

indicates that extracts derived from the storage mite *Ao* contain enzymes that are similar to allergenic enzymes derived from the house dust mites.

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## Increased concentrations of airborne grass allergen during lawn mowing

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Many individuals with perennial or seasonal rhinoconjunctivitis complain of increased symptoms when they are exposed to freshly cut grass and lawn mowing. Previous investigators have found that the history of increased nasal and ocular symptoms occurring with lawn mowing is significantly associated with positive skin test sensitivity and elevated specific IgE antibodies to grass pollens.<sup>1</sup> This study was undertaken to determine if airborne grass allergen is increased during lawn mowing.

### MATERIAL AND METHODS

#### Air sampling

Volumetric air sampling was performed at two separate sites in metropolitan Detroit during a 5-week period at the beginning of the grass-pollen season. A sample was collected before, during, and after lawn mowing with a volumetric air sampler (Quantec Air, Rochester, Minn.) onto a 9 by 9 cm polytetrafluoroethylene membrane filter (Quantec Air). The air sampler was 2 feet off the ground and located near the center of the lawn. Air was sampled at 8.5

m<sup>3</sup>/hr. The lawn mowers were gas-powered, rotary, mowers without attached grass collection bags or mulching capabilities. The air temperature and wind direction and speed at street level were recorded for each collection date. Samples were collected on days when rain had not occurred during the previous 24 hours.

#### Elution of samples

The collected air samples were eluted in 0.5 ml of 1% bovine serum albumin/phosphate-buffered saline, pH 7.4, and 0.002% Tween 20 for 24 hours. The filter paper and debris were separated from the supernatant with a standard serum filter (Labcraft, Houston, Texas; 16 by 9 mm).

#### ELISA

Grass content in the samples was determined by a biotin-avidin amplified ELISA, as previously described.<sup>2</sup> Polyclonal rabbit antigass antisera was prepared by immunization of rabbits with an equal part mixture of grass-pollen extract (grass mix [*Phleum pratense*, *Dactylis glomerata*, *Lolium spp*, and *Poa pratensis*], Hollister-Stier Laboratories, Spokane, Wash. 1:20 wt/vol, 50% glycerine) in Freund's adjuvant with standard immunization technique. The rabbit antigass antisera was diluted to 1:1000 in a 60 mmol/L carbonate/bicarbonate, pH 9.2, buffer and adsorbed to plastic microwells overnight at room temperature. The coated wells were then blocked with 0.1% Tween and 1.0% bovine serum albumin/phosphate-buffered saline. Sequentially, with interspersed washes, a study sample, biotinylated rabbit antigass sera, avidin labeled with alkaline-

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TABLE I. Pre, during, and postmowing values

| Site | Date | Pre*   | During* | Post*  | % Increase  |
|------|------|--------|---------|--------|-------------|
|      |      |        |         |        | Pre/during† |
| 1    | 5/4  | 0.0035 | 0.051   | 0.017  | 1457        |
|      | 5/11 | 0.0063 | 0.12    | 0.0047 | 1905        |
|      | 5/21 | 0.031  | 0.15    | low    | 484         |
|      | 6/8  | 0.0039 | 0.06    | —      | 1538        |
| 2    | 5/9  | 0.0035 | 0.012   | —      | 343         |
|      | 5/13 | 0.0071 | 0.028   | —      | 394         |
|      | 5/18 | 0.0047 | 0.077   | 0.0027 | 1638        |
|      | 5/27 | 0.0061 | 0.041   | —      | 672         |
|      | Mean | 0.0083 | 0.067   | 0.0064 | 807         |

\*Pre, during, and postmowing values for grass-allergen concentration are in international units per cubic meter of air sampled.

†Percent increase from before to during lawn mowing was calculated by dividing before to during values and multiplying by 100.

TABLE II. Other extracts assayed

| Extract assayed for<br>grass-allergen content<br>(1:20 wt/vol) | Concentration of grass<br>allergen detected<br>(IU/ml) |
|----------------------------------------------------------------|--------------------------------------------------------|
| Dog hair dander                                                | <25                                                    |
| Red maple                                                      | 50                                                     |
| Oak mix                                                        | 60                                                     |
| Birch mix                                                      | <25                                                    |
| Common cocklebur                                               | 40                                                     |
| Mold mix No. 1                                                 | <25                                                    |
| Mold mix No. 2                                                 | <25                                                    |
| Timothy grass                                                  | 94,000                                                 |

phosphatase, diethanolamine buffer, and dinitrophenol phosphate, was added. The resulting color change was read to maximal absorbance, approximately 2 hours later, at wavelength 405 nm. A standard curve was determined with sequential dilutions of timothy grass (*P. pratense*)—pollen allergenic extract that had been calibrated against the World Health Organization International Standard.<sup>3</sup> The assay was sensitive to 0.025 IU/ml. The interassay coefficient of variation was 18.3%. Values were reported from the mean of triplicate determinations for a study sample as international units per cubic meter of air.

