

Short communication

A brief method for analyzing Rotorod® samples for pollen content

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Abstract

Analyzing Rotorod® pollen samples can be time consuming when one uses the standard method of evaluating an entire collector rod. This present investigation explored an abbreviated analysis method which is indicated by Poisson statistical theory. The authors systematically analyzed 18 Rotorod pollen samples from Spring 1994 which contained 408–7394 pollen grains per rod. The atmospheric pollen concentration (pollen grains/m³) was calculated from the number of pollen grains contained on the entire rod surface (P_{total}) and a sub-area containing at least 400 grains ($P_{>400}$). The estimate of the atmospheric pollen concentration resulting from P_{total} and $P_{>400}$ for each rod did not vary by more than $\pm 9\%$ (mean, $2.0\% \pm 3.7$). These data indicate that pollen grains populated the sample rods rather uniformly, suggesting a mode of random recovery from the atmosphere. This study's results are consistent with the expectations concerning a Poisson process and support analyzing collector rods until a threshold number of pollen grains is counted.

Keywords: Rotorod Sampler; Pollen grains; Poisson statistics; Aerobiology

1. Introduction

The Rotorod Sampler is a rotation-impaction device used to recover airborne pollen (Grinnell et al., 1961). Two presumably identical atmospheric samples are produced per sampling period on small polystyrene rods (Solomon et al., 1980). Pollen grains impinged on the collector rods are identified with a light microscope and then expressed in terms of the volume of air sampled (Chapman, 1982). The result — often called a 'pollen count' — indicates the atmospheric pollen conditions during the period when the sample was collected.

Noll (1970) empirically demonstrated that rotating-arm impactors with operating characteristics like the Rotorod Sampler recover pollen-sized particles with > 85% efficiency. In addition, he found that particles were nearly uniformly distributed across the collector's surface without significant bias for the rod's slow or fast edges.

A uniform particle distribution offers a means to simplify analysis of Rotorod collector rods. Specifically, the pollen count determined by analyzing the entire rod should agree with results obtained by analyzing a fraction of the collector's surface. This present investigation compares pollen counts obtained using the former approach, the standard method, with the briefer latter method.

2. Materials and methods

A Rotorod Sampler Model 40 (Sampling Technologies, Inc., Minnetonka, MN) was installed 1.5 m above the flat rooftop of a three-story suburban office building. The site was unobstructed in all directions except for air-handling equipment located approximately 20 m to the northeast. Deciduous trees flanked the building south and west at a distance of approximately 50 m.

The Rotorod Sampler was operated according to the standard conventions suggested by the manufacturer (Brown et al., 1993). The sampler's clock was programmed to operate the Retracting Head 1 min every

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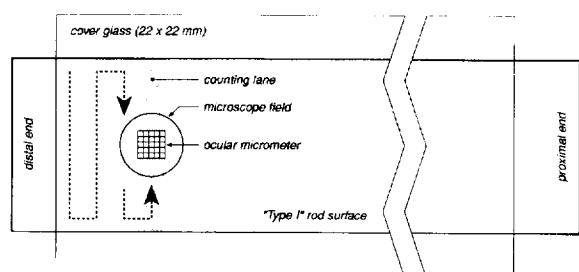


Fig. 1. Method for systematic microscopic analysis of Rotorod collector rods.

10 min (10% duty cycle). Clear polystyrene 'Type I' rods ($1.59 \times 1.59 \times 32$ mm) coated with a silicone grease (General Electric G-697; General Electric Co., Waterford, NY) were installed at approximately 16:00 h Monday through Friday. After removal from the retracting head, rods were stored at room temperature in dust-proof Transit Vials (Sampling Technologies) prior to analysis.

Eighteen samples from Spring 1994 were prepared for visual analysis in the standard manner. Rods were placed in the deep groove of a Stage Adapter (Sampling Technologies), stained with Calberla's Solution (Gay et al., 1941) and covered with 22-mm square cover glass (No. 1 1/2; VWR Scientific Inc., Media, PA).

Rods were analyzed at $200\times$ with a binocular microscope (Alphashot 2 YS2; Nikon Inc. Instrument Group, Melville, NY) equipped with a mechanical stage and eyepiece micrometer (MA6658; Swift Instruments, Inc., San Jose, CA). The width of the scale at this magnification was determined to be $355\text{ }\mu\text{m}$ with a stage micrometer

(MA663; Swift).

