

New Orleans asthma

IV. Semiquantitative airborne spore sampling, 1967 and 1968

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A semiquantitative survey of atmospheric spore concentrations in the city of New Orleans with the use of automatic intermittent rotoslide samplers was performed during the years 1967 and 1968. In comparison with our previous rotoslide pollen survey, spores were invariably detected in excess of pollen grains by a factor of 10 to 100. Relatively large spores of the deuteromycetes (fungi imperfecti) were easily identified morphologically. Identification of small spores was uncertain because of considerable group overlap and interspecies variation. Spores in this category were detected in highest quantity. Spores resembling large basidiospores were included in a third general category. Many myxomycete spores were also likely included in this category because of similar morphology. In all morphological categories, semiquantitative atmospheric spore counts were comparatively low in January and February. Counts increased sharply in March and April and increased variably throughout the summer and late fall months. Noticeable decreases were recorded in late November at or near the usual onset of local killing frosts. Petri plate colony identification, although performed only sporadically, was not as helpful as anticipated. Analysis of one typical large asthma epidemic (December, 1968) revealed high rotoslide catches in all major spore categories but no sharp increase in total cultural colony count or obvious change in colony composition.

Previous data demonstrated that sensitization of atopic individuals by a spectrum of natural inhalant allergens in association with certain climatologic conditions was involved in the genesis of New Orleans epidemic asthma.^{1,2} These findings suggested a need for a quantitative aerometric pollen survey of this area, which was recently performed.³

Airborne fungal spores of many genera have also been established as etiologic agents in allergic respiratory disease,⁴⁻⁷ and numerous surveys⁸⁻¹³ have demonstrated their ubiquity. Wheal and flare skin reactivity to allergenic extracts of fungi is routinely observed in Charity Hospital Allergy Clinic patients, and ecologic conditions of the New Orleans area are ideal for growth of fungi.¹⁴ These facts suggest that airborne spores may play a role in production of local asthma epidemics and warrant a systematic quantitative airborne spore survey.

The current report describes results of such a survey for the years 1967 and 1968 with the use of automatic intermittent rotoslide samplers.

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MATERIALS AND METHODS

The automatic intermittent rotoslide sampler of Ogden and Raynor¹⁵ was used. This sampler has been reported to yield more quantitative data than conventional gravity samplers because of its greater efficiency, known air-sampling volume, and larger collected quantities which allow greater statistical reliability.

Methods of collection, sampler locations, staining, and identification procedures are described in another report.³ These same samplers were used for pollen quantitation and atmospheric spore analysis. Two samplers were located in the central business district approximately 150 feet above ground level on top the Louisiana State University Medical School Student Dormitory and in the immediate vicinity of Charity Hospital. A second station with a single sampling device was located 8 feet above ground level on a golf course near the geographic center of the city. A rotoslide tetrabar containing four sampling slides was used, and samples were collected twice daily, in most cases at approximately 7 A.M. and 6 P.M. Two slides were stained with a modified Calberla's solution* for pollen quantitation and two with cotton blue stain† for spore identification and quantitation.

One rotoslide sampler used at the 150 foot elevation site was fitted with a cylindrical fiberglass slide shield and the second with an aluminum cover device which completely protected slides from exposure when not rotating. Except where indicated, all data in this report were obtained from the "shield" sampler located at the 150 foot sampling site.

Petri dishes containing Sabouraud's dextrose agar were exposed daily for 10 minute intervals at 7 A.M. and 6 P.M. at a previously described 150 foot central city sampling site.³ Plates were incubated at room temperature for 3 days, wrapped in sealed plastic bags, and stored at 4° C. for variable periods prior to subculture and colony counting. Only plates exposed during certain epidemic intervals were examined since it soon became apparent that plate counts did not aid in presumptive identification of the large numbers of spores collected on 24 hour rotoslide samplers.

RESULTS AND DISCUSSION

Morphological spore groupings

Continuous sampling for the years 1967 and 1968 indicated a wide range and variety of fungus spores on rotoslide samples. Spores were invariably detected in excess of pollen grains by a factor of 10 to 100. The study was designed to obtain continuous semiquantitative data, and no exhaustive attempt was made to identify single spores as to genus or species. However, there was little difficulty in identifying certain large spores of the deuteromycetes (fungi imperfecti).

Identification of small, round to oblong spores was uncertain because of considerable group overlap and interspecies variation. This is illustrated by the genus *Aspergillus* which, among its numerous species and strains, contains a variety of smooth, rugose, or echinate small spores of several colors and shapes, many of which cannot be distinguished from small spores of other species and genera.¹⁶

In an attempt to simplify spore grouping for estimations of seasonal fluctuations, we chose to group them into three broad categories as follows:

1. *Small, round to oblong spores approximately 1 to 6 × 1 to 12μ.* Among

*Modified Calberla's solution: 5 ml. of glycerine, 10 ml. of 95 per cent ethyl alcohol, 15 ml. of distilled water, 2 to 3 drops of saturated aqueous solution basic fuchsin, 2 drops of melted glycerine jelly.

