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A new sampler for airborne pollen: The rotoslide

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As experiments have shown that pollen capture by the standard Durham sampler is determined by several factors in addition to the concentration of pollen in the air, a study was conducted to determine what type of sampler would be the most acceptable replacement. Many types of samplers, including deposition surfaces, stationary and rotating impactors, suction, and filter devices, were tested in a wind tunnel and in the field under various meteorological conditions. Considerations of accuracy of sample, use under varying weather conditions, simplicity of operation, ease of counting, conversion of catch to a volumetric basis, and cost led to the development of the rotoslide, a rotating impactor which takes samples of pollen and other spores on the edge of standard glass microscope slides. Staining and other preparation of samples for study are similar to the methods in routine use. A special inexpensive holder positions the slides under a microscope. Identification and counting are done at 100x or at higher magnifications if desired. The rotoslide sampler is essentially independent of wind speed and direction and has a collection efficiency of approximately 64 per cent for particles in the size range of pollen. It samples approximately 60 L. per minute, but for long-period samples it may be operated intermittently to prevent overloading.

Many types of devices have been used to sample pollen in the atmosphere, but in recent years the Durham sampler¹ recommended by the Pollen and Mold Committee of the American Academy of Allergy has been in most general use

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in this country. Experiments have shown that pollen capture by this sampler is determined by several factors, in addition to the concentration of pollen in the air.² The combined effects of wind speed, turbulence, and orientation with wind direction may introduce large differences in catch between samplers exposed to the same pollen concentration. Uniform orientation with wind direction may be accomplished by mounting the sampler on a wind vane. An alternate method of overcoming directional bias which was tested extensively by us is the use of a square slide in a streamlined circular holder. However, errors due to different wind speeds and degrees of turbulence are still present with both these methods and no way of correcting for them has been found. In addition, it is impossible, under field conditions, to determine the volume of air sampled.

Another basic method of sampling airborne particles is by impaction on a cylindrical surface of suitable size oriented at right angles to the airflow. This is a reasonably efficient collector of particles in the pollen size range at any but low wind speeds.³ The collecting efficiency varies directly with wind speed, particle size, and particle density and inversely with cylinder diameter. The theoretical efficiency of a $\frac{1}{4}$ inch diameter cylinder for ragweed pollen varies from 10 per cent at one mile per hour to 80 per cent at 25 miles per hour. Therefore, an anemometer must be used in conjunction with it. In situations having variable wind speeds and variable concentrations of pollen, it is necessary to obtain the diurnal patterns for both these variables to properly weigh the correction factors.

Several devices sample measured air which is drawn into the sampler. Tests may indicate high efficiency in catching the pollen that enters the machine but we cannot assume this to be an accurate indication of the pollen concentration in the outside air. Unless the device is sampling isokinetically (the intake opening continuously oriented into the wind and the airspeed through the opening equal at all times to the wind speed in the free air approaching the intake) the system is subject to serious errors.⁴ Samplers drawing air through an aperture may not be suitable for conditions of variable wind speeds unless the diameter of the particles is less than 5 μ .

DEVELOPMENT

A method of sampling that reduces many of the above objections was devised at the Stanford University Aerosol Laboratory.⁵ This type of sampler collects particles by impaction on narrow surfaces whirled through the air at high speed and has the advantage of being practically independent of wind speed and direction. Their rotorod sampler uses a pair of small metal rods as collecting surfaces. Its major disadvantages for routine pollen sampling are: (1) It is difficult to prepare the samples for identification and counting, and (2) it cannot be operated continuously for a 24 hour day due to the large volume sampled and consequent overloading of particles. A modification of the rotorod sampler by experimenters at the University of Michigan is called the rotobar sampler.⁶

During the past few years we have used more than 60 rotorod units for sampling several types of pollen and have conducted many experiments with these and with numerous modifications to improve the taking and reading of

samples. These rods must be specially fabricated, are troublesome to store and position under the microscope, and their opacity makes pollen identification and counting difficult. These disadvantages may be partly overcome by attaching transparent tape to the leading faces; the tape with its sample are removed to a microscope slide. This procedure is not wholly satisfactory because of the difficulty in attachment of the tape to the rods and removal to the slide without modification of the sample. A modified rotorod* uses transparent plastic inserts for the collecting surfaces, which greatly improve visual examination of the samples.