The surface of each rod was systematically analyzed across the rod's short distance between the boundaries imposed by the cover glass. Successive counting lanes demarcated by the eyepiece micrometer were evaluated beginning with the end of the rod distal to the Retracting Head (Fig. 1). The micrometer was advanced proximally by using debris or pollen grains as reference points. The number of pollen grains counted in each lane was recorded.

The integral number of counting lanes required to achieve at least 400 pollen grains ($P_{>400}$) and the total number of grains (P_{total}) under the cover glass were calculated for each rod beginning with the distal-most lane. An estimate of the atmospheric pollen concentration was determined in both cases from the standard equation

$$\frac{\text{pollen grains}}{\text{m}^3 \text{ air}} = \frac{\text{pollen grains counted on surface of rod, } P_{>400} \text{ or } P_{\text{total}}}{(\text{counting lanes}) \times (3.66 \times 10^{-4} \text{ m}^3 \text{ min}^{-1}) \times (\text{min sampled})}$$

3. Results

Eighteen pollen samples from April and May 1994 were analyzed (Table 1). Seven samples were collected over a period of 3 days (Friday–Monday) while 11 represented 24-h samples collected on weekdays.

The integral number of counting lanes required to achieve $P_{>400}$ varied significantly between samples. In general, samples with a higher P_{total} required fewer lanes to achieve $P_{>400}$. For instance, $P_{>400}$ was achieved in just three lanes for the sample collected

Table 1

Atmospheric pollen concentration (pollen m^{-3}) and difference in concentration determined from Rotorod samples by counting the number of grains on the whole rod (P_{total}) and at least 400 grains ($P_{>400}$) on a variable fraction of the rod

Sample	Whole rod			Fraction of rod			Pollen/ m^3 (fraction:whole)
	P_{total}	Lanes	Pollen m^{-3}	P_{400}	Lanes	Pollen/ m^3	
8–11 April	1122	61	115.3	402	21	120.0	+4.1%
14–15 April	1022	61	320.1	404	25	308.8	–3.6%
15–18 April	3211	58	350.1	438	8	346.3	–1.1%
18–19 April	1016	62	316.4	412	24	331.5	+4.8%
20–21 April	408	62	125.3	401	60	127.3	+1.6%
21–22 April	464	58	152.3	404	52	147.9	–2.9%
22–25 April	2445	59	261.5	452	11	259.3	–0.8%
25–26 April	7394	55	2577.6	414	3	2645.9	+2.7%
2–3 May	982	62	302.6	404	26	296.9	–1.9%
6–9 May	1483	62	151.3	413	16	163.3	+7.9%
10–11 May	589	57	195.4	404	40	191.0	–2.3%
13–16 May	6230	58	678.6	432	4	682.3	+0.6%
17–18 May	1560	59	498.2	401	14	539.7	+8.3%
18–19 May	2040	61	634.5	432	12	683.1	+7.7%
19–20 May	1230	58	402.4	418	19	417.4	+3.7%
20–23 May	1370	59	147.2	404	17	150.7	+2.3%
25–26 May	588	59	190.4	402	40	192.0	+0.8%
27–30 May	589	59	64.7	405	39	67.3	+4.0%

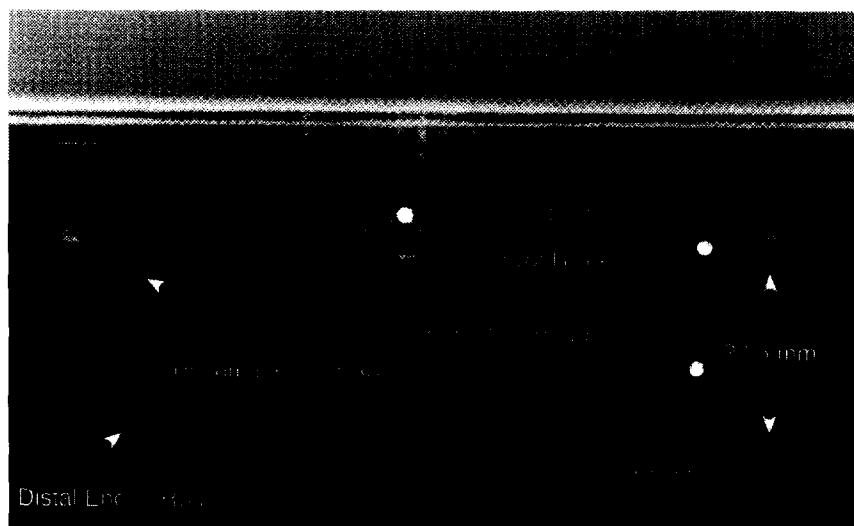


Fig. 2. Rotorod retracting head with plastic collector rods fully exposed.