†Lactophenol cotton blue stain: Phenol 20 Gm., lactic acid 20 ml., glycerine 40 ml., distilled water 20 ml.—gentle heating under hot water to dissolve, add 0.05 Gm. of cotton blue.

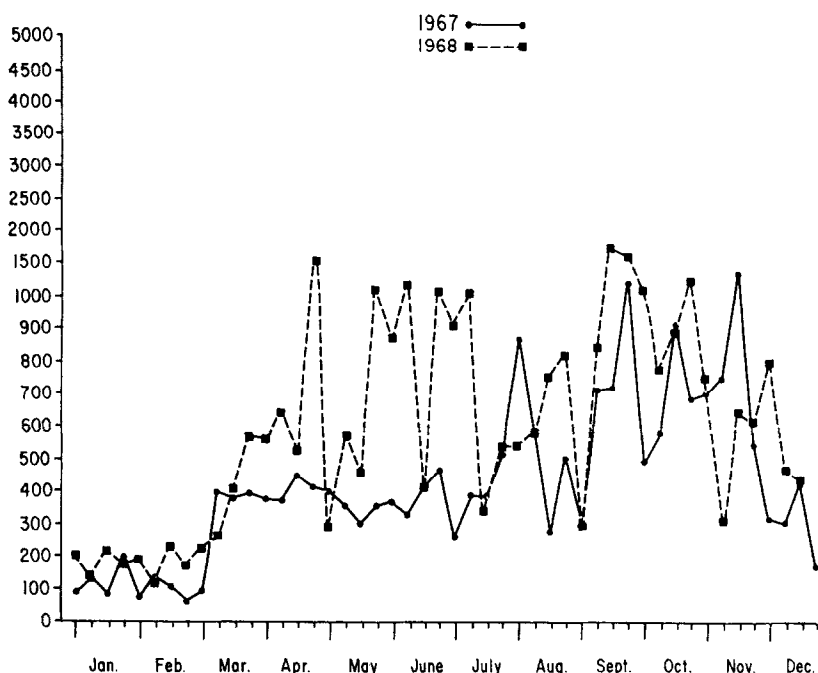


FIG. 1. Weekly mean semiquantitative roto-slide count of spores resembling large basidiospores, 1967 and 1968.

these were globose, smooth, or echinate spores and ovoid-elliptic, clear, or greenish spores belonging to the genera *Aspergillus* and *Penicillium*. Also included were the larger easily identifiable olivaceous *Hormodendrum*-*Cladosporium* complex, and some identifiable small zygomycete spores such as *Rhizopus*.

2. *Large spores, approximately 10 to 130 μ .* These included spores of the following easily recognizable deuteromycetes: *Helminthosporium*, *Fusarium*, *Curvularia*, *Trichothecium*, *Epicoccum*, *Alternaria*, and possibly the similarly appearing *Stemphyllium*. Ascospores of *Pyrenophora* and *Pleospora* were encountered infrequently, and many spores had characteristics intermediate between these genera and *Helminthosporium*. For over-all quantitative purposes they were classified as "spores resembling *Helminthosporium*."¹¹

3. *Spores resembling large basidiospores.* These included the characteristic large, orange-brown, glabrous, oval rust urediospores (25 to 30 μ) and septate teleutospores (the former far more common), together with the more abundant smut spores. Basidiospores are known to vary markedly in size,^{17, 18} and only large, brownish spores approximately 10 to 30 μ in diameter, ovoid to globose and reticulate, verrucose or spiny-walled, were included in this category. Many smaller basidiospores were placed in the "small, round to oblong" category on the basis of size, despite the presence of a typical apiculus at one end.

Myxomycete spores, which are approximately 8 to 18 μ in diameter, may be globose, smooth, spiny, or reticulate. They are commonly confused with

