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ENVIRONMENTAL CONTROL OF HOUSE DUST MITES:
STRATEGIES FOR LIMITING EXPOSURE TO HOUSE DUST MITES
AND THEIR ALLERGENS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctorate of Philosophy in the Graduate
School of The Ohio State University

By
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*****
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2002

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ABSTRACT

The purpose of this research were to improve the standard ELISA techniques used to quantify allergen exposure and to test novel control strategies both in laboratory and clinical studies aimed at lowering exposure to house dust mites.

Sample incubation time was found to impact allergen extraction efficiency. The greater than two-fold difference in Der f 1 allergen recovery may explain inter-laboratory variability for ELISA results. Dust samples should be incubated for 24 hours to optimize recovery and incubation time should be incorporated into the standard procedure for allergen quantification.

A significant challenge to understanding exposure to dust mites is the ability to quantify allergen in reservoir dust. This is in part due to a lack of a sufficient ELISA methodology with the sensitivity necessary to quantify airborne allergen levels. The sensitivity threshold for the standard ELISA for Der f 1 was reduced 50-fold using an amplified ELISA technique on small samples collected from the skin and hair coat of dogs. This improved sensitivity creates many opportunities for study of allergen exposure in both human and veterinary medicine.

Steam generated by a hard surface cleaner significantly reduced both mite and allergen levels after five passes over carpet and mattress test areas. The commercial label rate of permethrin (0.1% v/v) was verified to significantly reduce active dust mite populations as a residue on glass. The antimicrobial, Azoxystrobin, displayed no acaricidal activity when tested at 5.0% v/v and was non-synergistic with permethrin.

A double-blind placebo controlled clinical study on allergen avoidance was conducted in South Florida as part of a multiphase industry sponsored program. The study assessed the effectiveness and clinical benefits of commercial hot water extraction versus a consumer based cleaning. Benzyl benzoate was compared to
permethrin/Intercept for reducing house dust mites and their allergens in carpeted floors. Results indicated that both cleaning regimes reduced allergens, however, permethrin/Intercept and benzyl benzoate treatment of carpets failed to prevent allergen rebound and mite recolonization. No clinical benefits were noted in quality of life scores and spirometry among treatment group patients for the three month study period.

A double-blind placebo controlled study was conducted in the temperate climate of Columbus, Ohio. Active mite populations were sustained below established sensitization thresholds in both bedroom and living room carpets for one year following permethrin treatment and hot water extraction. Mite allergen levels were lower in permethrin treated living room carpets compared to controls, however these trends were not statistically significant. Allergen levels in bedroom carpet were not statistically significant from controls. Patients in the permethrin treatment group experienced improved lung function, possibly due to interventions. However, these trends were not statistically significant. The one year reduction in active mite populations from single a permethrin treatment is the longest reported to date of any environmental control study.

Overall, significant improvements were made in mite allergen quantification methods. The effectiveness of acaricides for environmental control of house dust mites is promising, however additional refinement of these strategies is needed to significantly improve the health and quality of life of allergic asthmatics.
Dedicated to my parents

Emmett and Anna
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xii</td>
</tr>
</tbody>
</table>

**Chapters:**

1. General Introduction
   - Asthma Prevalence: 1
   - Risk Factors in the Development of Asthma: 3
   - House Dust Mites and Allergic Disease: 4
   - Dust Mite Distribution and Ecology: 5
   - Mite Allergens: 8
   - Basis for Environmental Control: 9
   - Studies on Allergen Avoidance: 10
   - Chemical Agents: 11
   - Dissertation Overview: 13
   - References: 15

2. The effects of incubation time on the extraction of Der f 1 from household dust
   - Abstract: 26
   - Introduction: 27
   - Material and Methods: 28
   - Results: 29
   - Discussion: 31
   - References: 32
3. Detection of *Der f* 1 on the skin and hair coat of dogs using an amplified ELISA technique ................................................................. 33
   Abstract ........................................................................................................ 33
   Introduction .................................................................................................... 35
   Material and Methods .................................................................................. 37
   Results ............................................................................................................ 41
   Discussion ...................................................................................................... 45
   References ..................................................................................................... 46

4. Eliminating house dust mites and their allergens through high temperature treatment of textiles ....................................................... 50
   Abstract ........................................................................................................ 40
   Introduction .................................................................................................... 51
   Material and Methods .................................................................................. 53
   Results ............................................................................................................ 56
   Discussion ...................................................................................................... 59
   References ..................................................................................................... 61

5. Effects of permethrin and azoxystrobin on laboratory populations of the American house dust mite, *Dermatophagoides farinae* .......... 64
   Introduction .................................................................................................... 64
   Material and Methods .................................................................................. 67
   Results ............................................................................................................ 69
   Discussion ...................................................................................................... 80
   References ..................................................................................................... 81

6. Integrated management of house dust mite allergens: a feasibility study to determine the effectiveness of interventions in a high humidity climate ................................................................. 84
   Abstract ........................................................................................................ 84
   Introduction .................................................................................................... 86
   Material and Methods .................................................................................. 89
   Results ............................................................................................................ 93
   Discussion ...................................................................................................... 105
   References ................................................................................................... 108

7. A single permethrin treatment of carpeted floors reduces house dust mites and allergen in the homes of asthmatics ................................................................. 112
   Abstract ........................................................................................................ 112
   Introduction .................................................................................................... 114
   Material and Methods .................................................................................. 117
   Results ............................................................................................................ 120
   Discussion ...................................................................................................... 124
   References ................................................................................................... 130
LIST OF TABLES

Table  Page
3.1 Der f 1 recovery from skin and hair coat samples of 29 pet dogs......43
4.1 Mean mortality of American house dust mites, *Dermatophagoides farinae*, exposed to high temperature steam (97° C) treatment in medium cut pile nylon carpet and mini mattresses.................................................................57
4.2 Allergen reduction of Der f 1 allergen in medium cut pile nylon carpet compared to controls after high temperature (97° C) steam treatment..............................................................................................58
5.1 Mean percent mortality ± SEM of post-embryonic *D. farinae* life stages exposed to permethrin residues on glass at 96 hours........72
5.2 Mean percent mortality ± SEM of *D. farinae* adults exposed to azoxystrobin residues on glass at 96 hours.................................74
5.3 Mean percent mortality ± SEM of house dust mites exposed to permethrin and azoxystrobin in interaction assays indicating an antagonistic interaction by the addition of azoxystrobin to permethrin.................................................................78
6.1 Initial clinical characteristics of asthmatic patients used in this study (n=15).........................................................................................98
6.2 Summary statistics of living room allergen concentrations and percent changes from pre-interventions levels..........................100
6.3 Summary statistics of bedroom allergen concentrations and percent changes from pre-interventions levels..........................102
7.1 Home and clinical characteristics of patients enrolled in this study.........................................................................................121
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.1</td>
<td>Living room carpet Der f 1 allergen linear mixed-effects ANOVA model results</td>
<td>133</td>
</tr>
<tr>
<td>A.2</td>
<td>Bedroom carpet Der f 1 allergen linear mixed-effects ANOVA model results</td>
<td>133</td>
</tr>
<tr>
<td>A.3</td>
<td>Mattress Der f 1 allergen linear mixed-effects ANOVA model results</td>
<td>134</td>
</tr>
<tr>
<td>A.4</td>
<td>All site combined Der f 1 allergen linear mixed-effects ANOVA model results</td>
<td>134</td>
</tr>
<tr>
<td>A.5</td>
<td>Living room carpet mite count linear mixed-effects ANOVA model results</td>
<td>135</td>
</tr>
<tr>
<td>A.6</td>
<td>Bedroom carpet mite count linear mixed-effects ANOVA model results</td>
<td>135</td>
</tr>
<tr>
<td>A.7</td>
<td>Mattress mite count linear mixed-effects ANOVA model results</td>
<td>136</td>
</tr>
<tr>
<td>A.8</td>
<td>All site combined mite count linear mixed-effects ANOVA model results</td>
<td>136</td>
</tr>
<tr>
<td>A.9</td>
<td>Borg questionnaire linear mixed-effects ANOVA model results</td>
<td>137</td>
</tr>
<tr>
<td>A.10</td>
<td>Spirometry (FEV₁) linear mixed-effects ANOVA model results</td>
<td>137</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Effects of sample incubation time on HDM allergen (Der f1) extraction at 4 °C</td>
<td>30</td>
</tr>
<tr>
<td>3.1</td>
<td>Levels of Der f1 in samples collected from the skin and hair coat of 29 dogs</td>
<td>44</td>
</tr>
<tr>
<td>5.1</td>
<td>Mean percent mortality of <em>D. farinae</em>, exposed to six concentrations of permethrin in glass tubes at 96 hours</td>
<td>73</td>
</tr>
<tr>
<td>5.2</td>
<td>Probit analysis plot and calculated LD_{50} for female <em>D. farinae</em> from residual efficacy experiments</td>
<td>75</td>
</tr>
<tr>
<td>5.3</td>
<td>Mean percent mortality± SEM of adult <em>D. farinae</em>, exposed to varying concentrations of Azoxystrobin in glass tubes at 96 hours</td>
<td>76</td>
</tr>
<tr>
<td>5.4</td>
<td>Probit analysis plot and calculated LD_{50} for male American house dust mites exposed to varying concentrations of Azoxystrobin</td>
<td>77</td>
</tr>
<tr>
<td>5.5</td>
<td>Mean percent mortality± SEM of adult <em>D. farinae</em>, exposed to single and combined concentrations of Asoxystrobin and Permethrin in glass tubes</td>
<td>79</td>
</tr>
<tr>
<td>6.1</td>
<td>Mean square root transformed levels of house dust mite allergen in living room carpet before and after cleaning and application of BB and PI</td>
<td>99</td>
</tr>
<tr>
<td>6.2</td>
<td>Mean square root transformed levels of house dust mite allergen in bedroom carpet before and after cleaning and application of BB and PI</td>
<td>101</td>
</tr>
<tr>
<td>6.3</td>
<td>Mean± SEM Quality of Life scores by time within each treatment group pre-study and at 3 months post-study</td>
<td>103</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>6.4</td>
<td>Mean± SEM forced expiratory volume pre and post-study within each treatment.</td>
<td>104</td>
</tr>
<tr>
<td>7.1</td>
<td>Interaction plot for fourth-root transformed change in <em>Der f 1</em> in living room carpet</td>
<td>123</td>
</tr>
<tr>
<td>7.2</td>
<td>Interaction plot for fourth-root transformed change in mite levels determined from mite count assays for living room carpet</td>
<td>125</td>
</tr>
<tr>
<td>7.3</td>
<td>Interaction plot for square-root transformed changes in FEV$_1$ between treatment groups</td>
<td>126</td>
</tr>
</tbody>
</table>
Asthma Prevalence

Asthma is a complex disease that is characterized by a prolonged and fluctuating course punctuated by acute exacerbations. The National Asthma Education Program Expert Panel Report defines asthma as a lung disease with the following characteristics: (1) reversible airway obstruction (not completely in some patients, however) that resolves spontaneously or with treatment, (2) airway inflammation and (3) airway responsiveness to a spectrum of stimuli. The symptoms of the disease may include cough, wheezing, dyspnea and sputum production. Additionally, it may be associated with rhinitis, sinusitis, nasal polyposis and/or atopic dermatitis.¹

The American Lung Association estimates that 17.7 million Americans have asthma, including 5.6 million children.² Healthcare costs associated with this disease in 2000 were estimated at $12.7 billion.² Asthma prevalence in children is between 7% and 9.5%.³ Although less data are available for adults, prevalence is approximately 2.5%.⁴ In other studies, the sexual prevalence of asthma was higher at
7% in males and 5% in females. Worldwide asthma prevalence estimates encompassing all ages, ranges from 1.5% to more than 10%. Notable among these statistics is the increasing asthma prevalence in developed countries, especially the United States (up 29% from 1980 through 1987) Great Britain, Sweden, Finland, Norway, Switzerland, Denmark, New Zealand and Australia.

Morbidity and mortality associated with the disease have risen as well. It results in over 2 million pediatric visits annually. Additionally, hospitalizations have increased significantly in this country and abroad. In slightly less than 20 years, an astounding 200% increase in asthmatic hospitalizations has been noted in the United States. Similarly, in the period extending from 1964 to 1984, a ten-fold increase in asthma hospitalizations was noted in New Zealand. Increasing morbidity from childhood asthma is also reflected in school absences. In one study, 58% of parents reported their children to be absent from school because of wheezing, and 12% missed more than 30 days. In another study, 66% of children known to have asthma had school absence associated with their disease. In addition, to greater asthma morbidity, deaths from the disease have been increasing in the United States and globally. During the past decade, asthma deaths have increased over 6% in the United States. The increase in asthma mortality is highest among children, and in the United States the mortality is highest among African-American children of the inner city. Increased mortality has also been noted in the United Kingdom, Denmark, France, Germany, Canada, Australia, and New Zealand. An excellent summary of mortality figures, as well as other epidemiological data, has been published.
Risk Factors in the Development of Asthma

Stimuli for initiating the inflammation and airway hyper-responsiveness common to most asthmatics are both non-immunologic and immunologic. Non-immunologic excitants include viral infections, physical and chemical agents and exercise. Immunologic stimuli are primarily allergens. Atopy is defined as the abnormal production of IgE antibodies in response to exposure to environmental allergens as defined by elevated total serum IgE and/or positive skin prick test to common aeroallergens. Risks factors for the development of asthma have been thoroughly studied for house dust mite allergens and represent the most complete picture for causation of this disease. Three determinants of allergen specific immune responses are often considered when citing the importance of risk factors. These are: the particular susceptibility of an individual to allergens, the level of exposure and the immunological properties of the allergen itself.

The allergic response of an individual is genetically determined, although the exact pattern of inheritance is disputed. Researchers have known for many years that atopic diseases run in families. Analysis of the genetic causes of asthma have suggested dominant, recessive, co-dominant and polygenic models of inheritance for atopy and IgE levels. Genetic studies on twins have shown that IgE levels are higher for monozygotic rather than dizygotic twins. These studies also examined the contribution of environmental allergens to the causation of allergy and asthma and suggested that indoor allergens, specifically those of house dust mite origin, play a more important role than pollens. In addition, parental effects (imprinting) in the inheritance of atopy have been identified. One study noted an increased risk of atopy
in children whose mothers were atopic. Recent advances at the molecular level have identified chromosomal regions and gene candidates that include: genes involved in inflammation, effector molecules, immune recognition and regulation and genes that regulate lung development.

The major allergens associated with atopy and risk of asthma vary among communities according to climate, season and social factors. Exposure to indoor allergens in the etiology of the allergic asthma is more important than exposure to outdoor allergens because we spend more time indoors. Individuals in developed nations are estimated to spend as much as 90% of their time indoors. Indoor allergen exposure is thus perennial in nature with higher total exposure occurring compared to outdoor allergens such as pollens. In temperate and humid climates the greatest risk for asthma is associated with house dust mite exposure. Fungal Alternaria spp are the strongest risk factors in some arid climates, whereas in urban communities cockroach allergens are more important. Pet derived allergens, particularly cat (Fel d1) and dog (Can f1), are significant in a number of regions globally.

House Dust Mites and Allergic Disease

Dust mites are the single most important indoor allergen. Sensitization to dust mites occurs in 45% to 85% of asthmatics tested throughout the world. If mite concentration in house dust is greater than 100 mites/g of house dust, it is considered
a risk for the development of sensitization (development of IgE anti-mite allergen antibody) in allergic asthma. At 500 mites/g of house dust a significant risk factor exists for acute symptoms in most mite-allergic asthmatics.\textsuperscript{36}

Sensitization is a positive prick test with a wheal diameter of 5 mm or greater using an extract containing 20 to 70 $\mu$g of mite allergen per milliliter. Alternatively, if RAST testing is done to determine sensitization, a level of IgE antibody equivalent to or greater than 5 ng/ml should be considered positive.\textsuperscript{41} Exposure in early infancy is critical for sensitization. Studies of infants demonstrated that exposure to $<2 \mu$g $Der p$ 1/g fine dust in infancy caused neither sensitization to mites nor asthma.\textsuperscript{42} However of those exposed to greater than 10 $\mu$g/g in infancy, 50% become mite sensitized. The dose response relationship between exposure and sensitization was analyzed by meta-analysis of published studies.\textsuperscript{43} The results of this analysis indicated a significant correlation between cumulative exposure to allergen and the risk of sensitization.\textsuperscript{43} Therefore the risk for developing asthma in house dust sensitized children approximately doubles for every doubling of exposure level to mite allergen.\textsuperscript{44}

**Dust Mite Distribution and Ecology**

Two species of house dust mite from the family Pyroglyphidae, *Dermatophagoides farinae* and *D. pteronyssinus*, are a major source of indoor allergens in the United States as well as other countries.\textsuperscript{45} Both have worldwide distributions.\textsuperscript{46} The American house dust mite, *D. farinae*, is more resistant to desiccation and is the predominant species in temperate climates throughout the
The European house dust mite, *D. pteronyssinus*, is more abundant in coastal and higher humidity regions. *Euroglyphus maynei* has also been noted to have worldwide distribution. In tropical and sub-tropical climates the mite, *Blomia tropicalis*, is a known allergen producer.