25–26 April ($P_{\text{total}} = 7394$) compared to 60 lanes required for the sample obtained 20–21 April ($P_{\text{total}} = 408$).

The number of lanes required to achieve P_{total} varied by several lanes between samples (mean, 59 lanes ± 2). Although 22.5 mm of rod surface is available to collect atmospheric particles (Fig. 2), pollen grains and debris were generally not impinged on 750–1000 μm of the rod near the pivot block.

The estimate of the atmospheric pollen concentration resulting from $P_{>400}$ and P_{total} for each rod did not vary by more than $\pm 9\%$ (mean, $+2.0\% \pm 3.7$). The estimate offered by $P_{>400}$ exceeded P_{total} in 12 of 18 samples, of which nine occurred when $P_{>400}$ was achieved in less than 25 counting lanes.

4. Discussion

Particles impinged on a two-dimensional surface such as a microscope slide describe events occurring in the physical world. Traditionally, an estimate of λ , the actual particle concentration in an environment, can be determined by analyzing particles randomly distributed on a sample of λ of area A . Girshick et al. (1955) established in a theoretical framework, however, that if events occur in Poisson fashion one may also estimate λ by analyzing the number of particles, m , falling in a , a sub-area of A . Specifically, a estimates λ with a confidence interval (α) of 95% and a bounded error of estimate (γ) of $\pm 10\%$ when $m = 382$ particles. This estimate of λ provided by a should not vary significantly from the estimate furnished by evaluating all of A .

In this present investigation, the atmospheric pollen concentration (λ) was calculated from the number of

pollen grains (P_{total}) impinged on the surface of an entire collector rod (A). This result was contrasted with the pollen concentration determined by analyzing the number of pollen grains ($P_{>400}$) contained in a sub-sample (a) of the rod. For simplicity, sub-samples which contained at least 400 grains were used because they exceeded the minimum threshold of 382 particles required to bound the error of estimate at $\pm 10\%$.

Our data suggest that pollen grains are likely impinged on the collector rods in a random manner without significant bias for location along the collector surface. Namely, the estimate of the atmospheric pollen concentration provided by a was within $\pm 9\%$ of the estimate furnished by A . Although estimates of the pollen count derived from a exceeded those determined from A in two-thirds of the cases, the difference did not violate the prescribed tolerance for error.

Differences in the pollen count calculated from a and A necessarily resulted from differences in the density of pollen grains on each surface. Pollen grains more densely populated the distal end of the rod in the nine samples where $P_{>400}$ was achieved in less than 25 counting lanes. This finding and the exclusion of particles from 750–1000 μm of rod surface near the pivot block suggest certain sampling biases occurred.

Despite these biases, however, the pollen count furnished by a was an acceptable proxy for the value calculated from A regardless of the surface area analyzed. For instance, three counting lanes on the collector rod from 25–26 April yielded as satisfactory an estimate of λ as 60 lanes required by the sample from 20–21 April. These data suggest that an acceptable estimate of the pollen count results by counting a threshold number of pollen grains on a collector rod rather than evaluating a sample area of fixed dimensions.

These findings resolve certain ambiguities concerning Rotorod sample analysis. The manufacturer encourages users to evaluate both samples produced during each operating period to obtain valid results. Alternatively, a physician group recommends analyzing a single rod or a fraction of a rod to obtain expedient, yet meaningful, results (Segale, 1994). Neither case, however, elaborates the minimum length of rod required to obtain an acceptable estimate of λ .

Our data indicate that evaluating collector rods systematically until 400 pollen grains are counted is a satisfactory method for quickly estimating the atmospheric pollen count. The amount of time required to reach this threshold should not vary considerably between samples. This method overcomes seasonal variations in analysis time posed by the standard procedure.

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