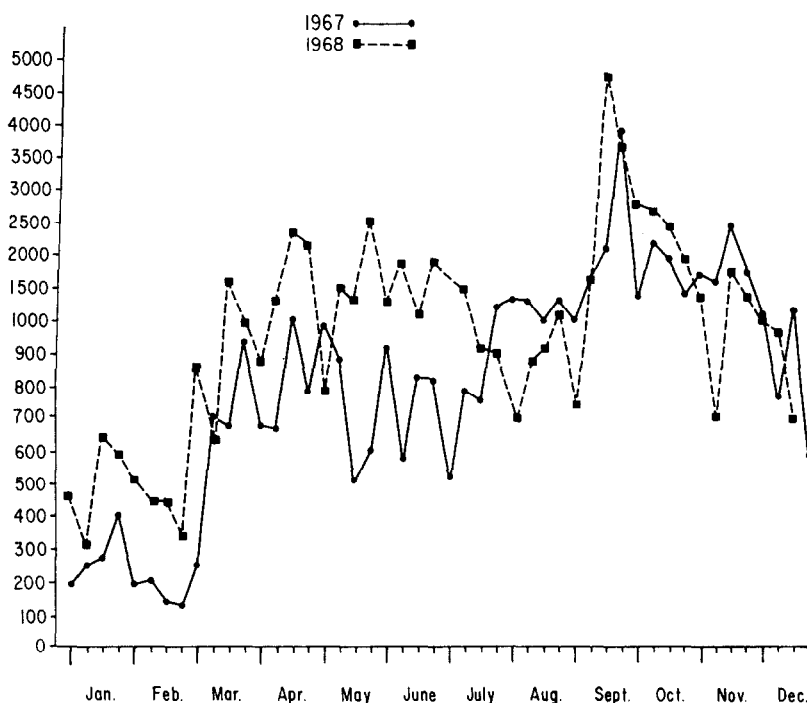


FIG. 2. Weekly mean semiquantitative rotoslide count of major "small spores," 1967 and 1968.

basidiospores and often difficult to culture on artificial media. It is likely that such spores were also included with the basidiospores.

Rotoslide counts in major spore categories

Figs. 1 to 5 illustrate 1967 and 1968 weekly mean counts of the major small spore, large spore, and "basidiospore-like" categories. Total spore and pollen rotoslide counts were comparatively low in January and February, the months of lowest mean temperature for both study years. It can be seen that in all categories atmospheric spore counts increased in March during the initial local warm spring period and near the peak local tree pollination season. Mean temperature was 50.5° F. for the combined months of January and February, 1967 and 1968, and 64.9° F. for March and April, the latter falling into the range of optimal growth temperature for vegetative parts of most fungi and possibly accounting for the observed increase in atmospheric spore concentrations. High counts in all major categories persisted with gradual but variable increases throughout the summer and fall months.

Decreases were recorded in all categories, beginning in late November at or near the usual time of local killing frosts and falling to minimal concentrations by late December or early January. Spores resembling large basidiospores were variable in concentration but approached "small spore" concentrations in magnitude (Figs. 1 and 2). "Small spores" were invariably detected in excess of

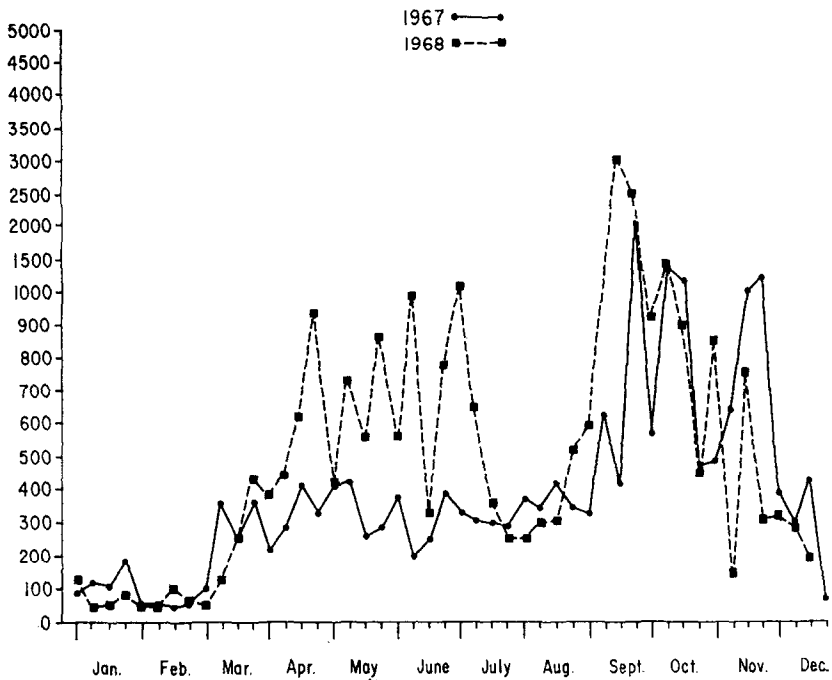


FIG. 3. Spores of Hormoderndrum-Cladosporium complex: 1967 and 1968 weekly mean semiquantitative roto-slide count.

