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Aerobiology of pollinosis

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Growing insight into tissue mechanisms of pollinosis has tempted us increasingly to overlook equally basic disease determinants in the functional anatomy and ecology of pollens. Therefore, it seems timely to reexamine these complex allergen carriers, considering their generation, release, transport, and mucosal deposition in the light of available study methods.

FLORAL GROUPINGS IN POLLEN BIOLOGY

Current data suggest that, except under extraordinary circumstances,¹ clinical importance in pollinosis is confined to flowering plants (namely, of the division Spermatophyta) with, primarily, *wind-dispersed* pollens. Although such (anemophilous) species form a floral minority everywhere, prodigious outputs of pollen continue to ensure their reproductive success.² Most higher plants (especially in tropical areas) exclusively use insects or bats, birds, etc., as self-directed vectors for pollen dispersal. Such species

Abbreviation used

EAR: Effective aerodynamic radius

(broadly termed entemophilous) produce relatively few pollen grains but have evolved large, brightly colored blossoms and sweet-scented nectars to attract (and facilitate transfer of pollen to) conveyer organisms. Plants with small, grouped flowers that are showy only in aggregate often are *amphiphilous* (i.e., have only a minority of their grains windborne, probably often dislodged by vigorously foraging insects). Familiar amphiphilous groups include the goldenrods, sumacs, elderberries, wild grapes, *native* cherries, and acacias. Since large groupings of these are necessary for substantial air contamination to result, the true place of each in pollinosis remains hypothetical. By contrast, highly productive anemophilous species present a common pattern of numerous, grouped, individually small and drab, scentless "florets." Fig. 1 demonstrates the organs of such a minute wind-pollinated flower in cross-section and emphasizes the relatively large, pollen-receptive *stigma* that commonly is present. Many anemophilous species (including grasses, elms, chenopods, and amaranths) exemplify this pattern, having both pollen-producing and ovule-bearing organs borne on a single recepta-

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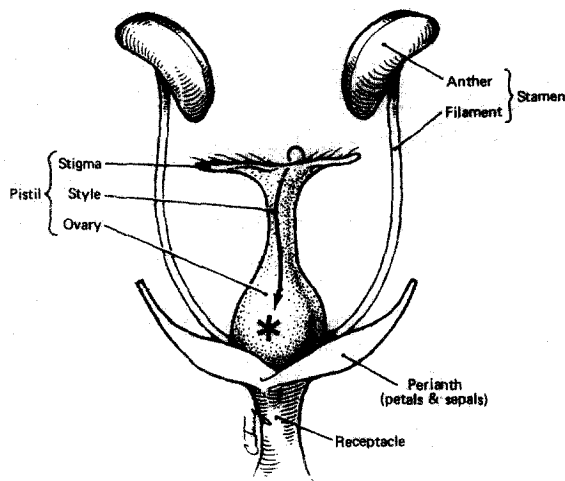


FIG. 1. Parts of a wind-pollinated (perfect) flower demonstrating typical adaptations. Note, especially, reduction in size of the petals and sepals. A pollen grain is demonstrated on the stigma, and the path of a pollen tube is designated by the sinuous arrow. The ovary is marked by an asterisk. (From Bierman CW, Pearlman DS: Allergic diseases of infancy, childhood, and adolescence. Philadelphia, 1980, WB Saunders Co.)

cle; such flowers are termed "perfect." Other wind-pollinated taxa have "imperfect" flowers in which the stamens and pistils are borne separately. A further distinction is made between "monoecious" species (e.g., oaks, birches, ragweeds) in which single individuals demonstrate both staminate and pistillate, imperfect flowers, and "dioecious" types where these are borne on different individuals (as among poplars, mulberries, and box elder). In temperate areas, at least, imperfect flowers are almost exclusively anemophilous. Single genera may display a range of the foregoing features. For example, among maples (*Aceraceae*) one finds entirely anemophilous species (e.g., *Acer negundo* [box elder]), progressively insect-pollinated amphiphilous species (*A. rubrum* [red maple], *A. macrophyllum* [western big leaf maple]), and essentially entomophilous taxa (*A. platanoides* [Norway maple]).

POLLEN MORPHOGENESIS AND REPRODUCTIVE FUNCTION

Familiar allergenic pollens develop in and are released from cavities, the *anther sacs*, in usually bean-shaped structures, the anthers. Commonly, each anther is fitted with a stalk or *filament*, the combination constituting a *stamen* (Fig. 1). The anther sacs are lined with a nutritive cell layer, the *tapetum*, and contain more or less numerous *pollen mother cells* that undergo meiosis and ultimately produce pollen

grains in tetrads. In most species these groupings of four separate to form single grains, termed "monads"; in some (e.g., broad-leaved cattail), however, they remain adherent and, in other taxa, may coalesce further to form complex *polyads* (e.g., closely-packed groups of 16 in *Acacia*). Pollen development proceeds in a thin, gelatinous *tapetal fluid* that serves as a medium of biochemical exchange between the tapetum and the developing pollen units.³ After its formation, each immature grain secretes about itself a cellulosic, relatively clear layer that will form the *intine* of the mature particle. Around this the tapetum will deposit a multi-layered *exine*, commonly with distinctive surface markings, composed of a highly durable but incompletely characterized polymer, *sporopollenin*.⁴ In some species small (i.e., 1 to 2 μ m) orbicules of exine substance (*Übisch bodies*) also are formed and may be dispersed with the release of the mature grains.^{5, 6} In any case it is clear that two separate stages of the plant life cycle contribute wall layers to the developing pollen: the exine from the anther (sporophyte wall) and the intine from the pollen protoplast itself, which will serve as the (male) *gametophyte* (literally, gamete-producing plant).⁷

Anthesis, the release of mature pollen from the anther, may involve various active and/or passive processes depending on the species considered. In a few taxa (e.g., nettles and paper mulberry) the anthers burst explosively when they are dry.⁸ More commonly, however, desiccation promotes cracking and retraction of the anther wall that creates gaps through which the moist pollen spills. A multistep scenario exemplifying this effect has been clearly documented for anthesis by male short ragweed florets.⁹ Regardless of the details, however, it appears that low humidity generally favors pollen release and aerial dispersion. Accordingly, many plants have evolved mechanisms to defer pollen release until suitably dry conditions prevail¹⁰; at such times peaks of airborne pollen prevalence often occur.

