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Immunochemical quantitation, size distribution, and cross-reactivity of Lepidoptera (moth) aeroallergens in southeastern Minnesota

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*With an immunochemical method, we analyzed outdoor air samples during a 3-year period for concentrations of the predominant local species of moth, *Pseudaletia unipuncta* (Haworth). Airborne particulates were collected on fiberglass filter sheets with an Accu-Vol sampler located 1.5 m above ground on the southeastern Minnesota prairie. Filter eluates analyzed by RIA inhibition contained concentrations of moth protein peaking in June and August to September of each year, with levels comparable to reported immunochemically measured levels of pollen and mold allergens. These peaks also corresponded with total numbers of moths captured in light traps. Moth-allergen activity was distributed in particle sizes ranging from 0.8 to $>4.1 \mu\text{m}$ when sized samples were obtained by use of an Andersen cascade impaction head. By RIA inhibition, there was cross-reactivity between *P. unipuncta* and insects of different genera, families, and orders, but not with pollens or molds. Forty-five percent of 257 patients with immediate positive skin tests to common aeroallergens had positive skin tests to one or more commercially available whole body insect extracts. Of 120 patients with allergic rhinitis believed to be primarily caused by ragweed sensitivity, 5% also had elevated specific IgE to moths. We conclude that airborne concentrations of Lepidoptera can be measured immunochemically and that moths may be a seasonal allergen in the United States. (J ALLERGY CLIN IMMUNOL 1988;82:47-54.)*

Moths and butterflies (order, Lepidoptera) have long been suspected to cause inhalant allergy on an occupational as well as on a seasonal basis.¹⁻⁷ Shed particles from these insects are easily airborne, but unlike pollen grains and mold spores, they have no specific morphologic characteristics. A recently described technique of high-volume air sampling has

Abbreviations used

RIA: Radioimmunoassay
TAW: True armyworm (*Pseudaletia unipuncta* [Haworth])
WHAW: Wheat-head armyworm (*Faronta diffusa* [Walker])

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proved useful in detecting amorphous animal and insect allergens,⁸ and Kino et al.⁹ have used this technique to measure insect aeroallergens in Japan.

Perlman⁴ found many years ago that a large percentage of patients referred for skin testing were sensitive to a variety of insects; further studies in India¹⁰ and Japan¹¹ have had similar results. Hundreds of moth and butterfly species are found in the United States, many in association with agricultural settings, but their role in seasonal allergy has not been studied.

We attempted to measure outdoor air quantities of moth allergen in the upper midwestern United States

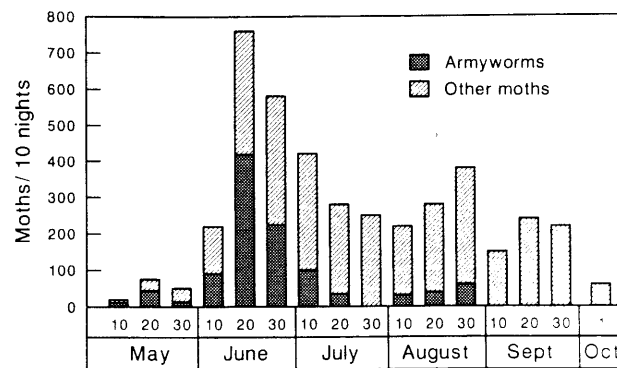


FIG. 1. Results of blacklight-trap capture near Ankeny, Iowa, 1984

by comparing the air-sampling technique described by Agarwal et al.¹² with counts of insects collected in blacklight traps. The prevalence of moth respiratory allergy was examined by screening patients referred for allergy skin testing for evidence of specific IgE to moths by epicutaneous skin testing. Stored serum from patients with allergic rhinitis considered to be primarily caused by ragweed were analyzed for moth-specific IgE by RAST.

MATERIAL AND METHODS

Insect capture

Light traps, which have been demonstrated to be an effective method to capture nocturnal insects,^{13,15} were used to capture moths at the Iowa State University Research Farm, Ankeny, Iowa. Insects were collected daily in a rural area during the growing season of 1984, grouped into 10-day intervals, and identified according to species by two of the authors (N. D. P. and W. B. S.).

