

THE VOLUMETRIC INCIDENCE OF ATMOSPHERIC ALLERGENS

IV. A PROPOSED STANDARD METHOD OF GRAVITY SAMPLING, COUNTING, AND VOLUMETRIC INTERPOLATION OF RESULTS

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SINCE the degree of contact of the sensitive person with air-borne allergens is strictly determined by the number of allergenic particles brought into his respiratory organs in a given volume of inhaled air during a very short period of exposure, it follows that the most logical measure of allergenic air contamination should be some convenient volumetric unit. This was first recognized by Blackley.¹ But so far no satisfactory volumetric sampling apparatus has been devised. It is true that several types of volumetric dust counters are altogether practicable for spot tests and short-run pollen sampling, but the usefulness of such instruments in allergy research has not been exploited, largely because we have had no basis for comparing the results yielded by them with the figures obtained from gravity slide tests. No reliable volumetric device has yet been found which will run unattended twenty-four hours a day, for months at a stretch, furnishing an unbroken series of seasonal air samples that can be easily counted. So the gravity method of sampling (adhesive coated slides held in a horizontal position) remains our only practicable source of seasonal aero-allergen data. I am convinced that we must make the best of it.

Efforts to interpret gravity slide figures in volumetric equivalents have from the start been hampered, first, by unwarranted assumptions as to the behavior of free-falling particles in outdoor air, second, by a completely erroneous table of conversion factors (based on Stokes' law) that stood unchallenged for nearly twenty years,^{2, 3} and, third, by the lack of a standard exposure apparatus for gravity slides.

LOCATION OF SAMPLING STATION

Since the greatest need for atmospheric pollen research is in the urban areas where the majority of the hay fever sufferers reside, it should be recognized at the outset that the "absolute pollen frequency of the air"⁴ differs widely at a given moment in various parts of any city and fluctuates greatly from hour to hour, as well as from day to day, in a given location. We must also recognize that buildings, trees, and other objects cause unavoidable air turbulence and unpredictable air currents that greatly influence the deposit of pollen on gravity slides. Therefore, since no ideal testing location can possibly be found or arranged, the only type of location that can be regarded as *standard* for obtaining a typical daily average measure of city-wide air contamination is the top of a tall building in the downtown section—a point farthest from possible local sources of air pollution. The roof chosen should be level and, if possible, free from obstructions. If not, the spot on it chosen for exposures should be as far from obstructions as possible. When gravity tests are made in residence parts

*From the Biological Laboratories of Abbott Laboratories.

of the city the flat roof of an apartment building would be most likely to yield data comparable to those of a downtown location. Porches and window ledges are not recommended, but special and unusual locations may, of course, be suitable for the study of special local exposure problems. Certainly in reporting results of any atmospheric survey the sampling location should be stated and described or pictured in detail.

A STANDARD GRAVITY SAMPLING DEVICE

The simplest possible apparatus for exposing gravity slides is a flat, unobstructed, horizontal plane on which a prepared slide may be laid face up. The major objections to this method are that it affords no protection from rain, that even a smooth horizontal surface is known to cause a ripple effect on air currents flowing over it, and that dust and other particles falling on the dry surface of any area larger than the slide may be subsequently swept directly onto the adhesive surface of the slide. In practice, such an exposure results in a heavier catch than an exposure at the same spot which allows air to flow under as well as over the slide.

The need of protection for the slide from the fall of soot and large airborne particles and from the direct wash of rain has led most workers to adopt some sort of cover for the slide. A popular form of shelter⁵ consists of a dome-shaped metal tunnel having openings cut in each side. The slide is usually placed in the center of this shelter flat on the floor. Such a device might recover comparable proportions of the pollen content of the air at each exposure if mounted on a wind vane so that the open end of the tunnel would always face the wind, but obviously without such a mounting the turbulence and the consequent effect on pollen impingement must differ greatly with any change in wind direction.

In my nation-wide studies at the U. S. Weather Bureau stations⁶ the slides have usually been held in a wooden clip placed about 1 foot beneath the Weather Bureau's instrument shelter which is some 4 feet square. These instrument shelters are usually located on top of tall business buildings. The deposits of ragweed pollen on slides exposed by any of the above methods (other exposure factors being similar) average considerably heavier than the deposits on slides exposed by methods to be described.

In two series of experiments⁷ carried out on a large four-story building in North Chicago (1942, 1943), I exposed slides in a horizontal position on top of a 3-foot iron rod, without a shelter of any sort. This very simple device offered the least possible interference to passing wind currents, but in spite of this the day by day deposit was far from uniform in proportion to the daily average ragweed pollen content of the air as measured by parallel tests with the Hawes volumetric impinger* or the Keitt volumetric filter,* or both run simultaneously.

