely to occur in a full-size mattress and therefore what appears as a sampling problem could be the mimicking of the real phenomena. Future work will investigate how often and how fast do the mites travel in the mattress surface, and whether that movement is guided towards food, environmental conditions, mates or whether it is random.
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APPENDIX A

FIGURES RELEVANT TO CHAPTERS 2, 3 AND 4

Figure A.1: The three wooden structures built to house the five mini-mattresses. Each wood frame was covered with clear plastic liner material on top and all sides.
Figure A.2: One of the fifteen misters used in the experiments. Although the mister has two units that dispense water only one was allowed to mist to create a circular water pattern covering the mattress surface.

Figure A.3: The hard plastic mesh and the white color fabric used to receive the misted water over the mattresses.
Figure A.4: The hard plastic mesh with the white color fabric glued to it.

Figure A.5: The misting system used for both studies.
Figure A.6: The difference in stitching patterns between the mini-mattresses for both studies.

Figure A.7: The three different layers that were cut out from the mattresses. On the left side is the ticking; on top right is the polyester layer and the semi-porous foam layer is on the bottom.
Figure A.8: The temperature and humidity probes used from the Onset Corp measured 4.5 x 6 cm.
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