These difficulties led to the development of the rotoslide, a sampler in which glass microscope slides in suitable holders replace the rods. The samples are collected on the thin leading edge. A special, easily constructed device positions the slide under the microscope for identification and counting. During the past 6 years our technicians have processed, without difficulty, more than 100,000 slide-edge samples. The rotoslide may be operated continuously for short-time sampling but its high efficiency causes it to overload rapidly. A simple, continuously operated model has proved useful for short-period sampling. For samples longer than 2 hours or so the device must be constructed to sample intermittently or sequentially. A satisfactory sequential model has been developed and tested, but its size, complexity, and the number of samples taken daily render it unsuitable for most routine pollen surveys. An intermittent model seems to have the most general usefulness and is described below. In Japan, Suzuki⁷ has independently developed a modified rotorod sampler which captures the sample on the broad face of the rotating glass slides.

DESCRIPTION

The intermittent rotoslide sampler (Fig. 1) is designed to take pollen samples representative of the average pollen concentration over a 24 hour period by automatically operating periodically for short periods. Pollen is collected on the edges of two microscope slides inserted in metal holders which are rotated by a small electric motor. The leading edge of each slide protrudes from its holder and is oriented so its face is always at right angles to the direction of motion. The motor is supported from a base section which terminates in a standard pipe flange by which the sampler may be mounted on any suitable pipe support. Also mounted on the base section is an aluminum box containing electrical components. Among these is a timing device which energizes the motor for 1 minute in each 12, thus giving a total sampling time of 2 hours each day.

Experience has shown that a slide edge is an efficient impactor of pollen even when not rotating if it happens to stop facing the airflow. Therefore, it is necessary to shield it from the wind when not rotating. For this purpose, a cylindrical shield is attached to a section of metal tubing which rides on the rod supporting the spinning motor. A reversible AC motor mounted inside the box is also actuated by the timer. On the shaft of this motor, which penetrates through the wall of the box, is mounted a pulley wheel to which a cable from

*Developed by Metronics Associates, Inc., Palo Alto, Calif.

**Fig. 1**

Intermittent rotoslide sampler.

the shield is attached. When the slides are rotating the shield is pulled down so they are exposed to normal airflow. When rotation ceases, the shield rises to shelter them from the wind. A rain guard is mounted above the slide holders.

All electrical components operate on 115 volt 60 cycle AC and are standard commercial products. The mechanical parts may be fabricated in any machine shop. Detailed blueprints, including a list of parts and materials, schematic drawings, and wiring diagram may be obtained.*

OPERATION

The sampler is easily and inexpensively installed in most situations. Where short-time (2 or 3 hours) samples are desired, the machine may be operated

*May be obtained from Oak Ridge Reproduction Service, P.O. Box 363, Oak Ridge, Tenn. 37831. The identification No. is CAPE-1093. Instructions for use of the sampler and names of manufacturers may be obtained from the American Academy of Allergy, 756 North Milwaukee Street, Milwaukee, Wis. 53202.