*The Hawes apparatus pulls approximately 0.1 cu. ft. of air per minute through a slit 10 mm. long and 0.2 mm. wide, impinging all particles on an adhesive slide held 2 mm. away from the slit. The slide is moved very slowly by clock work so that the deposit is left in a convenient swath. The Keitt apparatus pulls approximately 1 cu. ft. of air per minute through a nitrocellulose filter. The plug of nitrocellulose is dissolved and the pollen content is plated for counting.

Most of the gravity slides caught twice as much pollen per square centimeter as could possibly be accounted for by Stokes' law, but on some days the excess was as much as six times the calculated amount.

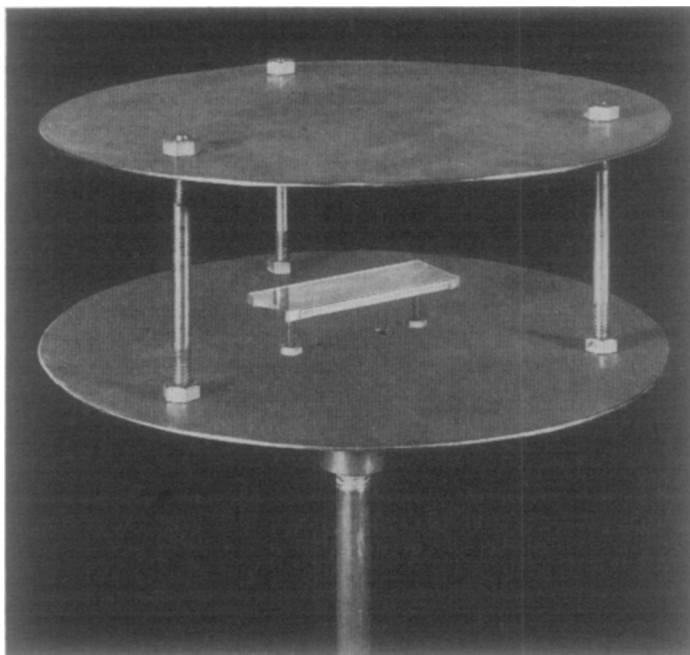


Fig. 1.—Standard air-sampling device. The apparatus consists essentially of two parallel planes of polished stainless steel, 9 inches in diameter, with the slide holder raised 1 inch above the lower plane. It is supported by a 30-inch metal rod on a tripod laboratory stand. Screw holes are provided in the base so that it may be fastened securely to any wooden floor.

The device pictured in Fig. 1 makes use of two parallel planes of polished stainless steel held in position by three narrow struts. The purpose of these planes, aside from the rain protection afforded by the upper one, is to cause an even horizontal flow of air between them regardless of the direction or velocity of the wind. As used in the 1944 experiments, the oiled surface of the slide was held *flush* with the surface of the lower plane which was itself oiled in order to fix all pollen and other particles falling on it. In the form shown here and used for making comparative exposures during 1945, the slide holder is elevated 1 inch above the lower plane, thus allowing the air stream to flow underneath as well as over the slide. This has resulted in a smaller deposit of pollens and spores (as compared with our parallel volumetric determinations), thus more nearly approaching the ideal calculated gravity deposit.

The need of a standardized method of exposure is strongly emphasized by the tabulated results (Table 1) of exposures with this improved, proposed standard device as compared with parallel exposures in shelters of varying design as noted. These shelters were, in each locality, the ones which have been used for a number of years by the individual local collaborators.