To realize their reproductive potential, pollen grains first must be conveyed to the stigma (Fig. 1) of a receptive flower, a translocation process properly termed "pollination." How compatibility between pollen grain and stigmatic surface is encoded and expressed is not clear, although a rapid exchange of chemical signals appears to occur.¹¹ The pollen-stigma interaction blocks matings across species boundaries and, within a species, determines individuals or clones that are self-fertile or self-sterile.¹² On a stigma of *compatible* type, a viable pollen grain may *germinate*, sending a projection of cell contents, thinly covered with intine, out through an aperture or

point of low resistance in the exine. This *pollen tube* grows rapidly and enzymatically digests tissues of the stigma and *style*, the relatively elongate connection between the stigma and ovary (Fig. 1). By the time a pollen tube is fully developed, mitotic division has occurred within the protoplast of the grain, producing *gametes* that can affect fertilization. Considering these diverse functions, it is not surprising that pollens are biochemically complex with enzymatic and other components that are rapidly releasable.¹³ The possibility that at least some pollen allergens may function as natural recognition substances has been increasingly considered.¹⁴ Furthermore, the microscopic localization of ragweed pollen allergens at relatively superficial mural sites is consistent with this view.^{14, 15}

DISPERSION OF POLLENS AND POLLEN ALLERGENS

Pollens may become airborne when they fall into an airstream or are scoured by turbulent eddies. Dispersion from many tree species is promoted by long, hanging flower clusters (*catkins* or *aments*) that readily present pollen to moving air currents. The flowering of most trees before leaf expansion, at a time when air movement through a forest canopy is relatively unobstructed, must facilitate this process. Similarly, floral clusters of many *herbaceous* (i.e., nonwoody) plants form projecting *spikes* (e.g., in ragweeds, sorrels, and docks) readily accessible to air. Where flowers are more sessile (e.g., in Russian thistle and *Kochia*), small or finely dissected leaves also occur, promoting air circulation. Pollens that have fallen onto dry vegetative surfaces also may be "catapulted" into the air when turbulent currents move these substrates.⁹ Whatever the dispersing force, pollen grains are projected through a relatively thin *laminar boundary layer* of still air into the constantly moving lower atmosphere. Since most common allergenic pollens are derived from huge *area sources*, separate particle "plumes" from discrete emission points usually cannot be traced. However, clearly, pollen prevalence at any site will reflect the strength and proximity of upwind sources and the activity of (diluting) crosswinds both vertical and horizontal.¹⁰ In addition, local surface effects may occur, including particle removal by vegetation screens,¹⁶ particle concentration in the lee of barriers,¹⁷ and creation of stagnation points by cul-de-sacs and wind screens.

Pollen levels also are influenced strongly by the depth of the volume of air in which free mixing can occur, and this, in turn, reflects the prevailing tem-

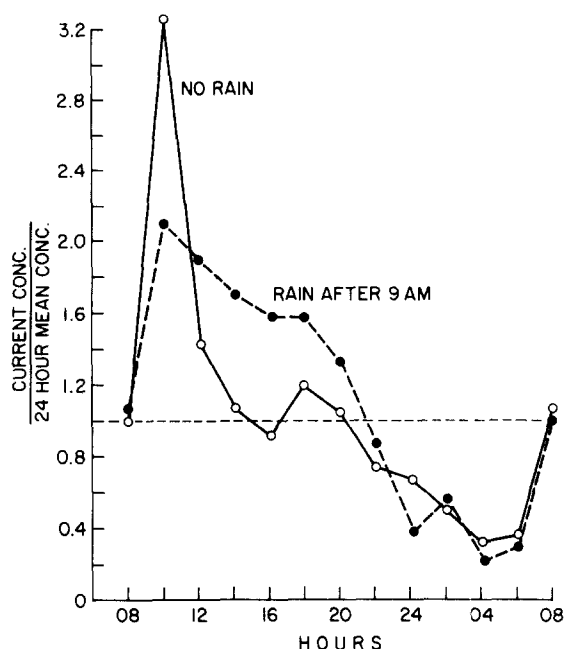


FIG. 2. Time-specific ragweed levels from Burkard trap deposits. Data are from 20 totally clear days (no rain) and for 20 days clear at sunrise but with rain after 9 A.M. in periods from August 20 to September 10 from several years. Data were normalized with respect to individual daily means and averages for each of the 12 times calculated. Clear days demonstrate peak dispersion followed by dilution in a deep mixing volume (*open circles*). Days with eventual rain demonstrated less hectic dispersion early, but levels remain high longer, probably caused by mixing in a shallow layer beneath an overcast (*closed circles*).

perature profile of the lower atmosphere. On clear, sunny days, surface warming promotes a (normal) gradient of progressively cooler air with increasing altitude. Under these conditions warm, pollen-loaded air "parcels" will rise briskly, and active mixing in a relatively deep layer results. When, instead, warmer air is present aloft, a *thermal inversion* is said to exist, and rising air will not ascend further. As a result mixing is limited to the relatively shallow layer below the inversion, and particles that are present will be concentrated closer to the ground (Fig. 2). During hours of darkness, the atmosphere cools, and denser, pollen-bearing air tends to *subside* (namely, move toward the surface) at times, creating modest, nightly "secondary" prevalence peaks.¹⁸ In addition, radiation of heat from the surface of the earth after sundown tends to promote a low-level thermal inversion, concentrating air pollutants in a shallow layer. Subsidence of particle-bearing air also may be prominent during thunderstorms when violent turbulence and the outwash from impacting raindrops probably serve