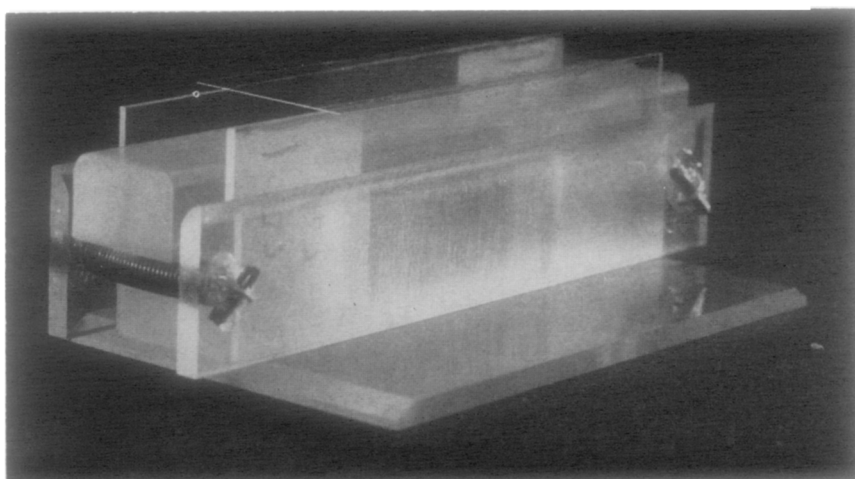
continuously by disconnecting a lead to the timer motor while the cams are in the operating position, or the simplified version may be used. A plastic bag is a satisfactory cover while the sampler is idle. Intermittent sampling proves to be satisfactory in nearly all locations. However, this sampler may be used intermittently only on the assumption that pollen concentration during its sampling cycles is representative of that prevailing during its longer idle cycles. Probably this assumption is valid at most sampling stations unless they are close to a large pollen source. Since pollen emission is not normally continuous or uniform, a sampling cycle may or may not coincide with a short period of peak emission. In either case, the concentration sampled may not be representative of a longer period mean. At reasonable distances from local sources, pollen is usually well mixed in the atmosphere and sharp discontinuities in concentration seldom occur. When atmospheric concentrations of pollen and/or other particulates are heavy, length of sampling must be restricted to avoid overloading, with consequent loss of accuracy and difficulty in counting.

In common with most other air samplers, the rotoslide is adversely affected by fog and rain. The rain guard shelters the collecting surfaces from rain only when wind speeds are light and gives no protection from fog. If rain or fog droplets penetrate the sampling area, they are efficiently impacted and tend to wash off pollen previously collected. No remedy for this difficulty is presently available except discontinuance of sampling in periods of precipitation or fog. If sampling is conducted under these conditions, the data should be used with caution.

Several years of field experience by us and others has revealed a few mechanical problems which can be minimized by proper maintenance. Internal rusting of the spinning motor, due to exposure in rain and fog, usually can be prevented by use of an oil-impregnated felt washer around the motor shaft. Failure of the shield to rise or lower may be avoided by keeping the sliding surfaces clean. Frequent inspection and occasional replacement of the lift cable may be necessary.

HANDLING THE SAMPLES

The sampling surface is one edge of a standard 25×75 mm. glass microscope slide. Care should be taken to obtain slides that are approximately one millimeter in thickness and without beveled or oblique edges. We have found that the more commonly used slides vary as much as 10 per cent from the nominal 1 mm. thickness, a greater variation than indicated by the manufacturer. Unless slides with little variation are obtained, we recommend that the thickness of the sampling edge be measured to the nearest 0.01 mm. with a micrometer calibrated in the metric system. The edge of the slide which is to receive the sample is coated with a suitable adhesive, such as silicone stopcock grease. This layer should be 5 to 15 μ thick, enough to retain the pollen but not so much that impacted pollen becomes buried. Silicone grease is easily applied with a finger and one soon learns how to leave the proper amount. If the greased slides are to be transported far or stored for some time before examining the samples, the slide box should be modified to prevent the greased upper edge from touching the cover. This is accomplished by choosing boxes (preferably of wood) with deep

**Fig. 2**

Slide positioner with slides and cover glass.

covers and by gluing strips of fine-cell polyurethane or other similar material to the inside of the cover to exert pressure on the edges of the slides close to the ends.

The greased and labeled slides are easily slipped into the holders on the sampler and as easily removed after the sample is taken. When ready to analyze the sample, one should place the pair of slides in the slide positioner (Fig. 2). This positioner is constructed of transparent plastic and holds the slides on edge, parallel and approximately 15 mm. apart, with the greased surface containing the pollen and other collected particles on the upper edges. A 22×50 mm. cover glass is prepared by painting two strips of Calberla's solution* on one face with a medicine dropper. The cover glass is then inverted and placed on the slides so the stain is on the slide edges containing the samples. These samples are ready for counting after a few minutes. The positioner is placed on the microscope stage and routine counting is done at 100 $\times$. Fig. 3 illustrates a portion of a slide-edge sample which includes ragweed pollen and other airborne particulates. For critical determination and separation of similar pollens, higher magnifications may be used.

