

THE VOLUMETRIC INCIDENCE OF ATMOSPHERIC ALLERGENS*†

V. SPOT TESTING IN THE EVALUATION OF SPECIES

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THE purpose of this paper is to call attention to a little-used method of air sampling and to demonstrate its value in supplementing our information on the dispersal of air-borne allergens. The term "spot test" as here used refers to a determination of the absolute frequency of atmospheric allergens, that is, the number of particles in a given volume of air at a particular moment in a designated location. By the simple techniques here described, sampling may be accomplished in less than five minutes. The method, therefore, yields information which cannot be obtained by the gravity slide method, which affords only an approximate *mean* of the aerial incidence of various particles over a relatively long period—usually twenty-four hours—and does not give any idea of the maximal or minimal concentrations during that period.

In studying atmospheric pollen frequency, it is highly desirable to learn as much as possible about major fluctuations in the air content throughout the day. This is particularly true because the allergic response to inhaled pollen may take place within a few seconds and persist for an hour or more without further exposure.

Spot testing may be used advantageously in studying the effects of certain weather factors on the dispersal of air-borne allergens as well as in assessing the air pollution of a given indoor or outdoor location where gravity testing is not practicable. It should prove helpful in discovering sources of strictly local plant offenders. In some areas the amount of any kind of pollen suspended in the upper air is so small that the results of gravity tests have no statistical meaning. Yet, in spite of the lack of general air contamination in such areas, there may be sufficient local contact with certain active pollens to cause severe and persistent allergic symptoms in a significant number of persons. Under such conditions intensive air research requires a far more flexible technique than that afforded by the gravity method.

HISTORICAL

Short time exposures, as already noted, are not feasible with gravity slides. A few 24-hour studies have been made by using one-hour¹ or two-hour² slide exposures, but such tests are possible only where pollen concentrations are very high. In Blackley's³ early atmospheric experiments he obtained air samples by means of an aspirator impinger which gave satisfactory volumetric readings in a period of an hour or less. As a result of tests made with this instrument he concluded, "In the middle portion of the day the amount of

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deposit obtained in a short period was always, or nearly always, largely in excess of the average for a longer period. Then the deposit obtained in twelve hours of the day was generally much in excess of that obtained during the twelve hours of the night. Occasionally, however, the night deposit would be large, whilst that of the following day would be small in amount. As I have said above, these facts show that the amount of pollen in the atmosphere was constantly changing." However, because of the difficulty involved in operating the device, it was abandoned in favor of the 24-hour gravity method.

Duke⁴ experimented with an electric fan impinger which enabled him to take five-minute air samples in any location where electric current was available. In a paper describing his technique (nonvolumetric) he referred to it as a "rapid and more accurate method of determining the pollen content of the air." He stressed the usefulness of the device, and attempted to standardize it by means of parallel gravity tests. But he neglected to carry out systematic research on any of the many problems for which the device was so obviously suitable.

In upper air sampling by aeroplane, very short exposures of oiled slides or agar plates are usually necessary because of the rapid vertical and lateral changes in the position of the plane, and because of the high rate of impingement caused by the speed of the plane. However, the pollen and fungus spore data which have been obtained from aeroplanes have seldom, if ever, been accurately volumetric, and have not as yet been coordinated with data obtained from stationary sampling devices.

The Owens' dust counter⁵ or the Wells' centrifuge⁶ could be used for spot testing, but no one has so far reported any aero-allergen research carried out by means of these or similar instruments. One probable reason why these devices have not been systematically exploited in this type of research is that until recently there was no reliable basis of volumetric comparison between spot reading and gravity slide figures. But now that a satisfactory gravity-volumetric technique has been devised,⁷ spot readings immediately assume an important place in pollen aerobiology.

APPARATUS AND TECHNIQUE

In assessing the extreme productive ability of wind-pollinated and even insect-pollinated plants and fungi, I have used two simple devices. One of these, the Hill dust pump, consists of an air pump which impinges all captured particles in three small spots on an oiled slide. The air intake of the pump is approximately 4.5 cubic inches per stroke. By regulating the number of strokes in accordance with the degree of air pollution at a given time and place, satisfactory volumetric samples can be obtained. On a few rare occasions a single stroke of the piston has been sufficient to test the content of visible clouds of pollen, while in other situations several hundred strokes were needed. Hand-operated impinging dust counters of several designs are available, but those with attached microscopes are not very well adapted to aero-allergen research.