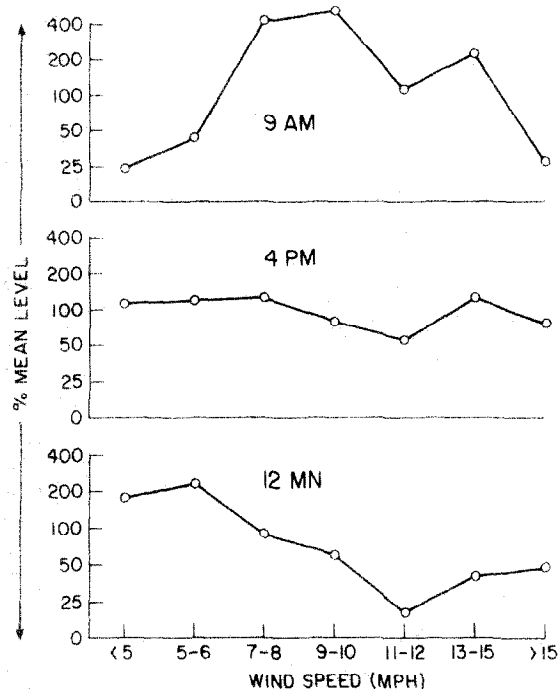


FIG. 3. Pollen prevalence in relation to wind speed for 9 A.M., 4 P.M., and 12 midnight time points in peak ragweed pollen periods. Each panel is based on at least 35 counts, and each wind speed level represents at least three counts. Data are normalized in relation to time-specific means. At 9 A.M., both calm and quite windy conditions are associated with wet weather and poor pollen dispersion. At midnight low wind speeds are correlated with clear skies and subsidence of pollen-laden air; with high winds, much pollen probably stays aloft.

also to refloat pollens.¹⁹ By contrast, in steady frontal rain, pollen removal predominates, as the 1 to 2 mm droplets effectively scavenge particles, an effect more closely proportional to the duration of rainfall than to its total depth.²⁰

Pollen emission, dispersion, and transport display a variety of distinctive and, often, apparently inconsistent effects when they are examined in relation to complex weather patterns. As a result it is generally impossible to characterize, in simple terms, the effects of single variables such as temperature, humidity, visibility, or wind speed on pollen prevalence. Overall, levels of airborne pollen are increased by warm, dry, clear conditions and fall during unseasonably chilly or wet periods.^{10, 21} For short ragweed specific temperature minima and humidity maxima beyond which anthesis is blocked have been defined.⁹ However data in Fig. 3 suggest that the relationship of ragweed pollen levels and wind speed is far less consistent and, in fact, varies among diurnal periods when it is studied at an urban site. It is clear that air

turbulence can refloat relatively dry pollen grains and that human activity potentially can cause similar effects.²²

Once entrained by prevailing winds, pollen grains may travel quite long distances. Recoveries from air samples at midocean are well documented¹⁰ as is extensive (intercontinental) transport (e.g., from Labrador to Greenland, a distance of several hundred miles). However, considering the vast numbers released, most windborne pollens are permanently lost within a few hundred meters of their sources. In the case of corn pollen (with grains often 100 μ m in diameter), this attrition occurs within meters. Not surprisingly, gravitational fallout is a major factor depleting pollen "clouds." Studies of pollens falling in still air have confirmed only small effects of particle densities (which usually are close to 1.0) and of air viscosity.¹⁰ For unlike particles, inequalities in fallout rates largely reflect differences in effective particle diameter, since the steady state (or terminal) settling velocity varies with diameter.² Fallout potential thus tends to rise logarithmically as progressively larger units are considered. This effect differentially influences the residence times of large and small particles in air as well as their susceptibility to capture by various sampling approaches.¹⁰

Although intact grains are assumed to be the primary carriers of pollen allergens, specific immunoreactive components have been found in other aerosol fractions. Observations of Busse et al.,²³ suggesting ragweed allergens associated with particles sized below 5 μ m, have been confirmed by use of "absolute filtration" and other appropriate means that exclude contamination by intact grains or microscopically visible pollen fragments.²⁴ Similarly, both whole ragweed pollen and antigen-E activities have been obtained by freezeout of atmospheric moisture from micronic particle-free fractions obtained during periods of ragweed pollination.²⁵ Recovered activity has been quantitated readily by direct skin tests as well as by RAST or ELISA inhibition²⁴⁻²⁶ and has been obtained both before and after the "ragweed season" per se.²⁶ However, the relative contribution of small aerosol-associated fractions to total pollen allergen burdens remains to be established. The origin of such pauci- and submicronic components is equally uncertain, and multiple sources may exist. Earlier reports²⁷ noting pollen allergen specificities extractable from somatic portions of ragweed plants have been confirmed and have suggested that these may become airborne in vegetative debris.²⁸ Allergens also might be eluted from pollen grains deposited on moist natural surfaces with dispersion of the resulting extracts in

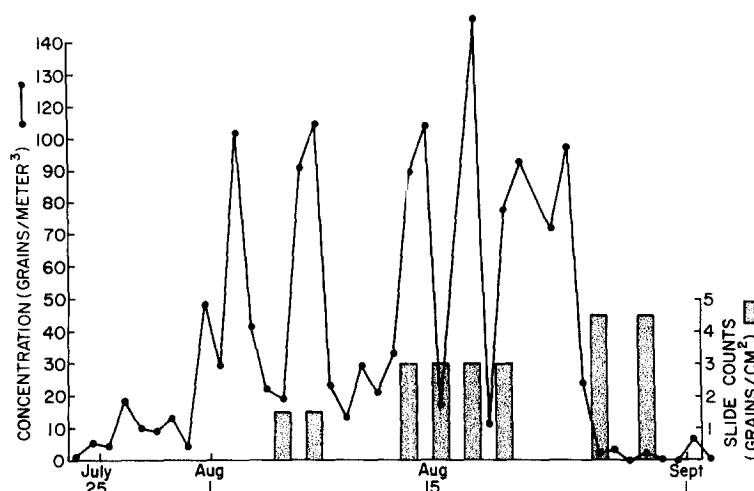


FIG. 4. Single site prevalence data for airborne nettle pollen in 1968 in Ann Arbor, Mich., from Burkard trap daily means (closed circles) and grains per square centimeter on a 24-hour gravity slide (shaded bars). Although nettle pollen achieves substantial levels, its small size (12 to 14 μm) determines sparse recoveries by fallout. The resulting counts give inexact clues to prevalence.