For mites to effectively colonize a home at least three of four requirements must exist. First, suitable nests for dust mites must be present (e.g.: carpets, sofas, mattresses, pillow and bedding). Secondly, human occupation must be present, which guarantees an abundant food source. Third, for optimal growth homes must be kept close to the optimal temperature of 25-30°C. Due to changes in housing construction and the widespread use of central heating and cooling during the last fifty years most homes in developed countries are kept near optimal temperatures range year round. Fecundity and development time (generation time: egg to adult) are temperature driven. The fourth requirement, indoor relative humidity, is the primary factor that determines survival of dust mites within a home. Humidity influences the rate of population increase at sub-optimal conditions and is correlated with allergen production by mites.

Water balance and survival of house dust mites in the home are not dependent on the total amount of water in air but rather on the interaction between temperature and humidity. The mechanism by which house dust mites extract water from the air is by absorption using a hyperosmotic salt solution containing sodium and potassium chloride secreted by the supracoxal glands. When air humidity falls below the critical equilibrium humidity (CEH: the humidity at which water loss is equal to water gain) of mites, water evaporates from the supracoxal gland secretions blocking
the opening of the gland and preventing water loss. At humidity above the CEH, salt crystals deliquesce and the solution absorbs water from the air. The CEH for *D. farinae* is 70% RH and for *D. pteronyssinus* 73% RH at 25° C.

There are many residential factors that are associated with high mite numbers and allergen levels. The highest population levels are often found in the bed, but carpet is considered the largest reservoir primarily due to the greater surface area provided for mite colonization. Floor level influences the level the allergen present in homes. Ground floors have greater Group 1 allergen levels than higher stories. Floors overlaying concrete slab foundations are more supportive to mite growth. Homes on concrete slabs are much cooler (3-6° C), which increases carpet relative humidity by 10-15% RH above ambient room humidity. Accordingly, carpet microhabitat RH may be sufficient to support mite growth even though room air is too dry. In addition, poor air exchange rates often elevate indoor RH’s. Thus the construction of more energy efficient housing during the last three decades has resulted in tighter damper homes. In response to this trend, specially designed homes with mechanical ventilation acting to increase air exchange with the outdoor environment and lower indoor humidity was examined. Mechanically ventilated homes reduced both indoor humidity and mite populations, and in some cases eliminated mites entirely.

Lastly, the presence of carpeted flooring in the home increases mite allergen load compared to smooth floors. The concentration of dust mite allergen increases with carpet age. Furthermore, *Der p 1* levels in dust from carpeted floors were 6-14 times higher than that of smooth floors. The presence of a dog is directly correlated
with higher mite allergen levels in carpet than homes without pets. The difficulty in modifying household risk factors associated with higher mite numbers and allergen levels have led physicians to recommend the removal of carpet from homes.

**Mite Allergens**

During the past twenty years immunochemical studies have identified 16 groups of allergens associated with the genus *Dermatophagoides*. Two groups in particular are major mite antigens: Group 1 (Der *p* 1 and Der *f* 1) and Group 2 (Der *p* 2 and Der *f* 2) allergens. Group 1 allergens are cysteine proteases that are derived from the mite alimentary tract and are released into the feces at levels of 10 mg/ml (0.2 ng per fecal pellet). The group 1 antigens are 24 kd heat liable glycoproteins that elute rapidly from fecal pellets. Greater than 90% of mite sensitive patient sera contain IgE antibodies to Group 1 allergens. Both Der *f* 1 and Der *p* 1 have very similar N-terminal amino acid sequences and there is significant cross-reactivity within Group 1 and Group 2 allergens but not between the two groups.

Group 2 allergens are associated with the mite bodies themselves and have a homology with primate epididymal or insect molting protein. Der *f* 2 and Der *p* 2 IgE antibodies have a 90% sequence homology and almost completely cross react. Allergens in Group 2 are more resistant to changes in temperature and pH than group 1 allergens. Commercially available monoclonal antibodies exist for Groups 1 and 2 allergens for quantification in enzyme linked immunoabsorbancy assays (ELISA).
The remaining allergen groups are: enzymes (Groups 3, 4, 6, 8, 9 and 15),
cytoskeletal molecules (Groups 10, 11 and 16), lipid transport molecules (Groups 13
and 14) and others (Groups 5, 7, and 12).60,61

**Basis for Environmental Control**

The ideal treatment for chronic diseases, such as asthma, is prevention. If that
is not achievable than the next aim should be to induce disease remission. The
concept of allergen avoidance dates back to the 17th century and clinical studies to the
1920s.68,69 Kern was the first to advise patients suffering from dust allergy to limit
dust exposure.70 In the late 1920s, many asthma patients kept in specially designed
climate chambers witnessed marked improvement in their asthma.71 The inspiration
for these experiments was the observations of significant improvements of asthmatics
moved to sanatoriums. Reductions in symptoms and IgE levels to dust mite allergen
occurred when allergic individuals were moved from the humid Mediterranean
French coast and the plains of Italy areas to the Alps where mite numbers are low.72,73
Children admitted to a high altitude asthma center experienced improved airway
hyperresponsiveness and reduced peak flow variability.74 These improvements were
reversed when patients were returned to their normal domestic environments. A goal
of environmental control interventions is to recreate a low allergen setting at home
without relocation to such institutions. Patient education is a fundamental component
in this process. Low patient adherences to recommendations make implementing
environmental control strategies challenging.
**Studies on Allergen Avoidance**

Attempts to create low allergen environments in the home have varied in approach and degree of success. Studies have emphasized hypoallergenic barriers, laundry, carpet removal, high efficiency vacuum cleaning, steam cleaning, dehumidification and increased ventilation and acaricides.

Clinical studies on allergen avoidance have focused on either the bedroom and mattress areas, or carpets throughout the home but not both. As already mentioned, the bed is often considered the most important site of allergen exposure due to airway proximity to the allergen source and time spent while sleeping. Physical barriers were first investigated when it was discovered that mites infested mattresses in large numbers. In studies on mattress encasement, long term reductions (15 to 65%) can be achieved in the amount of allergen present in the bed. Results from four clinical studies employing mattress encasements in concert with other interventions have shown clinical benefits. The first study examined mattress encasements with weekly washing of bedding, carpet removal and steps to reduce humidity over a period of one year. Mite numbers dropped 100 fold and treatment group patients used less medication while spirometry and bronchial hyperresponsiveness improved. A second study compared benzyl benzoate treatment of mattresses against encasement. Acaricidal treatment did not lower mite allergen levels; however the encasement intervention group experienced a significant reduction in allergen levels and airway hyperresponsiveness that lasted for 12-months. Another study with asthmatic children examined acaricidal treatment and vacuuming of mattresses, furniture and carpet as well as mattress encasement. These treatments resulted in substantial
reductions in mite levels, improved symptoms and lower medication use over 6 months. Lastly, three intervention groups were compared in a clinical study with 45 adult asthmatics. The three parallel study groups received either HEPA air filters, mattress and pillow encasements, or both. The group that received encasements only experienced a large drop in mite allergen levels in the mattress but only the patients that received both encasements and HEPA air filter showed improvement in airway hyperresponsiveness.

Carpets are the largest allergen reservoirs in the home based upon surface area. For this reason, medical specialists often recommend carpet removal and replacing them with hard surfaces. However, allergic sufferers rarely observe this advice. Of the 20 published clinical studies on allergen avoidance, four out of the seven studies that have shown clinical improvement removed carpets from the home. The remaining three exhaustively treated or cleaned the carpets. However, the clinical benefit of carpet removal is disputed because in the four trials where clinical improvement was noted, the studies incorporated other interventions as well. Low compliance with carpet removal has led many researchers to consider acaricidal treatment of carpets.

Chemical Agents

During the last three decades, several chemicals have been evaluated for their ability to kill mites in culture. However, only a few chemicals have been examined in
detail these include: benzyl benzoate, tannic acid, pirimiphos methyl, insect growth regulators and natamycin. Commercially, benzyl benzoate and tannic acid have been used extensively during the past decade for mite control.

Benzyl benzoate was first developed in Germany as a combined cleaning agent and acaricide. An advantage of using benzyl benzoate is that it is non-toxic and is widely used as a preservative in human food. In one investigation, carpet cleaning solution containing 3% benzyl benzoate effectively eliminated mites after a three day incubation period on glass slides.86 Another study examined the effect of benzyl benzoate on concentrations of Der p 1 and Der f 1 in mattress and carpet dust samples collected at 10, 30 and 60 days post-treatment. A significant reduction of mite allergens was noted only in the treated carpets at 30 and 60 days post-treatment.87 The use of a moist benzyl benzoate powder applied to indoor carpeting and upholstered furniture, and an aerosol foam of the chemical applied to the mattresses and bedding resulted in symptomatic improvement in a group of 25 patients with asthma, rhinitis and/or eczema.88 A double-blind, placebo-controlled trial was conducted examining application of benzyl benzoate foam to mattresses and upholstery and moist powder to carpeting in homes of D. pteronyssinus-sensitive asthmatic patients.89 A statistically significant improvement in airway hyper-responsiveness in the treatment group was demonstrated. However, no differences were noted for medication scores.

Tannic acid is commonly used as a textile treatment to denature mite glycoproteins. The chemical is present in tea, which is consumed, in large quantities by some individuals. The phenol groups of tannic acid polymerize mite allergen
making it less allergenic and more hydrophobic.\textsuperscript{90} One study compared both tannic acid and benzyl benzoate treatment on reducing mite allergen levels in mattresses over a one year period. The tannic acid group showed a statistically significant drop in mite allergen only at day 14 and month 8 in spite of frequent reapplication of the chemical. There was also a significant decrease in bronchial hyper-reactivity, but only at month 8, in the tannic acid group.\textsuperscript{91}

Another agent employed as a house dust mite acaricide is primiphos methyl. A single application of this organophosphate insecticide was found to reduce \textit{Der p} 1 in carpeting by 73\% and in soft furnishings by 50\%. Allergen reduction persisted for 6 weeks but no clinical evaluations were conducted in this study.\textsuperscript{92} Insect growth regulators (IGRs) have been employed as a strategy for reducing house dust mite populations. Their main advantage is safety. Some of these agents have been moderately effective, but their duration of efficacy was less than 60 days and effective concentrations were costly.\textsuperscript{93} The antifungal, natamycin, which interferes with mite reproduction, was examined in a placebo-controlled clinical study of asthmatics. There were no statistically significant treatment effects between study groups for mite allergen levels and patient clinical scores.\textsuperscript{94}

\textbf{Dissertation Overview}

Asthma incidence has increased since the 1980s especially in developed countries. HDM allergen exposure is established as a significant factor in the development of this disease. People spend as much as 90\% of their time indoors making HDM allergen avoidance strategies of primary importance. The motivation
for this work was to improve the health and quality of life for asthmatics. Taking the prevention approach, strategies aimed at reducing exposure to HDM allergen were evaluated \textit{in vitro} and in clinical studies.

In Chapter 2, the standardized procedure for extracting HDM allergen from dust samples for ELISA analysis was evaluated and improved. The standard procedure requires incubation of samples overnight at 4°C. Concerns after the Third International Workshop on Indoor Allergens and Asthma prompted researchers to examine the ELISA protocol in more detail to explain the large inter-laboratory variability witnessed among research laboratories. This chapter examined the effects of incubation time on HDM allergen recovery and proposes that time is a factor that contributes to this variability. Adoption of this improvement would help standardize this critical step in the allergen quantification.

In Chapter 3, a more sensitive amplified ELISA for \textit{Der f}1 was developed that decreased the lower detection limit of the standard assay. The assay was tested on small samples collected from the skin and hair coat of dogs. This work represents the first report of HDM allergen on the surface of canines. The importance of the new technique both in human and veterinary medicine are discussed as well as the role that HDM allergen may play in the causation of canine atopic dermatitis.

In Chapter 4, high temperature steam generated by a hard surface cleaner was examined as a novel means to eliminate active mite populations and allergen in both carpets and mattresses. Advantages and disadvantages of employing this technique as a HDM avoidance strategy are discussed.
In Chapter 5, the residual effectiveness of permethrin and the anti-microbial, Azoxystrobin, was examined against life stages of *D. farinae*. The dose response for both chemicals was determined *in vitro*. The synergistic properties of the two chemicals were tested. The results of these experiments verified the effectiveness of the label rate of permethrin and were justification for later use of this concentration in subsequent clinical studies using the acaricide.

In Chapter 6, permethrin and benzyl benzoate were compared in a small 15 patient, industry-sponsored clinical study in South Florida. The aim was to test both acaricides and two different cleaning techniques in a climate that represents a worst-case scenario for HDM environmental control. HDM allergens and patient clinical symptoms were monitored over a three-month period. The knowledge gained from this project will be applied in the future design of a statistically relevant study.

In Chapter 7, permethrin treatment and hot water extraction of carpets were examined in a double-blinded placebo controlled clinical study in Columbus Ohio. Clinical symptoms and HDM allergen levels were monitored at bi-monthly intervals over one year. The significance of the reductions in HDM population levels and allergens and the potential of permethrin as an intervention in environmental control of HDM are discussed.

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CHAPTER 2

THE EFFECTS OF INCUBATION TIME ON THE
EXTRACTION OF Der f 1 FROM HOUSEHOLD DUST

ABSTRACT

Scientists at the Third International Workshop on Indoor Allergens in Asthma recommended a quality control program to address the variability of ELISA assays while quantifying house dust mite (HDM) allergens. Previous studies have examined the effects of temperature and buffer type on allergen recovery. The purpose of this study was to examine the effects of incubation time on allergen extraction efficiency. HDM allergen-containing dust was prepared from Dermatophagoides farinae bulk culture material, weighed into 100 mg samples and incubated in replicates of five for varying time intervals (8-24 hours). Results indicated that allergen recovery increased with time of incubation with more than a two-fold variability (p=0.0001, ANOVA), independent of extraction temperature and buffer type, observed according to time of incubation. The reported seven-fold variability witnessed between laboratories may in part be explained by differences in incubation time. We recommend that samples be incubated for 24 hours to maximize allergen recovery and reduce variability.
INTRODUCTION

Research laboratories throughout the world measure house dust mite (HDM) allergens in dust samples as a risk factor for the development of asthma. The standard method for extracting allergen is to incubate samples either in phosphate buffered saline (PBS) overnight at 4° C or mixing end over end at room temperature for two hours in an orbital rotator. After the Third International Workshop on Indoor Allergens in Asthma in 1995, scientists recommended that a quality control program address the issue of variability of results observed among research laboratories in ELISA assays used for quantifying HDM allergens. Round one demonstrated a 6 – 7 fold variability in Der p1. Second round two tests confirmed that the variability was attributed somewhat to differences in dust extraction temperatures. Laboratories extracting dust samples at 4° C reported 40% less allergen than those extracting allergen at room temperature.

Participating laboratories (n=20) in round two examined only one sample at each temperature and it was suggested that mean values determined from multiple samples between laboratories would be consistent. Round three sent 6 dust samples to 23 laboratories for testing. The results indicated an 11 to 84 fold variability that could be explained in part due to temperature and buffer type differences. A follow up study examined extraction efficiency among three commonly used buffered solutions: phosphate-buffered saline (PBS), borate-buffered saline (BBS) and ammonium bicarbonate buffer (ABB). Samples were extracted at two temperatures and two incubation times (2 hours at 24° C and 16-18 hours at 4° C). Results indicated that both extraction temperature and buffer type influenced the amount of

27

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Der p1 measured in dust. BBS recovered approximately twice the amount of allergen compared to PBS or ABB at both extraction regimes. Altering the ionic strength or pH had no effect on Der p1 levels. The effects of time on allergen extraction have never been examined.

Previously discussed studies have examined temperature and buffer type at two time points. The protocol for allergen extraction requires an orbital rotator to extract allergen for two hours. If this equipment is not present then laboratories will incubate samples overnight at 4 °C. The time that samples are incubated overnight is not precisely defined in the accepted allergen extraction protocol. The variability witnessed among laboratories in allergen recovery from dust samples may be attributed to the incubation time. The purpose of this study was to examine the effects of time on allergen recovery.

MATERIALS AND METHODS

Dust samples were prepared by obtaining approximately 5 grams of dead Dermatophagoides farinae culture. Fine dust (approx. 10 grams) was collected by vacuuming domestic dwellings in Columbus, Ohio and added to the dead dust mite culture debris. The combined reference dust was thoroughly mixed for 30 minutes using a hematology mixer (Fisher Scientific). Samples for allergen extraction were prepared by fine sifting extract dust through a 425 μm and a 50 μm nylon mesh filter. Forty-five 100 mg samples were weighed and incubated at 4 °C in PBS. Samples were replicated five times per incubation period. Incubation times were: 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours. After incubation, samples were centrifuged at 3000
RPM for 20 minutes, and immediately analyzed for Der f 1 content by a double monoclonal antibody ELISA method. Allergen content was represented as micrograms Der f 1 allergen per gram of dust. Statistical analysis was performed by analysis of variance (ANOVA) on log transformed Der f 1 data [Microsoft Excel].