TABLE I. GRAVITY TEST COMPARISONS: RAGWEED SEASON, 1945

PLACE	DESCRIPTION OF OLD SHELTER	NUMBER OF DAYS	TOTAL CATCH		RATIO (NEW/OLD)
			NEW DEVICE	OLD SHELTER	
New York, N. Y., U. S. Weather Bureau, Central Park, ground level	6 inch vertical space between large covers, open at west and east ends	22	444	624	1.41
Brooklyn, N. Y., Jewish Hospital; roof; 5 stories	Metal tunnel; slide flat on floor	22	274	1,488	5.43
Philadelphia, Pa., Elverson Medical Building, 15th floor	Metal tunnel; slide elevated with air clearance under slide	48	2,817	3,798	1.35
Pittsburgh, Pa., Montefiore Hospital; roof; 4 stories	Wood tunnel, gable roof; slide elevated 1 inch on block	46	395	510	1.28
Pittsburgh, Pa., suburban residence, Penn Township; ground level	Same as above	50	2,574	2,371	0.92
Cleveland, O., Carnegie Medical Building; roof; 9 stories	Metal tunnel	43	657	804	1.63
Toledo, O., Medical Building; roof; 9 stories	Wood gable roof on two posts	50	2,998	6,034	2.01
Madison, Wis., Wisconsin General Hospital; roof; 6 stories	Wood roof supported by four corner posts	41	2,286	5,909	2.58
Milwaukee, Wis., U. S. Weather Bureau; P. O. Building; roof; 7 stories	Under U. S. Weather Bureau standard instrument shelter	4	378	1,573	4.16
North Chicago, Ill., Research laboratory; roof; 3½ stories	Shelter roof 24 in. square; 5 in. clearance	13	393	1,473	3.75
Chicago, Ill., Old P. O. Building; roof; 9 stories	Under U. S. Weather Bureau standard instrument shelter	30	645	1,704	2.61
Nashville, Tenn., Doctors Building	Parallel wood planes 18 in. by 21 in.; 5 in. clearance; 4 corner posts	44	1,914	2,424	1.27
Dallas, Tex., Medical Arts Building; 17 stories		70	9,512	11,356	1.19

CALIBRATION OF SAMPLING DEVICE

Finally, it was necessary to determine the average recovery of a given kind of pollen with this new proposed standard device as compared with the amount of that particular pollen in a cubic yard of air. A series of 17 tests (approximately 136 hours) were carried out using the gravity sampling device in parallel with the Hawes impinger during the ragweed season of 1945 (Table II). Part of the tests were made on the roof of a 3-story research laboratory building in North Chicago and part on the 7-story annex of the post-office building in Milwaukee. While air samples of ragweed, taken with the device, contained on the average nearly three times as much pollen as would be predicted by Stokes' law (using my experimental rate of fall for short ragweed for purposes of calculation), the daily figures were more consistent in their relation to the daily volumetric ragweed pollen determinations than those obtained with uncovered slides and much more consistent than those from gravity slides exposed by the old Weather Bureau method. The volumetric control tests made in parallel with each gravity test showed an average of 3.6 times as many ragweed pollen grains per cubic yard of air as were deposited on 1 sq. cm. of the gravity slides

TABLE II. EXPERIMENTAL RATING OF STANDARD SAMPLING DEVICE

PLACE	DATE 1945	GRAVITY: POLLENS PER SQ. CM. IN 24 HR.	VOLUMETRIC: POLLENS PER CU. YD. OF AIR, AVERAGE	RATIO GRAVITY/ VOLUME
North Chicago	August 20	34	110	3.2
North Chicago	August 24	100	254	2.5
North Chicago	August 26	9	10	1.1
North Chicago	August 27	169	273	1.6
North Chicago	August 28	111	681	6.1
Milwaukee	August 30	47	180	3.8
Milwaukee	August 31	222	821	3.7
Milwaukee	September 2	57	130	2.3
Milwaukee	September 3	52	115	2.2
North Chicago	September 5	66	407	6.2
North Chicago	September 6	30	202	6.7
North Chicago	September 7	113	159	1.4
North Chicago	September 9	68	478	7.0
North Chicago	September 10	45	201	4.4
North Chicago	September 11	45	183	4.1
North Chicago	September 12	16	38	2.4
North Chicago	September 19	10	55	5.5
Totals		1,194	4,297	
Ratio of totals				3.6

exposed for twenty-four hours. (For shorter exposure periods than twenty-four hours a larger equivalent area was counted).

STANDARD CONVERSION FACTORS

I suggest that the above factor—3.6—be regarded for present purposes as a standard conversion factor for ragweed air sampling *with this instrument* in areas where short ragweed is predominant. Thus an approximation of the average number of ragweed pollen grains per cubic yard of air for a twenty-four-hour period may be obtained either by counting an area of 1 sq. cm. on a twenty-four-hour slide and multiplying by 3.6 or by counting an area of 3.6 sq. centimeters. Since this latter area happens to be just twice the area that has been so widely used (1.8 sq. cm.), this part of the counting and interpolation will offer little difficulty.

If this ragweed factor proves to be acceptable it may be used as a pivot for establishing conversion factors for all other types of pollen found on slides exposed in the described standard apparatus. This can be done, as will be shown shortly, by means of the figures which I have already determined experimentally for the relative rates of fall of pollen grains of some 40 other species of hay fever plants.