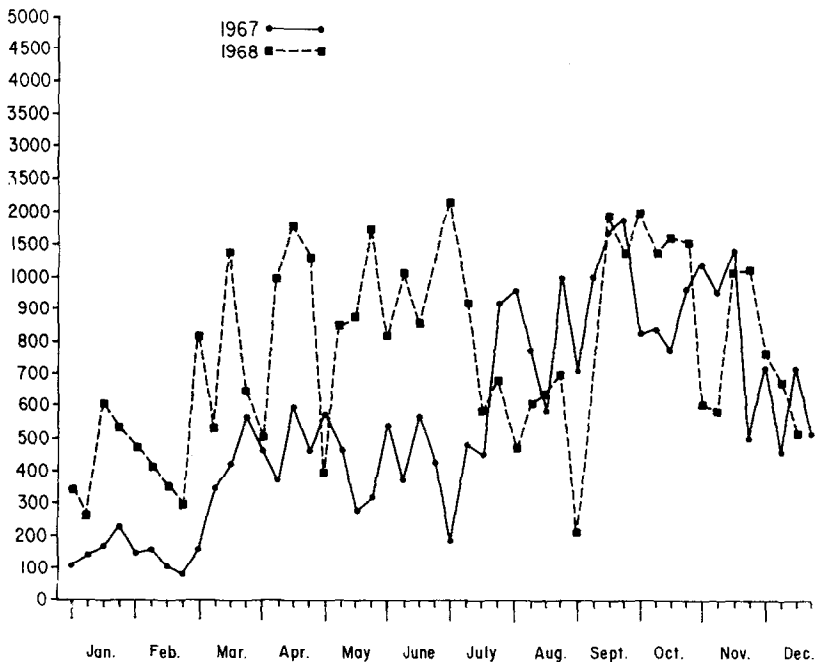


FIG. 4. Small spores and particles resembling *Aspergillus* and *Penicillium*: 1967 and 1968 weekly mean semiquantitative roto-slide count.

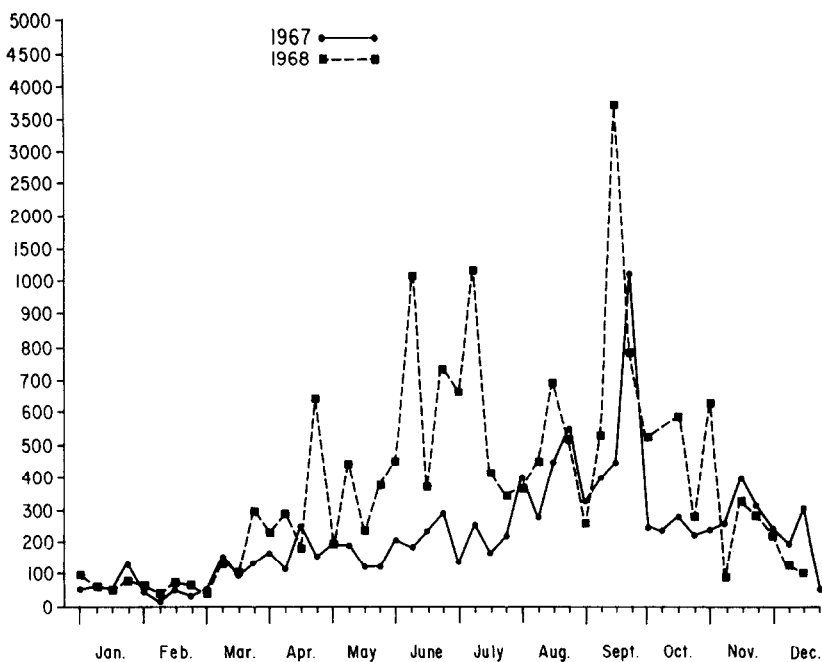


FIG. 5. Weekly mean semiquantitative rotoslide count of large deuteromycete spores and spores resembling fusiform ascospores, 1967 and 1968.

“large spores” and could be subdivided into two major categories, those resembling *Aspergillus* and *Penicillium* and those resembling *Hormodendrum-Cladosporium*, based on size, color, and morphologic characteristics.

Spores of the *Hormodendrum-Cladosporium* complex were easily recognizable as olivaceous or clear, oval, 4 to 8 × 8 to 22 μ particles often occurring in clumps. Mean weekly concentrations of these spores for 1967 and 1968 are illustrated in Fig. 3. It can be seen that concentrations generally paralleled those of the total small spore category and were particularly high during the fall months of September, October, and November, which are periods of warm, dry weather and of major asthma epidemics in New Orleans.

In contrast to the dark spores of many *Aspergillus* species, pure culture growths of several *Aspergillus* species revealed light-colored spores which were easily confused with those of *Penicillium*. Because of this variability, plus the similarity in size and morphology of *Penicillium* and *Aspergillus* spores, we chose to combine spores resembling those of these genera into a single subgroup. Staining was often unequally distributed on slides probably because of differences in thickness of the silicone grease coating. Since all particles compatible with descriptions of “small spore” types were quantitated, it is likely that some small unstained ash spheres resembling *Penicillium* spores were actually included in this category. Most refractile particles of this type were, however, excluded, and the total “*Penicillium*” count was likely not appreciably influenced by nonorganic particles. Atmospheric concentrations of this subgroup also increased during March and remained high through December (Fig. 4).

