

Immunochemical quantitation of airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: a two-year prospective study

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We conducted a 2 yr prospective study to measure atmospheric short ragweed and Alternaria allergens by RAST inhibition analysis of eluates from filter sheets exposed in air samplers. In both years ragweed pollen and Alternaria spore counts, obtained with a roto-slide sampler, correlated significantly with immunochemically measured airborne ragweed and Alternaria allergenic activity. Airborne levels of the purified allergens AgE and Alt-I were successfully quantitated; these levels correlated closely with total airborne ragweed and Alternaria allergenic activities, respectively, and also with ragweed pollen and Alternaria spore counts. Eluates from filter sheets exposed during late summer and fall produced positive wheal-and-flare skin tests in patients with fall hay fever. In both years immunochemical measurements of allergenic activity due to airborne short ragweed correlated closely with mean symptom score indices in groups of short ragweed-sensitive individuals. Measurable levels of atmospheric ragweed allergenic activity were noted before and after the ragweed pollination season, and at these times we noted small increases in mean symptom score indices in the short ragweed-sensitive groups. Thus immunochemical analyses provide important information concerning levels of environmental allergens. (J ALLERGY CLIN IMMUNOL 72:40-45, 1983.)

In patients with respiratory allergy it has been common practice to correlate the onset, duration, and severity of clinical symptoms with atmospheric pollen and fungal spore counts and their seasonal variations. However, pollen and spore counts may not adequately reflect the actual airborne allergen load, since previous data suggest that allergens may also be carried in airborne particles smaller than intact pollen grains.¹

Recently, we have developed an immunochemical method for quantitation of atmospheric allergens,² which involves collection of airborne particles ($>0.3 \mu\text{m}$) on a filter sheet, followed by extraction of the exposed sheets by descending elution. After dialysis, lyophilization, and reconstitution, the filter sheet

eluates are tested for allergenic activity in RAST inhibition assays using various solid-phase allergens. This technique not only offers a method of monitoring airborne allergens in amorphous particles that are not identifiable microscopically but also can be used to quantitate levels of the airborne purified allergens. Our 2 yr prospective survey of atmospheric SRW and *Alternaria* allergens by the immunochemical method includes the measurement of two purified allergens, AgE and Alt-I. In addition, we tested the in vivo allergenic activity of filter eluates by skin-testing patients with fall hay fever. Finally, variations in atmospheric SRW and AgE levels were compared with the symptom scores of SRW-sensitive patients.

MATERIALS AND METHODS

Atmospheric pollen and fungal spore counts

With a roto-slide sampler,³ 24 hr samples of outdoor RW pollen and *Alternaria* spores were measured daily from July 1 to October 10, 1980, and 72 hr samples were obtained from June 1 to October 10, 1981. Simultaneous atmospheric samples for the quantitation of SRW, *Alternaria*, AgE, and Alt-I allergen activity were obtained by continuously operating an upward-facing, high-volume air sampler (*vide infra*). The two samplers were operated 1½ to 2 m above ground, 5

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Abbreviations used

BCS:	Borate citrate saline
HSA:	Human serum albumin
RAST:	Radioallergosorbent test
RIU:	RAST inhibition unit
RW:	Ragweed
SRW:	Short ragweed

m apart on mowed grass yard located on the southeastern Minnesota prairie of an open rural site unobstructed by buildings or trees. The intermittent rotoslide sampler was set to operate automatically for 1 min every 12 min in 1980, but to avoid overloading, for only 30 sec every 12 min in 1981. Similarly, the high-volume air sampler was operated at 1.19 m³/min in 1980 and 0.75 m³/min in 1981. The results were expressed as pollen grains, fungal spores, or RIU of allergen per cubic meter of air sampled.