“jet” microdroplets produced by dewsplash or bursting bubbles.²⁹ However, it remains to be determined if these hypothetical effects operate widely in nature and whether additional pollen allergens are dispersible in aerosols smaller than intact grains.

MONITORING APPROACHES FOR AIRBORNE POLLENS

Despite more than a century of increasingly active study, pollen prevalence data continue to offer: (1) clues to potential offenders in new population centers, (2) evidence of extended exposure trends in communities with established data bases, and (3) insight into short-period exposure burdens for clinical correlation and investigative use. Sampling efforts have emphasized throughout the unbiased recovery of pollen grains on suitable surfaces for microscopic enumeration. Recent findings (noted above) of ragweed allergens in effectively amorphous aerosol fractions have suggested that immunochemical assays of bulk deposits might provide a better overall view of exposure burdens. Such collections may be made on large fibreglass filters by use of high-volume samplers with extraction of the deposits by descending elution.³⁰ Alternatively, small, open-ended membrane filters operated by laboratory pumps have provided, on direct elution, material suitable for studying ragweed activity in large and small aerosols.^{24, 31} At present, however, the relevance of immunochemical assays for other pollen allergens remains unclear, and, for ragweed, an ideal collection approach is still to be identified.

Attention therefore continues to center on collections of intact pollen grains for visual analysis.

Blackley's³² use of greased microslides as passive pollen collectors was widely adopted thereafter and reinforced by Durham's³³ description of a “standard” gravity sampler in 1946. However, despite this tradition and the method's evident simplicity, sampling by gravity slide displays well-documented limiting deficiencies,^{34, 35} most notably a total uncertainty as to the volume of air from which any pollen “catch” is derived. Deposition reflects a combination of passive fallout and accretion of particles projected onto the slide by eddies. These are processes that vary intractably with changes in wind speed, direction, and turbulence development. Without a common *vol-umetric* (volume-dependent) denominator, samples (gathered under different atmospheric conditions) cannot be compared; in addition, efforts to apply empirically derived conversion factors predictably have failed. A second critical defect of the gravity slide is its tendency to impose size-dependent collection bias with progressively smaller particles being increasingly underrepresented. As a result even *relative* prevalence levels cannot be deduced confidently for pollen types that differ in physical size.³⁶ Since gravity slides take relatively few particles overall, sampling errors also tend to be high, and, especially for smaller units, may entirely distort prevalence patterns (Fig. 4). Currently, gravity slides offer means to initially survey or study seasonal trends for larger, relatively numerous pollen types. More exacting goals

require volumetric devices that can provide data readily as "recoveries per cubic meter of air."

Rotating arm impactors, especially the intermittent rotorod (Ted Brown Associates, Los Altos Hills, Calif.), have served especially well as samplers for pollen. During rotorod operation, a greased, 1.3 mm wide surface, rotated through air at 2400 rpm, intercepts and captures particles. The rapid motion and circular path described serve to eliminate effects of wind speed and direction.³⁷ Although collection efficiency does rise with particle size (and with characteristic fall speed in still air, as previously described) the relevant correction factors *can* be calculated.³⁷ Furthermore, if a standardized sampling protocol is followed, recoveries made at different times and locations are readily *compared* even without the use of efficiency-related coefficients. Limited comparisons have suggested that silicone and Lubri-Seal (A. H. Thomas Co., Philadelphia, Pa.) stopcock greases are preferred adhesives for impactor surfaces.³⁸ However, under commonly prevailing field conditions, particle loading will diminish the collection properties of such coatings seriously during prolonged sampling runs.* This problem may be minimized by operating the rotorod for intervals shorter than those prescribed (e.g., 15 to 20 rather than 60 sec) during each 10-minute cycle without sacrificing effectiveness. Particles counted microscopically on the exposed rod surface are related to the volume of air swept out by that segment: volume "processed" per rotation = collecting area $\times$ circle of rotation = length $\times$ width $\times$ distance between rods $\times \pi$; volume per minute = volume per rotation $\times$ 2400 rpm; total volume = volume per minute $\times$ total minutes sampling. Data are best expressed as "particles recovered per unit volume (generally per cubic meter) of air processed."

Suction samplers, including *filters* and *impingers*, also are adaptable, with modest effort, to provide volumetric pollen collections. However, in all but essentially still air, the entrance orifices of these devices must be continuously wind-oriented.¹⁰ Operation without observing this precaution will greatly lower efficiency. The resulting collection errors rise with aerosol size as well as ambient air speed and reach totally unacceptable levels for pollens. Suction collectors also are best exposed with inflow rates that produce orifice velocities *equal* to those of ambient air (a condition termed *isokinetic*). Where the free stream speed is (relatively) excessive, factitiously high particle recoveries result; disparities of the reverse sort

lead to errors of undersampling. Since this problem is almost never addressed satisfactorily, it is fortunate that *anisokinetic* conditions cause relatively modest errors that are, in fact, least for particles of 20 μm (typical of ragweed pollen grains).³⁹

Filters often are favored in sheltered situations where arrays of small, inexpensive collectors are needed. Membrane filters may be cleared and examined microscopically or extracted with aqueous solvents for immunochemical assay. Bulk samples for antigen analysis may be collected with "high-vols" that process hundreds of cubic meters per day. In studies of industrial pollutants, these latter devices are traditionally exposed with their filters directed vertically, i.e., normal to most air flow; however, the extent of overall collection errors resulting have not been estimated, and effects on pollen recoveries remain entirely speculative.

A modified filtration approach, proposed by French workers, uses several layers of oiled gauze sheets forming large "masts" through which air passes.⁴⁰ After digestion of the gauze, huge particle samples are available for analysis, and resulting data are, qualitatively, very diverse. However, relating the catch to air volumes processed requires a recording anemometer (wind-speed gauge), and estimates of efficiency for various particle sizes remain to be defined.

