

Airborne ragweed allergens: Association with various particle sizes and short ragweed plant parts

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These investigations were undertaken to size airborne particles by use of a high-volume sampler and to measure the short ragweed allergen activity of airborne particles of different sizes. We found both in vitro and in vivo short ragweed allergen activity in particles of all size ranges including >6, 3 to 6, 1.8 to 3, 1 to 1.8, and 0.3 to 1.0 μm in diameter. Furthermore, we investigated various parts of the short ragweed plant as possible sources of allergen. Plant parts collected before, during, and after the pollination season demonstrated significant in vitro and in vivo allergen activities. We demonstrated allergen activity in various plant parts, especially the inflorescence, as late as November 30. Appreciable ragweed allergenic activity was also associated with particles <1 μm in diameter. Collectively, these observations suggest persistent ragweed plant debris in different sized particles as a source of allergen in the air before and after the ragweed pollination season. This may contribute to out-of-season symptoms observed in highly ragweed-sensitive individuals. (J ALLERGY CLIN IMMUNOL 74:687-93, 1984.)

Some patients with respiratory allergy who are highly sensitive to RW pollen often experience symptoms before and after the RW pollination season. Moreover, particles of the diameter of RW pollen (20 μm) are too large to penetrate to the bronchi and cause allergic reactions in the airways.¹ We have previously reported an immunochemical technique to measure atmospheric allergens,² which was successfully used for pollen and fungal allergens, including the purified SRW and *Alternaria* allergens AgE and Alt-I,³ as well as for mouse and guinea pig-derived allergens.^{4, 5} During these studies we found SRW, RRA, and AgE allergen activities in the atmosphere before and after the RW pollination season.³ Collectively, these observations suggest that there may be

Abbreviations used

AgE:	Antigen E from short ragweed
BCS:	Borate citrate saline
GRW:	Giant ragweed
HSA:	Human serum albumin
LHR:	Leukocyte histamine-release test
RIU:	RAST inhibition units
RRA:	Rapidly released allergens
RW:	Ragweed
SRW:	Short ragweed

airborne particles other than RW pollen carrying RW allergens, some of which may be smaller than RW pollen. The present investigations were undertaken to size airborne particles by use of a high-volume sampler and to measure the RW allergen activity in airborne particles of different sizes. Furthermore, we investigated parts of the SRW plant as possible sources of RW allergen.

MATERIAL AND METHODS

Collection of atmospheric samples and RW pollen count

With the use of a roto-slide sampler,⁶ 72-hour samples of outdoor RW pollen were measured from August 15 to 30,

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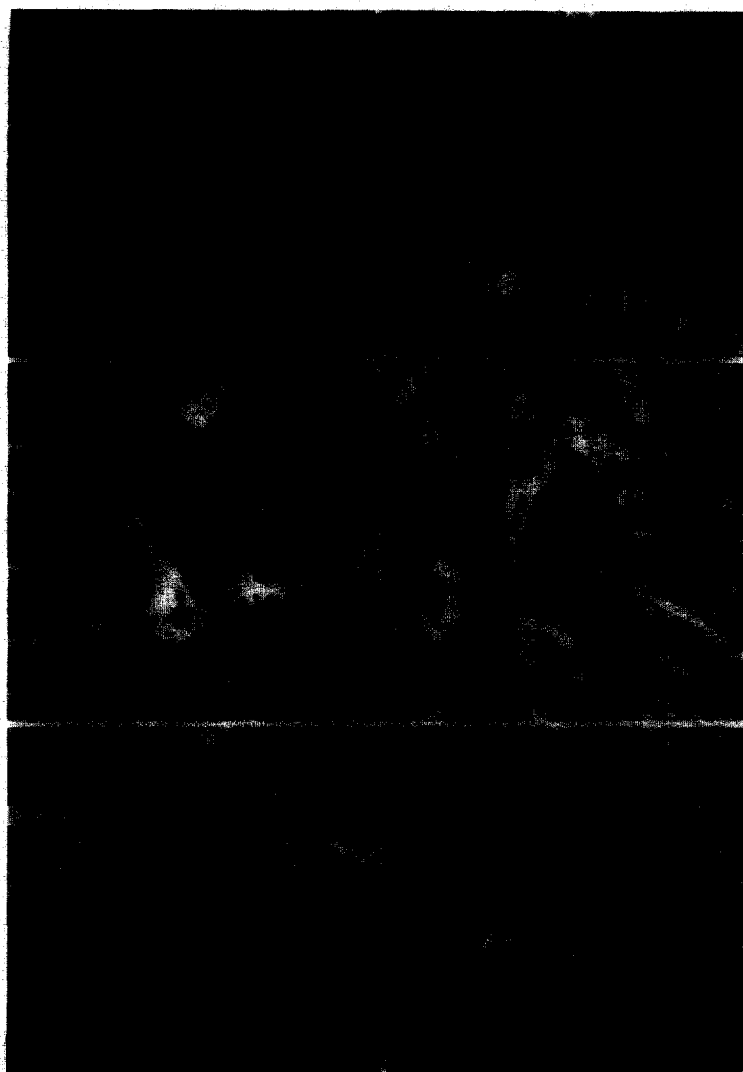