Preparation of extracts

Two of the most common nocturnal moths that are found in this region, *Pseudaletia unipuncta* (Haworth) (common name, TAW) and *Faronta diffusa* (Walker) (common name, WHAW), were used to prepare extracts. Dead insects were quick frozen over liquid nitrogen and dry ice and then lyophilized. The dried insects were defatted in ether and then pulverized and extracted overnight in a 1:20 wt/vol concentration in 200 mmol/L of ammonium bicarbonate. After extraction, the specimens were centrifuged, and the resulting supernatants were sterilized by passing through 0.22- μ m Millipore filters (Millipore Corp., Bedford, Mass.). After addition of an equal volume of glycerin, the extracts were stored at -20° C. Protein determinations were performed with the method described by Lowry et al.¹⁶ An

extract of TAW wing scales was prepared in the same fashion but was devoid of protein and RAST activity; it was not used for aeroallergen studies. Commercial moth (mixed *Heterocera* sp.), yellow jacket (*Vespa* spp.) and *Dolichovespula* spp.), short ragweed (*Ambrosia artemisiifolia* L.), and *Alternaria* extracts were purchased from Greer Laboratories, Lenoir, N.C. German cockroach (*Blattella germanica* Linnaeus), moth (mixed *Heterocera* spp.), butterfly (*Rhopalocera* spp.), caddis fly (*Trichoptera* spp.), and rye grass (*Lolium* sp.) extracts were purchased from Hollister-Stier, Spokane, Wash. Midge extract (*Chironomus plumosus*) was provided by Dr. Steve Kagen, Appleton, Wis.

Air-sampling technique

An Accu-Vol (General Metal Works, Inc., Cleves, Ohio) high-volume air sampler¹⁷ located 1.5 m above ground on the southeastern Minnesota prairie was used. This sampler was located approximately 200 miles north of the blacklight traps in an area with similar agricultural features and climate. Airborne particles were collected on 8 by 10-inch fiberglass filter sheets in 1983, 1984, and the first half of 1985; filter sheets were changed every 3 days. After June 1985, polytetrafluoroethylene filter sheets were used; these sheets were changed daily. The sampler was run continuously at a flow of 1.17 m³/min. In 1983 and 1984, samples were taken during the summer and early fall months only; samples were taken during all 12 months of 1985.

An Andersen cascade (Andersen Products Inc., Atlanta, Ga.) impaction head, which separates collected particles into five-size categories of mean aerodynamic diameter between 0.3 and 5.0 μ m, was used on selected samples to determine which particle sizes contained allergenic activity.

Air-filter samples were processed as previously described.¹² Briefly, one quarter of each sample was eluted during 8 hours with 100 mmol/L of ammonium bicarbonate

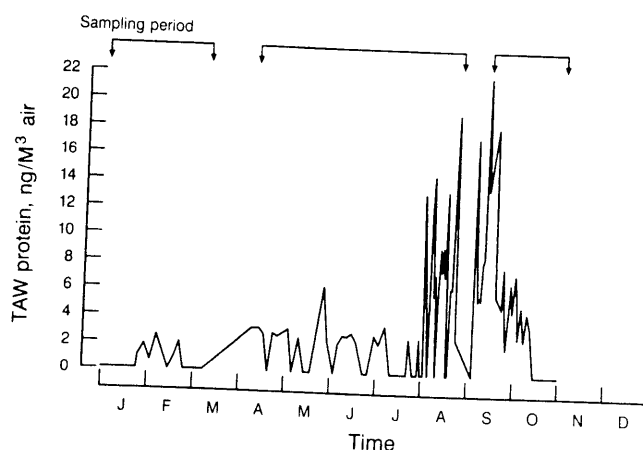


FIG. 2. Airborne quantitation of TAW allergen with Accu-Vol (General Metal Works, Inc.) air sampler at a flow rate of 1.17 m³/min for 1983 near Rochester, Minn.

buffer; the resulting eluate was lyophilized and then reconstituted in 1.5 ml of 0.5 mol/L of phosphate buffer and kept at -20° C for up to 24 months until it was assayed. The remainder of the air filter was saved for future studies.