At this point an important question will naturally arise concerning the fact that a considerable part of the deposit on any slide is evidently caused by impingement rather than by gravity. How can we then apply rate of fall data as though all of the deposit were the result of the action of gravity? The answer is that centrifugal force acts in much the same way as gravity in sorting particles of different weights. The heavier particles in the air are impinged in greater proportion than the lighter particles, the latter tending to remain in the air and be carried around the edge of the slide which intercepts the air current.

TABLE III. CONVERSION FACTORS FOR TYPICAL POLLENS

COMMON NAME	BOTANICAL NAME	DIAMETER IN MICRONS	EXPERIMENTAL RATE OF FALL AS COMPARED WITH SHORT RAGWEED	A	B
				FACTOR FOR 1 SQ. CM.	FACTOR FOR 3.6 SQ. CM.
Giant ragweed	<i>Ambrosia trifida</i>	19.25	0.93	3.87	1.08
Burweed marsh elder	<i>Iva xanthifolia</i>	19.3	1.07	3.36	0.93
Short ragweed	<i>Ambrosia elatior</i>	20.0	1.00	3.60	1.00
False ragweed	<i>Franseria acanthicarpa</i>	22.0	1.14	3.16	0.88
Marsh elder	<i>Iva ciliata</i>	23.0	1.34	2.69	0.75
Southern ragweed	<i>Ambrosia bidentata</i>	23.0	1.31	2.75	0.76
Western ragweed	<i>Ambrosia psilostachya</i>	26.4	1.59	2.26	0.63
Cocklebur	<i>Xanthium commune</i>	27.0	1.55	2.32	0.65
Russian thistle	<i>Salsola pestifer</i>	23.6	2.10	1.71	0.48
Palmer's amaranth	<i>Amaranthus palmeri</i>	25.8	2.10	1.71	0.48
Western water hemp	<i>Acnida tamariscina</i>	27.5	1.66	2.17	0.60
Mexican fireweed	<i>Kochia scoparia</i>	32.7	3.24	1.11	0.31
Annual sage	<i>Artemisia annua</i>	20.4	1.14	3.16	0.88
Tall wormwood	<i>Artemisia caudata</i>	21.0	1.10	3.27	0.91
Sagebrush	<i>Artemisia tridentata</i>	25.85	1.79	2.01	0.56
Nettle	<i>Urtica gracilis</i>	14.0	0.38	9.47	2.63
Red sorrel	<i>Rumex acetosella</i>	21.45	1.06	3.40	0.94
Hemp	<i>Cannabis sativa</i>	25.0	1.17	3.08	0.85
English plantain	<i>Plantago lanceolata</i>	27.5	1.69	2.13	0.59
Bluegrass	<i>Poa pratensis</i>	28.0	1.66	2.17	0.60
Bluegrass	<i>Poa pratensis</i>	30.0	1.97	1.83	0.51
Bermuda grass	<i>Capriola dactylon</i>	28.5	2.10	1.71	0.48
Orchard grass	<i>Dactylis glomerata</i>	34.0	3.14	1.15	0.32
Timothy	<i>Phleum pratense</i>	34.0	3.17	1.14	0.32
Rye	<i>Secale cereale</i>	49.5	4.86	0.74	0.21
Corn	<i>Zea mays</i>	90.0	20.7	0.17	0.05
Sycamore	<i>Platanus occidentalis</i>	22.22	1.18	3.05	0.85
Mountain cedar	<i>Juniperus sabinoides</i>	22.8	1.31	2.75	0.76
Hazelnut	<i>Corylus americana</i>	23.6	1.48	2.43	0.68
Birch	<i>Betula nigra</i>	24.6	1.90	1.89	0.53
Alder	<i>Alnus glutinosa</i>	26.0	1.66	2.17	0.60
Ash	<i>Fraxinus americana</i>	27.1	1.72	2.09	0.58
Cottonwood	<i>Populus virginiana</i>	30.0	1.72	2.09	0.58
Elm	<i>Ulmus americana</i>	31.2	2.31	1.56	0.43
Bur oak	<i>Quercus macrocarpa</i>	32.3	2.07	1.74	0.48
Shingle oak	<i>Quercus imbricaria</i>	33.1	2.48	1.45	0.40
Walnut	<i>Juglans nigra</i>	35.75	3.21	1.12	0.31
Beech	<i>Fagus grandifolia</i>	44.0	4.00	0.90	0.25
Hickory	<i>Carya ovata</i>	45.0	2.55	1.41	0.39
Scotch pine	<i>Pinus sylvestris</i>	52.0	2.82	1.28	0.35
Bull pine	<i>Pinus ponderosa</i>	60.0	3.95	0.91	0.25