FIG. 1. Scanning electron microphotographs of air filter sheets; original magnification $\times 1100$. **A**, Unexposed filter sheet. **B** to **F**, Exposed filter sheets from different sizing stages. **B**, Stage I ($>6 \mu\text{m}$); **C**, Stage II (3 to $6 \mu\text{m}$); **D**, Stage III (1.8 to $3 \mu\text{m}$); **E**, Stage IV (1 to $1.8 \mu\text{m}$); **F**, Stage I ($<1.0 \mu\text{m}$).

1981. Simultaneous atmospheric samples for quantitation of total SRW and AgE activity were collected by continuously operating an upward facing high-volume air sampler (Accu-Vol, General Metal Works, Cleves, Ohio) mounted with fiberglass filter sheets, which retained particles of $>0.3 \mu\text{m}$ size. In addition, a second identical high-volume air sampler was equipped with an Andersen multistage, multiorifice, cascade impactor (Andersen Samplers, Inc., Atlanta, Ga.); this sampler separated the airborne particles into $>6 \mu\text{m}$, 3 to $6 \mu\text{m}$, 1.8 to $3.0 \mu\text{m}$, 1.0 to $1.8 \mu\text{m}$, and 0.3 to $1.0 \mu\text{m}$ sizes. The characteristics and operating efficiency of this cascade impactor have been described previously.^{7, 8} The three samplers were operated 4 to 6 meters apart, on a mowed grass lawn located on the southeastern Minnesota prairie, in an open rural site unobstructed

by buildings or trees. To minimize overloading, the roto-slide sampler was set to operate automatically for 30 sec every 12 min rather than for the usual 60 sec every 12 min. The high-volume samplers were equipped with flow controllers that maintained constant airflow at 0.71 m^3 per minute.

Allergens

One gram of nondefatted SRW pollen (*Ambrosia elatior*; Greer Laboratories, Inc., Lenoir, N. C.) was extracted in 25 ml of BCS (32.5mM sodium borate and 34mM sodium citrate in 160mM sodium chloride, pH 8.2) at 4°C for 20 hr. After centrifugation the supernatant was mixed with an equal volume of glycerine and was used as a reference SRW extract in RAST-inhibition studies. AgE (reference sample

TABLE I. Sizing of ragweed allergen

		Total RIUs recovered					
		Staged samples*					
RW pollen count (gr/m ³)	Unstaged sample	I >6 µm	II 3 to 6 µm	III 1.8 to 3 µm	IV 1.0 to 1.8 µm	V <1.0 µm	
Aug. 15	ND	82615	14703	5428	—	—	40742
Aug. 18	72	87300	7998	5886	29261	13690	31510
Aug. 21	149	53109	32226	6321	8561	—	23133
Aug. 24	260	143504	28060	10829	5978	8923	54456
Aug. 27	66	69054	48099	10034	—	7458	13317
Aug. 30	ND	34633	24445	4294	—	—	4861

ND = Not done.

— = Not detected.