RIAs

To perform assays for the determination of moth-specific IgE, polystyrene microtiter wells (Immulon II, Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 100 µl of extract at a protein concentration of 10 µg/ml diluted in 100 mmol/L of sodium bicarbonate buffer titrated with 100 mmol/L of sodium carbonate to pH 9.2. After incubating overnight, the wells were washed three times with RAST wash (phosphate buffer and Tween 20). Unoccupied binding sites on the wells were then blocked for 4 hours with 0.01% human serum albumin (Sigma Chemical Co., St. Louis, Mo.).

Assay of IgE antibody to moth allergens was performed by incubating 50 µl of patient serum with equal amounts of 0.5 mol/L of phosphate buffer overnight in microtiter plates coated with TAW extract and then washing three times with RAST wash. One hundred microliters of ¹²⁵I-labeled antihuman IgE was added to each well and incubated overnight. After washing three times with RAST wash, each sample was counted for bound radioactivity for 1 minute on a Packard (Packard Instrument Co., Downers Grove, Ill.) gamma scintillation counter.

Air-filter eluates were analyzed by comparing the degree of inhibition of binding of moth-specific IgE by each eluate. The specific IgE source was a serum pool derived from four individuals selected for their high RAST values to TAW (data not presented). Two patients from Rochester, Minn.,

had summer and fall rhinitis and positive RAST to TAW. The other two patients, in addition to summer and fall rhinitis, also had rhinitis associated with swarms of midge (*C. plumosus*) in Wisconsin and had positive RAST and skin test to midge, as well as positive RAST to TAW (sera provided by Dr. Steve Kagen). None were clinically sensitive to insect venoms, none had been receiving immunotherapy of any kind, and none had frequent exposure to insects in their occupations or hobbies. The moth-allergen content in each sample was calculated by comparison with a standard inhibition curve of known protein concentrations of moth extract. The same RIA technique was used as described in the previous section. The following equation was used to determine moth allergen concentration per cubic meter of air sampled:

$$\text{Airborne moth allergen}(\text{ng}/\text{m}^3) = \frac{\text{Total moth allergen/air sample}}{\text{Total air volume sampled}}$$

Skin testing

Epicutaneous skin testing was performed by the prick method on 604 consecutive patients referred for skin testing to the Mayo Clinic Allergy Testing Laboratory. Testing was performed only if patients had not taken antihistamines for more than 24 hours, had no response to the negative control prick, and at least a 3 by 3-mm diameter wheal response with flare to 5 mg/ml of histamine phosphate. Included in the skin test battery of common aeroallergens were two commercial whole body moth extracts (Greer and Hollister-Stier), butterfly, caddis fly, and cockroach (all from Hollister-Stier) at 1:10 wt/vol.

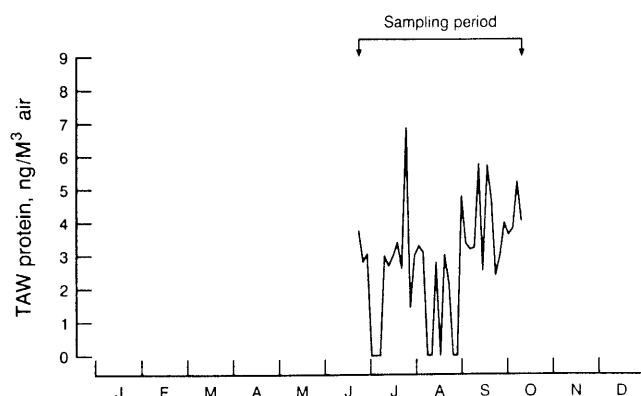


FIG. 3. Airborne quantitation of TAW allergen with Accu-Vol (General Metal Works, Inc.) air sampler at a flow rate of $1.17 \text{ m}^3/\text{min}$ for 1984 near Rochester, Minn.