Spore traps remain the most versatile collectors for a variety of recognizable particles, including pollens, and have been especially favored by Eastern Hemisphere workers.⁴¹ These devices aspirate measured volumes of air that are accelerated in passing through a narrow orifice or nozzle. The rapidly moving stream is then forced to make a sharp turn, and particles with too much momentum to change direction are thrown against a collection surface on the channel wall. Spore traps in wide use, such as the Burkard trap (Burkard Manufacturing Co., Herts, U.K.), are crisply wind-oriented and use orifice speeds that approximate modal outdoor wind velocities (often about 13 mph). Particle collections are made on adhesive-coated, slowly moving surfaces: microslides for 24-hour recoveries or transparent tape-coated drums for 7-day samples; both collections provide time discrimination that allows diurnal recovery patterns to be defined.

The Burkard (Hirst) trap draws in 10 L of air per minute from which particles are captured on a greased surface moving past the 14 by 2 mm orifice at 2 mm per hour. The resulting "24-hour" deposits, averaging 14 by 48 mm, represent 14.4 m³ of aspirate and may be mounted with (melted) hard glycerine jelly containing fuchsin, under 20 by 50 mm cover slips,

*Solomon WR: Unpublished observations.

for direct particle enumeration. The volume of air corresponding to an area examined is easily calculated. For example, in a complete longitudinal scan of 48 mm length, a 400 μm field will encounter particles from $400/14,000 \times 14.4 \text{ m}^3$ or 0.41 m^3 . Since deposition along the 14 mm path may not be entirely uniform, longitudinal scans probably should follow a route staggered from side to side rather than a straight line.⁴²

Absolute values for the collection capability of popular impactors and suction traps, operating in the field, remain to be clearly defined. However, for most pollens, there is reason to assume operating efficiencies for the Burkard (Hirst) trap(s) of well over 50%, whereas those of the rotorods appear slightly lower, even where surface loading is minimized. Side-by-side comparisons of these samplers have produced similar prevalence estimates (per cubic meter of air processed) for large pollens (e.g., hickory [*Carya*]); for the smaller ragweed grains, rotorod-based levels were already below those of the spore trap.⁴³ These modest differences, however, contrast with the substantially higher efficiency of wind-oriented suction collectors for smaller micronic agents, especially fungus spores and other agents well below 10 μm .

Although atmospheric mixing is a familiar effect, airborne particle levels often are nonuniform, especially near the surface. Therefore, thoughtful choices among collection points are necessary. Selection hinges on the precise goal of each sampling effort and must consider the proximity of local allergen sources, the accessibility of the site and of power lines, risks of vandalism, etc. In addition, free horizontal air circulation is essential, and points where vertical vectors predominate, such as roof edges and balconies, are best avoided. Similarly, cul-de-sacs and narrow flow channels forming "jets" or vortices among buildings and other obstructions are undesirable. Where attention is focused on "average background" levels, theory, at least, favors the traditional choice of an unobstructed rooftop at least 50 feet above grade without adjacent taller structures. The "optimal" elevation may be problematical, since, away from sources, some uniformity of lower atmospheric pollen levels has been demonstrated.⁴⁴ Sampling pursued nearer the ground and close to sources may reveal unexpected exposure risks not reflected by rooftop samplers.^{22, 45} Local effects may be especially prominent with delimited sources such as single, productive trees, urban plantings,⁴⁶ or ragweeds in midwestern grain fields.⁴⁷ Portable, compact devices, such as D.C.-powered rotorods, adapt well to studies indoors or near the ground, and a nonwind-oriented Burkard

(Hirst) trap has been marketed for calm air applications within enclosures. Personal collectors by use of small filters and compact pumps may reveal individual exposure patterns; however, the value of passive slide samplers^{48, 49} for this purpose has not been established.

POLLEN STRUCTURAL FEATURES IN IDENTIFICATION

Airborne pollens comprise an array of quasispherical particles with few exceptions ranging in size from 12 to 70 μm . Despite their celebrated "replicate" quality, grains of a single type may demonstrate an appreciable range of sizes, arrangements of apertures, etc. A surface layer of lipids (often termed pollenkit) contributes much of the (yellow to brown) color as well as the cohesiveness of pollen in aggregate. This coating usually is more prominent in entomophilous types. Size characterizations are on the basis of pollens mounted in aqueous media (namely, expanded grains). However, during dispersion, the particles "collapse" and assume the smaller and less regular outlines of dry grains. The behavior of such particles may be predicted by determination of EAR. For a given pollen type, EAR is the radius of that unit density sphere that, at equilibrium, will fall in still air with the same velocity as the grains in question. Although EAR values are not widely considered, they do effect collection potential as well as airway penetration and can differ substantially from the measured radii of expanded grains.

The microscopic appearance of pollen is determined primarily by its three concentric domains: the (central) protoplast, the intine, and the (outermost) exine. The protoplast contains genetic material and various organelles. Starch granules, especially, can convey a distinctive appearance when they are numerous, as in sorrel and dock pollens (*Rumex*). The elastic intine is largely transparent and fails to stain with widely used dyes. However, local thickenings in this layer may become evident by inducing visible changes in refractivity. The shape and surface features of the exine contribute most to the distinctive appearance of specific pollens. Furthermore, the affinity of exine for (red) dyes such as basic fuchsin and phenosafranin allows these to be used to enhance microscopic details. Shape or arrangement alone will distinguish several commonly recovered pollen categories. Examples include the flat tetrads of broad-leaved cattail (*Typha latifolia*), the densely packed, 16-membered polyads of *Acacia*, the large grains, each with two lateral air-filled bladders typical of pines, spruces, firs, and some exotic conifers, and the

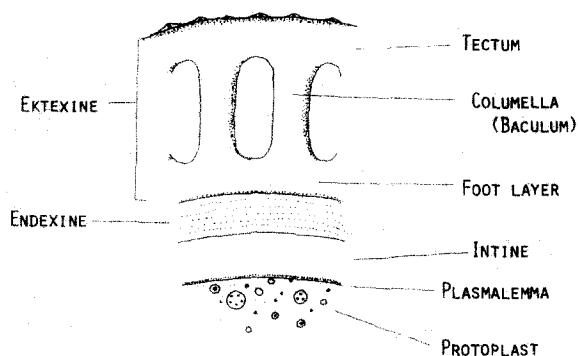


FIG 5. Pollen grain structural components diagramed in cross-section. As indicated at *left*, the three outer, stainable exine layers are often considered together as *ektexine*; an inner, lamellar, *endexine* is less readily stained. The plasmalemma is the limiting membrane of the protoplast.