*Amounts recovered from various stages of a second Accu-Vol (General Metal Works) sampler equipped with an Andersen (Andersen Samplers, Inc.) sizing head located beside the first sampler.

no. A-601-902-185) was obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

We prepared RRA from SRW pollen^{9, 10} by extracting 5 gm of defatted SRW pollen in 120 ml of 150mM sodium chloride, pH 7.0, for 2 min at room temperature with continuous stirring. The extract was recovered by rapid filtration under vacuum by use of a Buchner funnel with Whatman no. 3 filter paper, dialyzed against distilled water by use of Spectrapor no. 3 membrane (nominal molecular weight cutoff = 3500), and lyophilized; recovery totaled 200 mg.

The solid-phase allergens for RAST-inhibition studies were prepared by coupling various allergens (crude SRW pollen extract, AgE, and RRA) to CNBr-activated microcrystalline cellulose particles.¹¹ The IgE serum pool used for RAST-inhibition studies was obtained from 12 nonimmunized subjects with high levels of IgE antibodies to SRW pollen.

Collection of SRW plant parts

SRW plants were collected from June through November 1981 from the same roadside locations. The SRW plants, separated into various parts, were washed thoroughly in distilled water, cut into small pieces, lyophilized, pulverized to obtain fine powders, and defatted with diethylether by use of a Soxhlet apparatus. The defatted plant powders were checked for SRW pollen microscopically and extracted in 125mM of ammonium bicarbonate containing 15mM of sodium azide (pH 7.5) with continuous stirring for 72 hr at 4° C. After centrifugation the supernatants were dialyzed (Spectrapor no. 3 dialysis membrane) and lyophilized. The extracts were weighed, reconstituted in 50mM of ammonium bicarbonate, and were assayed for SRW pollen allergens.

TABLE II. Skin test results with eluates from different sizing stages

	Controls		RW-sensitive patients		
	1	2	1	2	3
Stage I	10 ¹	—	10 ⁴	10 ⁴	10 ⁵
II	—	—	10 ³	10 ³	10 ⁴
III	—	—	10 ³	10 ³	10 ⁵
IV	—	—	10 ³	10 ³	10 ⁴
V	—	—	10 ³	10 ³	10 ⁵
SRW standard	—	—	10 ⁷	10 ⁷	10 ⁸
<i>Alternaria</i>	—	—	—	—	50 PNU/ml

Numbers listed are the reciprocals of highest dilution giving a 2+ skin test reaction.

— = Less than 2+ reaction at 1:10 dilution (RW) or 500 PNU/ml (*Alternaria*).

Quantitation of atmospheric SRW allergens

The exposed filter sheets from high-volume air samplers were processed by descending elution,² and the eluates were dialyzed against distilled water by use of Spectrapor no. 3 membranes, lyophilized, and reconstituted in 1 ml of BCS. The allergen content in the eluates was measured by mini-RAST-inhibition assays¹² by use of solid-phase SRW pollen extract, AgE, and RRA allergens. The SRW reference extract (1:500 w/v) was arbitrarily assigned an allergen content of 10⁵ RIU/ml and was tested in varying concentrations (300 to 5000 RIU) in SRW RAST-inhibition assays to generate standard inhibition lines. Similarly, AgE and RRA were tested in increasing amounts in respective RAST-inhibition experiments to generate standard inhibition lines. Total SRW allergen activity in the air (per cubic meter) and

TABLE III. Total SRW-allergen activity in plant parts (estimated RIUs/mg dry weight)*

	May 30	August 1	September 7	November 30†
Inflorescence	ND	4240	96550	14010
Leaves‡	ND	590	5830	490
Stems‡	ND	980	990	650
Roots‡	ND	4490	2350	750
Seedlings	4050	ND	ND	ND
Seeds/bracts	ND	ND	ND	4530

ND = Not done.

*Standard reference extract of short ragweed pollen (1:500 w/v) contains 10⁵ RIUs/ml dry weight.

†After a snowfall.

‡Estimated values only; slope of RAST-inhibition curve did not parallel that of SRW reference standard, making assignment of absolute values impossible.

TABLE IV. Amount of AgE and RRA of ragweed in ragweed plant parts

Plant part	Date of collection	AgE content (μg/gm of powder)	RRA content (μg/gm of powder)
Inflorescence	Sept. 7, 1981	559	60,888*
Leaves	Sept. 7, 1981	39	551
Stems	Sept. 7, 1981	3.5	739
Roots	Sept. 7, 1981	31.7†	7,475
Seeds and bracts	Sept. 26, 1981	18.3	3,189

*Nondialyzable solids.