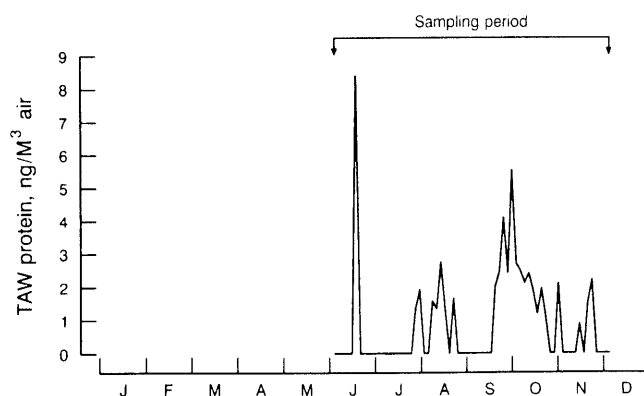


FIG. 4. Airborne quantitation of TAW allergen with Accu-Vol (General Metal Works, Inc.) air sampler at a flow rate of $1.17 \text{ m}^3/\text{min}$ for 1985 near Rochester, Minn.

RESULTS

Light trap capture

The highest numbers of moths were captured in a bimodal distribution with peaks in early summer and early fall (Fig. 1). TAW and WHAW were two of the most common insects collected during this period, together composing 30% of the Lepidoptera collected, and were predominant in early summer.

Airborne Lepidoptera allergen concentrations

The airborne concentrations of particulate TAW allergen were determined by RIA inhibition of air-filter

cluates during the years 1983, 1984, and 1985 (Figs. 2, 3, and 4). Moth allergen peaks were demonstrated in June through October in 1983 and 1984. Analysis of 1985 demonstrated similar peaks, with low concentrations measured during winter and early spring, increasing concentrations through the summer, and peaks in late summer and early fall. These data correspond to the light trap capture numbers for 1984. Much higher concentrations of TAW were found in 1985 as compared to the previous years.

Cross-inhibition studies performed with the same four-member serum pool demonstrated significant

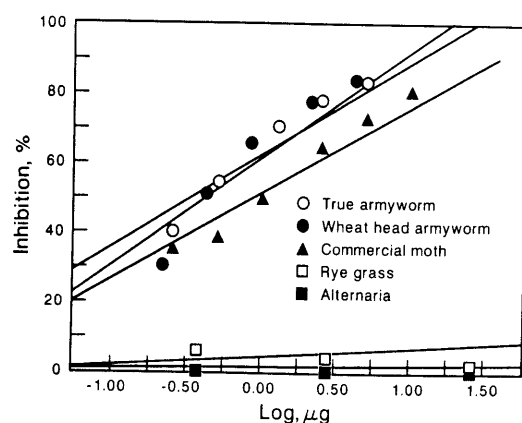


FIG. 5. RIA inhibition with TAW as solid phase and inhibitors of TAW, WHAW, a commercial moth extract, rye grass, and *Alternaria* with a serum pool of four patients with summertime rhinitis and a positive RAST to TAW.

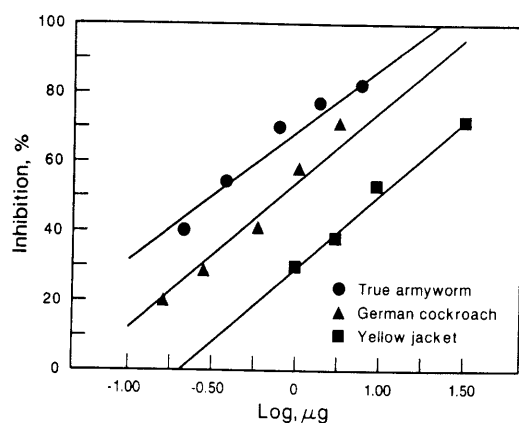


FIG. 6. RIA inhibition with TAW as solid phase and inhibitors of TAW, cockroach, and whole body yellow jacket with a serum pool of four patients with summertime rhinitis and a positive RAST to TAW.

cross allergenicity among TAW and other insects. Extracts of TAW, WHAW, as well as commercial moth, yellow jacket, and cockroach extracts, cross-reacted (Figs. 5 and 6). Two control extracts, *Alternaria* and rye grass, as well as short ragweed (data not presented), did not cross-react. The correlation coefficient for each RAST-inhibition curve was >0.98 , with points with inhibition values between 15% and