RESULTS

Results of mean Der f 1 ± SEM values by incubation period are graphically depicted in Figure 2.1. The following mean allergen levels were recovered for each incubation time: 8 hours (76.66±4.0 µg/g), 10 hours (78.85±2.9 µg/g), 12 hours (87.51±8.3 µg/g), 14 hours (112.48±17.21 µg/g), 16 hours (112.04±18.5 µg/g), 18 hours (142.9±24.4 µg/g), 20 hours (170.0±20.0 µg/g), 22 hours (190±0.0 µg/g) and 24 hours (190±0.0 µg/g). Allergen recovery increased with length of incubation period. Greater than two-fold variability, independent of extraction temperature and buffer type, was noted between samples incubated for 8 hours and those 18 hours and longer. The two longest incubation times were above the detection threshold (190 µg/g) of the assay. Incubation time was statistically significant (p=0.0001, ANOVA).
Figure 2.1: Effects of sample incubation time on HDM allergen (Der f1) extraction at 4 °C. Extraction time had a statistically significant impact on allergen recovery (P=.0001, df=4, one factor repeated measures ANOVA [Microsoft Excel]).
DISCUSSION

Based on these results, there is a clear need for a global consensus on a standardized methodology for quantifying house dust mite allergen. Known sources of variability are extraction buffer type and temperature, which make inter-laboratory comparisons of allergen recovery results problematic. Other obvious sources of variability may be allergen standards and plate-to-plate differences among ELISAs. Published results on plate-plate variability with in labs are less than 10% and results obtained using different standards are very similar. The reported seven fold variation in Derp1 cannot be completely explained by temperature alone.

The results of this study show that extraction time is statistically relevant on Der f1 allergen recovery. The present protocol for allergen extraction requires dust samples to be incubated overnight at 4°C with overnight not being specifically defined. The differences in incubation time used across laboratories for allergen extraction may be contributing to the high variations witnessed among laboratories. A consensus should be reached on a standard procedure for allergen extraction that all laboratories should follow. Doing so should reduce the variability in HDM allergen analyses and enable multi-center comparison studies. We recommend that dust samples be incubated for 22 hours at 4°C to maximize allergen recovery and reduce variability. Since both Der group 1 HDM allergens are similar in their nature with respect to origin and protein structure, this recommendation should be applicable to Der p1 as well. Extraction time and temperature should be incorporated into a standard operating procedure for allergen quantification. Until a standard protocol is agreed upon, research laboratories should indicate in the methods section their dust extraction conditions.
extraction conditions. A future external quality control study examining standardized extraction time (24 hours), temperature (4º C), and buffered solution (PBS) would be necessary for the inclusion of these findings into a recommended protocol.

REFERENCES


CHAPTER 3

DETECTION OF Der f 1 ON THE SKIN AND HAIR COAT OF DOGS
USING AN AMPLIFIED ELISA TECHNIQUE

ABSTRACT

Objective: We hypothesized that house dust mite allergens are present on the skin and in the hair coat of dogs and this source of allergen may act as a reservoir for allergen exposure in hypersensitive dogs. Our objective was to document and quantify the level of the house dust mite allergen, Der f 1, in small skin and hair coat dust samples employing a new amplified ELISA technique.

Sample Population: Domestic canines

Procedure: Dogs were weighed and body surface area in square meters was determined. Skin and hair coat dust samples were obtained by vacuuming dogs. Collected dust was analyzed using both a standard and amplified ELISA technique.

Results: Results from the amplified ELISA indicated that Der f 1 was detected in 69% of dogs compared to 21% using the standard technique. Using an amplified technique, we were able to lower the detection limit of the standard assay to 0.01 ng/ml, a 50-fold difference.
Conclusions: This study is the first to report the presence of house dust mite allergen on the skin and hair coat of dogs. Identifying environmental levels of house dust mite allergens in the home and on the dog will help to identify the relationship between immunologic findings and environmental exposures in dogs with atopic dermatitis.

Clinical Relevance: Employing new methods to lower the detection limits of standard ELISA techniques for house dust mite allergens has many advantages in the study of allergic diseases in both human and veterinary medicine.
INTRODUCTION

Atopic dermatitis (AD) is defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features, and associated most commonly with IgE antibodies to environmental allergens.¹ The house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* are amongst the most common and important allergens in dogs with AD.²³ The veterinary teaching hospital at The Ohio State University has reported hypersensitivity to *D. farinae* in over 68% of dogs with AD.⁴ In humans, sensitivity to dust mites is frequently observed in patients with bronchial asthma and allergic rhinitis.⁵ Sensitization to dust mites occurs in approximately 51% of children with atopic dermatitis and 60% of adult asthmatics.⁶⁻⁷ Risk levels for human exposure to dust mite allergen have been proposed with levels greater than 10 μg *Der* Group 1 per gram of dust representing a significant risk for the development of asthma in atopic individuals.⁵

Variables for exposure to mites and allergen are the concentration or quantity of allergen in reservoirs in or around the home. Generally accepted techniques for estimating exposure to dust mite allergens include: mite counts, assays of specific mite allergens and measurement of mite fecal matter (guanine).⁸ Immunoassays are sensitive, specific and can produce quantitative results of a given allergen in absolute units. Monoclonal antibodies for mite allergen Groups 1 and 2 are commercially available. Allergens are generally measured from samples collected by vacuuming reservoirs of settled dust within the home such as: carpet, bedding, and upholstered furniture. Allergen quantification may be limited by the ability to collect sufficient amounts of dust, extraction protocol and sensitivity of immunochemical methods.
employed. In analysis of *Der* Group 1 allergens in small samples obtained from air sampling, allergen levels are generally below the detection limit of 0.2 μg/g or 10 ng/ml for the standard ELISA assay.\(^9\) Airborne mite allergen levels are commonly in the 30 pg/m\(^3\) range.\(^10\) Recently, an amplified ELISA technique was developed for *Der* Group 2 allergens to overcome this obstacle.\(^11\) The amplified technique increased the sensitivity of the standard ELISA for *Der* Group 2 fifteen fold lowering the detection limit to 300 pg/ml. These results indicated for the first time that a significant portion (20.6%) of *Der* p 2 was associated with small particles that were in the respirable range (1.1-4.7 μm). In this technique, an enzyme cycling system was used to amplify the colorimetric signal generated by an alkaline phosphatase label. Two enzymes, diaphorase and alcohol dehydrogenase, interconvert NADH into NAD with each turn of the cycle generating a colored formazan molecule.

The human scalp has been reported as a reservoir for house dust mites\(^12\), and the house dust mite allergen *Der* p 1 has been detected in human hair dust.\(^13\) We hypothesized that house dust mite allergens are present on the skin and in the hair coat of dogs and this source of allergen may act as a reservoir for allergen exposure in hypersensitive dogs. We sought to document the presence and quantify the level of the house dust mite allergen, *Der* f 1, in skin and hair coat dust samples collected from dogs. Further we sought to perform the amplified ELISA technique to quantify *Der* f 1 allergen that was below the detection limit of the standard ELISA assay.
MATERIALS AND METHODS

Dogs

Dust samples from the skin and hair coat were collected for this study from 2 groups of dogs. Dogs in group A were pets belonging to staff and students of the College of Veterinary Medicine, The Ohio State University, while group B dogs were pets presented to a private veterinary practice for boarding and grooming. The selection of dogs for inclusion into the study was made irrespective of the dog’s presenting problem, and irrespective of the individual characteristics (such as breed, age, and sex) of the dog.

Detailed questionnaires on the clinical background and history were completed for dogs in group A. The questionnaires obtained data on insecticides used on pets and in the home, frequency of dog bathing, the location and characteristics of the dog’s resting areas, and historical features of any chronic skin or ear disease. A diagnosis of AD was made according to accepted criteria.\(^{14}\) All dogs with AD had positive reactions on intradermal testing and/or allergen-specific IgE serology to allergens that were suspected or known to be in their environment, and which were consistent with the nature of the disease in the individual patient.

Dust Sampling Technique

Dogs were weighed and body surface area in square meters was determined using the standard equation: \(^{15}\)

\[
m^2 = (10.1 \times \text{body weight in grams}^{2/3}) \times 10^{-4}
\]
Based on the standardized protocol of vacuuming 1 m² for 2 minutes when collecting samples of dust for allergen identification and quantification from environmental areas, we calculated the time of vacuuming of each dog according to calculated body surface area. A dog with 1 m² of body surface area would be vacuumed for 2 minutes, and dogs with less than 1 m² body surface area were vacuumed for 12 seconds for each 0.1 m² body surface area. The entire body was vacuumed. Some dogs were lightly sedated with xylazine at 1.0 mg/kg IV, and atropine at 0.02 mg/kg IV to facilitate sample collection.

Skin and hair coat dust samples were collected using a Filter Queen® HEPA vacuum cleaner (HMI Industries Inc., Cleveland, OH). Samples were stored sealed in plastic bags at 4°C until the Der f 1 allergen ELISA was performed. The vacuum cleaner head and tubing distal to the filter were thoroughly cleaned and scrubbed in boiling water between sample collections.

**Standard Der f 1 ELISA**

House dust mite allergen (Der f 1) concentrations were determined using a standard ELISA technique. All collected samples were sieved through a 355 μm diameter filter to remove hair and 100 mg weighed and extracted in 2.0 ml PBS-T overnight at 4°C. For samples that weighed less than 50 mg, 1.0 ml PBS-T was added. For those samples between 50-100 mg the appropriate amount of buffer was added to make a 50 mg/ml sample dilution. ELISAs were performed as follows: Immunlon II microtiter plates were coated with 200 ng/well Der f 1 specific monoclonal antibodies (6A8: Indoor Biotechnologies, Charlottesville, VA) in 50mM

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carbonate-bicarbonate buffer, pH 9.6, at 4° C over night. Microtiter plates were then incubated with 100 μl of 1% BSA-PBS-T for one hour. Next, 100 μl of doubling serial dilutions from 250 ng/ml to 0.5 ng/ml of reference Der f 1 or 1:10-1:80 dilutions of skin and hair coat dust samples were added in duplicate wells and incubated for 1 hour. Three negative controls consisted of duplicate wells that lacked one of the following: primary antibody, secondary antibody, or Der f 1. Following incubation, wells were incubated with 100 μl 1/1000 dilution of biotinylated anti-Der f 1 monoclonal antibodies (4C 1-Indoor Biotechnologies, Charlottesville, VA) and allowed to incubate for one hour. Wells were then washed three times and coated with 100 μl 1/1000 dilution steptavidin-peroxidase solution and allowed to incubate for 30 minutes. Bound biotinylated-labeled antibody was detected by adding 100 μl 1 mM 2',2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)(ABTS) with the addition of 1 μl H₂O₂/ml ABTS. The reaction was stopped after 5 minutes with 100 μl of sodium azide and the optical density read using a microplate spectrophotometer (Model # MRX Revelation, Dynex Technologies Inc., Chantilly, VA) at 405 nm. Der f 1 allergen concentration was determined by interpolation from reference curves by StatLIA® Immunoassay Software (Brendan Scientific Corp., Grosse Point Farms, MI) and reported as ng of Der f 1 per ml sample. Indoor Biotechnologies assay standards were used and interassay coefficients of variation were typically under 15%.
**Amplified Der f1 ELISA**

Amplification ELISA was performed using the AmpliQ amplification kit (Dako Ltd, Cambridgeshire, UK) on samples that were negative for the presence of *Der f1* in the standard ELISA technique. Immulon flat bottom II plates were coated with 200 ng/well *Der f1* specific monoclonal antibody overnight at 4°C. Plates were washed three times and incubated in 1% BSA in wash buffer for 1 hour at room temperature. Wash buffer in this technique differed from the standard ELISA and consisted of tris-buffered solution with detergent containing 15 mmol/l sodium azide as a preservative. After an additional three washes, plates were incubated for one hour with 100 μl of doubling serial dilutions from 5 ng/ml to 0.01 ng/ml of reference *Der f1* or 1:10 and 1:20 dilutions of skin and hair coat dust samples. Three negative controls consisted of duplicate wells that lacked one of the following: primary antibody, secondary antibody, or *Der f1*. The plates were washed five times and incubated for one hour with 100 μl 1/1000 dilution of biotinylated anti-*Der f1* monoclonal antibodies (4C1-Indoor Biotechnologies, Charlottesville, VA). Dilutions were made with wash buffer instead of BSA-PBS-T as in the standard ELISA. PBS is known to inhibit the conjugate enzyme used in the AmpliQ kit and affect sensitivity. Wells were then washed three times, coated with 100 μl of a 1/1000 dilution streptavidin-alkaline phosphatase, and allowed to incubate for one hour. A final five washes were performed and then the AmpliQ amplification kit was used. One hundred μl of amplification reagent A followed by 100 μl of amplification
reagent B were added to each well. The plates were developed and read when the first standard reached an OD of 2.7 at 490 nm. *Der f* 1 allergen concentration was determined and reported as previously described.

**Statistical Analysis**

Regression analysis was performed to determine the correlation between recovered dust and the amount of *Der f* 1 present in samples.

**RESULTS**

A total of 29 dogs was sampled, with 16 dogs in group A for which historical and clinical data were available, and 13 dogs in group B. Mean weight for all samples collected from dogs was 37.42 mg with a range of 14-75 mg. One dog in group B had less than 10 mg of recoverable dust collected and was excluded leaving 12. In group A dogs with background data, the majority 11/16 (69%) were short hair breeds. The most common breeds were mixed 6/16 (38%) and greyhound 4/16 (25%). Only one dog spent a significant amount of time outdoors. Dogs spent the majority of their time indoors in dogs beds 5/16 (32%), on the floor 4/16 (25%) or couch 4/16 (25%).

Using the standard ELISA technique, *Der f* 1 allergen was detected in the skin and hair coat dust samples from 6/29 (21%) dogs (Figure 3.1; Table 3.1). The mean concentration of *Der f* 1 in the 6 positive samples was 16.16 ng/ml with a range of 5.61 – 31.24 ng/ml (Table 3.1). Negative samples were then retested for the presence of dust mite allergen using the amplified technique. An additional 14 dogs were
positive (Figure 3.1; Table 3.1). The mean level quantified was 0.36 ng/ml with a range of 0.19-2.20 ng/ml (Table 3.1). Combining both techniques, 20/29 (69%) of dogs tested positive for Der f 1. Regression analysis revealed no correlation ($r^2 = 0.009$, $p=0.68$) between the amount of dust collected and the level of quantifiable Der f 1.

Comparison of the historical and clinical data between dogs in group A, with and without Der f 1 in their skin and hair coat dust samples, did not reveal any differences between each sub-group. Routine use of insecticides on dogs for flea control was performed on 11/12 group A dogs with positive samples, and 4/4 dogs with negative samples. Fipronil was the insecticide used in most cases 12/16 (75%). The mean time since the last bathing prior to sampling was 3 weeks in dogs with positive samples, and 5.3 weeks in dogs with negative samples. Only one dog with a positive sample had AD and house dust mite hypersensitivity, whereas 6/12 (50%) dogs with positive samples had AD. Thus no association between the presence and the level of Der f 1 allergen in skin and hair coat dust samples and the presence of atopic disease was apparent.
<table>
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<th>Amplified ELISA Der f1 (ng/ml)</th>
<th>Recovered dust (mg)</th>
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Table 3.1: *Der f* 1 recovery from skin and hair coat samples of 29 pet dogs.
Figure 3.1: Levels of *Der f1* in samples collected from the skin and hair coat of 29 dogs. Employing the standard technique only 6/29 (21%) samples were positive. The amplified technique detected measurable allergen levels in an additional 14 samples. No trend was observed between the amount of allergen present with the amount of dust collected ($r^2 = 0.009$, $p=0.68$).
DISCUSSION

This is the first report of house dust mite allergen on the skin and hair coat of dogs. It has recently been reported that the major allergen of *D. farinae* for dogs is *Der f* 15\(^\text{18}\), and that *Der f* 1 is not a major allergen in this species.\(^\text{19-20}\) As such, it may be more important to examine skin and hair coat dust samples for *Der f* 15. However, detection reagents for this allergen are not commercially available. Despite the fact that *Der f* 1 is not the major allergen for dogs, we believe it is still relevant to quantify this allergen as a marker for mite exposure.

With the lower detection limit achieved in the amplified assay the potential of the dog skin and hair coat serving as a reservoir for exposure of house dust mite allergens must be considered. Recent evidence supports an epidermal route of allergen challenge and entry in canine AD.\(^\text{21-24}\) Three observations support this hypothesis. First, Langerhans cells are commonly seen in clusters and in greater numbers in atopic skin lesions in clinically normal atopic versus normal non-atopic dogs and Langerhans cells in lesional atopic skin exhibit membrane bound IgE. Secondly, gamma-delta T-cells are specialized lymphocytes in mucosal and epithelial immunity and canine lesional skin commonly express the gamma-delta T-cell receptor. Lastly, intact and degranulated eosinophils are only seen below the stratum corneum of lesional atopic skin. The documented presence of house dust mite allergens on the surface microenvironment on the skin of dogs are in support of hypotheses on the epidermal route of allergen entry. Levels of quantifiable mite allergen from the skin and hair coat of dogs in this study were similar to mean *Der p* 1 allergen levels (22 ng/ml) found in hair dust samples from the scalps of humans.\(^\text{12}\)
Identifying environmental allergen levels in the home and on the dog will help to identify the relationship between immunologic findings and environmental exposures in dogs with AD. An improved technique with lower detection limits creates many opportunities to understand allergic diseases in both human and veterinary medicine. Using an amplified technique, we were able to lower the detection limit of the standard assay for *Der f* 1 to 0.01 ng/ml, which is a 50-fold improvement. Compared to the standard ELISA that uses steptavidin-peroxidase as a label, colorimetric signal amplification was achieved using alkaline phosphatase and an enzyme cycling system. A similar technique could be developed with the appropriate sensitivity for *Der f* 15 to study exposure and sensitization in canines.