Another convenient device is an "air whip"—a 36-inch aluminum rod for swinging a slide in a circle with the oiled face forward. By means of repeated parallel tests, this device has been carefully calibrated against the Hill dust impinger just described, as well as against the power-driven slit impinger devised by Hawes and associates,⁸ which has been used extensively in my own previously reported volumetric studies.^{7, 9, 10, 11} No great difficulty has been encountered in standardizing the number of turns and the rate of rotation of the device nor the number of trips to be counted across the slide to give the approximate equivalent of the pollen content of a cubic yard of air. For ragweed pollen and those of greater size I usually make 200 turns at the rate of 100 per minute. In counting, it is only necessary to cover ten trips across the slide and to multiply the result by four. This technique allows any amount of variation according to the density of the air content. For very light fungus spores, the pick-up with this device is not as accurate as with the pump. With either device the tests can be made in a very short time. Under some conditions the dust pump is more convenient, under others the whip is preferable. Neither instrument is bulky or heavy, and counting of the samples with either is not difficult.

In securing the spot records shown in the table, every effort was made to take samples of particular species under ideal natural conditions of maximum pollen production and dispersal, numerous successive tests being made on the same occasion or on different days during anthesis. Usually the samples were taken early in the morning at the time of maturation of pollen, and, when possible, during a heavy wind on the leeward side of producing plants. Unfavorable weather conditions or other exigencies prevented adequate testing of certain species. Some common important wind-pollinated plants have not yet been tested because they do not occur or do not reach maximum development in the Chicago area where all this work has been done.

DISCUSSION OF DATA

The astonishing record for giant ragweed was obtained on Aug. 21, 1945, near Highland Park, Illinois, in the middle of a country road that runs directly through a pure stand of some 30 acres of giant ragweed. The tests were made at about 9 o'clock in the morning during the first few minutes of a sudden line squall which was preceded by ideal ripening conditions without wind. In those few minutes the windstorm dislodged the total pollen output for the day. A great yellow cloud 50 feet high was easily visible at a distance of 200 yards. The degree of air contamination on the highway at the moment of sampling may be best appreciated by the observation that while making the tests I was inhaling more than 6,000 pollen granules with every breath, or more than 100,000 granules a minute—more than a whole season's intake for the average city apartment resident. But in spite of this and other similar dense clouds of giant ragweed pollen in the country on that day, the gravity slide at the Weather Bureau station in Chicago recorded a 24-hour average of only 60 ragweed pollen grains per cubic yard of air.

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Ragweeds				
Short (<i>Ambrosia elatior</i>)	5628	Indianapolis	78,000	In wheat stubble. Inadequate record.
Giant (<i>Ambrosia trifida</i>)			9,600,000	In a visible cloud of pollen 20 feet from weeds
Cocklebur (<i>Xanthium spp.</i>)	8	Kansas City, Mo.	440	Test made in very weedy corn-field
Burweed Marsh Elder (<i>Iva xanthifolia</i>)	556	Scottsbluff, Nebr.	160,000	Test made adjacent to small patch
Composites				
Goldenrod (<i>Solidago canadensis</i>)	5		192	Three feet above weeds
Sunflower (<i>Helianthus annuus</i>)	-		12	On leeward side of large acreage
Wild Lettuce (<i>Lactuca canadensis</i>)	-		0	In large patch
Fleabane (<i>Erigeron canadensis</i>)	-		0	In large patch
Chamomile (<i>Anthemis cotula</i>)	-		0	In large patch
Chicory (<i>Cichorium intybus</i>)	2		184	In heavily grown vacant lot
Coneflower (<i>Lepachys columnifera</i>)	-		0	In patch of many acres
Daisy Fleabane (<i>Erigeron ramosus</i>)	-		60	Test on windy day in large field
Ox-eye Daisy (<i>Chrysanthemum leucanthemum</i>)	-		504	Three feet above blossoms in high wind
Coreopsis (<i>Coreopsis lanceolata</i>)	-		0	Leeward side of large patch
Dandelion (<i>Taraxacum officinale</i>)	-		2	Ten-acre lawn. Heavy crop. Windy.
Dog Fennel (<i>Eupatorium capillifolium</i>)	107	New Orleans	-	
Unidentified composite	177	Oklahoma City	-	
Grasses				
Orchard Grass (<i>Dactylis glomerata</i>)	-		2,000,000	In a visible cloud of pollen
Canada Bluegrass (<i>Poa compressa</i>)	-		460,800	Four feet above heavy growth
Redtop (<i>Agrostis palustris</i>)	-		328,000	Leeward side of meadow
Timothy (<i>Phleum pratense</i>)	-		66,000	In a large field. Unsatisfactory record.
Bluegrass (<i>Poa pratensis</i>)	-		14,000	In a large field. Unsatisfactory record.
Millet (<i>Setaria italica</i>)	-		9,840	In a large field
Corn (<i>Zea mays</i>)	-		4,640	Roadside, leeward side of field.