85%. Logit transformation of this data revealed similar slope ratios for all five curves: TAW, 1.54; WHAW, 1.86; commercial moth extract, 1.35; German cockroach, 1.78; and yellow jacket, 1.64. The slope ratio of a midge-extract inhibitor (data not presented) was 1.14. Comparison of paired slopes yielded the following p values: TAW-WHAW, 0.14; TAW-commercial moth, 0.18; TAW-German cockroach,

TABLE I. Percent of TAW allergen activity in airborne particles of defined sizes

Date of sample July 4-7, 1985		
Size	% TAW	% WHAW
>0.8	3.3	2.6
0.8 to 1.4	43.5	38.8
1.4 to 2.3	3.7	1.9
2.3 to 4.1	18.2	12.1
>4.1	31.2	44.7

0.12; TAW-yellow jacket, 0.32; and cockroach-yellow jacket, 0.40.

Particle size

TAW allergenic activity occurred in all particle sizes, ranging from <0.5 to 5.0 μm , and was predominant in particles between 0.8 and 1.4 μm and >4.1 μm (Table I).

Skin test results

Six hundred four patients with a variety of clinical symptoms underwent epicutaneous skin testing to a battery of inhalant allergens as part of their clinical evaluation at Mayo Clinic. Negative results were obtained in 347 patients, including 46 patients with only one positive test (wheal size ≥ 3 by 3 mm). Of 257 patients with positive reactions to two or more allergens, 116 (45%) had positive reactions to one or more of the five inhalant insect allergens tested. Only five of these patients reacted to only the insect extracts; none of these patients had received whole body insect immunotherapy, and none were sensitive by history to insect venoms. None had occupational or a recreational exposure to insects. Approximately the same percentage of patients reacted to extracts of other common aeroallergens of this region (Table II).

RIA results

RIA of IgE to TAW was positive (defined as counts per minute >400% of control value) in 5% of 120 patients with ragweed-allergic rhinitis whose serum had been stored after use in a previous study.

Lability of extract

The TAW and WHAW extracts stored at -20°C appeared stable during an 8-month period. There was no change in binding of the serum pool during this time (data not presented). Lowry protein determinations of the TAW extract were 5.37 and 5.07 mg/ml in freshly prepared and 8-month-old extracts, respectively. The WHAW extract yielded protein concentra-

TABLE II. Skin test results

Total patients tested	604
Positive tests ≥ 2 extracts	257
Positive tests	
Insects*	
(1-5 positive)	116 (45%)
5 positive	32 (12%)
4 positive	17 (7%)
3 positive	13 (5%)
2 positive	16 (6%)
1 positive	38 (15%)
Short ragweed	129 (50%)
<i>Alternaria</i>	82 (32%)
Miscellaneous grasses	122 (48%)

*Moth, Hollister-Stier and Greer Laboratories; cockroach, caddis fly, and butterfly, all Hollister-Stier.

tions of 8.29 and 8.52 mg/ml fresh and after 8 months, respectively.

DISCUSSION

The role of Lepidoptera in seasonal allergic symptoms is less clear than their role in occupational settings. Perlman¹ found that 50% of patients referred for allergy evaluation had positive scratch skin tests to a variety of whole body insect extracts. Shivpuri et al.,¹⁰ who studied inhaled insect allergy in more than 1000 patients in India with respiratory allergy, found that >25% had positive skin tests to moths, as compared to <10% for control subjects.¹⁰ Kino and Oshima¹¹ demonstrated that more than half of a group of 66 patients in Japan with asthma had positive skin tests to moths and butterflies, and approximately one third had positive bronchial challenges.

The study of moths as seasonal allergens has been hampered by the lack of a method to quantify airborne insect allergens. The technique of measuring airborne allergens by RAST inhibition of eluates obtained on high-volume air samplers as described by Agarwal et al.¹² is ideal for the study of amorphous insect aeroallergens. Ostrom et al.¹⁷ used this technique to measure indoor whole body honeybee airborne allergens in a honey-packing plant. Swanson et al.⁸ was successful in measuring indoor cockroach and mite airborne allergen concentrations, and Tee et al.¹⁸ have described the immunochemical measurement of airborne housefly allergens.