Understanding the clinical presentation and the mechanism of sensitization both in human allergic diseases and canine AD requires more knowledge on specific routes of allergen exposure. Although the exact clinical relevance of low levels of *Der f* 1 on the skin and hair coat of dogs is unknown, the amplified assay may provide a useful tool to experimentally test this question. Presently, we are conducting studies to determine the ecological relationships between house dust mites, their allergens, and dogs within the household microenvironment with emphasis on exposure and clinical manifestations.

REFERENCES


CHAPTER 4

ELIMINATING HOUSE DUST MITES AND THEIR ALLERGENS THROUGH HIGH TEMPERATURE STEAM TREATMENT OF TEXTILES

ABSTRACT

We evaluated the effects of high temperature steam generated by a hard surface cleaner on live-mite populations of American house dust mites, *Dermatophagoides farinae* Hughes and their allergens in carpet and mattress. To access mortality, carpet swatches and mini mattresses were inoculated with 50 adult mites and exposed to two steam treatment regimes. Statistically significant mite mortality (100%) in both carpet and mattresses exposed to either treatment regime was observed (P<0.05). The effects of steam treatment on *Der f 1* was examined by artificially seeding carpet with exhausted mite culture. A 100% reduction in allergen was observed with five passes and 61.4% with two passes of the hard surface cleaner. These results demonstrate the potential of using a hard surface cleaner as a novel method to eliminate house dust mite populations in a residential setting. Steam treatment of textile needs further testing before being recommended in allergen reduction strategies but appears particularly promising.

50

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INTRODUCTION

House dust mite (HDM) allergens are recognized as a major risk factor for sensitization and the development of allergic asthma in all areas of the world where the climate is conductive for mite population growth. As high as 85% of asthmatics show an antibody response to house dust mite allergen. Exposure to HDM allergens plays an important role in childhood sensitization and the doubling of HDM allergen level exposure doubles the risk of HDM sensitized children developing asthma.

Exposure in the household environment often occurs after disturbance of an area. These reservoirs are often carpets, soft furnishings and bedding. Group I HDM allergens are heat labile glycosylated proteins with cysteine protease activity produced by mites during digestion and released into feces. Group 2 allergens are nonglycosylated proteins that are more resistant to changes in pH and temperature. Evidence suggests that group 2 allergens originate from the male mite reproductive tract. The stability of HDM allergen under simulated natural condition has been examined. Neither heating of Der p1 to 60° C nor the storage of allergen samples for four years influenced allergen decay. Another study examined HDM allergen decay after exposure to varying temperature (15°, 20° and 25° C) and relative humidity (33, 55, and 75%). HDM mite group 1 allergens did not decay after being exposed to these conditions for 18 months. The inability of HDM allergen to degrade naturally shows that they are stable proteins in which the allergenicity of the initial protein remains constant over an extended period. Since HDM allergens decay slowly if at
all naturally, they tend to accumulate within indoor settings until removed. With the advent of central heating and cooling, dwellings are usually closed year around, which facilitates allergen accumulation to potentially harmful levels.

Allergen avoidance programs should combine strategies for mite extermination with allergen removal. Novel ways to physically remove or prohibit exposure to HDM allergens have had varying degrees of success. Prior allergen avoidance strategies have emphasized physical barriers on the bedding, “steam” cleaning and highly efficient particulate air filters to minimize exposure. The effectiveness of HDM physical control measures such as encasing mattresses with hypoallergenic barrier material and hot water extraction of carpet has been assessed. Encasing mattresses reduced Der f1 and Der p1 by 99% and improved bronchial responsiveness in mite sensitive asthmatic children when combined with immunotherapy. Domestic “steam cleaning” has been examined as a means to remove mites and allergen from carpet. Der p1 levels were reduced by 86.7% after steam cleaning carpets. Recently, a clinical study examined the effectiveness of commercial steam cleaning of carpets and a hot air (110° C) -steam treatment-hot air treatment of mattresses as a novel commercial based means to eliminate house dust mite and their allergens. The study reported a four-fold improvement in bronchial hyperresponsiveness. However, HDM group 1 allergens were above 2 μg/g in both active treatment groups at the end of the study. It is important to note that steam cleaning is a misnomer as temperatures employed during conventional steam cleaning are not 100° C but are closer to warm tap water (50° C) at the carpet surface. The thermal death point for dust mites is at or above 50° C.
thermal lethal limit, the temperature required to kill mites is similar between air and water, with mortality of mites exposed to 60° C occurring within ten minutes. The objective of this study was to determine the effectiveness of high temperature steam generated by a hard surface cleaner on eliminating house dust mites and their allergens in carpet and mattresses.

MATERIALS AND METHODS

Carpet Study

Mite mortality due to high temperature steam generated by the EcoVap®, a consumer based hard surface cleaner, was assessed in vitro in carpet. Mites from bulk cultures maintained at The Ohio State University were seeded into a black medium pile carpet. Two treatment groups consisted of two or five passes with the EcoVap® over a carpet surface in addition to an untreated control. Passes consisted of moving the steam attachment forward the length of the carpet and then back again. Three carpet sections measuring 30.48 x 96.52 cm were cut from a new carpet roll. The sections were thoroughly vacuumed using a Filter Queen® HEPA vacuum cleaner to remove dirt and debris. A 60-mm x 15-mm petri dish served as a template to draw a circle onto the carpet backing. A single-edge razor blade was used to carefully extract the 60-mm x 15-mm carpet sample from the larger carpet section. Then 50 adult American house dust mites were inoculated into each carpet core. Mites were inoculated using a fine (000) artist's brush. Each treatment group plus controls was replicated three times.
Once carpet cores were seeded with dust mites they were returned to the larger section of carpet and EcoVap® steamed with either two or five passes each. Control cores were inoculated, and then placed back into the larger section and removed promptly for mite counting. The heat escape method was employed to determine mite mortality in samples. Briefly, mite counting was performed by placing either control or steam treated carpet cores into 100-mm x 15-mm petri dishes covered with a Parafilm® lid with a hole covered by cigarette paper to allow for air exchange. Petri dishes containing the mite-inoculated carpet cores were inverted Parafilm® side down and exposed to an incandescent light source to drive living mites onto the Parafilm® surface where they were counted. This process was repeated three times until all mites were recovered. Mites were considered alive if in response to gentle brushing failed to move one body length. Mortality was represented as a mean ± standard error margin (SEM) for each group. A t-test was performed using StatView statistical software on the mean mortalities at a 95% confidence level to determine statistical significance between treatment types and controls.

**Mattress Study**

Mini-mattresses were constructed similar to a normal mattress consisting from top to bottom of: fabric ticking, foam padding, foam padding, and fabric ticking. Again there were two treatments consisting of two or five passes with the EcoVap® over the mattress surface, and an untreated control. Mattresses measured 10 inches square and were identically constructed. First, the center of each mattress was marked by tracing an inverted 100-mm x 15-mm petri dish with a marker. Next, 50
adult house dust mites were inoculated into the center of this circle on each mattress. The mattress was then steamed with either two or five passes. The controls were not exposed to steam. Three replicates per group were examined. The surface ticking of each mattress was first visually inspected and mite mortality was assessed via stereomicroscope. Then mattresses were disassembled and mite mortality determined on the foam padding using a stereomicroscope. Mortality was expressed as mean ± SEM. A t-test of proportions was performed on mean mortalities to determine statistical significance between treatment groups with a 95% confidence interval (Stat View SE, 1993).

Allergen Denaturing Study

The effects of the EcoVap® on HDM allergen, Der f1, were examined by treating carpet in the home of a volunteer. Reference dust consisting of dead mite culture was thoroughly mixed with fine dust collected from a vacuum cleaner bag. One meter square sections of carpet were marked and treated with two or five passes of the EcoVap® and an untreated control. The entire carpet surface was vacuumed with a HEPA Filter Queen® vacuum cleaner before inoculation of dust into carpet sections. Reference dust (500 mg) was then evenly distributed into each carpet section and worked into carpet using a carpet rake. Immediately after steaming carpet with the EcoVap®, the same vacuum was used to collect allergens on a specially designed allergen trap. The allergen trap consisted of a hinged chamber containing a filter disk and placed inline between the vacuum hose and a non-bristle vacuum upholstery attachment. This procedure was performed three times for treatment and
control groups. Allergen was extracted from dust in phosphate buffered saline with Tween 20 (PBS-T) overnight at 4°C, and then centrifuged at 3000 RPM for 20 minutes. The allergen containing fraction was then stored at -20°C until analyzed.

Allergen quantification was performed using a standard enzyme linked immunoabsorbency assay (ELISA). ELISA methodology was according to a standard procedure. Briefly, Immunlon II microtiter plates were bound with specific monoclonal antibody (Mab) (6A8: Indoor Biotechnologies). After washing plates with PBS-T twice microtiter plates were saturated with 100 μl of 1% BSA-PBS-T. Next, 100 μl of doubling serial dilutions from 250 ng/ml to 0.5 ng/ml of reference *D. farinae* or 1:10-1:80 dilutions of house dust samples were added and allowed to incubate for 1 hour. Following incubation, plates were washed 5 times with PBS-T and wells were coated with biotinylated specific Mab and allowed to incubate, then washed and coated with steptavidin-peroxidase solution. Bound biotinylated-labeled antibody was detected by using 2', 2'-azino-bis (3-ethyl-benzthiazoline) sulfonic acid as a chromogen and H₂O₂ as a substrate. The reaction was stopped after 5 minutes with 100 μl of sodium azide and the optical density read using a spectrophotometer at 410 nm. *Der f 1* concentration was determined by interpolation from reference curves and reported in μg/g of antigen.

**RESULTS**

Mortality of house dust mite exposed to either two or five passes of the EcoVap® are listed in Table 4.1. Both two and five passes over carpet caused 100% mortality of house dust mites. Both treatments were statistically significant from
untreated control mortality of 10% (P<0.05, t-test [Stat View SE, 1993]). The EcoVap® performed similarly on mattresses. Treatments with an EcoVap® on mattresses caused 100% mortality of house dust mites compared to 9.79% for untreated controls. This result was statistically significant (P<0.05, t-test [Stat View SE, 1993]). The dead mites observed in these experiments appeared turgid with their lateral dermal opisthonomal glands stained black. The results of high temperature steam treatment on HDM allergen in carpets are listed in Table 4.2. Two passes of the EcoVap® reduced Der f1 allergen in carpet dust by 61.04%. Five passes caused total elimination of HDM allergen (P<0.05, t-test [Stat View SE, 1993]).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>% Carpet Mortality ± SEM</th>
<th>% Mattress Mortality ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two passes w/ EcoVap</td>
<td>100 ± 0.0a</td>
<td>100 ± 0.0a</td>
</tr>
<tr>
<td>Five passes w/ EcoVap</td>
<td>100 ± 0.0a</td>
<td>100 ± 0.0a</td>
</tr>
<tr>
<td>Untreated Controls</td>
<td>10 ± 1.2b</td>
<td>9.79 ± 1.9b</td>
</tr>
</tbody>
</table>

Table 4.1: Mean mortality of American house dust mites, Dermatophagoides farinae, exposed to high temperature steam (97° C) treatment in medium cut pile nylon carpet and mini mattresses. Mean mortalities followed by different letters were significantly different (p<0.05, t-test [Stat View SE, 1993], n=3, 50 mites per replicate).
Table 4.2: Allergen reduction of *Der f*1 allergen in medium cut pile nylon carpet compared to controls after high temperature (97° C) steam treatment. Statistical significance is indicated by (*) from controls (p<0.05, t-test [Stat View, 1993], n=3).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Der f1 Concentration (µg/g)</th>
<th>Allergen Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two passes w/ EcoVap</td>
<td>0.21± 0.07*</td>
<td>61.04</td>
</tr>
<tr>
<td>Five passes w/ EcoVap</td>
<td>0.00± 0.0*</td>
<td>100.00</td>
</tr>
<tr>
<td>Untreated Controls</td>
<td>0.53± 0.10</td>
<td>N/A</td>
</tr>
</tbody>
</table>
DISCUSSION

House dust mite allergens are glycoproteins that are subject to breakdown at certain temperatures. A glycoprotein is simply a protein with a bound glucose or sugar molecule. To break down or denature allergens three approaches can be used: 1) high temperatures, 2) mildly acidic solutions, or 3) surfactants. The fact that high temperature will denature mite allergens is well documented. However, the means to deliver this heat in a cost-effective way has not been available. The EcoVap® provides a new, relatively inexpensive means to eliminate allergens and house dust mites on textile surfaces and mattresses. This dual effective makes the EcoVap extremely attractive for use in house dust mite management practices.

Results from in vitro experiments on carpet and mattresses, show the potential of high temperature steam as a novel method to eliminate house dust mite populations in a residential setting. The high temperature produced by the EcoVap (205° F) eradicated dust mites upon contact. In both experiments, not a single house dust mite survived the exposure to steam treatment. Two methods of treatment provided different effects on dust mites allergens. Two passes eliminated 61% of the allergen, where as, five passes eliminated 100% of the heat labile Der f1 allergen. Heat generated with the two passes was not sufficient to completely denature the allergen. The recommended treatment should be five passes with the EcoVap® over carpeted flooring. These results are applicable to house dust mite allergens present in mattresses as well.

High temperature steam generated by an EcoVap® has many advantages over conventional steam cleaning, mattress encasements and chemicals. Hot water

59
extraction is effective in removing surface allergens and killing mites in carpet if temperatures are sufficiently high. However, hot water extraction does not penetrate deep enough into carpet or furniture fiber where large mite populations exist and a significant portion of mites will survive. Residual water after hot water extraction may in fact promote mite and mold growth producing the opposite desired effect. In this experiment, treated textiles had minimal residual water and were completely dry after three hours of treatment. Since all mites were killed upon contact with the high temperature steam, concerns surrounding the brief increase in humidity are eliminated. Wet cleaning of fixed carpets does not kill nor remove all of the mites and populations can rebound within 1-3 months after cleaning. Evidence suggests that reinfestation occurs by passive dispersal of mites on infested clothing and scalp hair. Therefore, professional hot water extraction of the entire home should be performed 4-6 times per year, making this approach costly to the allergic individual. Steam treating carpets could be performed on a similar interval but at a fraction of the cost. The recommended strategy for mite control in mattresses is to encase bedding in a hypoallergenic barrier material. HDM allergen accumulates to harmful levels on mattress covers over time. Therefore, covers should be washed frequently to eliminate build up of allergens. Furthermore, there are few risks associated with employing high temperature steam. This is an important advantage over using acaricides as many HDM allergic individuals have chemical sensitivities as well. One risk associated with the equipment is mishandling or carelessness during operation, which could result in burns. However, when directions are followed the
machine can be safely used. Another concern is removal of stain blockers from textile surfaces. A recommendation to retreat fabric with stain blockers after using the EcoVap\textsuperscript{\textregistered} is advisable.

Ultimately, the success or failure of HDM environmental control strategies rests patient compliance. Education on the benefits/risks of each control strategy will improve compliance. Tailoring an individualized strategy that works best for each patient will improve adherence. In conclusion, the results from these experiments show that the EcoVap\textsuperscript{\textregistered} provided 100\% eradication of house dust mites in mattresses and carpeted flooring. Compared to other methods the steam heat provides a user friendly, safe and inexpensive means to control house dust mites and eliminate their allergens.

REFERENCES


CHAPTER 5

EFFECTS OF PERMETHRIN AND AZOXYSTROBIN
ON LABORATORY POPULATIONS OF THE
AMERICAN HOUSE DUST MITE, Dermatophagoides farinae

INTRODUCTION

Environmental control strategies for limiting exposure to house dust mites should have three goals: (1) reduce live mite populations; (2) control of reservoir allergens in beds, carpets, soft furnishings and clothing; and (3) reduce human exposure to both. Many factors must be considered when recommending a particular intervention strategy. These include the cost of the interventions to the patient, the ease of which it can be implemented, the safety of the chemicals employed, and the potential benefits to the patient. Numerous strategies have been examined that employ chemicals to kill mites, thorough cleaning to remove allergen, and physical manipulation of the home environment to reduce allergen exposure and mite populations; and these have been reviewed elsewhere.

Methods that have been proposed to kill mites include the use of extreme temperatures both hot and cold, steam cleaning, HEPA vacuuming, reduced humidity, and acaricides. The use of liquid nitrogen to freeze mites in carpet has been
examined; however this strategy is not practical and is expensive. Washing stuffed toys, bedding and clothing in hot water (>55° C) effectively removes allergen and kills mites. Domestic hot water extraction has been examined as a means to remove mites and allergen from carpet. Der p1 levels were reduced by 86.7% after cleaning carpets. Results of a recent clinical study examined the effectiveness of commercial steam cleaning of carpets and a hot air (110° C) treatment of mattresses as a novel commercial based means to eliminate house dust mite and their allergens. The study reported a four-fold improvement in bronchial hyperresponsiveness but group 1 allergens were above 2 μg/g in both active treatment groups at study conclusion. Vacuuming alone will remove only surface allergen and mites and may increase exposure if the vacuum has inadequate exhaust filtration. One study reported weekly vacuuming can reduce mite allergen levels by up to 48% when performed over a 5 week period. Reducing indoor humidity below 50% in temperate climates using dehumidifiers and air conditioning has been shown to reduce mite and allergen levels. However, in high humidity regions this method is unproven.