†Estimated value only; slope of RAST-inhibition curve did not parallel that of AgE standard, making assignment of absolute value impossible.

plant parts (per milligram of dry weight) were interpolated from the standard inhibition lines based on the inhibition produced by 50 μm of reconstituted atmospheric filter eluates and plant extracts. Similarly, the amounts of AgE and RRA in plant parts (per milligram of dry weight) were also interpolated. Selected extracts from different parts of the SRW plant were used in varying quantities in different RAST-inhibition assays to generate dose response lines to compare their slopes with reference standards.

In vivo allergen activity

Serial dilutions of SRW plant parts and pooled eluates from filter sheets of different stages were made in Sörensen's buffer containing 0.5% of HSA.¹¹ To determine the end point dilutions demonstrating 2+ positive reactions, we performed skin tests with these extracts on nonallergic volunteers and SRW-sensitive patients. Histamine and SRW extract (Greer Laboratories, Inc.) were also skin tested on each control subject. The intradermal skin tests were performed and graded as described by Norman.¹³

RESULTS

Sizing of airborne particles

We verified the sizing efficiency of the Andersen multistage, multiorifice, cascade impactor by examining an unexposed filter sheet and exposed filter sheets from each sizing stage under a scanning electron microscope (Fig. 1). These photomicrographs provided information regarding the sizing efficiency of the sampler and the relative distribution of particles of different sizes in the air. There was minimal overlap of particles on different sizing stages. All the unstaged filter sheets of August 15 to 30, 1981, demonstrated significant SRW allergen activity. The sized filter eluates also demonstrated SRW allergen activity in all the stages at one time or another during this period (Table I). We further evaluated the presence of SRW allergen on stages I to V by skin testing eluates from these stages on two healthy volunteers and three SRW-sensitive patients. Eluates from all the sizing ranges demonstrated in vivo allergen activity in SRW-sensitive patients at dilutions of at least 1000-fold higher than on healthy controls (Table II). All patients had symptoms only during RW season. These patients and healthy volunteers were also skin tested with *Alternaria* extract (a coseasonal allergen), and only one allergic patient demonstrated a mild positive reaction.

Sources of SRW allergens other than RW pollen

We checked various SRW plant parts other than pure pollen for the presence of SRW allergen activity. All plant parts collected during different weeks demonstrated varying amounts of total SRW antigens (Table III). Different SRW plant parts collected on September 7 and 26 demonstrated varying quantities of AgE and RRA components (Table IV). The data in

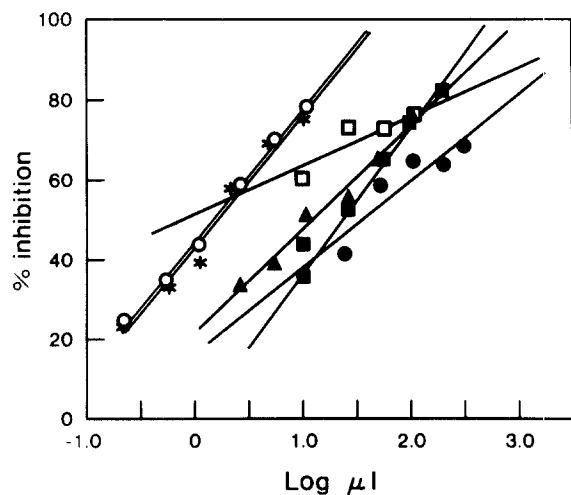


FIG. 2. Dose response lines obtained with various plant part extracts (1 mg/ml) and SRW standard (1:500 w/v) in total SRW RAST-inhibition assay. SRW standard (*), inflorescence (○), leaves (▲), stems (●), roots (□), and seeds and bracts (■).