Formula for deriving factors in columns A and B:

$$\text{Factor A} = \frac{3.6}{\text{comparative rate of fall}} \quad \text{Factor B} = \frac{A}{3.6}$$

This I have verified a number of times by whirling an oil-coated slide through the air, oiled face forward, at a rate of approximately 50 miles per hour. Such a slide picks up a much smaller proportion of the lighter particles in the air, such as *Alternaria*, than of the heavier ragweed pollen grains as compared with the number of each in the air as shown by simultaneous volumetric sampling. Moreover, in the course of my four seasons' experience with gravity sampling controlled with volumetric tests, I have found that the more buoyant particles such as nettle pollen grains and *Alternaria* spores actually produce lighter "gravity"

deposits than ragweed, while heavier pollen grains such as Mexican fireweed, western water hemp, and hemp produce heavier deposits in about the expected proportion.

As a result of these findings I feel justified in deriving standard conversion factors (as shown in Table III) from figures for comparative rates of fall as originally published in the third paper of this series.⁸

It must be noted that these factors apply only to gravity slide data from exposures in the apparatus described. Standardization of any other gravity sampling device which in the future may seem more acceptable than this one will require at least one season of daily volumetric control tests, preferably during a season of ample ragweed pollen incidence, and the development of a whole new set of conversion factors. In spite of variations in pollen grain size and specific gravity which necessarily affect the accuracy of the conversion factors here presented, it is quite likely that errors from these sources are much smaller than the inherent errors in any gravity sampling method. Extreme accuracy is an impossible goal in this field of research.

DISCUSSION

Factors are given for eight species of ragweed, six of which are of more or less restricted distribution. The amount of giant ragweed pollen in the air in most areas will hardly justify the use of a higher factor during the early part of the ragweed season. If in some areas the average size of air-borne ragweed pollen grains throughout the whole season or during the greater part of the season proves to be appreciably more or less than 20 microns, an adjusted factor should be used. The only other possible call for adjustment of the ragweed factor would be in the case of an experimenter finding the actual ratio of ragweed pollen per cubic yard of air to the number per square centimeter of slide at his place of sampling to be more or less than 3.6. Such a local factor could be established only by a long series of parallel volumetric and gravity tests.

The above suggestions apply to the use of all other factors for the typical pollens listed in Table III. If, for example, the poplar pollen grains found on the slides in a particular locality average smaller or larger than the 30-micron cottonwood pollen which I have tested, a different local conversion factor will need to be calculated. This may be derived as follows, assuming that local poplar species measures 32 microns in polar diameter (expanded in Calberla's solution or other aqueous medium).

$$\frac{32^2}{30^2} = \frac{2.09}{x}, \text{ solving}$$

$$x = \frac{30^2 \times 2.09}{32^2} = 1.84$$

Obviously no common factor can be used for grass pollens since the size of grass pollen granules varies considerably with the prevailing grass pollen in the air. The factor for redbow (diameter, 26 microns) will likely be about 2.8 and Canada bluegrass (diameter, 30 microns) about 1.97—as for bluegrass of that same diameter.

The set of factors in column *A* of Table III are to be used when the unit slide area counted is 1 sq. cm., but in case it is deemed more convenient to count a slide area of 3.6 sq. cm., by which method ragweed figures will not need to be converted, the factors for other pollens will be found in column *B*.

Factors for those types of fungus spores which may be identified on gravity slides are not yet available except for *Alternaria*. From extensive data on volumetric incidence and relative gravity deposit of ragweed pollen and *Alternaria* spores given in the second paper of this series, we seem justified in using *A* and *B* factors for *Alternaria* which are 4.14 times those of ragweed—factor *A*, 14.90; factor *B*, 4.14.

SUMMARY

This paper stresses the need of a standardized method of sampling, counting, and volumetric conversion of data for air-borne allergens. As a result of five seasons of experimentation with various gravity sampling devices controlled by parallel volumetric tests, the following proposals are submitted:

1. The basic index of atmospheric contamination in a given community should depend on aerobiologic data secured from air samples taken on the unobstructed top of a tall downtown building.

2. A practicable gravity sampling device (slide shelter) is pictured and described, and suggested as a tentative standard device. It has been used in a number of places during the past season and has been carefully rated as to the average proportion of ragweed pollen which it recovers from a cubic yard of air.

3. A method of volumetric conversion of pollen and fungus spore counts is submitted with definite factors for about 40 pollens and one fungus spore. Two sets of conversion factors are listed so that counting may be done on the basis of 1 sq. cm. of slide area or on 3.6 sq. centimeters.

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