Acaricides employed for control of house dust mites either in laboratory or in clinical studies have included: benzyl benzoate, permethrin, disodium octaborate tetrahydrate (DOT), pirimiphos methyl, natamycin, caffeine, and insect growth regulators. Some studies have shown a benefit from benzyl benzoate application, whereas others have seen no benefits from its use. In the only clinical study employing permethrin, both active house dust mite population and allergen levels were reduced over a period of one year. DOT is marketed in the United States as a dust mite control product. Pirimiphos methyl, an organophosphate insecticide, was
shown to reduce dust mite allergen levels in carpeting by 73% and furnishings by 50%, but failed to persist longer than 6 weeks. This chemical is not marketed for dust mite control and is not registered for indoor use. Both natamycin and caffeine inhibit mite growth in laboratory experiments but neither are available commercially. Insect growth regulators (IGRs) have also been examined for control against house dust mites. Methoprene and hydroprene, which are both juvenile hormone mimics, were found to suppress mite populations by 98% and 89% after 30 days. However, activity was short lived and degraded after 90 days. The effective concentration of the IGRs (50,000 ppm) used was some 33-50 times greater than what is generally used for cat-flea control in homes.

Chemical control of house dust mites is often dismissed as an ineffective intervention in reducing allergen exposure among asthmatics due to both real and perceived risks of chemical exposure to acaricides. Permethrin provides a long-term control measure to reduce dust mite populations that requires less frequent reapplication compared to other currently available products. Azoxystrobin is a new synthetic broad-spectrum fungicide with activity against of variety of plant pathogenic fungi.

The objectives of this research were to determine the in vitro rate responses for permethrin and Azoxystrobin against mobile life stages of the American house dust mite, and secondly, to determine if Azoxystrobin enhances or decreases the residual effectiveness of permethrin against house dust mites. Additional research will examine the dose responses of these chemicals against house dust mites in carpet
with and without daily rigor. Residue analysis of permethrin treated glass tubes and
carpet employing gas chromatography will be performed to determine dose of
permethrin per surface area.

MATERIAL AND METHODS

Dose Response Assays

The glass shop at the Ohio State University uniformly cut glass tubes
measuring 10 mm in diameter and 2.5 cm in length. Tubes were cleaned using
phosphoric acid to removal all surface residues and then treated with either
permethrin or Azoxystrobin. Concentrations of permethrin and Azoxystrobin used in
dose response experiments were 5.0%, 1.0%, 0.50%, 0.10%, 0.05%, 0.01%.
Concentrations were prepared by serial dilution from technical-grade permethrin
(cis:trans ratio, 40:60; purity, 94.5%) and Azoxystrobin (97.8%, purity) obtained
from Syngenta Inc. HPLC grade hexane was used as a solvent for permethrin
dilutions and Azoxystrobin was diluted using acetone. Tubes were exposed to
permethrin and Azoxystrobin by dipping and then dried at room temperature on a
hematology roller before testing. Post-embryonic stages (larvae, nymph, adult male
and female) were exposed to varying concentrations of permethrin in glass tubes to
obtain a dose response for each life stage and LD50 were applicable. In the
Azoxystrobin experiment, only adults were tested. The tube ends were covered with
circular (10 mm) 40μm nylon mesh to prevent mite escape. Prior to inoculation,
mesh was treated with an identical concentration of test compound as the tube and
allowed to dry before being adhered to tubes with Duco® cement. Each mite stage
tested was replicated five times with ten individuals per replicate for each test concentration, plus solvent only controls. All mite transfer was done using a fine artist paintbrush. Glass tubes after inoculation were maintained in humidity chambers (75% R.H. and 22°C supersaturated NaCl)\textsuperscript{21} and mortality was determined at 96 hours post inoculation. Mites were scored dead if they were desiccated or failed to move one body length in response to gentle probing. Data were expressed as mean percent mortality ± SEM. A student test of proportions was performed to determine if treatment means significantly differed from controls. When a sufficient dose response occurred for a particular life stage probit analysis was conducted to calculate LD\textsubscript{50} using software provided by Dr. Hsin Chi (Laboratory of Theoretical Ecology, National Chung Hsing University, Taiwan).

**Interaction Assays**

The effect of Azoxystrobin on the activity of permethrin was examined to determine if any synergism or antagonism was occurring in a combined application. Glass tubes were treated as previously discussed with 5.0% Azoxystrobin, 0.05% permethrin and a combined 5.0% Azoxystrobin/0.05% permethrin treatment. Adult dust mites were exposed for 96 hours to these test concentrations and mortalities were calculated to determine the synergistic or antagonistic effect of Azoxystrobin on the ability of permethrin to kill dust mites. Expected mortality of the combined chemicals against dust mites was determined from the observed mortality of each individual chemical according to equation [a]: Expected mortality= O\textsubscript{a} + O\textsubscript{b} (1-O\textsubscript{a}).\textsuperscript{22}
The expected and observed values were analyzed by t-test with a synergistic or antagonistic interaction between permethrin and Azoxystrobin defined as a departure of the observed from the expected mortalities.

RESULTS

Dose Response Assays

The dose response data of house dust mites exposed to permethrin can be found in Table 5.1 and graphically depicted in Figure 5.1. The six permethrin concentrations tested were statistically significant (P<0.05; t-test [Microsoft Excel]) from controls for males, nymphs and larvae. Residual efficacy was greater than 90% for all concentrations tested for males, nymphs and larvae and 100% for the three highest permethrin doses tested (5.0%, 1.0%, and 0.5%). Female mortality decreased as the dose of permethrin decreased. Efficacy of females exposed to permethrin were above 90% for the three highest concentrations and then fell to 76.0% at 0.1%, 38.0% at 0.05%, and then 20.0% at 0.01%. The two weakest permethrin doses (0.05%, 0.01%) were both insignificant from adult female controls (P>0.05; t-test [Microsoft Excel]). Adult females were the only life stage that displayed a dose response to the concentrations of permethrin tested in this experiment. Therefore, no probit analysis was performed for males, nymphs and larvae. Probit analysis of the dose response of females calculated a LD$_{50}$ for permethrin at 0.1178% (Chi square value=19.99, heterogeneity factor=6.66, d.f.=3) as depicted in Figure 5.2.
Results on the residual effectiveness of Azoxystrobin are listed in Table 5.2 and graphically depicted in Figure 5.3. Only adults were tested in these dose response assays because results from the permethrin dose response experiments indicate males, nymphs and larvae act similarly with regard to acaricide exposure. Only one Azoxystrobin dose was significantly different from controls in this experiment. Male mortality exposed to 5.0% Azoxystrobin was 74.4% compared to 17.4% for controls a statistically significant reduction of 54% (P<0.05; t-test [Microsoft Excel], n=5, 10 mites per replicate). No Azoxystrobin only doses were statistically significant from controls (P>0.05). Since females did not display any residual efficacy in this experiment, a probit analysis to determine an LD_{50} to Azoxystrobin could not be performed. The calculated LD_{50} of males exposed to varying doses of Azoxystrobin was 4.39% (Chi square value=21.30, heterogeneity factor=5.32, d.f.=4) as shown in Figure 5.4. However, the results of the probit analysis should be interpreted with caution since it was based on one statistically significant dose.

Interaction Assays

A combined treatment of permethrin and Azoxystrobin was examined to determine if Azoxystrobin acted to enhance the acaricidal ability of permethrin against both adult life stages of house dust mite. The results of the dose response assays indicated that the maximum concentration of 5.0% Azoxystrobin should be combined with a 0.5% permethrin concentration. The efficacy results of the individual chemical tests as well as the combined chemicals are listed in Table 5.3.
and graphically depicted in Figure 5.5. Azoxystrobin tested individually was not statistically significant from controls for both adult stages (P>0.05; student t-test of proportions [Microsoft Excel]). Mortality of males (96.0±4.0) was similar to 0.5% permethrin as in the dose response assays. Female mortality (78.0±11.1) was 40% higher than the mortality observed (48.0±5.8%) in the dose response assay. However, the observed mortalities of males and females to permethrin were both statistically significant from controls (P<0.05; t-test [Microsoft Excel]). The expected mortality of adults to a combined Azoxystrobin and permethrin treatment was calculated from the observed mortalities using equation 1. The expected mortality for females was 80.56% and 96.93% for males respectively. Observed mortality of males exposed to a combined treatment was 48.0±5.8 % and 84.0±5.1% for females. A statistically significant antagonistic effect was observed in the combined treatment for females (P<0.05; t-test [Microsoft Excel]). The observed mortality was reduced by 32.56% from the expected mortality of 80.56%. The combined product displayed a neutral interaction on males reducing observed mortality by a statistically insignificant 12.93% (P>0.05; t-test [Microsoft Excel]).
<table>
<thead>
<tr>
<th>Stage</th>
<th>Control</th>
<th>5.0%</th>
<th>1.0%</th>
<th>0.5%</th>
<th>0.1%</th>
<th>0.05%</th>
<th>0.01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>34.4±5.7</td>
<td>92.4±3.4</td>
<td>96.0±4.0</td>
<td>90.0±3.2</td>
<td>76.0±5.1</td>
<td>38.0±9.7*</td>
<td>20.0±5.5*</td>
</tr>
<tr>
<td>Male</td>
<td>16.0±5.1</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>90.0±4.5</td>
<td>96.0±2.5</td>
</tr>
<tr>
<td>Nymph</td>
<td>13.7±5.2</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>98.0±2.0</td>
<td>100±0.0</td>
<td>94.0±2.5</td>
</tr>
<tr>
<td>Larvae</td>
<td>22.4±5.8</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>100±0.0</td>
<td>94.02.5</td>
<td>100±0.0</td>
<td>96.0±2.5</td>
</tr>
</tbody>
</table>

Table 5.1: Mean percent mortality ± SEM of post-embryonic *D. farinae* life stages exposed to permethrin residues on glass at 96 hours. Within a row, means followed by (*) were not significantly different from respective controls (P>0.05; t-test [Microsoft Excel], n=5, 10 mites per replicate).
Figure 5.1: Mean percent mortality of American house dust mites, *Dermatophagoides farinae*, exposed to six concentrations of permethrin in glass tubes at 96 hours (n=5, 10 mites per replicate).
<table>
<thead>
<tr>
<th>Stage</th>
<th>Control</th>
<th>5.0%</th>
<th>1.0%</th>
<th>0.5%</th>
<th>0.1%</th>
<th>0.05%</th>
<th>0.01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>20.0±4.4</td>
<td>24.7±2.9</td>
<td>22.9±9.7</td>
<td>21.8±2.5</td>
<td>20.7±4.9</td>
<td>21.0±7.0</td>
<td>14.5±2.3</td>
</tr>
<tr>
<td>Male</td>
<td>17.4±6.1</td>
<td>74.4±4.2*</td>
<td>26.0±3.5</td>
<td>26.0±5.1</td>
<td>29.7±1.3</td>
<td>29.3±7.8</td>
<td>29.6±6.7</td>
</tr>
</tbody>
</table>

Table 5.2: Mean percent mortality ± SEM of *D. farinae* adults exposed to Azoxystrobin residues on glass at 96 hours. Within a row, means followed by (*) were significantly different from respective controls (P<0.05; t-test [Microsoft Excel], n=5, 10 mites per replicate).
Figure 5.2: Probit analysis plot and calculated LD$_{50}$ for female American house dust mites from residual efficacy experiments. Calculated LD$_{50}$ was 0.1178% permethrin (Chi-square value=19.99, heterogeneity factor= 6.66, d.f.=3).
Figure 5.3: Mean percent mortality± SEM of adult American house dust mites, *Dermatophagoides farinae*, exposed to varying concentrations of Azoxystrobin in glass tubes at 96 hours. (*) indicates a significant difference from respective controls (P>0.05; t-test [Microsoft Excel], n=5, 10 mites per replicate).
Figure 5.4: Probit analysis plot and calculated LD$_{50}$ for male American house dust mites exposed to varying concentrations of Azoxystrobin. Calculated LD$_{50}$ was 4.39% Azoxystrobin (Chi-square value=21.30, heterogeneity factor= 5.32, d.f.=4).
<table>
<thead>
<tr>
<th>Stage</th>
<th>Control</th>
<th>5.0% AZ</th>
<th>0.05% P</th>
<th>5.0% AZ + 0.05% P</th>
<th>Calculated Expected Mortality</th>
<th>Interaction (observed - expected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>22.0±4.9</td>
<td>11.7±5.7</td>
<td>78.0±11.1*</td>
<td>48.0±5.8*</td>
<td>80.56</td>
<td>-32.56*</td>
</tr>
<tr>
<td>Male</td>
<td>24.0±2.5</td>
<td>23.3±6.0</td>
<td>96.0±4.0*</td>
<td>84.0±5.1*</td>
<td>96.93</td>
<td>-12.93</td>
</tr>
</tbody>
</table>

Table 5.3: Mean percent mortality ± SEM of house dust mites exposed to permethrin and Azoxystrobin in interaction assays indicating an antagonistic interaction by the addition of Azoxystrobin to permethrin. Within a row, means followed by (*) were significantly different from respective controls or represented a significant interaction (P<0.05; t-test [Microsoft Excel], n=5, 10 mites per replicate).
Figure 5.5: Mean percent mortality ± SEM of adult American house dust mites, *Dermatophagoides farinae*, exposed to single and combined concentrations of Azoxystrobin and Permethrin in glass tubes at 96 hours (n=5, 10 mites per replicate).
DISCUSSION

Results from the dose response assays clearly demonstrate the *in-vitro* ability of permethrin to reduce house dust mite populations. The dose response results indicate that the effectiveness of permethrin on the various life stages is subject to relative size and surface to volume ratios. The adult females were the most resistant to permethrin treatment and are the life stage with the largest surface to volume ratio. This is in agreement with the results of previous work examining the responsiveness of dust mites to acaricides. A dose response was not obtained for males, nymphs and larvae at the concentrations of permethrin tested. Therefore, the role surface to volume ratio plays in the responsiveness of these life stages to permethrin could not be determined. The dose response results confirm that a concentration of 0.1% would significantly reduce active house dust mite populations and are justification for examination of this acaricide in clinical studies on allergen avoidance. The results of the residual effectiveness of Azoxystrobin in the dose response assays indicated that the chemical has no acaricidal properties. The high mortality observed in males exposed to 5.0% Azoxystrobin can likely be explained by handling during inoculation. Results in the interaction assay of 5.0% Azoxystrobin against males confirm this notion. The efficacy of a combined permethrin/Azoxystrobin treatment indicated that the residual effectiveness of permethrin is diminished by combining these products together in a single treatment. A possible explanation for this is that Azoxystrobin covered permethrin lowering exposure to permethrin. This result is not desirable as a higher dose of permethrin would be necessary to effectively kill dust mites in a combined product.
Additional research will examine the dose responses of these chemicals against house dust mites in carpet. Treatments will employ precision spray equipment to accurately and uniformly deliver precise doses per unit area of carpet. Residue analysis of permethrin treated glass tubes and carpet employing gas chromatography will be performed to determine dose of permethrin per surface area. This will be achieved to determine what concentration and application rate will provide adequate mite reduction on textiles. The results of this work will be further tested in a real world pilot study and combined with information gained from the results of Chapters 6 and 7 to conduct a large clinical study both in a high humidity and temperate climate.

REFERENCES


CHAPTER 6

INTEGRATED MANAGEMENT OF HOUSE DUST MITE ALLERGENS: A FEASIBILITY STUDY TO DETERMINE THE EFFECTIVENESS OF INTERVENTIONS IN A HIGH HUMIDITY CLIMATE

ABSTRACT

This research was undertaken at the request of industry partners as a multiphase program to evaluate the effectiveness of reducing allergen exposure as an overarching strategy to manage asthma. The idea was to combine allergen removal procedures (either commercial truck-mounted or consumer-based equipment) with chemical treatment to control house dust mites and mold in the challenging high-humidity environment of South Florida. Fifteen dust mite allergic asthmatics living in the Ft. Lauderdale area were enrolled into a double-blind, placebo controlled study with three groups of five patients.

Homes were subjected to two different cleaning methods followed by application of active ingredients to control mites and/or mold. Both treated groups received allergen barriers on bedding, HEPA vacuums and HEPA whole-house air filters. Treatment group one received a consumer-based wet vacuum cleaning of bedroom and living room carpets followed by a benzyl benzoate treatment on those
same areas. The other treatment group received a commercial steam cleaning followed by a permethrin and Intercept application of carpeted surfaces. Group three was a sham-cleaned placebo. Dust mite allergens and mold spore presence were assessed pre and post-cleaning/treatment and monthly over ninety days and compared to sham cleaned/treated controls. Clinical patient monitoring was conducted to determine if an integrated approach influenced asthma symptoms.