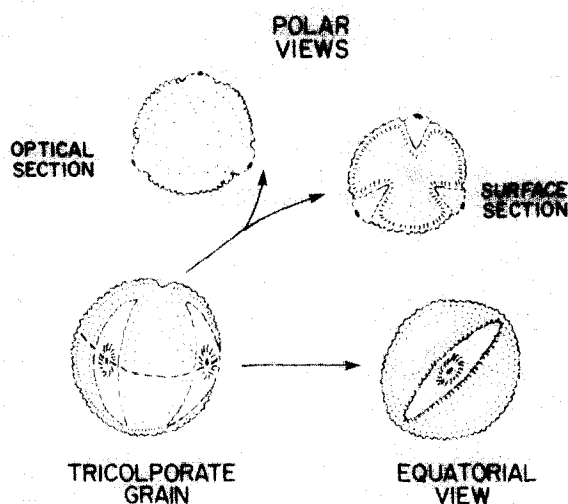


FIG 6. Schematic depiction of a tricolporate grain in equatorial and polar positions; for the polar position, tangential (*surface*) and interior (*optical*) sections are demonstrated. Views were created by changing the focal plane.

capsule-shaped grains of the carrot family (Umbelliferae).

As diagrammed in Fig. 5, the exine is several layered, although the outermost portion, or *tectum*, may be intact, incomplete, or virtually absent. These variations as well as diversity in exterior ornamentation produce an array of distinctive surface features. Many grain types are quite smooth (*psilate*), whereas other grains have minute projections roughening the surface (a condition termed *scabrate* or *verrucate*). When grains have large club-shaped excrescences, the condition is termed *clavate*. Surface projections

also may coalesce to form spines, i.e., producing an *echinate* surface; netlike arrays, a *reticulate* one; coarse ridges, *rugulate*; linear bands, *striate*; etc. In addition, most pollen types have characteristic patterns of apertures in the exine; when these are essentially round, these faults are considered *pores*; when these are elongated, they are *furrows*. Apertures tend to be spaced regularly so as to divide the grain surface in orderly fashion. Pores, whether three or many, commonly are arranged along a specific meridian, designated the "equator" of the grain. Furrows, also, tend to be aligned with their *centers* along such an equatorial line. An obvious analogy regards the areas farthest from the equator as two *poles*; grain regions are named accordingly. In "polar" and "equatorial" views grains are positioned, respectively, with one pole or with a segment of equator nearest the observer's eye (Fig. 6). In exact polar position, the equator forms the circumference of the grain; in equatorial position, the perimeter includes the poles. Appearance also is strongly affected by whether the focal plane chosen is tangential (a *surface* section) or deep (an *optical* section).

Grains come to rest in characteristic positions determined by the ratio of their (inter)polar and equatorial diameters, respectively P and E. Grains with P/E diameters < 1 are termed "oblate" and commonly are observed to rest on a flattened pole; when the P/E is > 1 , the *prolate* condition, most rest on their equators, presenting their long axes to the microscopist.

Anemophilous grains may have (one or more) pores alone (a condition designated *porate*), furrow alone (*colpate*), or furrows with central pores (*colporate*). Fig. 7 displays a system and terminology for pollen apertures. In addition to being arranged meridionally, pores may be scattered over the grain surface, a *periporate* condition (as in chenopods and amaranths); rarely they are restricted to one hemisphere (e.g., in walnut [*Juglans*]). Pores and furrows are covered by thin, nonstaining membranes, derived from intine and/or inner exine, and often bulge into (or through) the apertures. Pore membranes each may be capped by an *operculum* (a disc of stainable exine), a feature typical of the single pores of grass pollens; other types demonstrate the membrane dotted with exine fragments. Pores with projecting margins are said to be "collared" as in birch (*Betula*) grains; by contrast, those pores of hickory (*Carya*) are uncollared or *simple*. Among members of the birch family (Betulaceae) and other taxa with triporate grains, inner wall characteristics of the collared pores furnish points of distinction. An additional feature of value is the *aspis* (plural, *aspidēs*; adjective, *aspidate*), a

broad refractile "shield" of inner wall substance surrounding pores of some grains including those of birches, mulberries (*Morus*), Australian pine (*Casuarina*), etc.

Table I uses the morphologic features described to classify a number of commonly encountered North American (and a few exotic) pollens. The scheme is distinctly exclusive and *not* intended as a key for identification; however, several illustrated manuals may assist in approaching "unknowns."⁵⁰⁻⁵⁴