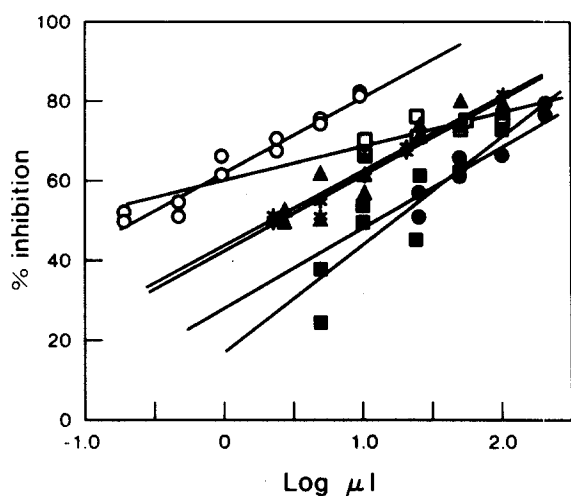


FIG. 3. RAST-inhibition lines in AgE RAST with use of various plant-part extracts (1 mg/ml) and AgE standard (1 μg/ml) as fluid phase inhibitors. AgE standard (*), inflorescence (○), leaves (▲), stems (●), roots (□), and seeds and bracts (■).

Table III suggest the male inflorescence as the most probable source of ragweed antigens found in small particles as well as ragweed antigen found before and after the RW pollination season.

The slope of the inhibition lines produced by standard extracts or by different SRW plant parts by use of total SRW, AgE, and RRA solid-phase allergens are compared in Figs. 2, 3, and 4. The specificity of

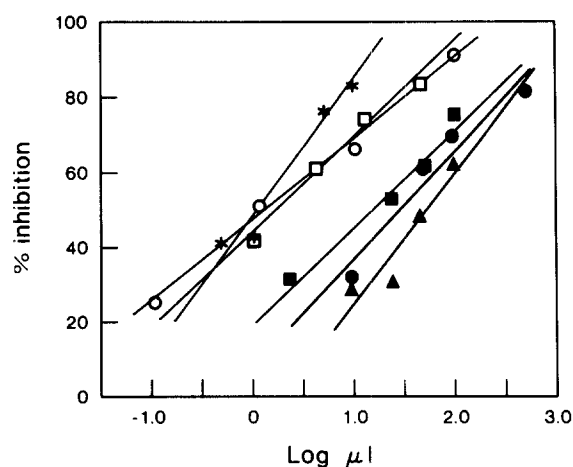


FIG. 4. RAST-inhibition lines in RRA RAST obtained with RRA standard (*) (1 mg/ml) and various plant-part extracts (1 mg/ml); inflorescence (○), leaves (▲), stems (●), roots (□), and seeds and bracts (■).

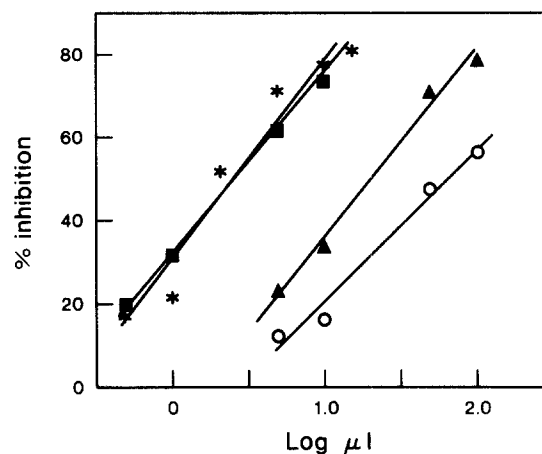


FIG. 5. RAST-inhibition lines in SRW RAST with use of various fluid phase inhibitors including SRW standard (1:500 w/v) and extracts (1 mg/ml) from inflorescence collected on August 1, September 7, and November 30, 1981. SRW standard (*), inflorescence collected on August 1 (○), September 7 (■), and November 30 (▲).

the RAST inhibition produced by the SRW plant parts was checked by use of these extracts as inhibitors in the honeybee venom RAST. None of the extracts produced significant inhibition (>15%) at the highest concentration used in SRW, AgE, and RRA RASTs. In the situations where the slopes of the inhibition lines were not parallel to that of the standard extract, as was the case with extracts of roots, stems, and leaves in the total SRW RAST (Fig. 2) and with root extract in the AgE RAST (Fig. 3), the allergen con-

TABLE V. Skin tests results with various SRW plant parts collected before and after RW pollination season