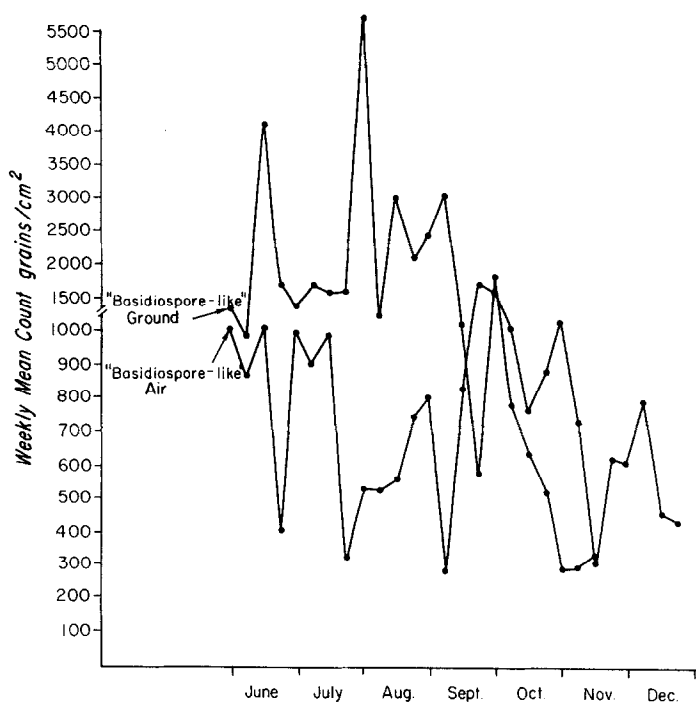


FIG. 6. Comparison of suburban ground and central city elevated rotoslide catches of spores resembling large basidiospores, June to December, 1968.

Quantities of large deuteromycete and ascomycete spores were insufficient for clear-cut recognition of seasonal fluctuations, but concentrations also increased from late March through November in a pattern similar to that of the "small spore" category (Fig. 5).

Effect of sampler elevation and location on rotoslide spore catches

Sampler elevation has been reported not to influence rotoslide pollen concentrations,¹⁹ but sampling site location is of considerable importance.^{20, 21} Although two sampler sites and elevations were used in this study, all results listed in Figs. 1 to 10 were reported from a single centrally located sampler at elevation of 150 feet except where indicated as "ground level" sampler. Our central city sampler rotoslide counts may not be representative of other city areas and they may not necessarily reflect a representative average of counts obtained at many city locations. In a previous study, however, results of a single rotoslide sampler accurately reflected average counts obtained with several conventional gravitational Durham samplers placed at several locations.²²

We³ previously noted considerably higher *Ambrosia* pollen counts at a suburban ground level (8 feet) sampling site when compared with the 150 foot level central city site employed in this study. This difference was attributed primarily to location and source proximity, since our suburban sampling site was in a low-lying area surrounded by considerable *Ambrosia* growth in nearby developing areas.

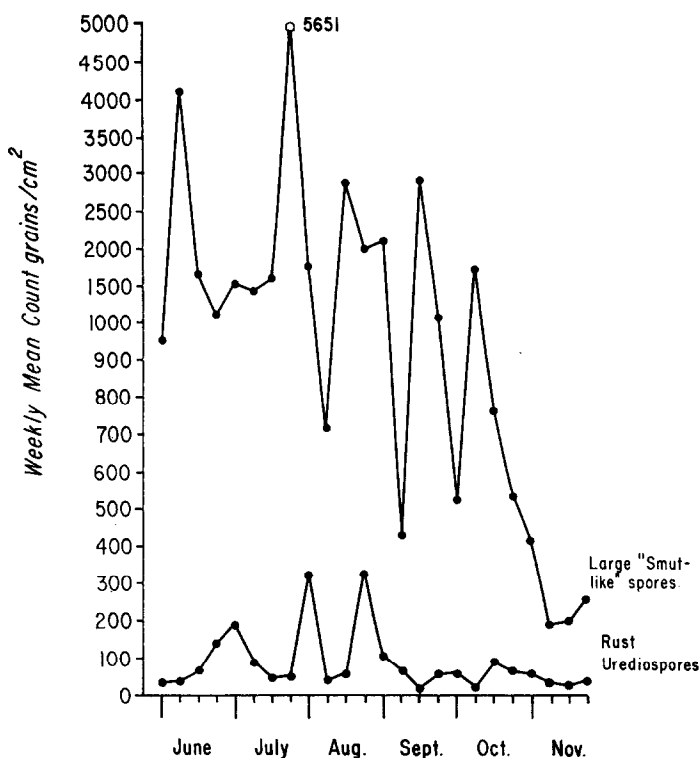


FIG. 7. Large basidiospores: ground samples, 1968. Relative concentration of rust urediospores and large "smut-like" spores on suburban ground rotoslide sampler (compare with elevated sampler catch, Figs. 1 and 6).