Allergens

SRW pollen (*Ambrosia elatior*) and *Alternaria alternata* powder (source strain No. 16086; American Type Culture Collection, Rockville, Md.) were purchased from Greer Laboratories, Inc., Lenoir, N. C. Nondefatted SRW pollen (100 mg) was extracted in 25 ml BCS (0.0325M sodium borate and 0.034M sodium citrate in 0.16M sodium chloride, pH 8.2) on a magnetic stirrer at 4° C for 20 hr. After centrifugation the supernatant was mixed with an equal volume of glycerine and was used as reference SRW extract in RAST inhibition studies. An AgE reference sample No. A-601-902-185 was obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Crude *Alternaria* extract and the purified Alt-I fraction were prepared in our laboratory as described previously.⁴

The solid-phase allergens for RAST experiments were prepared by coupling various allergens (crude SRW pollen extract, 70% (NH₄)₂SO₄-precipitable fraction of crude *Alternaria* extract, AgE, and Alt-I) to CNBr-activated microcrystalline cellulose particles.⁵ The IgE antibody serum pool used for the SRW and AgE RAST inhibition studies consisted of equal volumes of defibrinated plasma from 12 nonimmunized subjects with high levels of IgE antibodies to SRW pollen. Similarly, the IgE antibody serum pool used for the *Alternaria* and Alt-I RAST inhibition studies was obtained from seven nonimmunized subjects with high levels of IgE antibodies to *Alternaria*.

Quantitation of atmospheric allergens

Atmospheric SRW, *Alternaria*, AgE, and Alt-I allergens were measured by an immunochemical technique.² Briefly, airborne particles (>0.3 µm diameter) were collected on a filter sheet by using a high-volume air sampler (Accu-Vol; General Metal Works, Inc., Cleves, Ohio). These sheets were processed by descending elution, and the eluates were dialyzed, lyophilized, and reconstituted in 1 ml of BCS. The allergen content in the eluates was measured by mini-