CONVERSION OF COUNTS

Pollen counts obtained from the roto-slide sampler are converted to volumetric measurements by dividing the count by the volume of air sampled and by the sampler efficiency. The volume is obtained by multiplying the linear air-flow past the slides by the cross-sectional area of the sampling surface. If 50 mm. of the edge are counted on each of the two 1 mm. wide slides, the cross-sectional

*Calberla's solution: 25 c.c. 40 per cent alcohol, 5 c.c. glycerin, 2 or 3 drops saturated aqueous solution of basic fuchsin. We add 2 or 3 drops of glycerin jelly to make it more viscous. Some may prefer to use a stain in glycerin jelly.

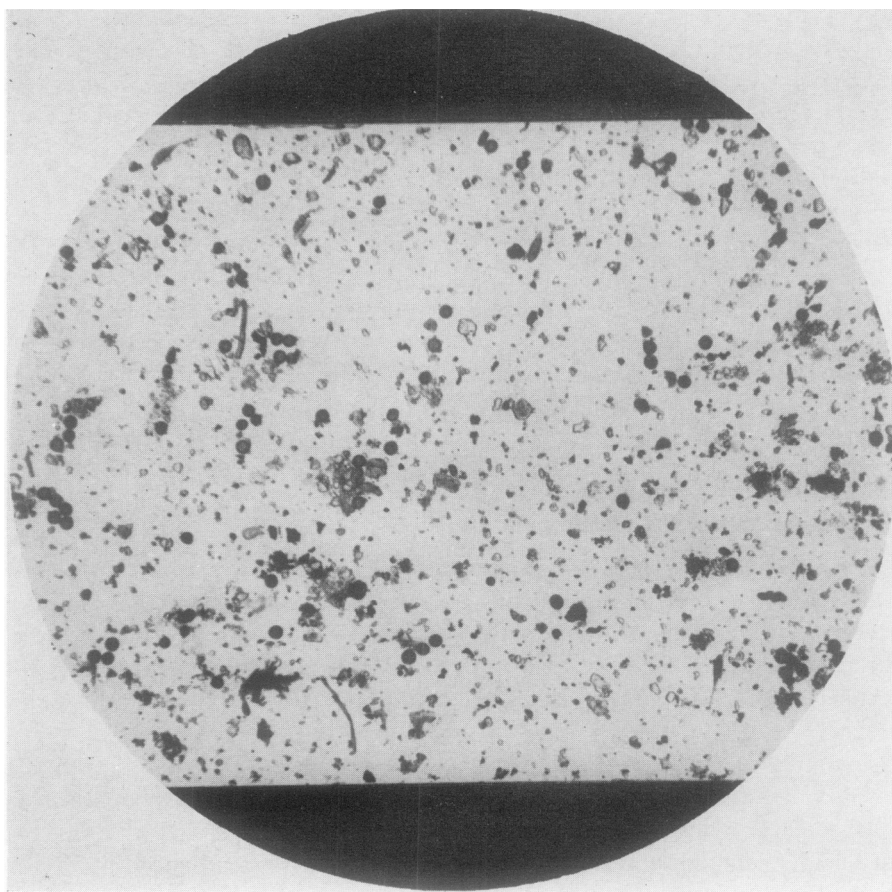


Fig. 3

Field of view of typical slide-edge sample. Most of the circular black particles are ragweed pollen grains which in the actual sample are red in contrast to nearly all the other particles.

area = $100 \text{ mm.}^2 = 1 \times 10^{-4} \text{ M.}^2$ The linear airflow is found by multiplying the speed of rotation in revolutions per minute by the circumference of the circle the sampling edge travels and by the number of minutes sampled. The product will be in linear measure. The speed of rotation used should be the actual speed of the slide holder, which may be more or less than the rated speed of the motor.