Specificity of the assay was determined by assaying other extracts (dog hair dander, red maple [*Acer rubrum*], oak mix [*Quercus rubra*, *Q. virginiana*, and *Q. alba*], birch mix [*Betula papyrifera*, *B. nigra*, and *B. pendula*], common cocklebur [*Xanthium strumarium*], mold mix No. 1 [*Alternaria alternata*, *Aspergillus* spp, *Penicillium* spp, *Hormodendrum* spp, and *Helminthosporium interseminatum*] and mold mix No. 2 [*Curvularia* spp, *Fusarium vasinfectum*, *Pullularia pullulans*, *Mucor racemosus*, and *Rhizopus nigricans*], Hollister-Stier Laboratories, 1:20 wt/vol or 1:10 wt/vol, 50% glycerine) at 1:1000 dilutions in the above assay.

## RESULTS

The airborne grass-allergen concentration increased approximately 800% for the eight collection dates between the premowing and during-mowing samples. The mean of the premowing ( $n = 8$ ) and during-mowing ( $n = 8$ ) grass-allergen concentrations was 0.0083 IU/m<sup>3</sup> of air and 0.067 IU/m<sup>3</sup> of air, respectively. The during-mowing grass-allergen concentration was significantly higher than the premowing concentration ( $p < 0.05$ ). The postmowing grass-allergen concentration dropped quickly to premowing values (mean, 0.0064 IU/m<sup>3</sup> of air) (Table I).

There was a trend for a greater increase in grass-allergen concentration between the premowing and during-mowing samples with a greater wind speed at street level ( $r = 0.66$ ;  $p = 0.08$ ). The wind direction and air temperature did not correlate with the concentration of grass allergen measured (data not presented).

The other extracts assayed for grass-allergen content demonstrated a trace or undetectable grass allergen (Table II). By comparison, mean concentrations of grass allergen detected in the premowing and during-mowing samples were 70.3 IU/ml and 567 IU/ml, respectively.

## DISCUSSION

It appears that the concentration of airborne grass allergen was increased approximately eightfold during lawn mowing and rapidly decreased immediately after lawn mowing. Other investigators have demonstrated that a fivefold to tenfold increase in allergen concentration will provoke symptoms in sensitive individuals during nasal challenge.<sup>4,5</sup> The increase in ocular and nasal symptoms noted by grass-sensitive patients dur-

ing lawn mowing was likely to be the result of increased airborne grass-allergen concentrations associated with lawn mowing.

It is unlikely that the assay detected other, nongrass allergens. When various tree, weed, dog, and mold extracts were assayed, trace or undetectable grass allergen was measured. The grass allergen detected in red maple, oak mix, and common cocklebur extracts may represent cross-reactivity between grass and nongrass allergens or contamination of the extracts with grass allergen.

The peak grass-pollinating period in the area studied was the last week of May through the first week of June. Consistently increased grass-allergen concentrations were noted in the during-mowing samplings from early May through June. Possible explanations include grass-pollen grains settling onto lawns and then being propelled back into the air by lawn mowers

or that nonpollen parts of the grass plant contain allergenic proteins.

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## Allergenicity screening of "hypoallergenic" milk-based formulas

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Presently available, extensively hydrolyzed, milk-based formulas, like Nutramigen (Mead Johnson, Evansville, Ind.), have a poor taste, sometimes limiting their use. Recently, formulas containing less extensively hydrolyzed milk proteins have been produced. Various amounts of remaining antigenic determinants are left in these products, and there are reports of serious adverse reactions from the use of commercially available hydrolyzed whey protein-based formula in cow's milk-allergic children.<sup>1</sup>

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#### Abbreviations used

SPT: Skin prick test

DH: Degree of hydrolysis

We used the SPT with blinded extracts of various hydrolyzed formulas (new or already on the market) in children with a history of cow's-milk allergy as the first step in the clinical evaluation of rest allergenicity of formula products aimed for use in milk-allergic children or as a part of an allergy-prevention program.

#### MATERIAL AND METHODS

Four girls and 12 boys, aged 3 to 13 years (median, 8 years), with a history of immediate-type cow's-milk allergy, participated. Fourteen age-matched milk-tolerant subjects served as control subjects in the SPT part of the study. All parents gave informed consent,