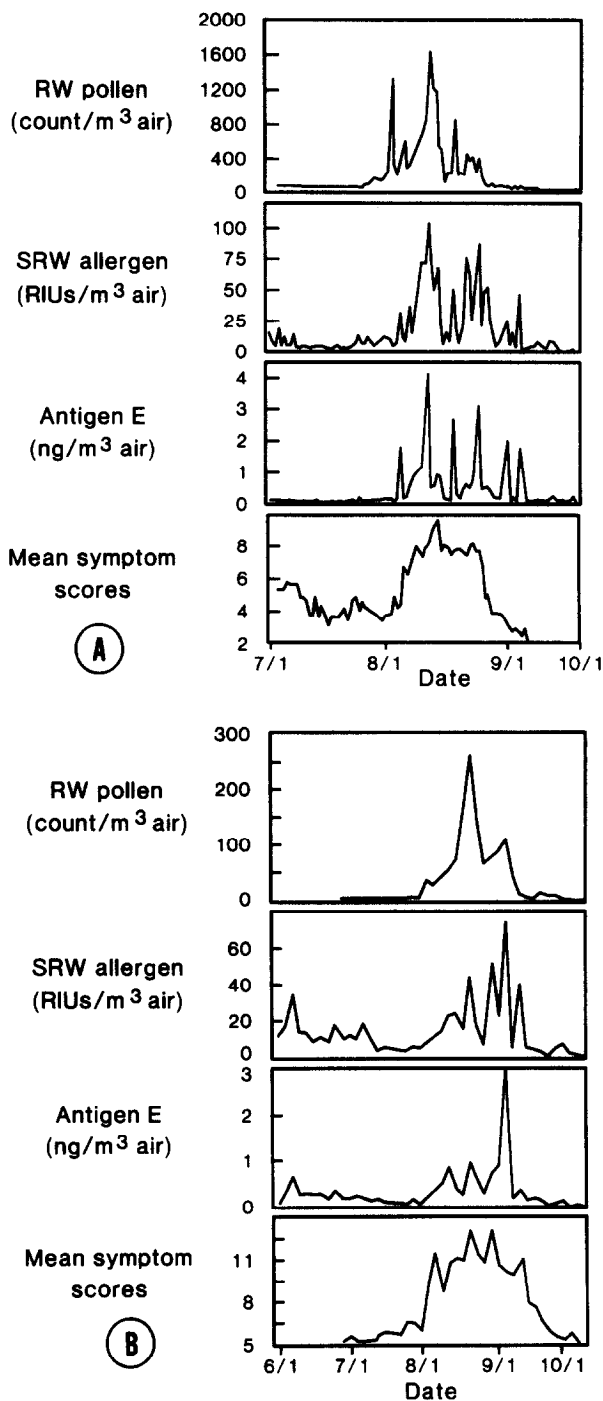


FIG. 1. Atmospheric RW pollen counts, SRW allergenic activity, AgE content, and average symptom scores of SRW sensitive individuals. **A.** Results of 24 hr samples from July 1 to October 1, 1980. **B.** Results of 72 hr samples from June 1 to October 10, 1981.

RAST inhibition assay⁶ using the various solid-phase allergens described previously. The SRW and *Alternaria* reference extracts were arbitrarily assigned an allergen content of 10⁶ RIU/ml and were tested at varying concentrations (300 to 5000 RIU) in each RAST inhibition assay to gen-

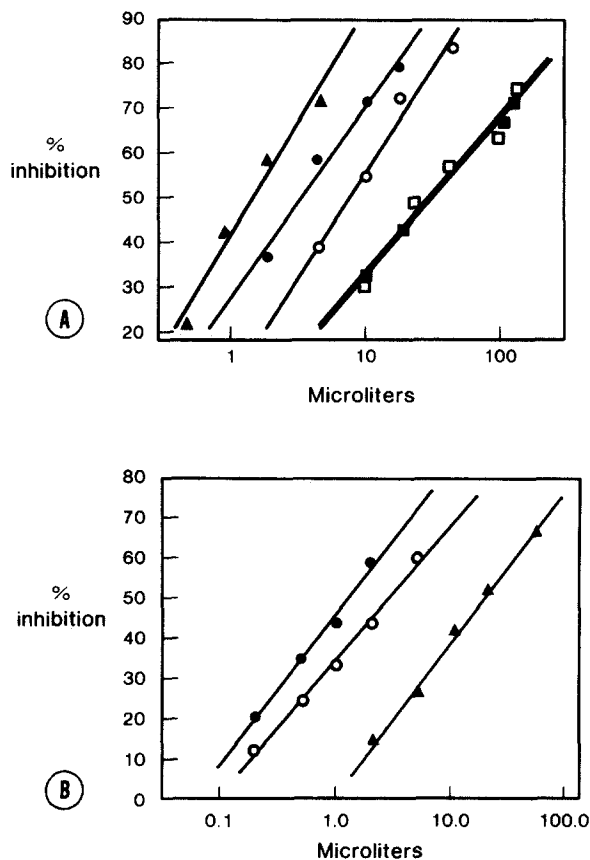


FIG. 2. RAST inhibition lines produced by SRW and AgE standards and pooled filter eluates. **A**, SRW inhibition lines. ▲, SRW standard (1:500, w/v), arbitrarily assigned 10^6 RIU/ml. **B**, AgE inhibition lines. ▲, AgE standard, 1 μ g/ml. ■, Pool 1 from 8/6/80 to 8/13/80; ●, pool 2 from 8/20/80 to 8/26/80; ○, pool 3 from 9/3/80 to 9/9/80; □, pool 4 from 9/15/80 to 9/21/80. Pools 1 and 4 were not available in sufficient quantities for use in the AgE RAST inhibition assay.

erate standard inhibition lines. Total SRW and *Alternaria* allergen activity values (per cubic meter) in the air were interpolated from these standard curves on the basis of the inhibition produced by 50 μ l of the reconstituted atmospheric filter eluates. Similarly, AgE (2 to 53 ng/tube) and Alt-I (50 to 1400 ng/tube) were used, respectively, to generate standard inhibition lines in AgE and Alt-I RAST inhibition assays, and the quantity of these purified allergens per cubic meter of air was calculated as above.