For example, with a rotation speed of 1,650 r.p.m. and a radius of rotation of 5.7 cm. giving a circumference of 0.3581 M., the linear length in meters will be $1,650 \text{ r.p.m.} \times 0.3581 \text{ M. per revolution} = 591 \text{ M. per minute}$. When this is multiplied by the cross-sectional area, the volume equals $591 \text{ M. per minute} \times 1 \times 10^{-4} \text{ M.}^2 = 0.0591 \text{ M.}^3 \text{ per minute}$.

The theoretical impact efficiency⁸ of a 1 mm. diameter cylinder traveling at the rotoslide speed (nearly 10 meters per second or 22 miles per hour) is approximately 96 per cent for fresh ragweed (*Ambrosia artemisiifolia*) pollen. However, the slide edge is not a true cylinder; turbulence caused by the rotary motion and by the slide holder disturbs the normal air flow and the retention

efficiency of the sticky surface is less than 100 per cent. Therefore, we would expect the actual efficiency to differ from the theoretical efficiency. This was confirmed by the wind tunnel tests reported below which resulted in a recommended value of 64 per cent for airborne ragweed pollen. This will differ for other types of particles. One source of error in the above calculations is the variation in slide thickness mentioned previously. The corrected count obtained by dividing the actual count by the slide thickness should be used. Efficiency will be changed only about 1 per cent by these variations.

For an example of count conversion, if N ragweed pollen grains are taken in a 2-hour sampling period, the concentration would be:

$$\chi = \frac{N}{\frac{0.64 \times 0.0591 \text{ M}^3 \times 120 \text{ minute}}{\text{minute}}} = \frac{N}{0.64 \times 7.1 \text{ M}^3} = \frac{N}{4.5 \text{ M}^3}$$

If $N = 100$, $\chi = 22$ grains per cubic meter.

TESTING OF THE ROTOSLIDE SAMPLER

Field comparisons with other samplers

Evaluation of the sampling efficiency of the roto-slide sampler is difficult in the absence of any reliable standard for accurate measurements of pollen concentrations in the field. However, numerous comparisons have been made with other types of samplers, and the sampler was calibrated in a wind tunnel where the concentration may be measured accurately by use of a filter sampling isokinetically.

Comparisons with the Durham sampler were made with the roto-slide and with its predecessor, the rotorod. These comparisons are only qualitative in nature, since it is impossible to define the volume of air sampled by a horizontal slide and its catch is known to be highly dependent on wind speed and orientation. In nine 24 hour sampling periods in a position exposed to relatively high wind speeds (355 feet above the ground) the rotorod captured an average of 188 times as many pollen grains per square centimeter of sampling surface as the Durham sampler and 156 times as many as a modified Durham, with the use of a square slide in a circular, streamlined holder. At a height of 37 feet where lighter wind speeds prevail, the rotorod took 370 and 414 times as much pollen as the two Durham samplers. In forty-nine 24 hour sampling periods on a flat roof at a height of 140 feet, a roto-slide took an average of 43 times as much as a Durham sampler. The chief advantage of this much larger catch, aside from the fact that it can be converted to a volumetric measure, is the greater statistical reliability given to the results.

In 1962, a large number of field comparisons were made between the roto-slide and the slide-edge-cylinder sampler,⁹ a vane-mounted device which combines the principle of impaction on the side of a cylinder with the ease of counting on a microscope slide. The latter was subsequently calibrated in wind tunnel tests with measured concentrations and found to have an average total collection efficiency almost equal to its theoretical impaction efficiency at wind speeds of 1 meter per second and over. Thus, these field tests of the roto-slide may be regarded as quantitative comparisons with a sampler of known efficiency.