*References are noted and credit is thus extended for gravity records which are quoted or interpolated from published reports other than those of the author. All other gravity figures are from the author's own records made over a period of more than two decades. These studies have been made possible by assistance in air sampling by the observers of the United States Weather Bureau, the Canadian Meteorological Service, and numerous cooperating allergists and interested laymen in all parts of the United States and Canada.

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES (CONT'D)

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Oats (<i>Avena sativa</i>)	—		1,360	Three feet above blossoming heads
Sudan Grass (<i>Holcus sorghum sudanensis</i>)	—		704	Leeward side of heavy growth
Western Wheat Grass (<i>Agropyron smithii</i>)	—		520	Leeward side of small patch
Wheat (<i>Triticum sativa</i>)	—		80	Three feet above heads
Unidentified mixed grasses	250	Kansas City, Mo.	—	
Unidentified mixed grasses	770	Minneapolis ¹²	—	
Chenopodiales				
Mexican Firebush (<i>Kochia scoparia</i>)	165	Scottsbluff, Nebr.	86,400	Three feet above weeds
Russian Thistle (<i>Salsola pestifer</i>)	110	Aberdeen, S. Dak.	—	
Western Water Hemp (<i>Acnida tamariscina</i>)	528	Omaha	1,800	Leeward side of heavy growth
Water Hemp (<i>Acnida tuberculata</i>)	—		12,000	Three feet above weeds
Lamb's Quarters (<i>Chenopodium album</i>)	15	Indianapolis	768	In large weed patch
Pigweed (<i>Amaranthus retroflexus</i>)	15	Cleveland	800	In large weed patch
Miscellaneous Weeds				
Hemp (<i>Cannabis sativa</i>)	116	Omaha	—	
Hop (<i>Humulus japonica</i>)	134	Philadelphia ¹³	—	
Nettle (<i>Urtica gracilis</i>)	56	Omaha	46,665	Leeward side of small patch
	184	Minneapolis ¹²		
English Plantain (<i>Plantago lanceolata</i>)	30	Philadelphia ¹³	3,840	Four feet above heavy growth
	136	Minneapolis ¹²		
Red Sorrel (<i>Rumex acetosella</i>)	25	Yonkers ¹⁴	50	Unsatisfactory spot record
	574	Minneapolis ¹²		
Trees				
Pine (<i>Pinus spp.</i>)	387	Grand Lake, Colo.	12,000	Under Austrian pine tree
Alder (<i>Alnus spp.</i>)	200	Richmond, Va. ¹⁵	—	
Elm (<i>Ulmus americana</i>)	618	Worcester, Mass. ¹⁶	15,000	Under tree, windy
	1,755	Minneapolis ¹²		
Elm (<i>Ulmus crassifolia</i>)	176	Dallas	—	
Cottonwood (<i>Populus virginiana</i>)	302	Dallas	930	Under trees. Unsatisfactory
	3,287	Minneapolis ¹²		
Box Elder (<i>Acer negundo</i>)	909	Minneapolis ¹²	1,168	Under tree
Sugar Maple (<i>Acer saccharum</i>)	—		300	Under tree
Soft Maple (<i>Acer saccharinum</i>)	—		256	Under tree
Ash (<i>Fraxinus americana</i>)	370	Minneapolis ¹²	12,000	Under tree
Sycamore (<i>Platanus occidentalis</i>)	597	Philadelphia ¹³	—	
Oak (<i>Quercus spp.</i>)	650	Yonkers ¹⁴	1,000	Unsatisfactory record
	2,809	Minneapolis ¹²		
Walnut (<i>Juglans nigra</i>)	51	Charlotte, N. C.	—	
Hickory (<i>Hicoria spp.</i>)	125	Kansas City, Mo.	—	
Birch (<i>Betula papyrifera</i>)	238	Worcester, Mass. ¹⁶	1,400	Under tree
Pear (<i>Pyrus communis</i>)	—		948	Under tree
Willow (<i>Salix nigra</i>)	—		198	Under tree

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES (CONT'D)