The ground level sampler usually trapped greater quantities of spores resembling large basidiospores than did the 150 foot elevation sampler, as illustrated for the months of June through December of 1968 in Fig. 6. During the warm humid summer months, high concentrations in this category were detected on the ground sampler, but with dry, cool conditions beginning in mid-September this trend was no longer noted. Subdivision of this category into "rust" (predominantly urediospores) and "smut-like" subgroups revealed high spore concentrations in the latter category (Fig. 7). Large "smut-like" spores dominated the rotoslide catch in the large basidiospore category, but rust urediospores were prominent from late July through mid-September. Analysis of ground level samplers for small spores was performed only at selected intervals because of difficulty in quantitating their enormous numbers and adequately visualizing the many spores which were at times layered in the silicone grease slide coating.

Day versus night rotoslide catches

Fungi have long been noted to display rhythmic production and release of spores,²³⁻²⁵ and patterns common to certain genera have been described.²⁶ Basidiospores and ascospores have been reported in highest concentrations at night, and many small basidiospores and ascospores may be discharged into the atmosphere

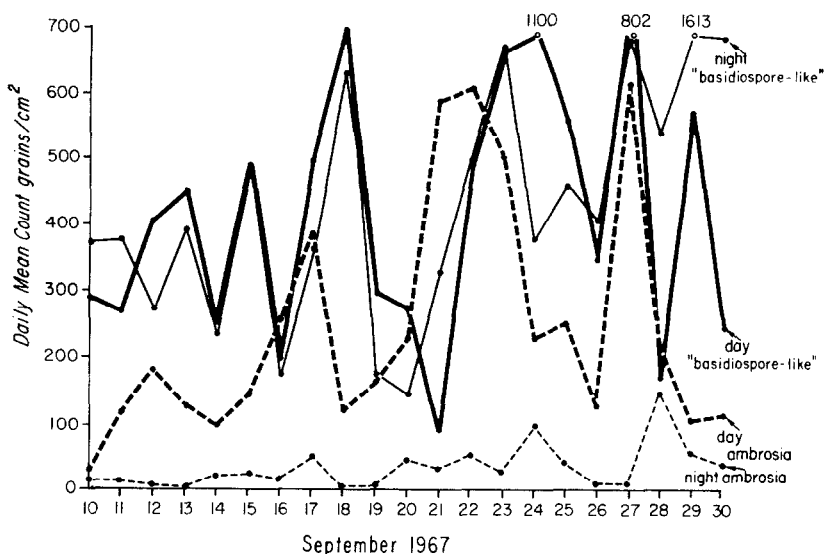


FIG. 8. Night versus day rotoslide catches of spores resembling large basidiospores; comparison with Ambrosia, September 10 to 30, 1967.

during periods of rain or high humidity. "Dry spores" of many deuteromycete species and of some asexual basidiomycetes are, however, blown into the atmosphere by wind currents and reach peak atmospheric levels in daytime during periods of maximal solar heating.²⁷

We noted some differences between our night and day rotoslide sampler collections. Fig. 8 illustrates an arbitrarily chosen 20 day period from September 10 through September 30, 1967, near the peak Ambrosia season, for comparison of fungus spore rhythmicity with diurnal Ambrosia pollen periodicity. As anticipated, Ambrosia pollen, which is known to be released in largest quantity near 9:00 A.M. standard time,²⁸ was detected almost exclusively on day samples. Spores resembling large basidiospores were, however, equally distributed between day and night samples. On certain occasions the rotoslide sampler was effective in trapping large nocturnal influxes of these spores, as seen on the nights of September 24 and 29, 1967. Such basidiospore "showers" were often associated with or preceded by periods of overt precipitation. Small spores were generally detected in higher concentrations on day samples except under conditions of overt daytime precipitation (Fig. 9).

Shield versus closed cover rotoslide sampler catches

The continuous rotoslide sampler with a fiberglass cylindrical "shield" slide protector consistently trapped greater number of "small spores" than did the closed "cover" sampler (Fig. 10). However, larger particles such as Ambrosia pollen and large basidiospores were collected in similar concentrations on both samplers, suggesting that the rotation speed of both samplers was similar. Particles below 15μ are known to be recovered poorly by turbulent impaction via a tendency to follow airflow around leading slide edges.²⁹ Since the "shield"