CYCLES AND DISTRIBUTION OF POLLEN SOURCES

Summaries of pollen prevalence by region and season also are available.^{51, 53, 55, 56} However, since they must attempt to generalize to large areas from one or a few stations and often rely on data of limited scope, these collations offer imprecise guides to exposure conditions. Throughout temperate areas, trees and other woody species, which form their buds in the preceding year, are among the earliest to undergo anthesis in each growing season. Pollen shedding by trees in North America extends over a period from mid-December (when mountain cedars bloom in the Southwest) to late June (when the last boreal forest conifers are exhausted). Where harsh winters prevail, bud loss can be extensive, and flowering conditions can be unpredictable, making *tree* pollination especially variable in date and intensity from year to year. Flowering by nonwoody species with perennial rootstocks (e.g., grasses, sorrels and docks, plantains, hemp, nettles, etc.) generally follows that of trees from late spring to midsummer. In warm southern and West Coast areas, bud formation and flowering by grasses may occur through much of the year; anthesis of perennial *forbs* (broad-leaved, nonwoody species) also is prolonged. Anemophilous annuals, which must complete their life cycles in a single season, generally shed pollen in midsummer to late summer, depending on their seed germination dates; this group includes most ragweeds, Russian thistle, and *Kochia*. In the Midwest, short ragweed (*Ambrosia artemisiifolia*) seeds germinate in mid-May, and the eventually resulting male florets shed pollen about 10 wk later. Determinants of pollen output by anemophilous species are not well understood but appear to include temperature, humidity, rainfall, and light intensity.^{9, 57, 58} During rapid vegetative growth, drought stress appears to diminish pollen output by short ragweed populations.³⁶ Furthermore, Davies and Smith⁵⁷ were able to estimate the annual start and eventual intensity of grass pollen exposure from measurements of accumulated spring temperature. Floral

POLLEN APERTURAL TYPES

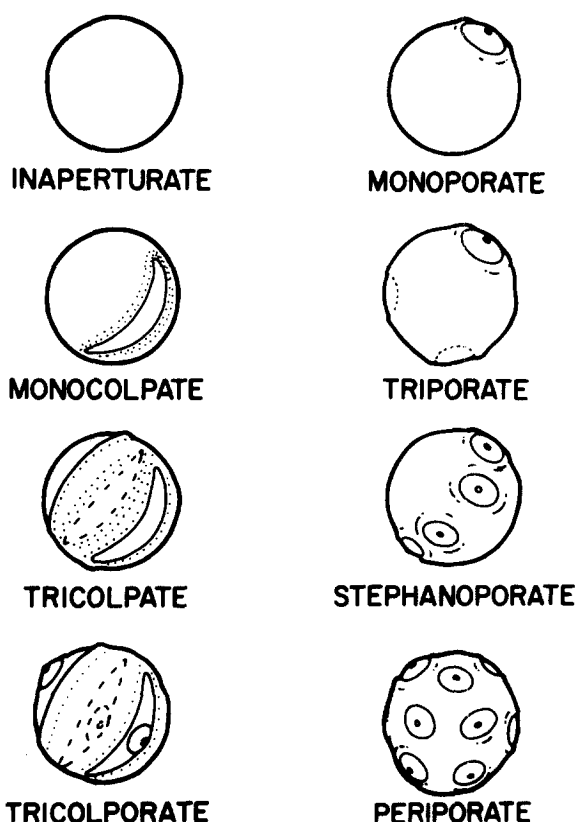


FIG. 7. Nomenclature for aperture arrangements of common windborne pollens; other patterns are encountered rarely. Four or more equatorial pores are designated a "stephanoporate" condition.

development by short ragweeds is known to require a specific period of nighttime darkness (a trait termed *negative photoperiodism*) and is not initiated until days shorten appropriately.⁹ This adaptation appears to determine the observed earlier flowering of short ragweed at lower latitudes (e.g., in Florida) than at its more northern center of abundance. Photoperiod also may impose a northern range limit for ragweeds that must be excluded from areas where the interval from suitably short days to killing frost is too brief to allow seed "setting." Despite effects of light on floral development, *mature* ragweed florets will shed pollen normally in total darkness and under drought conditions. Similarly, although anthesis is greatly depressed below 50° F, it proceeds quite well in intense summer heat. As previously noted, effects of rain and high humidity are marked and complex. These points do not suggest that *accurate, long-range* predications of day-to-day variations in pollen prevalence will soon be achieved.

TABLE I. Morphologic features of some common pollens

-
- I. Bearing two lateral bladders—pine spruce, fir, true cedar, *Podocarpus*
 - II. Inaperturate (no pores or furrows)
 - A. Poplars and aspens (*Populus*), scabrate exine
 - B. Junipers (*Juniperus*); "cedars" (*Cupressus*); cy-press (*Taxodium*); yews (*Taxus*), thick intine, stellate protoplast
 - C. Hemlock (*Tsuga*), exine coarsely verrucate, 70 to 80 μm
 - III. Bearing a single pore only (monoporate)
 - A. Grasses, single, sharply defined operculate pore
 - B. Sedges, prolate, subtriangular, "poroid"* on broad end
 - IV. Bearing two pores, mulberry (*Morus*), pores aspidate
 - IV. Bearing two pores—mulberry (*Morus*), pores as-
 - A. Hickory (*Carya*), pores simple; no aspidate
 - B. Birch (*Betula*); Hazelnut (*Corylus*); Bayberry (*Myrica*); Australian pine (*Casuarina*), pores aspidate, collared, >20 μm ; Nettle (*Urtica*); Pellitory (*Parietaria*), aspidate <18 μm
 - C. Chenopod, Amaranth type, periporate, pore mm long
 - VI. Bearing five pores
 - A. Elm (*Ulmus*), pores simple, exine surface undulating
 - B. Alder (*Alnus*), pores collared, aspidate
-

*Pore with indistinct margins.

†Box elder demonstrates a diffusely granular surface much like the oaks.

TABLE I. Cont'd

-
- VIII. Bearing one furrow (monocolpate)—palms, *Ginkgo*
 - A. Walnut (*Juglans*), pores all on 1 hemisphere, grains flattened
 - B. Plantain (*Plantago*), pores simple, 6 to 11, pore membranes operculate
 - C. Chenopod, Amaranth type, Periporate, pore number >15
 - VIII. Bearing one furrow (monocolpate), palms, *Ginkgo*
 - IX. Bearing three furrows without pores
 - A. Oak (*Quercus*), size >25 μm , exine verrucate
 - B. Maple (*Acer*), size >25 μm , exine striate†
 - C. Willow (*Salix*), grains distinctly prolate, one dimension >20 μm , exine reticulate
 - D. Sycamore (*Platanus*), size <20 μm , exine granular
 - X. Bearing four furrows—Ash (*Fraxinus*), exine finely reticulate
 - XI. Bearing furrows with central pores (colporate)
 - A. Beech (*Fagus*), grains >32 μm , exine finely granular
 - B. Sorrel, dock (*Rumex*), three or four long furrows with elliptical pores, interior starch granules evident
 - C. Sage (*Artemisia*), exine thicker midway between furrows, baculae prominent
 - D. Mesquite (*Prosopis*), exine smooth, grains subtriangular, pore membranes bulging
 - E. Tree of heaven *Ailanthus*; Olive (*Olea*), exine reticulate
 - F. Marsh Elder (*Iva*), exine spiny, <22 μm
 - G. Cocklebur (*Xanthium*), exine minutely spiny, <24 μm
-