In vivo allergen activity

Serial dilutions of the filter sheet eluates from July 28, September 6, and October 7, 1980, were made in Sörensen's buffer containing 0.5% HSA.⁵ To determine the end-point dilutions giving positive reactions, we skin-tested three nonallergic volunteers and three patients with fall hay fever; all the latter group had positive skin tests to SRW extract, and two of the three had co-existing *Alternaria* sensitivity. As controls, the eluate from an unexposed filter

sheet, histamine, and SRW extract (Greer) were also skin-tested on each subject. The skin tests were performed and graded as described by Norman.⁷

Patient symptom scores

Each year a different group of SRW-allergic individuals were studied: 11 in 1980 and 10 in 1981. All patients had a history of seasonal allergic rhinitis corresponding to the local RW pollination season, had not received previous immunotherapy to SRW, and had positive skin tests and elevated IgE antibody levels to SRW. Each patient maintained symptom score diaries prior to, during, and after the RW pollination season, along with records of medications used to control their allergy symptoms (July 1 to September 21, 1980, and July 1 to October 10, 1981). The average symptom scores were calculated and compared with both the RW pollen count and the atmospheric SRW and AgE contents.

Statistical tests

The results were evaluated by regression analysis,⁸ with a Hewlett-Packard 9845B desk top computer.

RESULTS

Ragweed allergen survey

Results of atmospheric RW pollen count, SRW allergen activity, and AgE content for the years 1980 and 1981 are shown in Fig. 1, *A* and *B*, respectively. We found a significant correlation between the RW pollen count and the atmospheric SRW allergen activity (1980: $r = 0.69$, $p < 0.01$; 1981: $r = 0.64$, $p < 0.001$). Similarly, a significant correlation was obtained between the atmospheric AgE content and the RW pollen count (1980: $r = 0.57$, $p < 0.001$; 1981: $r = 0.52$, $p < 0.01$). Atmospheric AgE content showed a highly significant correlation with the atmospheric SRW allergenic activity in both years (1980: $r = 0.77$, $p < 0.001$; 1981: $r = 0.85$, $p < 0.001$).

The specificity of the RAST inhibition method for measuring atmospheric allergen activity in the filter eluates was tested by using selected filter sheet eluates as inhibitors in a honeybee venom RAST, a nonatmospheric allergen-antibody system. These eluates showed insignificant ($< 15\%$) inhibition of the honeybee venom RAST. To test for possible qualitative differences in atmospheric SRW allergenic activity during the pollination season, eluates obtained during four separate 6-day periods in August and September 1980 were pooled separately. These eluate pools were added in increasing quantities as inhibitors in the SRW and AgE RAST, to compare the slopes of the resulting inhibition lines with the slope produced by the reference SRW or AgE standard. All four filter sheet eluate pools showed dose-related RAST inhibition lines in SRW RAST whose slopes were not

TABLE I. Endpoint skin test titrations with filter sheet eluates in SRW-sensitive and control subjects

Test extract	RW pollen (per m ³)	Reciprocal of highest dilution giving a 2+ intradermal test					
		Controls			Patients*		
		1	2	3	1	2	3
Filter eluate							
7/28/80	11	—	50	—	5,000	50	50
9/6/80	377	—	50	—	50,000	5,000	5,000
10/7/80	2	—	50	—	5,000	5,000	50
Unexposed filter extract		—	—	—	—	—	—
SRW reference extract		—	—	—	5 × 10 ⁶	5 × 10 ⁷	5 × 10 ⁶

— = less than 2+ with 1:50 dilution.

*Patients 1 and 3 were also sensitive to *Alternaria*.

statistically different ($F_{4,12} = 2.70$; $p > 0.05$; NS) from the inhibition curve obtained with the reference SRW extract (Fig. 2, A). Similarly, the two filter sheet eluate pools tested in AgE RAST produced dose-related inhibition lines. The slopes of these inhibition lines were not statistically different ($F_{2,8} = 2.29$; $p > 0.1$; NS) from the inhibition line produced by the AgE standard (Fig. 2, B).

