Dyed Pollen Grains and Spores as Tracers in Dispersion and Deposition Studies[1]

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1. Introduction

Due to the large number of possible combinations of meteorological variables, it is seldom possible to duplicate experiments in atmospheric dispersion under substantially identical field conditions, or to replicate trials with no variables altered other than those chosen by the experimenter. For instance, it might be desirable to conduct a series of tests varying the height of release but keeping other factors constant. Such tests could not be made consecutively without the risk of changing meteorological conditions, but could be accomplished by the simultaneous emission of physically identical but distinguishable tracers from the heights of interest. None of the tracer systems in current use has this capability. Although it is theoretically possible to distinguish two or more fluorescent or radioactive substances of similar size and shape, the radiation emissions of such materials usually overlap enough to make such separations difficult if not impractical for field use. A simple solution to this problem has been developed at Brookhaven National Laboratory, where pollen grains and spores stained distinctive colors are successfully used in multiple-source dispersion and deposition experiments.

2. Spores

Many pollens and spores are suitable for use as tracers. Pollens commonly range in size from about 15 to 100 μ in diameter. Many are spherical although often with roughened surfaces. Others are ellipsoidal, and asymmetrical shapes also occur. Detailed descriptions of pollen grains are given by Wodehouse (1935), Hyde and Adams (1958) and Erdtman (1954). Fungus spores vary from a few microns upwards and occur in a variety of shapes. Those of most interest as tracers, however, are small and essentially spherical. Spores of Club Moss (*Lycopodium*), frequently used as tracers by European workers, are shaped like truncated pyramids with hemispherical bases. Their largest dimension is about 32 μ. Although reliable measurements are few, the specific gravity of most spores seems to be near 1.0 when fresh but is usually less when in a dried state. Of prime importance is the fact that pollen grains and, to a somewhat lesser degree, fungus spores of a given species are essentially monodisperse. Gregory (1961) summarizes much available information on pollen and spore size and terminal velocity. Only a few of the potentially useful types have been tested for staining or used in field experiments at Brookhaven. Characteristics of these, selected for a wide range of size and terminal velocity, are listed in Table 1. The pollen diameters are from Wodehouse and the diameter of *Lycopodium* from Gregory. The diameters of the fungus spores are from our determinations. The experimental values of terminal velocity were obtained from Gregory and the calculated values were computed from Stokes law assuming a specific gravity of 1.0. Both should be regarded as approximate.

Lycopodium and smut spores as well as many pollens are available at modest cost from commercial drug firms, particularly those serving allergists. If large quantities are desired, it may be necessary to make advance arrangements to have the required supply in stock. The materials are usually available both untreated and defatted. The former is recommended since some types have proven more difficult to stain when defatted. The puffball spores are usually not listed but would be

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TABLE 1. Characteristics of pollens and spores tested as tracers.

Genus	Shape	Surface	Mean dia. (μ)	Terminal velocity (cm sec^{-1}) Calculated	Experimental
Ragweed (*Ambrosia*)	Spheroidal	Spiny	18	1.0	0.6
Timothy (*Phleum*)	Spheroidal	Smooth	34	3.5	1.0
Rye (*Secale*)	Ellipsoidal	Smooth	40×62	8.0	6.0–8.8
Corn (*Zea*)	Spheroidal	Smooth	95	28.0	
Club Moss (*Lycopodium*)	Modified pyramidal	Slightly roughened	32	3.0	1.8–2.1
Puffball (*Lycoperdon*)	Spheroidal	Slightly roughened	3.5	0.03	0.05
Puffball (*Calvatia*)	Spheroidal	Rough	4.5	0.06	
Corn Smut (*Ustilago*)	Spheroidal	Rough	6.5	0.13	0.3

collected to order. Several of the species listed can be collected in the field if the experimenter is so inclined.

3. Dyes and staining techniques

Lycopodium spores and the pollens listed are easy to color with a variety of biological stains. Some dyes, however, change color with pH changes in the neutral region and are not recommended. Several that have given good results with pollen grains and *Lycopodium* spores are: Basic Fuchsin (red), Fast Green (green), Methylene Blue Chloride (blue-green), Methyl Violet (violet), Orange II (orange), Brilliant Cresyl Blue (blue), Uranine (fluorescent yellow-green) and Rhodamine B (fluorescent orange-red). Fungus spores are more difficult to stain and only Brilliant Cresyl Blue has given usable results with the spores tried.

The staining procedure is simple. The particles are added to a saturated aqueous solution of the dye with a small quantity of wetting agent such as Aerosol C-61 from American Cyanamid Company. If the latter produces objectionable foam, an anti-foam agent such as Dow Corning Antifoam B is added. If the saturated solution stains the particles too dark a color it may be diluted as required. The mixture is then stirred or agitated until the stain has dyed the particles and all clumps are well separated. This may take from half an hour to a day or so. Vibrations produced by a commercial ultrasonic cleaner have given excellent results in de-agglomerating clumped particles but a laboratory stirrer is usually quite adequate. Technical discussions of biological stains, their characteristics and uses are given by Conn (1961) and Gurr (1962) should they be needed.

4. Emission and sampling techniques

At Brookhaven, the particles are released into the atmosphere by compressed air-operated atomizing nozzles utilizing the staining solution as a carrier (Raynor and Smith, 1964). This solution may be diluted to some extent without affecting the color of the particles if a larger volume is desired. When emitted into the air at any but high humidities, the liquid quickly evaporates, leaving the dry particles subject only to atmospheric motions and their normal settling velocity. If particles of two or more colors are to be released from the same container, it is desirable to remove them from the staining solution and mix them in a liquid in which the dyes are not soluble to prevent the colors from mixing. Toluene has proven successful for field use but is not recommended indoors because too great a concentration of the vapor becomes explosive. If the particles are to be dispensed dry they may be separated from the liquid by filtration, evaporation or other suitable methods. In this case, however, re-agglomeration of many of the particles usually occurs and methods of separating them again must be used.

Any device suitable for sampling particles in this size range may be used for measuring air concentrations of these tracers. Since the colored particles must be identified visually under a microscope, the sampling surface should be compatible with this technique. For instance, membrane filters which retain particles on the surface are acceptable, but fibrous filters in which much of the collected material is within the filter would be unsuitable. Glass microscope slides used as collecting surfaces in a number of sampling devices are ideal from the counting standpoint. At Brookhaven, a rotating impactor sampler which collects particles on the edge of microscope slides is commonly used (Raynor and Smith, 1964).

Deposition surfaces must also be suitable for microscope examination unless a technique is developed for removing the particles from the collection surface for analysis. Slides or plates of glass or sheets of transparent gummed film are probably the best choices. Techniques for measurement of these particles deposited on natural surfaces such as grass or soil have not been developed.

5. Applications

At Brookhaven, dyed pollens have been used extensively as tracers in experiments designed to study the behavior of particles in a forested region. A number of experiments have involved simultaneous releases from a single height but several distances upwind of the forest, while others included emissions from several heights at a single distance. These experiments would have been impossible without this technique. Dyed pollens have also proven useful in single-source experiments since the collected particles can more readily be distinguished from other material on the sampling surfaces.

6. Conclusions

The use of dyed pollens and spores as tracers permits multiple-source dispersion and deposition experiments to be conducted with physically identical but readily separable monodisperse particles in a wide range of sizes. Necessary techniques have been developed and tested and suitable instrumentation is available for application of those tracers to field studies.

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