DOSE-RESPONSE CONSIDERATIONS IN POLLINOSIS

Efforts to establish dose-response matrices for pollinosis or even to define natural "threshold" exposures have confronted two long-standing obstacles: (1) the severity range for individual sensitivities appears to be quite broad and (2) symptoms often reflect concurrent natural exposure to several different allergens. Two further concerns have emerged from recent research: (3) responses to pollen allergens seem to change (generally increase) with ongoing, short-term exposure and (4) challenge probably involves aerosol fractions besides intact grains. Whether total airborne pollen allergen and specific pollen levels are linked systematically remains unclear. However, it seems safe to assume that positive dose-response relationships for whole pollen exist in at least part of the exposure range.

Airborne pollen burdens generally increase out-of-doors. Interior levels vary with ventilation rates, surface disturbance, and concentrations in free air.

Traditional avoidance maneuvers that emphasized remaining quietly in still, sealed rooms have been replaced by ad lib activity in closed interiors served by effective central or window air conditioners.^{59, 60} The value of air cleaners in allergen avoidance has been suggested⁶¹ but, overall, remains unsubstantiated.⁶² Particle penetration into structures may reflect crackage (or infiltration between structural components) as well as bulk flow through open windows and doors.⁶³ Indoors, occupant activity and air recirculation may promote continuing exposure risks well after the corresponding outdoor peaks have subsided.

Pollen exposures in nature reflect both well-mixed background levels and the emissions of discrete sources at specific times. Thus, on August 30 in southern Michigan, ragweed pollen exposure is a certainty, but it will be especially intense if the day is warm and dry, the winds light, and one is in the vicinity of grain fields (heavily infested by ragweeds that emit just after sunrise) at midmorning.^{9, 47} Determinants of prevalence for other pollen types are

less well-known, although many weedy sources clearly favor sites disturbed by construction, agriculture, livestock, highway deicers, etc. In addition, limited human activity on natural substrates has been demonstrated to (re)entrain pollens (and spores), an effect more evident in old fields than in lawn areas and least prominent in woodlots.²² However, there is little evidence that *entemophilous* pollens are dispersed similarly by modest disturbances. Moving vehicles provide another common setting for pollen exposures, which with (one or both) front windows open, have been more intense in rear seat areas.⁶⁴ Limited data imply that closure of auto windows can substantially lower interior pollen levels, and this approach becomes a practical option with available air conditioning.

Direct studies of the natural interactions between pollens and human tissues *in vivo* have not been reported; however, deposition on mucous membranes followed by allergen elution is assumed. Since the aerodynamic size (namely, the EAR) of a particle will determine its level of respiratory penetration, pollen-sized units seldom are expected to pass beyond the glottic level.⁶⁵ This theoretically based formulation has been supported by use of technetium-tagged ragweed grains inhaled by normal humans and followed by external scanning, allegedly sensitive to emissions of single grains.⁶⁶ Particles were absent essentially from tissues beyond the carina, despite large amounts of radiolabeled particles in the nose, pharynx, esophagus, and stomach. Rapid *in vitro* elution of specific allergens from ragweed pollen has been described.¹³ However, effects of upper airway and gastric mucosal fluid components on this process remain unstudied, although distinctive changes in pollens, differentially induced by atopic salivary secretions, have been suggested by a single group.⁶⁷

Since the human nostrils are directed *downward*, they may function as relatively inefficient "suction traps" for pollens in horizontal air currents; indeed, subtle racial differences in large particle capture may exist. In any case, it appears simplistic to assume that respiratory "dose" will equal minute volume $\times$ ambient concentration (per unit volume) consistently in moving air. Pollen deposition on ocular surfaces also is to be anticipated and should rise with increasing pollen size and wind speed; clarification of these effects is overdue.

Except for imprecise generalizations, symptom responses to defined pollen challenges have not been characterized either in the field or laboratory. By use of a panel of known sufferers contacted daily, it was noted that clinical grass pollenosis would occur in essentially all subjects at risk when early season pol-

len levels climbed to daily averages of 20 grains/m³.⁵⁷ Similar systematic observations have not been made for ragweed pollen, although threshold levels of from 10 to 50 grains/m³ in nature have been proposed empirically. In patients undergoing laboratory challenges out of season, sneezing and mast cell-mediator release have been observed promptly after intranasal introduction of as few as seven ragweed pollen grains as a fluid suspension.⁶⁸ By contrast, sensitive subjects, exposed *passively* to native ragweed pollen in a chamber, required *at least* 40 min at levels of 800 to 1200 grains/m³ to demonstrate measurable nasal changes when these subjects were tested extraseasonally.⁶⁹ Furthermore, with sequential challenges, progressively shorter time periods were required to produce the same response end point.* The finding accords with the "priming" effect described by Connell⁷⁰ with defatted pollens and recently confirmed by Bacon et al.⁷¹ by use of aqueous pollen extracts. Indications of a decreasing exposure threshold for sustaining clinical pollinosis also have been implicit in seasonal trends of morbidity and pollen prevalence incidentally summarized by Norman⁷² in discussions of immunotherapy. On a daily basis, Goodwin et al.⁷³ observed peaks of ragweed pollenosis severity at 6 A.M., well before the daily rise in ambient levels and, in fact, during a circadian "trough" period of pollen prevalence. Such disparities remain unexplained but suggest the potential value and intriguing opportunities that innovative studies of pollen aerobiology continue to offer.

*Solomon WR: Unpublished observations.

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