In 41 tests lasting from 1 to 4.5 hours, a rotoslide sampler running continuously was compared with two slide-edge-cylinder samplers at the same positions. Samples were taken over a wide range of pollen concentrations from 1 to more than 10,000 grains per cubic meter and at wind speeds averaging from 1.4 to 6.1 meters per second. Ratios of concentration computed from the rotoslide catch, after correcting for theoretical impaction efficiency, to concentration computed from the average slide-edge-cylinder catch averaged 0.93. This indicates an actual collection efficiency of 89 per cent. Considerable scatter was present in the results but much of it can be attributed to those cases where the concentration was very low and catches small. The correlation coefficient between the rotoslide concentration and the average cylinder concentration was 0.89. For comparison, the correlation coefficient between the two cylinders was 0.95.

Twenty-two other comparisons were made at a location distant from a pollen source and consequently at low pollen concentrations, all less than 100 grains per cubic meter. Wind speeds ranged from 0.4 to 3.2 M. per second and were often low enough to render doubtful the determination of cylinder impaction efficiency. Two rotoslides sampling intermittently on different time cycles were compared with two adjacent slide-edge cylinders. Sampling periods were 24 hours long. Ratios were taken of the concentration computed from the average rotoslide catch to the concentration computed from the average cylinder catch. The mean was 0.73, indicating an actual collection efficiency of 70 per cent. The results, however, are affected by poor agreement between the two cylinder samplers due to the light wind speeds. The correlation coefficient between the two cylinders was 0.71, between the two rotoslides 0.94, and between the average of each 0.67.

A third series of daily comparisons was made at four levels of the meteorology tower (37, 75, 150, and 355 feet) for 15 days, thus giving 60 comparisons. Here a single rotoslide sampling intermittently was compared to the average of two slide-edge cylinders. The mean ratio was 1.10, indicating an actual efficiency of 106 per cent. These results are questionable since the high wind speeds sometimes prevented the shield on this earlier model from rising and lowering. Thus, the slides were sometimes subject to wind impaction during their idle cycle, increasing their catch. The correlation coefficient between the two cylinders was 0.98 and between their average and the rotoslide, 0.89. These tests indicate generally good agreement between the two types of samplers but cannot be regarded as accurate calibrations. Extensive field comparisons between slide holders as here illustrated (Fig. 1), in which the two slides are 180° apart, with holders accommodating four slides 90° apart, indicate no difference in efficiency. During the past few years while this sampler has been rigorously tested by us, a large number of these instruments has been used for various types of aerobiological studies in this country and abroad with generally favorable results.

Wind tunnel calibration

In order to determine the efficiency of the rotoslide sampler more precisely than was possible in the field, a series of 11 tests were made in a 2 foot diameter

wind tunnel at speeds ranging from 0.6 to 10.0 M. per second. Ragweed pollen was dispersed in a sealed room containing the wind tunnel and circulated by four large fans plus the wind tunnel fan, until a uniform concentration was obtained in the room. Checks of the mixing technique were made by taking samples across the profile of the tunnel. A filter sampling isokinetically was mounted in the center of the tunnel approximately 5 feet from the entrance. The roto-slide was mounted 1 foot downstream at the same height. Sampling periods ranged from 5 to 10 minutes in length. The catches from all samplers were reduced to pollen grains per cubic meter of air sampled.

Results indicate that the efficiency of the roto-slide varies linearly with wind speed from 68 per cent at zero speed to 49 per cent at 10 meters per second. This decrease with wind speed is in accord with impaction theory. Since most pollen sampling takes place under low wind speeds, we recommend a value of 64 per cent for operational use when wind speeds are not known. Determinations of efficiency in sampling other pollens are also being studied.

A series of tests was also made with the roto-slide having a crossarm twice the normal length but operating at one-half the normal rotational speed, thus having the same linear speed at the sampling surfaces, to determine if this configuration would reduce the turbulence caused by the device and improve the efficiency. Results indicate that the efficiency was improved somewhat but the scatter in the experimental points and the variation with wind speed were greater. Therefore, this modification is not recommended.