Kino et al.⁹ used a similar immunochemical technique to measure silkworm wing, caddis fly wing, and chironomid whole body allergens in the atmosphere in Kyoto, Japan, during a period of 1 year. Airborne concentrations of insect allergens reached peaks in the spring and fall and reached unmeasurable levels in the

winter. The aeroallergen levels measured for the three insects were similar (ranging between 1 and 9 ng/m³ for much of the spring and fall, with a single peak of 18 ng/m³ for chironomid in October). The possible cross-reactivity of these species was not addressed.

TAW and WHAW are pests of corn and other crops in the midwestern United States. Like most Lepidoptera, they are nocturnal insects. Light traps are good indicators of moth presence because they will attract the insects from neighboring areas. Allergens from these insects might become airborne through several means. The scales from these moths are easily airborne and could be released into the atmosphere during flight or when particles from dead moths are dispersed during activities in the fields, such as during cultivation. Windy, dry weather would appear to encourage dissemination of insect aeroallergens.

We used whole body extracts in our aeroallergen quantitation studies because our scale extracts did not contain antigen. However, if our hypothesis that dead insects in the soil reenter the atmosphere is true, then whole body antigens are not irrelevant. Indeed, major aeroallergens from two other arthropods, the dust mite¹⁹ and the locust,²⁰ come from the gastrointestinal tract and not the exoskeleton. Moreover, we found little allergenic activity in our extract prepared from shed scales with our serum pool.

We found airborne concentrations of moth allergens during a 3-year period that correlated well with known patterns of insect life cycles and agricultural schedules. There was much day-to-day variability in moth aeroallergen levels, which has also been demonstrated in the measurement of short ragweed and *Alternaria* aeroallergens, similar to the day-to-day variability in pollen and mold spore counts.²¹ Of interest was the high concentration found in October 1985, during a time of year that numbers of moths have decreased. It is possible that the harvesting activity during this time period dispersed into the atmosphere large quantities of moth protein that had settled into the soil earlier in the season. We are uncertain that higher concentrations observed overall in 1985 are significantly different from those in 1983 and 1984; since all eluates were processed at the end of 1985, this finding could be due to loss of potency in the 1983 and 1984 eluates, as well as the use of Gore-Tex filter sheets in the latter half of 1985. Further studies are needed to evaluate the changes in aeroallergen concentrations from year to year, as well as the stability of air filter eluates over time.

A large percentage of patients who received skin testing for a variety of indications had positive reactions to one or more insect allergens. The clinical correlations in these patients with positive and neg-

ative tests were not analyzed, but the rate of positive reactions to inhalant insect allergens was similar to that for other aeroallergens common in the upper Midwest. Even 5% of a population of allergic rhinitis patients formerly believed to be sensitive primarily to ragweed had serum IgE antibody to TAW. Two of the sera used for our IgE source in our RIA assays came from this population, and insect skin tests were not done in these patients.

Various species of moth, as well as such divergent insects as German cockroach and yellow jacket, cross-react by RAST inhibition. Cross-reactivity between moth and chironomid midge was less clear. Since there is cross-reactivity by RAST inhibition with this serum pool and a TAW solid phase between several types of insects, we may actually be measuring aeroallergens that are common to many families of insects. Further studies are needed to determine the extent of cross-allergenicity of insects.

We conclude that high-volume air sampling is an excellent technique for outdoor airborne insect allergen quantitation and that the seasonal variation in aeroallergen levels correlates with the numbers of insects captured. We found insect aeroallergen levels, as well as total insect numbers, similar to those measured in Japan. Further studies are needed to determine the diurnal, as well as yearly, variations in insect aeroallergen levels, the extent of immunochemical cross-reactivity of moths with other insect families, indoor moth aeroallergen levels, and the clinical significance of moth sensitivity in atopic patients with seasonal symptoms.

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