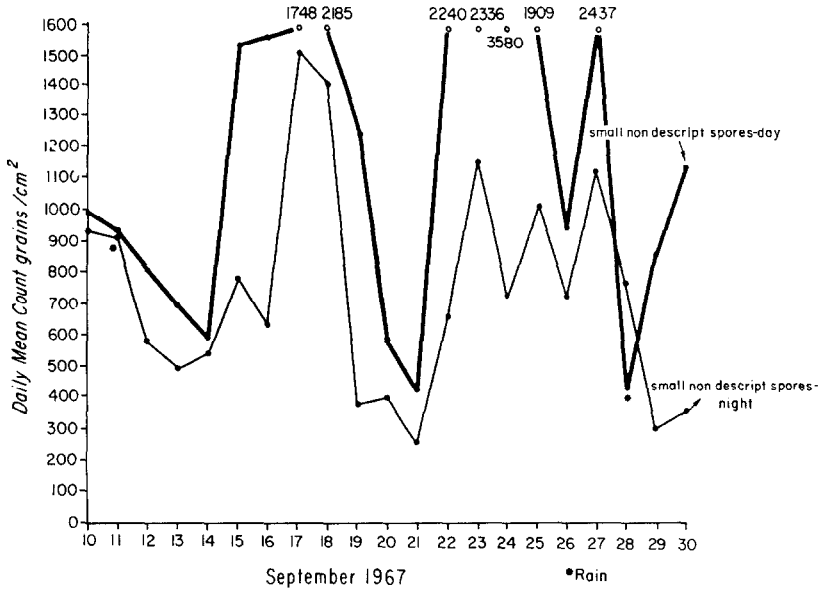


FIG. 9. Night versus day roto-slide catch of "small spores," September 10 to 30, 1967.

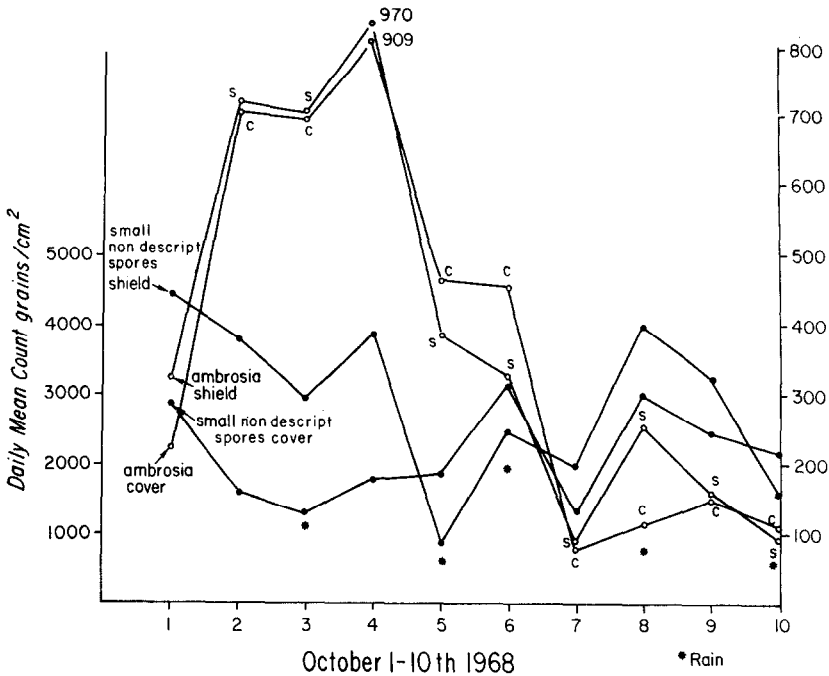


FIG. 10. Comparison of "small spores" and Ambrosia catches of two roto-slide samplers at same location and elevation but with different slide-shielding devices (closed cover versus cylindrical shield), October 1 to 10, 1968.

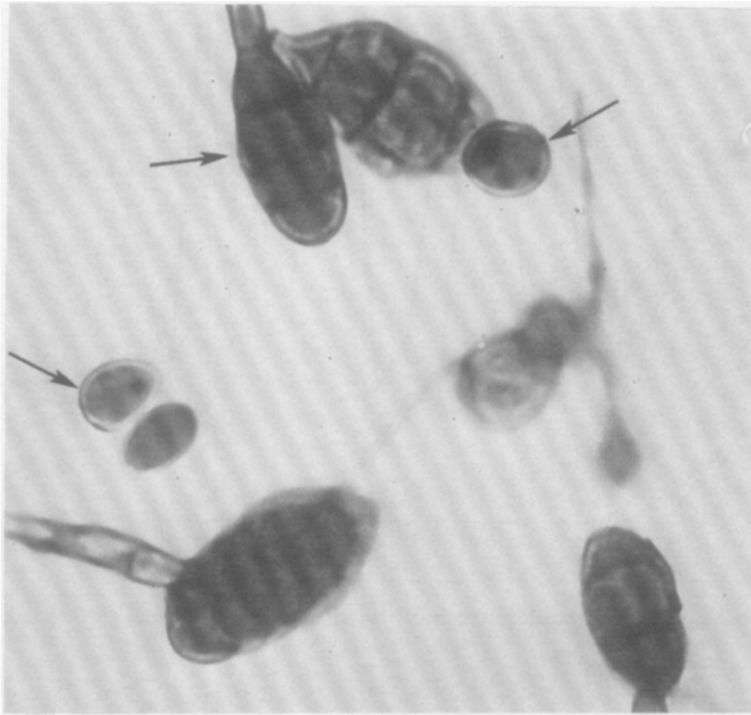


FIG. 11. Slide culture of pure growth of mature *Alternaria* sp. grown from 150 foot elevation on exposed Petri dish colony subculture. One arrow points to classic spore. Two remaining arrows indicate conidia resembling other form genera.