In vivo allergen activity

Table I shows the results of endpoint skin test titration with atmospheric eluates on the SRW hay fever patients and nonallergic volunteers. We found in vivo allergen activity in the atmospheric eluates. Skin test results showed marked differences in skin test endpoints between patients and controls.

Alternaria allergen survey

The filter sheet eluates from both years were assayed by *Alternaria* and Alt-I RAST inhibition to quantitate atmospheric *Alternaria* and Alt-I allergen activity. The results of these studies along with *Alternaria* spore counts for 1980 and 1981 are shown in Fig. 3, A and B, respectively. A reference crude *Alternaria* extract and Alt-I were used as internal controls. A highly significant correlation was found between the *Alternaria* spore counts and the *Alternaria* allergen content in both years (1980: $r = 0.57$, $p < 0.001$; 1981: $r = 0.72$, $p < 0.001$). Similarly, *Alternaria* spore counts correlated well with the atmospheric Alt-I content (1980: $r = 0.69$, $p < 0.001$; 1981: $r = 0.58$, $p < 0.001$). Atmospheric Alt-I content also showed a highly significant correlation with the atmospheric *Alternaria* allergen activity in both the years (1980: $r = 0.84$, $p < 0.001$; 1981: $r = 0.68$, $p < 0.001$). These findings support our earlier observations that Alt-I is a major allergenic fraction of *Alternaria*.^{4, 9}

Symptom scores and atmospheric SRW allergens

The average 24 hr symptom scores (1980) and 72 hr symptom scores (1981) showed significant correlation with RW pollen count (1980: $r = 0.58$, $p < 0.001$; 1981: $r = 0.83$, $p < 0.001$) and atmospheric SRW allergen (1980: $r = 0.59$, $p < 0.001$; 1981: $r = 0.63$, $p < 0.001$). These results suggested that the immunochemical method of measuring atmospheric allergens is a specific and sensitive technique that correlates well with the symptoms of the patients.

DISCUSSION

During a 2 yr study period (1980, 1981) we observed year-to-year variations in the RW pollen and *Alternaria* spore counts in the air in southeastern Minnesota, even though the roto-slide sampler was operated at the same site and the pollen and spore counting was done by the same person both years. These variations may have been due to changes in meteorological factors from year to year and/or the change in the sampling time from 24 hr in 1980 to 72 hr in 1981. Nevertheless, we found a significant correlation between the atmospheric RW pollen count and the quantity of atmospheric SRW allergen. Similarly, the number of airborne *Alternaria* spores showed highly significant correlations with the atmospheric *Alternaria* allergen in both years. The filter sheet eluates failed to produce significant inhibition in a nonatmospheric allergen system, establishing the specificity of the immunochemical assay for airborne allergens. Furthermore, the slopes of inhibition lines produced by pooled atmospheric filter sheet eluates were not statistically different from the slopes produced by the reference SRW or AgE standard, suggesting that there were no qualitative differences in the atmospheric SRW allergen activity during the RW pollination season.