Comparison of slides from a single sampler

The roto-slide sampler actually takes two samples at each exposure. Theoretically, the two slides should catch the same amount of pollen since they sample the same concentration. However, the catches on the two slides usually differ, sometimes widely. Occasionally, this may be explained by very low concentrations or short sampling periods but more often must be attributed to unknown factors which may include the following: (1) differences in retention due to unequal greasing, (2) differences in slide thickness, (3) displacement of particles in handling or staining the samples, (4) counting errors, and (5) other random variations in sampling. These factors are difficult to control in routine field sampling but an attempt should be made to minimize them. The following data illustrate the variation found by us and others.

In a series of eleven 5 minute wind tunnel tests with average concentrations ranging from 2,000 to 26,000 ragweed pollen grains per cubic meter, the slides were carefully greased as uniformly as possible. The absolute percentage difference between the two slides ranged from 0 to 21 per cent with an average of 6 per cent. In a later series of 33 tests, the mean difference was 10 per cent. In preceding field comparisons, where slides were greased in large numbers and presumably less uniformly, results showed poorer agreement.

Thirty-eight field tests lasting from 1 to 4.5 hours under a wide range of pollen concentrations were made with continuously operating roto-slides. Absolute percentage differences ranged from 0 to 74 and averaged 15. In forty-one 24 hour exposures at low pollen concentrations with roto-slides operated intermit-

tently, differences varied from 2 to 244 with a mean of 29 per cent. In a third similar series but under higher wind speeds, differences in 60 tests varied from 0 to 196 and averaged 23 per cent. Forty-three daily exposures of intermittent rotoslides in Albany, N. Y., by Janet V. Hayes, gave differences from 0 to 89, averaging 10 per cent. A series of 243 daily exposures of similar intermittent rotoslides in the city of Baltimore, by Dr. Marie Britt Rhyne, yielded differences ranging from 0 to 209, averaging 32 per cent. Pollen concentrations were generally moderate.

A series of eight indoor tests with artificially dispersed pollen was made to study the effect of thickness of grease. One slide of each pair was greased as lightly as possible, the other given a medium coating estimated at 5 to 10 μ thick. The more thickly greased slide took from 1.6 to 75.5 times as much pollen as the other, with an average of 13.5. The above data indicate the importance of adequate and uniform greasing of slides.

Janet V. Hayes assisted in the field evaluations and development of techniques for handling samples. Lester A. Cohen assisted in the wind tunnel tests.

REFERENCES

1. Durham, O. C.: The Volumetric Incidence of Atmospheric Allergens. IV. A Proposed Standard Method of Gravity Sampling, Counting, and Volumetric Interpolation of Results, *J. ALLERGY* 17: 78, 1946.
2. Ogden, E. C., and Raynor, G. S.: Field Evaluation of Ragweed Pollen Samplers, *J. ALLERGY* 31: 307, 1960.
3. Gregory, P. H.: Deposition of Air-borne Lycopodium Spores on Cylinders, *Ann. Appl. Biol.* 38: 357, 1951.
4. Watson, H. H.: Errors Due to Anisokinetic Sampling of Aerosols, *Am. Indust. Hyg. A. Quart.* 15: 21, 1954.
5. Perkins, W. A.: The Rotorod Sampler, Second Semiannual Report, CML 186, Aerosol Laboratory, Stanford University, Palo Alto, Calif., 1957.
6. Harrington, J. B., Gill, G. C., and Warr, B. R.: High-Efficiency Pollen Samplers for Use in Clinical Allergy, *J. ALLERGY* 30: 357, 1959.
7. Suzuki, H.: An Improved Rotorod-Sampler for the Study of Aerobiology in Piricularia, *Ann. Phytopath. Soc. Japan* 31: 296, 1965.
8. Brun, R. J., and Mergler, H. W.: Impingement of Water Droplets on a Cylinder in an Incompressible Flow Field and Evaluation of Rotating Multicylinder Method for Measurement of Droplet-Size Distribution, Volume-Median Droplet Size, and Liquid-Water Content in Clouds, Technical Note 2904, Washington, 1953, National Advisory Committee for Aeronautics.
9. Ogden, E. C., Raynor, G. S., and Vormevik, J. M.: Travels of Airborne Pollen, Progress Report No. 4, New York State Museum and Science Service, Albany, N. Y., June, 1963.