		Reciprocals of highest dilution giving a 2+ reaction			
		Controls		SRW-sensitive patients	
	Date collected	1	2	1	2
Seedlings	May 30	---	---	10 ³	10 ³
Inflorescence	Aug. 1	---	---	10 ³	10 ⁴
	Nov. 30	---	---	10 ³	10 ³
Stems	Aug. 1	10	---	10 ³	10 ³
	Nov. 30	---	---	10 ³	10
Leaves	Aug. 1	---	---	10 ³	10 ³
	Nov. 30	---	---	10 ³	10 ³
Roots	Aug. 1	---	---	10 ³	10 ³
	Nov. 30	10	---	10	10
SRW standard		---	---	10 ³	10 ³

*Less than 2+ when skin tested at 1:10 dilution of 1 mg/ml solution.

tents of these extracts could not be quantitated absolutely. Accordingly, the allergen contents of these extracts listed in Tables III and IV were recorded as estimates only. The slopes of the inhibition lines produced by the SRW standard and by extracts of inflorescence collected before, during, and after the RW pollination season in the total SRW RAST are demonstrated in Fig. 5.

The SRW allergen activity in SRW plant parts, collected before and after the RW pollination season, was also verified in vivo by skin testing these extracts on two healthy control subjects and two SRW-sensitive patients. The results are presented in Table V. Except for the root sample obtained on November 30, the plant-part extracts demonstrated positive reactions in patients at dilutions of at least tenfold higher than on healthy control subjects.

DISCUSSION

It has been known that only a negligible number of particles as large as the intact SRW pollen grains (20 μ m) penetrate to the bronchi and thus are unlikely to cause symptoms of bronchial asthma. However, RW-induced asthma is an accepted clinical diagnosis in patients who experience their symptoms of dyspnea during RW pollination season and who lack sensitivity to other allergens in the air at that time. Bruce et al.¹⁴ found that of 29 RW-sensitive asthma patients who demonstrated positive skin tests, LHR, and inhalation challenge tests, only 13 patients had symptoms that correlated with the atmospheric RW pollen counts. It has also been reported that patients with a high degree of sensitivity to SRW often experience

mild hay fever symptoms both before and after the RW pollination season.³ Furthermore, by immunochemical analysis of atmospheric samples, we found significant amounts of total SRW antigen and AgE before and after the RW pollination season.³ Results of sizing the particles clearly demonstrated that the RW antigen activity is present in particles of different size ranges varying from 0.3 μ m to >6 μ m, as detected by in vitro and in vivo tests. These observations confirm the results of Busse et al.¹ that RW antigens are carried in particles smaller than the intact RW pollen, as well as the results of Solomon et al.¹⁵ who demonstrated by immunochemical means that ragweed antigenic determinants were associated with airborne particles between 0.8 μ m and 5 μ m in diameter. We considered the possibility that these antigens are carried in soil dust particles coated with RW antigens. However, extracts of soil samples collected at a single site from June 1981 to November 1981 demonstrated very little allergen activity (data not shown).

SRW plant parts collected before, during, and after the RW pollination season demonstrated significant SRW allergen, AgE, and RRA activities (Tables III and IV). The extracts of inflorescence, stems, and leaves of SRW plants collected before and after the RW pollination season also demonstrated significant in vivo allergen activity when these extracts were skin tested on RW-sensitive patients (Table V). Only root extracts collected before the RW season demonstrated significant in vivo allergen activity. Published findings on these points are at variance. Lowell¹⁶ was unable to detect allergen activity in SRW leaves,

stems, and seeds by direct skin testing in RW-sensitive patients. Rebhun et al.,¹⁷ however, demonstrated by direct skin testing that GRW allergens were present in all parts of GRW plants. Larson and Gleich¹⁸ found a moderate amount of AgE in RW plant anthers before the RW pollination season. Shafiee and Staba¹⁹ were able to isolate two allergenically active fractions from SRW leaf cells grown in tissue cultures. These workers²⁰ also isolated AgE from leaves and stems of the SRW plant collected before the RW pollination season.

The demonstration of SRW allergenic activity in SRW plant parts in our study, especially the inflorescence, as late as November 30 would suggest persistent SRW plant debris as a source of antigen in the air before and after the pollen season. Another possible source of RW allergen may be other botanical matter cross-reactive with SRW, contributing to out-of-season as well as seasonal airborne SRW antigen activity.

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