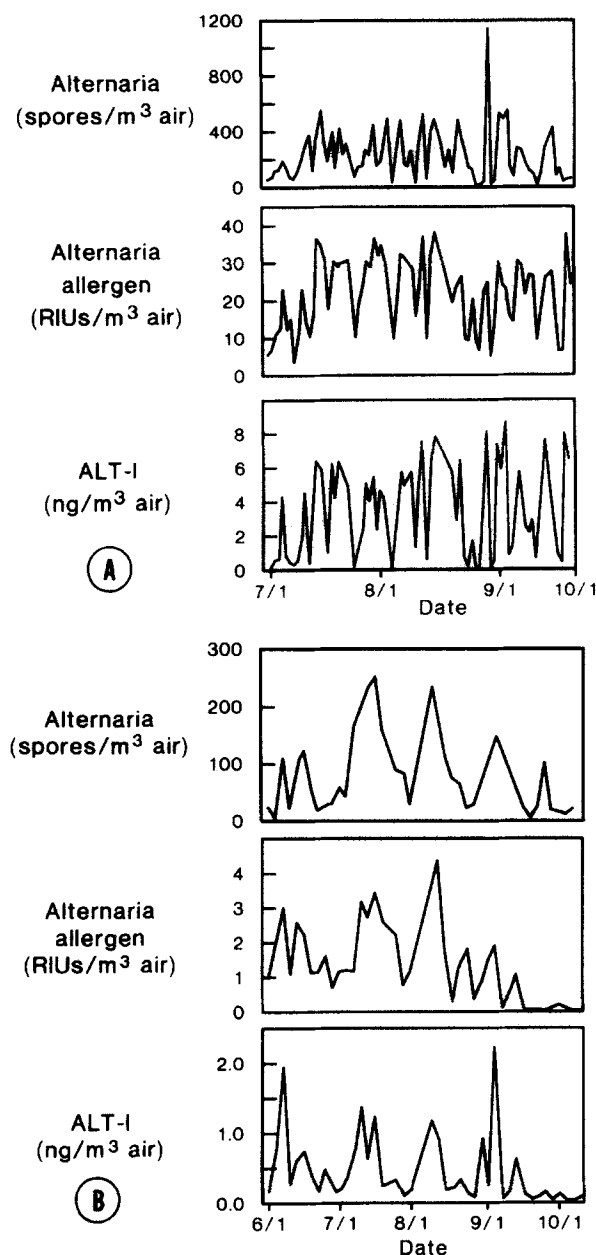


FIG. 3. Atmospheric *Alternaria* spore counts, *Alternaria* allergenic activity, and Alt-I content. **A**, Results of 24 hr samples from July 1 to October 1, 1980. **B**, Results of 72 hr samples from June 1 to October 10, 1981.

For the first time we were also able to quantitate purified allergens in the air, such as AgE and Alt-I, further establishing the fact that we were measuring relevant allergens. We found a highly significant correlation between the atmospheric AgE content and the SRW allergen activity. AgE content also showed a highly significant correlation with the RW pollen count. Similarly, Alt-I content showed a significant correlation with the atmospheric *Alternaria* allergen content, as well as with the prevalence of airborne

Alternaria spores. These studies established that the immunochemical technique measured specific atmospheric allergens independent of their morphological features.

We confirmed in vivo the allergenic activity of the filter eluates by skin-testing patients with fall hay fever. The filter eluates produced positive skin tests in these patients that were markedly different from those obtained in nonallergic volunteers.

Although there were highly significant overall correlations among the RW pollen counts and the total SRW and AgE allergen activity, there were some days when the RW pollen count was low but SRW and AgE allergen activities were elevated (June and July 1981; Fig. 1, B). We suggest that this activity may have been produced by sources of SRW allergen other than intact pollen grains. Such sources could include portions of the RW plant other than the inflorescence,¹⁰ or perhaps other plants, or soil. In addition, there were some days when the RW-sensitive individuals showed mild symptoms during periods that were outside the SRW pollination season (July 1980; Fig. 1, A). Even though we did not find any RW pollen in these weeks, we did identify measurable SRW allergen activity in the filter sheet eluates. This finding may be explained by the presence of RW allergen from nonpollen sources, as mentioned above, or by possible inclusion of a larger number of patients with co-seasonal *Alternaria* allergy in the 1980 study group. Unfortunately, symptom scores of RW-sensitive patients for June 1981 were not available to correlate with the SRW and AgE activities observed during this period. Due to differences in the volume sampled by the two samplers (0.75 to 1.19 m³/min for high volume vs. 0.058 m³/min for the roto-slide sampler), it is also possible that there were too few pollen grains in the air to be detected by the roto-slide sampler but that these were caught on the filter sheet by the high-volume air sampler.

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