Hypoallergenic cleaning reduced mite allergen levels by 98%. However, a substantial allergen rebound was observed after cleaning in both treatment groups. Allergen levels for dust mites in both treatment groups were lower than controls for the duration of the study but were above established sensitization thresholds. No statistically significant improvement in clinical outcomes was observed due to the short duration of the study and small sample size. At the outset it was anticipated that the combination of patient variability and chronic nature of asthma would make statistically significant symptom improvements unlikely. Most importantly, we wanted to evaluate the cleaning strategies side-by-side and test the ability of active ingredients to prevent allergen production by mites and/or molds. Future studies will assess the clinical benefits of employing these techniques over a longer period with a larger patient cohort.
INTRODUCTION

Houses can accumulate large quantities of mite, mold and pet allergens in the dust of beds, carpets, and soft furnishings. All of these sites function as potential reservoirs for allergens. Exposure to indoor allergens is considered more important than exposure to outdoor allergens based on where people spend the majority of time. Indoor exposure is often perennially high and increases with the housing modernization typical of developed countries. House dust mites represent the greatest risk for asthma in temperate and humid regions in the United States. In more arid climates the strongest allergy risk factor is the fungal species Alternaria.

Many studies have demonstrated the relationships between the risk of sensitization and the levels of allergen exposure. A dose response relationship between mite allergen and sensitization has been established at 2 μg Der Group 1 allergen per gram of dust, or 100 mites/g being important risk thresholds for asthma. At 10 μg/g of Der Group 1 allergen/g of dust and/or 500 mites/g the risk for asthma symptoms in dust mite sensitive individuals is very high. The role of exposure in the progression from sensitization to established asthma is not well defined by the medical community. In certain susceptible individuals investigators hypothesize that there is no safe minimum level of exposure. Scientific evidence suggests that disease severity increases with exposure to dust mites.

Asthma is a chronic respiratory condition characterized by airway inflammation and intermittent episodes of bronchospasm that can be provoked by a variety of stimuli. The American Lung Association estimates that 17.7 million Americans have asthma, including 5.6 million children. Asthma is recognized as the...
most important chronic disease of children in the U.S. Poor children are 40% more likely to be hospitalized for asthma than other children and they experience more frequent symptoms.\textsuperscript{6} Healthcare costs associated with this disease in 2000 were estimated at $12.7 billion.\textsuperscript{5} The trend for asthma treatment has been to rely more on pharmaceutical solutions as reactive rescue remedies. Despite these advances, the national morbidity and mortality rates are not improving. In view of these statistics implementing safe environmental control measures that minimize exposure should be a priority especially within the household.

Environmental control should be considered as the first line of treatment for asthma and allergic rhinitis. The goals for such strategies should be to prevent disease onset and induce remission. Intervention tactics should reduce live mite populations, reduce mite allergen levels and decrease human exposure to both.\textsuperscript{7} Numerous chemical and physical control methods have been examined with varying degrees of success.\textsuperscript{1,7,8} The American Thoracic Society recommends the following as effective mite control methods: weekly vacuum cleaning of carpets and upholstered furniture, removal of wall-to-wall carpeting, applying zippered plastic covers on mattresses, pillows, and box springs, weekly washing of bedding and soft toys in hot (\textgreater130°F) water, removal of curtains, stuffed toys, and books from the bedroom, and maintaining absolute humidity below 7g/kg or 50% RH at 72°F.

Prior research has suggested that carpet acts as a reservoir or culture medium for dust mites that affect airborne concentrations of house dust mite allergen.\textsuperscript{9} These findings have been widely incorporated into the advice given to asthmatics by recommending carpet removal.\textsuperscript{1,7} However, the clinical benefits of carpet removal
have not been demonstrated. The role of carpets as either an allergen sink or source for exposure should be determined before carpet removal is included in the general strategy of allergen avoidance strategies.

Vacuuming alone is ineffective against dust mites. Our operating paradigm is that when carpets are not removed then a hypoallergenic cleaning should be employed followed by chemical treatment to kill mites. Several methods have been proposed for controlling dust mites in fabric-based carpet, furniture and bedding. They include using high and/or low temperature, reduced humidity, using encasements and applying various acaricides.¹

Permethrin is a widely used home and garden insecticide that is effective against a variety of pests in or around the home and is also an effective acaricide. Intersept® is a broad spectrum anti-microbial that is marketed and distributed by Interface Research Inc for the control of indoor mold and mildew. Benzyl benzoate has been widely tested in controlled studies with HDM sensitive asthmatics. Products containing this active ingredient have been sold in North America for the last decade. The objectives of this study were, first, to test the effectiveness of two different cleaning processes to remove mite allergens from carpets in the homes of asthma patients. The second objective was to test and compare the effect of permethrin and Intersept® versus benzyl benzoate to a placebo control group, on dust mite allergen rebound and mold colonization following cleaning. The target was to decrease allergen to below the 2 µg Der group 1 allergen per gram of dust. The third
Objective was to determine the effect of treatment on quality of life measures and spirometry readings. Lastly, we wanted to test these control measures in a high humidity environment that is favorable for mite and mold growth.

MATERIALS AND METHODS

Patient Selection

In a double-blind, placebo controlled study patients were selected from the clinical practice of the Cleveland Clinic Florida, Ft. Lauderdale. Both male and female subjects were included and their ages ranged from 18-79 years. Inclusion in the study was based on three positive criteria: 1) asthma symptoms; 2) positive skin test for dust mite extract; and 3) presence of dust mites in their carpet. Patients with a history of asthma symptoms for at least one year occurring weekly were contacted after responding to an advertisement in the Ft. Lauderdale Sun Sentinel or posters in the clinic. Hypersensitivity to HDM allergen (Der p 1/f 1) was determined by an immediate epicutaneous skin prick test with dust mite standardized extract. Prior to study intervention, the Acarex® test kit was tested to estimate how well it corresponded to allergen levels. The kit is semi-quantitative and tests for the presence of guanine the major excretory product of mites. As a prescreening step, bedroom carpet was vacuumed and the dust tested using the kit. Those containing a screening level of medium or high were enrolled. Those not meeting this criterion were excluded. Patients were excluded if diagnosed with lower respiratory tract
disease other than asthma and with upper respiratory disease other than allergic rhinitis and sinusitis, or any medical or surgical disease that would reduce compliance. Patients who smoked were excluded.

**Clinical Assessment**

A physician examined patients at baseline and three months. Symptoms and medication uses were recorded. Daily peak flow readings (AM and PM) were requested for inclusion in a diary. Spirometry (FEV₁) and Borg quality of life questionnaires (QOL) were performed at baseline and the end of month three. The potential for confounding effects due to occupational and environmental allergen exposure were not controlled nor was medication use.

**Experimental Design**

Fifteen patients were enrolled who met the specific clinical criteria for enrollment and whose carpet was positive. The study consisted of three study groups of five patients each. Patients were sequentially assigned into a one of the three study groups according to their bedroom carpet (BRC) allergens levels from highest to lowest. This was determined via ELISA analysis of collected BRC dust for *Der* group 1 mite allergen. Study group one consisted of benzyl benzoate (BB) treatment of living room carpet (LRC) and BRC following by cleaning with a Bissell ProHeat® wet vacuum of these areas at baseline. Hypoallergenic mattress barriers were installed on the mattress and a 3M Filtrete Ultrallergen filter® was placed into the central heating and cooling system. Patients were given a Bissell Lift-off® HEPA
vacuum cleaner and were instructed to vacuum carpeted areas weekly. Patients enrolled into study group two received LRC and BRC cleaning with a truck mount system by a professional cleaning company at baseline. After cleaning, the LRC and BRC of group two patients received a Permethrin/Intersept® (PI) treatment and patients were given a Bissell Lift-off® HEPA vacuum with instructions for weekly use. Microklean filters® supplied by American Air Filter Corporation were fitted onto the central heating and cooling system and mattress were encased with hypoallergenic barrier material. The third patient group was a placebo control (PC) that received a sham cleaning at baseline. Designing a sham cleaning was an important component of the study. Patients were to believe they were receiving a thorough cleaning followed by some active ingredient to manage allergen production. For our sham cleaning, talc powder was applied and raked in similarly to the benzyl benzoate treatment. The suction was disconnected from the Bissell Lift-Off® vacuum so mite allergen would remain.

Dust Sampling and Allergen Quantification

Dust samples were obtained pre- and post-cleaning, and at 1, 2, and 3 months from the LRC and BRC. Dust was collected by vacuuming 1 m² for two minutes using a Bissell Liftoff® HEPA vacuum with a special dust collection device (Fussnecker Corp, Springfield OH) that holds a cotton fabric filter to catch debris. Samples were placed into Ziploc® bags for shipment to Ohio State University, then were transferred to Falcon® 50 ml conical tubes and stored at 4°C until assayed. Samples were not analyzed when the total quantity of dust was less than 10 mg.
Allergen (Der p 1 and Der f 1) was quantified using a standard enzyme linked immunoabsorbancy assay (ELISA). Der f 1 and Der p 1 concentrations were determined by interpolation from reference curves by StatLIA® Immunoassay Software (Brendan Scientific Corp., Grosse Point Farms, MI) and reported as µg of Der Group 1 per gram of dust for Der p 1 and Der f 1.

Mold Sampling

The microbiology laboratory of Interface Research, Inc. performed mold analysis of samples. RODAC (Random organism detection agar contact) plates filled with Malt extract agar (Difco) were imprinted onto carpeted surfaces in the bedroom and living areas of the test residences. The plates were then labeled, sealed with tape, and transported to the laboratory within 24 hours. RODAC plates were incubated at 30° C for 72 hours and inspected for the development of fungal colonies. Fungal colonies were counted and the number was reported as CFU’s/28cm². Identification of the three most prominent fungal genera was performed by touching the colony with the adhesive side of clear mailing tape (3M). The clear tape with adhered fungal elements was positioned adhesive side down into a drop of lactophenol cotton blue dye on a microscope slide. A Nikon light microscope (400X) was used to view the morphological characteristics of the conidiophores (spore bearing structures) and conidia (asexual spores). Identification was based on photographic images and keys.
Statistical Analysis

In the Der Group 1 allergen analyses, the baseline concentrations were compared between the three groups to determine the validity of the study assignment protocol. A one-way analysis of variance (ANOVA) model was used for this comparison. To test for changes in allergen concentrations in response to treatment, a repeated measures ANOVA model was used. In addition, a repeated measures ANOVA was performed to determine the effect of time and treatment, and the interaction between the two on allergen concentrations. Normality assumptions of the ANOVA were violated and transformations to the allergen data had to be applied. An unstructured covariance matrix was used in the data transformations. Significant effects were followed by multiple comparisons using Tukey's method.

In both the QOL and FEV₁ analyses, baseline values were compared among the three study groups to determine if the groups were similar at baseline. A repeated measures ANOVA was performed to determine the effect of time and treatment, and the interaction between time and treatment, on QOL and FEV₁ measures. Any significant effects were followed by multiple comparisons using Tukey's method.

RESULTS

Thirty-five patients were screened of which 16 qualified and 15 were entered into the study in March of 2001. There were no differences between the three treatment groups at baseline in either the LRC or BRC Der f 1 concentrations as determined by ELISA during the enrollment phase. Patients were similar with respect to duration of asthma and spirometric scores determined during the month.
before initiation. The Acarex® test corresponded well to Der Group 1 allergen levels. The results indicated the following ranges: no result=below 100 ng/ml, weak=150-600 ng/ml, medium =600-1700 ng/ml, strong=1700+ ng/ml Der 1. Baseline mean values and ranges for ages and spirometric scores of patients enrolled in the study are listed in Table 6.1. One patient withdrew from the SI treatment group at month 3.

**Effectiveness of Treatment on Mite Allergenic Load**

ELISA analysis of collected dust from LRC and BRC carpet revealed below detectable levels of Der p1 and therefore Der f1 levels were used for within and between group comparisons. A repeated measures ANOVA model was fit to the LRC and BRC Der f1 concentrations and the assumptions of normality and equal variances appeared to be violated at each time point. A square-root transformation was then applied to the Der f1 concentration. When the models were run using the transformed data, the variances of the residuals stabilized and the normality assumption was met at all but one time point for both LRC and BRC. Time was the only statistically significant effect (P-value for LRC analysis = 0.0144 and P-value for BRC analysis = 0.0010).

**Living Room Carpet Der f1 Levels**

Multiple comparisons for the LRC model revealed significant differences in Der f1 concentrations between pre-treatment and post-treatment (adjusted P = 0.0334), and post-treatment and months 2 (adjusted p = 0.0130) and 3 (adjusted P = 0.0305) (Figure 6.1). Commercial cleaning removed 98.1% of Der f1 allergen at
baseline and was more effective than the 69.0% reduction by the Bissell ProHeat®
wet vacuum cleaner (See Table 6.2). However, the sham cleaning, which was
performed in the placebo group, removed 96.0% of allergen making the reductions
witnessed in both active treatment groups insignificant when compared to the placebo
group. Unfortunately, LRC dust samples for the one-month post treatment and
cleaning were not collected. Allergen levels in LRC reservoir dust in both treatment
groups were below placebo group levels at both months 2 and 3. However, LRC Der
f 1 levels exceeded established thresholds of 2 μg/g of Der f 1 in both treatment
groups.

Bedroom Carpet Der f 1 Levels

Multiple comparisons for the BRC model indicated that the differences in Der
f 1 concentrations were significant between pre-treatment and post-treatment
(adjusted P = 0.0013), and post-treatment and months 1 (adjusted p = 0.0089) and 3
(adjusted P = 0.0599) as shown in Figure 6.2. Mean reduction following cleaning
was higher in the PI group (-97.7%) compared with the BB group (-78.1%) and
placebo groups (-72.0%) (see Table 6.3). Mean Der f 1 levels were reduced below
established threshold levels of 2 μg/g following commercial cleaning in only the PI
treatment group. Allergen levels rebounded dramatically in both treatment groups at
months one, two and three post intervention. Allergen levels were above the
recommended sensitization threshold of 10 μg/g Der Group 1 for all three time points
following interventions in both treatment groups.
Mold Levels

Only the PI treatment group contained an antimicrobial treatment. No attempt was made to quantify fungal allergen levels in dust collected from carpets. The following general observations were made from the fungal analysis performed. A reduction of recoverable fungi was noted in the pre-versus post-cleaning samples at baseline. A definite "cleaning effect" was noted. None of the carpets in any of the homes demonstrated active colonization by fungi as determined by direct microscopy of acetate tape mount specimens from LRC and BRC. Most samples were culture positive due to spore settling or normal traffic patterns. Averages of recovered fungi from the three sample groups showed no significant differences over the three month period. Mean recovered fungi were 19.5 cfu/28 cm in the Placebo group versus 19.1 cfu/28 cm for BB group and 19.5 cfu/28 cm for the PI group respectively.

It is important to note that Intersept as well as the acaricides are not sporicidal. Therefore, a gradual increase of recoverable fungi over time would be expected. However, this could be affected by filtration efficiency, vacuuming frequency and efficiency, and traffic patterns and sampling locations within the various homes.

Types of fungal genera recovered were typical of the Southeastern US. The most frequently encountered species listed in order of prevalence were: Cladosporium, Curvularia, Penicillium/Aspergillus and Alternaria.

Clinical Outcomes

Treatment groups differed with respect to QOL scores at baseline (p = 0.0206) and the multiple comparisons indicated that the difference was between the PI and
BB groups. The repeated measures ANOVA model indicated that only time was statistically significant, with baseline values higher compared to month 3 (P = 0.0044). Figure 6.3 contains mean QOL scores± SEM by time within each treatment. The results must be interpreted with caution because the groups were not similar at baseline. It is possible that the PI group had a great influence on the results since their baseline values were higher than the other groups and they experienced a marked decrease at 3 months. There was little overlap between the scores at baseline and 3 months in this group. However, in the other two groups, scores at baseline and 3 months are very similar, which indicates no effect of time. There were no baseline differences in FEV1 levels between the three groups, and there were neither time nor treatment effects on FEV1 over the course of the study (Figure 6.4).
<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>22</td>
<td>78</td>
<td>57</td>
</tr>
<tr>
<td>FEV1 percent predicted</td>
<td>51</td>
<td>115</td>
<td>81</td>
</tr>
<tr>
<td>FVC percent predicted</td>
<td>55</td>
<td>112</td>
<td>84</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>59</td>
<td>103</td>
<td>78</td>
</tr>
<tr>
<td>Percent change in FEV1 post</td>
<td>-5</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>Number of positive skin tests</td>
<td>1</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Sex: 11 females, 4 males

Table 6.1: Initial clinical characteristics of asthmatic patients used in this study (n=15).
Figure 6.1: Mean square root transformed levels of house dust mite allergen in living room carpet before and after cleaning and application of BB and PI. Time was statistically significant (P=0.01, ANOVA), no statistically significant effects were observed among treatment groups (P>0.05). Within group ANOVA noted significant differences in Der f 1 concentrations pre-treatment and post-treatment (adjusted P = 0.0334), and post-treatment and months 2 (adjusted P = 0.0130) and 3 (adjusted P = 0.0305).
Table 6.2: Summary statistics of living room allergen concentrations and percent changes from pre-interventions levels (n=5 unless noted).