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Wild Plum (<i>Prunus americana</i>)	-		32	Leeward side of bushes
Paper Mulberry (<i>Broussonetia papyrifera</i>)	335	Charlottesville, Va. ¹⁷	-	
Miscellaneous Entomophilous Plants				
Sweet Clover (<i>Melilotus alba</i>)	-		992	Leeward side of heavy growth
Red Clover (<i>Trifolium pratense</i>)	-		0	In large field
Black Mustard (<i>Brassica nigra</i>)	-		96	Two feet above the weeds
Opium Poppy (<i>Papaver somniferum</i>)	-		27	Two feet above the blossoms
Knotweed (<i>Polygonum spp.</i>)	-		0	Above heavy growth
Asparagus (<i>Asparagus officinalis</i>)	-		0	Above heavy growth
Tamarisk (<i>Tamarix gallica</i>)	-		0	Beside shrubs
Wild Rose (<i>Rosa blanda</i>)	-		0	Six inches from flowers
Lilac (<i>Syringa vulgaris</i>)	-		0	Beside shrubs
Fungi				
<i>Alternaria spp.</i>	14,600	Moorhead, Minn.	1,360	In a cornfield. Inadequate record
			675,000	In dust from combine (harvester-thresher)
<i>Hormodendrum spp.</i>	20,000	Minneapolis	869,000	In dust from combine (harvester-thresher)
<i>Helminthosporium spp.</i>	30	Moorhead, Minn.	177,327	In dust from combine (harvester-thresher)
Stem Rust (<i>Puccinia graminis</i>)	6,800	Goodland, Kan.	-	
	10,380	Moorhead, Minn.	-	
Corn Smut (<i>Ustilago zeae</i>)	500,300	Wayzata, Minn. ¹⁸	-	

No such ideal opportunity has subsequently presented itself for testing short ragweed. There was no visible cloud of pollen over the stubble of the old wheat field where I got my best short ragweed sample. The cocklebur spot figure is probably a fair one. By comparing it with records for any of the other ragweeds and with those of insect-pollinated composites one may safely conclude that under ordinary conditions the possibility of specific exposure to cocklebur pollen is almost as remote as of exposure to pollen of goldenrod.

In considering the orchard grass record cognizance must be taken of the fact that the sample was secured under conditions similar to that which produced the high figure for giant ragweed. Visible clouds of pollen could be seen blowing across the street from a vacant lot that supported an almost pure growth of orchard grass. No visible clouds of pollen were encountered while testing any of the other heavy producing grasses. It was extremely difficult to obtain high spot counts of corn pollen even on windy days either outside a cornfield or between the corn rows. The western wheat grass record is probably typical of the performance of most of the wild grasses.

Water hemp spot records much higher than mine could probably be obtained in the cornfields and creek bottoms of Iowa and eastern Nebraska. Under

favorable conditions the western species (*Acnida tamariscina*) actually rivals the productive performance of the ragweeds. I doubt whether much better pigweed or lamb's quarters records can be obtained. The English plantain figure is also as high as could reasonably be expected.

Results with the tree tests were as a whole quite disappointing, as weather conditions were often very unfavorable. For some species higher counts have actually been obtained with 24-hour gravity slides, particularly at Minneapolis, where Dr. Rosendahl's sampling station must have been located in a grove of oak or cottonwood trees.

The testing of miscellaneous insect-pollinated plants other than composites has proved what we already suspected—that most such plants are negligible sources of air contamination. The case for rose pollen as an air contaminant is very poor indeed.

My only worth-while spot records for the fungi were obtained in the heavy clouds of dust produced by a combine while in operation in an oat field. These findings should be supplemented by tests in elevators and feed mills as well as in undisturbed grain fields. My highest *Alternaria* spot record made under natural conditions in Illinois was 13,600, which is only slightly less than my highest 24-hour average as found by gravity testing in the center of Moorhead, Minnesota.

SUMMARY

By using two simple sampling devices I have tested the allergen producing ability of more than 60 plant species and several fungi, including many of the more common hay fever offenders and a number of related insect-pollinated species.

As a result of repeated spot testing of the air in the immediate vicinity of single plants, small plots or extensive acreages, some very striking figures for maximum production have been obtained. These are tabulated and in almost every case compared with maximum gravity data (stated on volumetric basis) as previously reported for these plants in areas where they are most abundant.

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DISCUSSION

DR. MATTHEW WALZER (Brooklyn).—As an allergist and as a member of the Pollen Survey Committee of this society, I am indebted, in many ways, to Mr. Durham. It is indeed an unusual state of affairs to have so many who are interested in the treatment of hay fever depend on the contributions of a single man for so much of the information which makes such treatment possible.

This is the fifth in a series of studies by Durham which, undoubtedly, will be historic in this field. It affords a very quick way to obtain, in a few minutes, information about what is happening at a particular spot at a particular time under particular conditions. If you tried to get that information from the gravity slide, it would, of course, be impossible for reasons which have already been pointed out.

These spot-testing procedures have wide applications and advantages. They can be used indoors as well as outdoors. They give us considerable information about certain agents which some think are very important aero-allergens; but, when tested with these instruments, prove to be insignificant.

Of course, procedures of this kind would not be very practical without standard techniques with which they may be compared. That emphasizes the importance of the attempts of the Pollen Survey Committee to standardize counting techniques so that we may all talk the same language and understand each other. It is probably superfluous to point out that these methods for obtaining spot information are not intended to replace the standard pollen counting techniques which Mr. Durham as Technical Chairman of the Pollen Survey Committee is doing so much to promote.