sampler allowed continuous atmospheric slide exposure through the upper and lower ends of its cylindrical slide guard, some settling of small spores onto silicone slide surfaces during nonsampling intervals could have accounted for their observed higher concentration on these samplers. "Shield" samplers, however, were often less efficient than were "cover" samplers during rain and heavy fog, presumably because of partial slide washout during sampling periods while slides are rapidly rotating in wet air. In Fig. 10 it can be seen that 3 of 5 days of overt precipitation were associated with a reversal in the usual pattern of high "shield" versus "cover" sampler small spore concentrations.

Disadvantages of the cover sampler were its large and bulky slide cover which obscured slide exposure from one side during sampling, with likely restriction of air motion about the sampling edges, and the tendency of the cover to malfunction during periods of high wind velocity. Other automatic rotating impaction samplers, the "fly shield" Rotobar³⁰ and swing-shield rotoslide,³¹ were introduced after commencement of our study and should obviate many of the above-mentioned sampler slide cover difficulties in future studies of this type.

Petri plate colony counts

Early 1967 Petri plate colony identification was not as helpful as anticipated and was abandoned except for certain epidemic intervals. Sharp hourly changes

Table 1. Comparison of 24 hour rotoslide spore catches and Petri plate colony counts during early December, 1968, asthma epidemic

December, 1968	Rotoslide spore count				Colony count (24 hr. A.M. and P.M.)		
	Total	Large	Small	"Basidiospore-like"	Total colonies	Genus	No.
2	1,883	170	1,299	424	9	Cladosporium	3
						Aspergillus	1
						Bispora	1
						Humicola	1
						Hormodendrum	2
						Fusarium	1
3	768	96	310	380	10	Hormodendrum	4
						Cephalosporium	2
						Bispora	2
						<i>M. sterilia</i>	2
4	1,348	70	562	716	Overgrown with <i>Mucor</i> , inadequate subculture		
5	1,780	168	886	696	8	<i>Mucor</i>	1
						Monosporium	1
						Dematiaceous	1
						Fusarium	3
						Penicillium	1
						Helicosporium	1
6	3,024	392	1,468	1,152	14	Sepedonium	1
						Cephalosporium	1
						Hormodendrum	4
						Penicillium	1
						Verticillium	1
						Phoma	1
						Sporobolomyces	1
						Oospora	1
						Aspergillus	1
						Fusarium	1
						Monosporium	1
7	1,176	84	714	378	15	Fusarium	2
						Phoma	1
						Sporobolomyces	1
						Hormodendrum	2
						Alternaria	3
						Graphium	1
						Curvularia	1
						Macrosporium	1
						Unidentified	2
						Paecilomyces	1

in spore counts have previously been noted using volumetric samplers,^{21, 32} but plate colony counts have not reflected with any degree of accuracy the incidence of airborne spores during a given 24 hour period.⁹ No doubt this is related to the widely known inefficiency of small spore recovery by sedimentation.

Our plates were often overgrown during the 72 hour incubation period, and several genera characterized by the production of small round or oblong spores were identifiable only after multiple subcultures. This rendered colony counts of little use in presumptive identification of the large number of similar small single spores detectable on 24 hour rotoslide samples.

We also noted that slide culture preparations of a particular genus often revealed some conidia resembling other genera, which would likely further

decrease the ability to accurately identify single small spores on rotoslides or to compare colony count and roto-slide results. This situation is illustrated in Fig. 11, which is a single spore slide culture of pure growth *Alternaria* sp. obtained from an exposed Petri dish colony. Several conidia, if airborne as single spores, would likely be identified as other genera (arrows) on roto-slide or conventional gravitational samplers.

Table I illustrates a comparison of 24 hour roto-slide spore catches and Petri plate colony counts during a typical moderately large asthma epidemic involving 123 adult emergency room admissions over a 3 day period in early December of 1968. Petri plate colony counts varied from 9 to 15 with no definite relationship to semiquantitative roto-slide spore counts. It is interesting that total roto-slide counts seemed to correlate with the asthma admission rate, which increased sharply from December 5 through the morning of December 6, whereas such a relationship was not evident with Petri plate counts. The increase in our "basidiospore-like" category on roto-slide samples might also be missed on routine Sabouraud's dextrose Petri plate analysis in view of the special synthetic nutrient media requirements of these organisms. The over-all interrelationship between roto-slide spore counts and asthma epidemics will be discussed in a following article.

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