<table>
<thead>
<tr>
<th></th>
<th>BB Group</th>
<th>PI Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Living room Carpet Der f 1 (μg/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>12.4</td>
<td>16.9</td>
<td>18.4</td>
</tr>
<tr>
<td>Range</td>
<td>1.1-37.5</td>
<td>0.61-52</td>
<td>0.53-3.3</td>
</tr>
<tr>
<td>Post-Clean Mean</td>
<td>3.9</td>
<td>0.32</td>
<td>0.78</td>
</tr>
<tr>
<td>Range</td>
<td>0.2-8.7</td>
<td>0.0-0.7</td>
<td>0.0-3.3</td>
</tr>
<tr>
<td>% change</td>
<td>-69.0</td>
<td>-98.1</td>
<td>-96.0</td>
</tr>
<tr>
<td>Month 1 Mean</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 2 Mean</td>
<td>10.8</td>
<td>6.2</td>
<td>33.2</td>
</tr>
<tr>
<td>Range</td>
<td>0.6-29.6</td>
<td>1-14.9</td>
<td>0.2-100</td>
</tr>
<tr>
<td>% change</td>
<td>-13.0</td>
<td>-63.3</td>
<td>+80.4</td>
</tr>
<tr>
<td>Month 3 Mean</td>
<td>12.4</td>
<td>26.9*</td>
<td>42.5</td>
</tr>
<tr>
<td>Range</td>
<td>0.7-28.1</td>
<td>0.7-100</td>
<td>0.3-100</td>
</tr>
<tr>
<td>% change</td>
<td>0.0</td>
<td>+59.2</td>
<td>+131.0</td>
</tr>
</tbody>
</table>

* (n=4), NC=No samples were collected during month 1.
Figure 6.2: Mean square root transformed levels of house dust mite allergen in bedroom carpet before and after cleaning and application of BB and PI. Time was statistically significant (P=0.001, ANOVA), no statistically significant effects were observed between treatment groups (P>0.05). Within group ANOVA revealed significant differences in *Der f* 1 concentration between pre-treatment and post-treatment (adjusted P = 0.0013), and post-treatment and months 1 (adjusted P = 0.0089) and 3 (adjusted P = 0.0599).
### Table 6.3: Summary statistics of bedroom allergen concentrations and percent changes from pre-interventions levels (n=5 unless noted).

<table>
<thead>
<tr>
<th></th>
<th>BB Group</th>
<th>PI Group</th>
<th>PC Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bedroom Carpet Der f 1 (µg/g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Clean Mean</td>
<td>30.6</td>
<td>24.7</td>
<td>45</td>
</tr>
<tr>
<td>Range</td>
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<td>12.6</td>
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<td>0-30.6</td>
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<td>-97.7</td>
<td>-72.0</td>
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<td>22.9</td>
<td>27.7</td>
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<tr>
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<tr>
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<tr>
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<td>0.9-100</td>
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<td>% change</td>
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<tr>
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<tr>
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<td>-4.7</td>
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* (n=4)
Figure 6.3: Mean± SEM Quality of Life scores by time within each treatment group pre-study and at 3 months post-study. ANOVA model indicated that only time was statistically significant, with baseline values higher compared to month 3 (P = 0.0044).
Figure 6.4: Mean± SEM forced expiratory volume pre and post-study within each treatment. ANOVA indicted no statistically significant (P>0.05) effect for both time and treatment in FEV\textsubscript{1} indices over the course of the study.
DISCUSSION

Our objectives in the study revolved more around understanding processes than testing hypotheses, thus the small sample sizes and short duration. We wanted to analyze each of the components (e.g. cleaning strategies, active ingredients) that may reduce allergen in a home environment while monitoring mite-sensitive asthma patients. More importantly, we needed to evaluate our ability to measure allergen and monitor patient symptoms. Our goal was also to find out whether a group of companies and two academic units could function as a funding and research team. A considerable amount of time was spent just organizing a framework for operation. So forming the consortium was an important although non-scientific aspect of our project.

We detected no statistically significant differences in dust mite allergen among the benzyl benzoate, permethrin/Intersept® and control groups on bedroom and living room carpet. In addition, there were no significant changes in asthmatic status, as determined by spirometry. Due to the small sample size generalizing these results to larger populations should be done only with great caution. Since clinical benefits of environmental interventions occur gradually\(^1,5\), and successful environmental studies that have improved the asthmatic status of patients lasted longer than 3 months\(^1\), we did not anticipate a clinical benefit in this study. Two reports have provided estimates on the samples sizes required to show statistically valid changes in clinical outcomes. From the repeated measures analysis of settled dust allergen it has been estimated that 50 patients would be required per treatment group to detect a 38% change in allergen levels.\(^{16}\) For the detection of a two fold
difference in allergen levels, 35 patients per group would be required in cross
sectional studies and 30 patients per group in parallel randomized placebo controlled
studies.\textsuperscript{17}

Failure to reduce mite allergen levels in environmental control studies are
numerous.\textsuperscript{1,18} Some studies have shown a benefit from benzyl benzoate \textsuperscript{19,20}
application, whereas others have observed no benefits from its use.\textsuperscript{21-24} In the only
study using permethrin, both active house dust mite population and allergen levels
were reduced over one year.\textsuperscript{25} The reasons for conflicting results with anti-mite
products are several. We detected a significant reduction in allergen levels for the
control group after sham cleaning. This surprising outcome was a major factor in
confounding our statistical analysis when comparing the two treatments with the
sham control. One idea is that the pre- and post-intervention sampling was conducted
over the same square-meter area, which resulted in the allergen reduction. In other
words the sampling may have reduced the allergen in the control, not the sham
cleaning. The allergen rebound after baseline was greatest for the sham control
group, suggesting that baseline sampling accounted for the initial drop in allergen.
Weekly vacuuming can reduce mite allergen levels by up to 48\% when performed
over a 5 week period.\textsuperscript{26}

Regardless of any sampling impact, the allergen rebound following cleaning
was noteworthy. Previous ‘successful’ tests of benzyl benzoate and permethrin were
conducted in more temperate climates, not in the high humidity setting we selected,
and which represents a worst case scenario for mite managing allergen. The high
average humidity and temperature of South Florida results in far higher mite numbers
and allergen levels\textsuperscript{27} compared to homes in central Ohio\textsuperscript{25}. More work is required but we predict that more frequent acaricidal treatment will be necessary to mitigate HDM populations in warmer, more humid climates where only a brief or no heating season occur.

Further complicating the analysis was the time of year that we started and finished the study (April-June). As this is the beginning of the rainy season and indoor humidity begins to increase. Significant mite population increases typically occur during the spring and early summer months.\textsuperscript{28} Thus the study timing as well as the local high humidity environment represented a severe test of these interventions.

Lastly and maybe most importantly, not all carpeted areas of the homes were cleaned and treated. We suspect that a significant amount of allergen was tracked from untreated areas into cleaned/treated bedroom and living room carpets. Also, a small (20.6\%) but significant proportion of Der p 2 HDM allergens can be carried on airborne particles of less than 4.7 μm.\textsuperscript{29} Since we did not collect live mite samples we can only speculate that allergen tracking may have occurred.

In this study commercial cleaning in the permethrin/Intercept group was superior to the Bissell consumer based cleaning in the BB group. Both cleaning regimes were effective in reducing allergen levels below established threshold levels of 2 μg Der Group 1 per gram of dust. Permethrin and benzyl benzoate are both effective laboratory acaricides. However, the results show that they are ineffective at lowering HDM allergens below sensitization thresholds over a three month period. Results of this study underscore the need to examine EPA-approved acaricides in
larger (greater than 30 patients per group) clinical studies extended over a longer period. In the future, investigators should compare the effectiveness of environmental control interventions between warm high humidity and temperate climates. Doing so will provide insights for specific allergen management regimes tailored for each climate.

REFERENCES


108


CHAPTER 7

A SINGLE PERMETHRIN TREATMENT OF CARPETED FLOORS REDUCES HOUSE DUST MITES AND ALLERGEN IN THE HOMES OF ASTHMATICS

ABSTRACT

Background: The purpose of this study was to test the effectiveness of a single permethrin treatment of carpeted floors followed by conventional hot-water extraction to lower house dust mite allergen levels in the homes of asthmatics. The clinical benefits of this strategy in the management of asthma after one year were determined.

Methods: Permethrin was applied to bedroom and living room carpets in the homes of 15 asthmatic patients followed by hot water extraction of carpets at month four. Dust mite levels and allergen (Der f 1, Der p 1) concentration were measured and compared to a placebo control group of 13 asthmatics to determine the clinical benefits of the intervention.

Results: A sustained reduction in mite population levels (p=0.0617) and a statistically significant reduction in Der f 1 allergen concentration (p=0.022) occurred in living room carpet due to treatment. A significant reduction in active mite...
populations was observed (p=0.0468) in bedroom carpet. Spirometry results between study groups were not significant (p=0.0632).

**Conclusions:** Permethrin treatment of carpet lowers both active house dust mite populations and allergen levels. Further studies are needed to examine permethrin in combination with accepted allergen avoidance measures as part of an integrated management approach of indoor allergens. The application frequency required for permethrin makes the chemical an excellent candidate for mite control by overcoming the challenges of patient noncompliance.
INTRODUCTION

Asthma related to house dust mite (HDM) allergens is a growing medical concern with worldwide incidence nearly doubling during the last twenty years.\textsuperscript{1} HDM allergens are recognized as a major risk factor for sensitization where climate is conductive to mite population growth.\textsuperscript{2,3} Allergen exposure plays an important role in sensitization especially during childhood.\textsuperscript{4} The risk that HDM sensitized children will develop asthma increases two-fold with the doubling of mite allergen exposure.\textsuperscript{5} Exposure to more than 2 $\mu$g $\text{Der p 1}$/gm of dust may present a risk for early sensitization\textsuperscript{6}, with a longer sensitization induction period occurring at lower levels.\textsuperscript{7} A dose-response relationship between IgE-mediated hypersensitivity and allergen exposure has been established. Mite density of 100-500 mites per gram of household dust, or 2 $\mu$g $\text{Der p 1}$/gm of dust is considered a significant risk for sensitization. Extended exposure to more than 500 mites per gram of dust or 10 $\mu$g $\text{Der p 1}$/gm is associated with an increased incidence of extrinsic asthma.\textsuperscript{8,9} These thresholds provide valuable benchmarks for evaluating the relative success or failure of dust mite allergen reduction strategies.\textsuperscript{9,10}

Physical and chemical control, or a combination of both have been examined for lowering HDM allergen exposure.\textsuperscript{3,11,12} Although clinical improvement has been observed using these approaches, reduction strategies for HDM allergens often remains secondary to pharmaceutical treatment. Recommended tactics generally include protective barriers, frequent HEPA vacuuming and chemical intervention employing acaricides.\textsuperscript{13} An integrated approach to HDM allergen management is worthy of consideration especially where it incorporates effective cleaning and
maintenance with chemicals that control allergen producers. When this strategy is undertaken the issues that must be addressed are patient adherence to recommendations (compliance) and safety of proposed environmental control strategies.

Until recently, HDM control using traditional agricultural acaricides had been dismissed as a viable approach because of the perceived risks. This idea is being revisited as several compounds have excellent mite-killing properties, are very safe and potential indoor half-life is measured in years rather than days, weeks or months. Permethrin is a broad spectrum synthetic pyrethroid that is used against an array of pests on nut, fruit, vegetable, cotton, ornamental, and cereal crops. It is used commercially in greenhouses, in or around the household, and for the control of animal ectoparasites, biting flies, and cockroaches.\textsuperscript{15}

Permethrin is traditionally known as an acarine repellant; however this ingredient is also an excellent acaricide. Household carpet samples sprayed with 0.1\% (v/v) permethrin are significantly resistant to mite inoculations.\textsuperscript{14} Kill rates for the introduced mites approached 80\% at 48 hours with statistically significant long-term residual activity present 48 months post-treatment.\textsuperscript{14} A currently used safe application is permethrin treatment of uniforms that help protect military personnel from ectoparasites like chiggers, ticks and body lice. In one study, effectiveness increased with time after multiple applications.\textsuperscript{16} Permethrin fabric-adherence and stability were positive attributes as treated clothing repelled body lice even after twenty hot water washes.\textsuperscript{17}
Human health risks of employing permethrin as an acaricide are minimal. Permethrin oral LD$_{50}$ in rats and rabbits is greater than 4000 mg/kg.$^{15}$ Dermal LD$_{50}$s are greater than 4,000 mg/kg on rats and more than 2,000 mg/kg on rabbits.$^{15}$ As a dermatological agent in an alcoholic base, less than 2% was absorbed in man.$^{15}$ The major metabolites of permethrin, $cis$ and $trans$ dichlorovinyl acid, are almost entirely excreted in the urine within 72 hours in free or glucuronide conjugated forms.$^{15}$ Permethrin is metabolized and excreted faster than it can be absorbed through the skin, thereby negating tissue storage. A post-marketing study of 1% permethrin cream rinse for the treatment of head lice examined 47,578 treatments with pediculicides and detected no serious, unexpected adverse events in the 18,950 patients treated with permethrin.$^{18}$ Human dermatological usage of permethrin cream has not resulted in any significant skin sensitizations.$^{19}$

The purpose of this study was to determine the effectiveness of permethrin to lower HDM (Dermatophagoides spp.) populations and their associated allergens in the homes of dust mite sensitive asthmatics. An additional objective was to determine the clinical benefits to permethrin application by monitoring patient outcomes over a one-year period. A single, post-treatment hot water extraction was performed to remove these offending allergens. Our hypothesis was that permethrin would remain active to suppress mite populations for 12 months. With this outcome, we predicted that patient spirometry and quality of life would improve. The ramifications of long-term mite control and patient compliance are discussed.
MATERIALS AND METHODS

Patient Recruitment

Asthmatic subjects aged 18 to 75 years were recruited by advertisements in local newspapers and from The Ohio State University Hospital between September 1997 and March 1999. Permission to conduct this study was obtained from the biomedical research institutional review board at The Ohio State University and all patients gave written informed consent. Twenty-eight patients fulfilled the asthma criteria established by the American Thoracic Society. Patients with a history of symptoms for at least one year, and a documented 15% rise in FEV₁ after inhaled albuterol were included in the study. Dust mite hypersensitivity was performed by immediate skin prick using intradermal dust mite extract (Holister Stier, Spokane Wash) and atopy was defined as a greater than 4-mm skin wheel response to HDM allergen. Fifty dust mite sensitive asthmatics were pre-study patch tested using a glycerinated permethrin extract with no skin reactions. Patients excluded were those with lower respiratory disease other than asthma, upper respiratory other than allergic rhinitis, sinusitis or any other medical or surgical disease, cigarette smokers or those who planned to move in the next twelve months.

Study Design

In a double-blind placebo controlled randomized design, twenty-eight adult asthma patients were sequentially allocated to one of two groups. Study group randomization was accomplished by drawing numbers. Patients in study group 1 received (0.1% v/v) permethrin treatment at baseline and hot-water extraction of...
carpets (n=15) at month four. Permethrin was applied to carpets using a standard
pump-up sprayer. Group 2 participants received a sham treatment at baseline and hot
water extraction of carpets at month four (n=13). Clinical parameters for each patient
and their homes were monitored at two-month intervals over 12 months. Patients
were examined three times by a physician and seen by a nurse who conducted an
interim history and chest auscultation at four additional visits. Monitored clinical
parameters included spirometry, BORG assessment and daily peak flow meter (AM
and PM) measurements. Home parameters included living room and bedroom carpet
Der f 1 and Der p 1 allergen and mite density.

Avoidance Measures

Avoidance measures (HDM and allergen mitigation) focused on living room
and bedroom carpets. This included a permethrin application to both settings at
baseline followed by the hot water extraction at month four by a professional
company (Serv Pro Inc., Columbus, OH). Permethrin treatment of the patients
mattress was also performed initially. The technician applying treatment and the
participating families were blinded to the treatment type. A sham treatment was
performed in the placebo group using water.

Dust Sampling, Mite Counts and Allergen Quantification

Dust samples were obtained at baseline and 2, 4, 6, 8, 10, 12 months post
permethrin treatment from the living and bedroom carpets as well the mattress
between September 1997 and February 2000. Dust was collected by vacuuming 1 m²
for two minutes using a Filter Queen® HEPA canister vacuum cleaner (HMI Industries Inc., Cleveland, OH). Two samples were taken from each site, one to be analyzed for mean number of mites/gram of dust and the other to quantify Der group 1 allergen content. Samples were sealed in plastic bags and stored at 4°C. Samples were excluded from analysis when the amount of dust was <10 mg.

Mites were collected by placing a cotton fabric filter between a small five-inch non-brush upholstery attachment and the hose with the vacuum set on low power. Mites were quantified within one week using a modified dust flotation method and expressed as house dust mites per gram of dust. Allergen (Der p1 and Der f1) was quantified using a standard enzyme linked immunoabsorbancy assay (ELISA). Der f1 and Der p1 concentrations were determined by interpolation from reference curves by StatLIA® Immunoassay Software (Brendan Scientific Corp., Grosse Pointe Farms, MI) and reported as µg of Der Group 1 per gram of dust.

Data Analysis

Allergen levels, mite counts, Borg scale and FEV1 were analyzed for statistical significance. For each outcome, a linear mixed effects model was fit to the data. A random intercept term was included because initial values were not part of the entry criteria; therefore the baseline values ranged greatly. The model fit with and without this term was then assessed, and the two models were compared using a likelihood ratio test. If the outcome was highly skewed and a transformation was not found to
correct the problem, the differences from baseline were then assessed. If the differences from baseline were modeled, then the baseline value was also included as a covariate.

RESULTS

The patients in both study groups were similar with regard to demographics, asthma history, baseline pulmonary function tests, and medication use and HDM allergen content. Baseline parameters for patients enrolled in each study group are listed in Table 7.1. Not all patients completed the one-year study. Patient compliance was low and similar to previously published adherence (20-50%) in environmental clinical control studies. Compliance in our study was 70% for dust sampling compared to 40% for office visits at study completion.

Allergen Level Change in Response to Treatment

Most homes were negative for Der p 1 allergen and thus no statistical analysis was conducted. All homes positive for Der p 1 were also Der f 1 positive. These measurements were highly skewed and transformation of the raw data did not improve the non-normality problem. Histograms of the change from baseline at each time interval using a fourth-root transformation suggested that the normality assumption was met. A linear mixed effects model was used to examine the interactions between time and treatment. Results indicated a significant interaction (P=0.022) in living room carpets between time and treatment for Der f 1 (Figure 7.1). Mean treatment group living room Der f 1 allergen levels were higher than the control group at baseline; however, this difference was not of statistical relevance
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Table 7.1. Home and clinical characteristics of patients enrolled in this study.
Parameter estimates for time and treatment indicated that treatment group Der f 1 levels decreased over time compared to the control. A mean reduction of 97.8% was observed at month two for the treatment group. Mean reduction in Der f 1 levels was 14.77 μg/g, from 15.26 at baseline to 0.49 μg/g. Reduction of allergen content in the living room was maintained below 2 μg/g Der f 1 for the duration of the study. There was no interaction between time and treatment type for mattress and bedroom carpet allergen levels or all sites combined.

**House Dust Mite Population Change in Response to Treatment**

Mite counts were highly skewed, and transforming the raw data did not improve the distribution assumption. As with the Der f 1 data, the changes in the fourth-root transformed mite counts from the fourth-root transformed baseline mite counts were normally distributed. A sustained reduction of HDM populations was achieved with permethrin treatment compared to placebos. Results of the linear mixed effects model using living room carpet indicate that the interaction between time and treatment were not significant (P=0.0617; Figure 7.2). Parameter estimates of the living room mite population data indicate that the pattern of change over time was different for the treated and control groups. The effect of treatment was significant for the bedroom carpet data (P=0.0468). The negative parameter estimate indicated that the permethrin group had a significantly lower mite count compared to the control. The effects of time and treatment were not significant for the mattress data (P=0.5495).
Figure 7.1: Interaction plot for fourth-root transformed change in $Der f 1$ in living room carpet. Allergen levels between treatment groups were statistically significant (P=0.022).
Clinical Outcomes

The distributions of the FEV\textsubscript{1} measures were normally distributed, so a transformation was not applied. The baseline scores were different between the two groups, although not statistically different. Results from the analysis indicate that no statistically significant effects between the treatment group FEV\textsubscript{1} (P=0.0632; Figure 7.3). Raw BORG scale scores were skewed and transformations of the raw data did not improve the distribution. Therefore, the changes in the square-root transformed scores from the square-root transformed baseline scores were analyzed. Results from the linear mixed effects model indicated that there was no effect of treatment (P=0.9423), time (P=0.9030), or an interaction between the two (P=0.1716).

DISCUSSION

Significant and sustained reductions in HDM populations and allergen levels were achieved over one year using a single, low concentration application of permethrin. This acaricide reduced HDM populations in both living room and bedroom carpets. A one year sustained reduction in mite numbers by a single permethrin application is the longest reported for any chemical previously examined for HDM control. Permethrin has several advantages over other ingredients such as benzyl benzoate or boron-containing products. Permethrin could be applied annually by a professional as opposed to a patient-based application such as benzyl benzoate that requires frequent reapplication. Benzyl benzoate is typically applied as a fine powder and vacuumed off carpets after 24 hours. Concerns arise using this method, as there is significant potential for inhalation of fine benzyl benzoate dust.
Figure 7.2: Interaction plot for fourth-root transformed change in mite levels determined from mite count assays for living room carpet. A reduction in mite numbers was noted between treatment groups but this trend was statistically significant (P=0.0617).
Figure 7.3: Interaction plot for square-root transformed changes in FEV₁ between treatment groups. Patients in the permethrin treatment experienced an improvement in lung function however this trend was not statistically significant (P=0.0632 compared to the placebo group.
In contrast, results of this study show the excellent fabric adherence ability of permethrin. Conventional hot water extraction performed four months post treatment failed to dislodge and remove permethrin and reduce its effectiveness. The fabric adherence properties of permethrin should allow regular hot water extraction to be incorporated into an integrated approach without the need for frequent reapplication. The low effective permethrin concentration (0.1% v/v) needed to achieve a sustained reduction in mite numbers is five times less than the US Environmental Protection Agency allows for home usage. Potential adverse health effects are also minimized with a low effective concentration of permethrin. Overall, these benefits can be passed on to the patient in cost savings, while also minimizing their direct role in managing environmental interventions.

Hot water extraction employed four months post permethrin treatment maintained Der f 1 allergen levels in living room carpet below established sensitization thresholds for at least 12 months. This is a noteworthy accomplishment and means the patient compliance issue becomes less critical from a mite allergen standpoint. Significant mite population reduction in living room carpet corresponded to the observed reduction in Der f 1 allergen. However, HDM allergen levels were not reduced in bedroom carpets. There are several plausible explanations. Mattress and blanket ages in the absence of hypoallergenic encasements, are associated with higher HDM allergen levels in mattress surface dust. In addition, the amount of mite allergen in mattresses and floor dust are significantly correlated. Although we treated mattresses in the active treatment group we did not clean nor did we attempt to adjust for the age of the mattresses between patients. Since we obtained bedroom
carpet samples by vacuuming directly next to the bed, allergen fallout from the mattresses may have skewed our results. The statistically significant reduction in mite numbers in the bedroom support this scenario. It is therefore reasonable to assume that clinical outcomes were impacted in those patients without hypoallergenic mattress encasings, lessening the overall clinical statistical significance of our findings. Mattress encasement was not a part of the treatment strategy because we wished to determine the effectiveness of a single permethrin treatment on reducing HDM allergen exposure.

Explanations for our failure to reduce allergen and mites in mattresses are several. Mattress cleaning was not performed as a part of the hot water extraction or routine vacuuming, so allergen levels should therefore not decrease. In fact, allergens actually increase with mattress age. We did not attempt to screen out patients with old mattresses. In addition, hot water washing of mattress covers and bedding is an essential component of bedroom HDM allergen reduction.

In an earlier study, Der p 1 increased over a six-month period on mattress covers with a significant reduction only after the covers were hot water washed weekly.25 We did not make recommendations to patients on bed linen or mattress encasement washing frequency. Nor did we treat pillows with permethrin. Interestingly, Der p 1 levels in homes of patients were mostly lacking and low when present. We observed a travel correlation to Florida by household occupants in those houses that were positive. These results are in agreement with previous studies21, indicating that D. farinae is the dominant mite species in drier temperate regions such as Ohio.
Asthma is a multi-faceted chronic disease and much remains to be learned about the underlying causes. Dust mite ecology needs to be studied more thoroughly in carpets, rugs, upholstered furniture and mattresses. Although the data presented here indicating that a sustained reduction in dust mite allergen is attainable; we achieved only a borderline significant difference in the asthma outcomes of patients in a small cohort study. This may indicate insufficient time to appreciate clinical improvement. Low compliance underscores the need for more patient education and a strategy that relies less on patient compliance. A patient population of greater than 50 individuals studied over a longer period with improved compliance will certainly help clarify unanswered questions.26

Our results emphasize the need for an integrated approach that incorporates established strategies such as mattress encasement with frequent laundering, professional hot water extraction of carpets, HEPA vacuuming and air filtration, with acaricidal treatment. The advice given to asthmatics to replace carpet and fabric upholstered furniture with hard floors and furniture is not generally followed in the United States.

Professional hot water extraction followed by permethrin treatment has the potential to provide a long term, cost effective and low maintenance approach to reducing HDM populations and allergens in household carpet. These benefits, coupled with the minimal exposure risks, make permethrin treatment of carpet an approach that could be incorporated into the advice given to asthmatics. A professionally managed integrated plan endorsed by managed care may be needed for
environmental allergen control strategies to be effectively implemented and maintained. A comprehensive integrated approach has not been examined in a clinical study.

REFERENCES


10. Munir AKM. Risk levels for mite allergen: are they meaningful, where should samples be analyzed? Allergy 1998; 53:84-7.


APPENDIX A

DATA RELEVANT TO CHAPTER 7

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Fixed effects: LRC.diff ~ month * TREAT + LRC.base + HWD
Number of Observations: 127
Number of Groups: 28

TABLE A.1: Living room carpet Der f 1 allergen linear mixed-effects ANOVA model results.

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<td>0.3432016</td>
<td>0.2078435</td>
<td>77</td>
<td>1.651251</td>
</tr>
<tr>
<td>month</td>
<td>-0.0223068</td>
<td>0.0234066</td>
<td>77</td>
<td>-0.953012</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.2068974</td>
<td>0.2641136</td>
<td>20</td>
<td>-0.783365</td>
</tr>
<tr>
<td>BRC.base</td>
<td>-0.0614960</td>
<td>0.0185924</td>
<td>20</td>
<td>-3.307591</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.0069165</td>
<td>0.0316137</td>
<td>77</td>
<td>0.218781</td>
</tr>
</tbody>
</table>

Fixed effects: BRC.diff ~ month * TREAT + BRC.base
Number of Observations: 102
Number of Groups: 23

TABLE A.2: Bedroom carpet Der f 1 allergen linear mixed-effects ANOVA model results.

133
<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.1687444</td>
<td>0.2340173</td>
<td>90</td>
<td>0.721076</td>
<td>0.4727</td>
</tr>
<tr>
<td>month</td>
<td>-0.0261937</td>
<td>0.0268346</td>
<td>90</td>
<td>-0.976117</td>
<td>0.3316</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.0480556</td>
<td>0.3091486</td>
<td>23</td>
<td>-0.155445</td>
<td>0.8778</td>
</tr>
<tr>
<td>MAT.base</td>
<td>-0.0172841</td>
<td>0.0045084</td>
<td>23</td>
<td>-3.833734</td>
<td>0.0008</td>
</tr>
<tr>
<td>HWD</td>
<td>0.1099386</td>
<td>0.3121809</td>
<td>23</td>
<td>0.352163</td>
<td>0.7279</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.0167606</td>
<td>0.0370969</td>
<td>90</td>
<td>0.451807</td>
<td>0.6525</td>
</tr>
</tbody>
</table>

Fixed effects: MAT.diff ~ month * TREAT + MAT.base + HWD
Number of Observations: 119
Number of Groups: 27

TABLE A.3: Mattress Derf1 allergen linear mixed-effects ANOVA model results.

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.4055035</td>
<td>0.2177543</td>
<td>98</td>
<td>1.862207</td>
<td>0.0656</td>
</tr>
<tr>
<td>month</td>
<td>-0.0408623</td>
<td>0.0245105</td>
<td>98</td>
<td>-1.667134</td>
<td>0.0987</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.1454210</td>
<td>0.2866650</td>
<td>24</td>
<td>-0.507286</td>
<td>0.6166</td>
</tr>
<tr>
<td>ALL.base</td>
<td>-0.0122226</td>
<td>0.0021276</td>
<td>24</td>
<td>-5.744727</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HWD</td>
<td>-0.3764713</td>
<td>0.2726571</td>
<td>24</td>
<td>-1.380750</td>
<td>0.1801</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.0156610</td>
<td>0.0341299</td>
<td>98</td>
<td>0.458863</td>
<td>0.6473</td>
</tr>
</tbody>
</table>

Fixed effects: ALL.diff ~ month * TREAT + ALL.base + HWD
Number of Observations: 128
Number of Groups: 28

TABLE A.4: All sites combined Derf1 allergen linear mixed-effects ANOVA model results.
### TABLE A.5: Living room carpet mite count linear mixed-effects ANOVA model results.

<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-0.1574676</td>
<td>0.1212777</td>
<td>94</td>
<td>-1.298405</td>
</tr>
<tr>
<td>month</td>
<td>0.0311400</td>
<td>0.0119650</td>
<td>94</td>
<td>2.602592</td>
</tr>
<tr>
<td>TREAT</td>
<td>0.2355058</td>
<td>0.1614636</td>
<td>24</td>
<td>1.458570</td>
</tr>
<tr>
<td>LRC.base</td>
<td>-0.0640046</td>
<td>0.0241759</td>
<td>24</td>
<td>-2.647454</td>
</tr>
<tr>
<td>HWD</td>
<td>-0.1888141</td>
<td>0.1876707</td>
<td>24</td>
<td>-1.006092</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>-0.0316133</td>
<td>0.0167175</td>
<td>94</td>
<td>-1.891027</td>
</tr>
</tbody>
</table>

Fixed effects: LRC.diff ~ month * TREAT + LRC.base + HWD
Number of Observations: 124
Number of Groups: 28

### TABLE A.6: Bedroom carpet mite count linear mixed-effects ANOVA model results.

<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.1441837</td>
<td>0.1122057</td>
<td>77</td>
<td>1.284994</td>
</tr>
<tr>
<td>month</td>
<td>-0.0085249</td>
<td>0.0102918</td>
<td>77</td>
<td>-0.828312</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.3348910</td>
<td>0.1580610</td>
<td>20</td>
<td>-2.118745</td>
</tr>
<tr>
<td>BRC.base</td>
<td>-0.2912835</td>
<td>0.2120036</td>
<td>20</td>
<td>-1.373955</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.0137277</td>
<td>0.0143123</td>
<td>77</td>
<td>0.959157</td>
</tr>
</tbody>
</table>

Fixed effects: BRC.diff ~ month * TREAT + BRC.base
Number of Observations: 102
Number of Groups: 23

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<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.0305960</td>
<td>89</td>
<td>0.257642</td>
<td>0.7973</td>
</tr>
<tr>
<td>month</td>
<td>-0.0009464</td>
<td>89</td>
<td>-0.092469</td>
<td>0.9265</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.1330016</td>
<td>23</td>
<td>-0.848538</td>
<td>0.4049</td>
</tr>
<tr>
<td>MAT.base</td>
<td>-0.0744216</td>
<td>23</td>
<td>-1.277720</td>
<td>0.2141</td>
</tr>
<tr>
<td>HWD</td>
<td>-0.2306098</td>
<td>23</td>
<td>-1.346735</td>
<td>0.1912</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.0084000</td>
<td>89</td>
<td>0.600780</td>
<td>0.5495</td>
</tr>
</tbody>
</table>

Fixed effects: MAT.diff ~ month * TREAT + MAT.base + HWD
Number of Observations: 118
Number of Groups: 27

TABLE A.7: Mattress mite count linear mixed-effects ANOVA model results.

<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.0324166</td>
<td>109</td>
<td>0.254413</td>
<td>0.7997</td>
</tr>
<tr>
<td>month</td>
<td>0.0080958</td>
<td>109</td>
<td>0.562185</td>
<td>0.5751</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.0821246</td>
<td>24</td>
<td>-0.476316</td>
<td>0.6382</td>
</tr>
<tr>
<td>ALL.base</td>
<td>-0.0572945</td>
<td>24</td>
<td>-2.749106</td>
<td>0.0112</td>
</tr>
<tr>
<td>HWD</td>
<td>-0.1914445</td>
<td>24</td>
<td>-1.113521</td>
<td>0.2765</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>-0.0055777</td>
<td>109</td>
<td>-0.278931</td>
<td>0.7808</td>
</tr>
</tbody>
</table>

Fixed effects: ALL.diff ~ month * TREAT + ALL.base + HWD
Number of Observations: 124
Number of Groups: 28

TABLE A.8: All sites combined mite count linear mixed-effects ANOVA model results.
<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.1933826</td>
<td>0.2179154</td>
<td>73</td>
<td>0.887421</td>
</tr>
<tr>
<td>month</td>
<td>-0.0016674</td>
<td>0.0136393</td>
<td>73</td>
<td>-0.122251</td>
</tr>
<tr>
<td>TREAT</td>
<td>0.0115717</td>
<td>0.1580132</td>
<td>20</td>
<td>0.073233</td>
</tr>
<tr>
<td>BORG.base</td>
<td>-0.0540019</td>
<td>0.0236008</td>
<td>20</td>
<td>-2.288134</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>-0.0266145</td>
<td>0.0192766</td>
<td>73</td>
<td>-1.380664</td>
</tr>
</tbody>
</table>

Fixed effects: BORG.diff ~ month * TREAT + BORG.base
Number of Observations: 98
Number of Groups: 23

<table>
<thead>
<tr>
<th>Value</th>
<th>Std.Error</th>
<th>DF</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>29.13899</td>
<td>9.903750</td>
<td>72</td>
<td>2.942217</td>
</tr>
<tr>
<td>month</td>
<td>-0.97560</td>
<td>0.707582</td>
<td>72</td>
<td>-1.378777</td>
</tr>
<tr>
<td>TREAT</td>
<td>-0.33681</td>
<td>7.561715</td>
<td>23</td>
<td>-0.044542</td>
</tr>
<tr>
<td>fev1.base</td>
<td>-0.20953</td>
<td>0.125184</td>
<td>23</td>
<td>-1.673783</td>
</tr>
<tr>
<td>month:TREAT</td>
<td>0.53983</td>
<td>1.001421</td>
<td>72</td>
<td>0.539063</td>
</tr>
</tbody>
</table>

Fixed effects: fev1.diff ~ month * TREAT + fev1.base
Number of Observations: 100
Number of Groups: 26

**TABLE A.9**: Borg questionnaire linear mixed-effects ANOVA model results.

**TABLE A.10**: Spirometry (FEV₁) linear mixed-effects ANOVA model results.
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144

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