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Airborne pollen: A brief life

William R. Solomon, MD *Ann Arbor, Mich*

The transfer of pollen, whether it is transported by insects or carried by the wind, from floral anther to recipient stigma is the critical reproductive event among higher plants. In this scenario, the pollen grain functions as a fully constituted life-cycle stage, capable of growth (albeit limited) and delivery of gametes. Pollen is prepared for this role by an intricate developmental process with dual sources of structural elements and chemical constituents, including allergens. The resulting complexity relates, at least in part, to the requirements of an unforgiving recognition process at stigmatic surfaces and of active growth before the achievement of gametic union. Recently, the basic participants in pollen-stigma interactions have been defined, and they provide a striking counterpoint to human histocompatibility concerns. Pollen development offers a useful tableau in terms of which to reexamine forces affecting pollen prevalence and their interactions. Development also provides clues to the sources and significance of more minute bioaerosols now known to carry pollen allergens. (*J Allergy Clin Immunol* 2002;109:895-900.)

Key words: Pollen, tapetum, anthesis, pollen transport, pollen-stigma interactions, pollen allergens, paucimicronic, submicronic

The dispersion of replicate units in massive abundance assures the success of wind pollination as well as its all-too-familiar human health effects. This reproductive strategy is not an innovation of modern hay fever plants but has deep roots in the fossil record, antedating the evolution of targeted vectors (eg, insects).¹ Although no more than 10% of today's flowering species are wind-pollinated (ie, anemophilous), they are a highly successful lot. Aerial dispersion of spores by horsetails, mosses, ferns, and club mosses, though seldom a health concern, is a comparable but far more ancient success story.

A grasp of the function (and related chemistry) of pollen requires suspension of preconceptions, especially those based on imperfect fungi; both groups do share size-determined, passive, aerial transport, but there the similarities end. Although it is microscopic, the pollen grain functions as a discrete, self-contained (sexual)

Abbreviation used

PCP: Pollen-coat protein

stage in the plant's life cycle (viz, the male gametophyte [sperm-producing agent]) in which generation of gametes by meiosis occurs. The corresponding female gametophyte is the multinucleate ovule, localized in the floral ovary (Fig 1). Both mating types are multiply produced mitotically by most flowering plants, each functioning as an (asexual) sporophyte (literally, "spore plant") stage. As a complete entity in the plant life cycle, pollen must receive a full genetic endowment for sporophyte structure-function as well as for the grain's unique reproductive mission.

EARLY GROWTH AND DEVELOPMENT

Pollen grains develop within anther sacs from specialized mural progenitor ("pollen mother") cells, precursors of the inner protoplast (Fig 1). Maturation proceeds in a liquid medium (tapetal fluid) secreted by a lining layer of the anther sac cavity (termed *tapetum*). Pollen wall components reflect a dual origin: the pectocellulosic intine is secreted by the protoplast in which nuclear and metabolic components reside; the overlaying exine is tapetum-derived, a complex polymer of squalene units with the less-than-inspired name of *sporopollenin*.² This sturdy material confers on the exine its avidity for basic dyes (eg, fuchsin and phenosaffranin) as well as remarkable resistance to lysis at pH extremes (eg, glacial acetic acid treatment in lake sediment analysis). An active bidirectional chemical exchange in tapetal fluid seems certain, and tiny (0.30-2.0 μ m) surface modules of superfluous exine substance might be visible as "Übish bodies," or orbicules, on high magnification.* In the aggregate, such surface material, derived from secretory tapetum, might be one source of pollen allergen described in paucimicronic—and smaller—particle fractions (discussed below).³

PREPARATION FOR THE JOURNEY

The release of mature grains (anthesis) is largely pas-

From the Division of Allergy, Department of Internal Medicine, University of Michigan Medical School.

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Reprint requests: William R. Solomon, MD, Division of Allergy, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109-0380.

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*Normal exine structure appears to be assembled from similar sporopollenin modules.

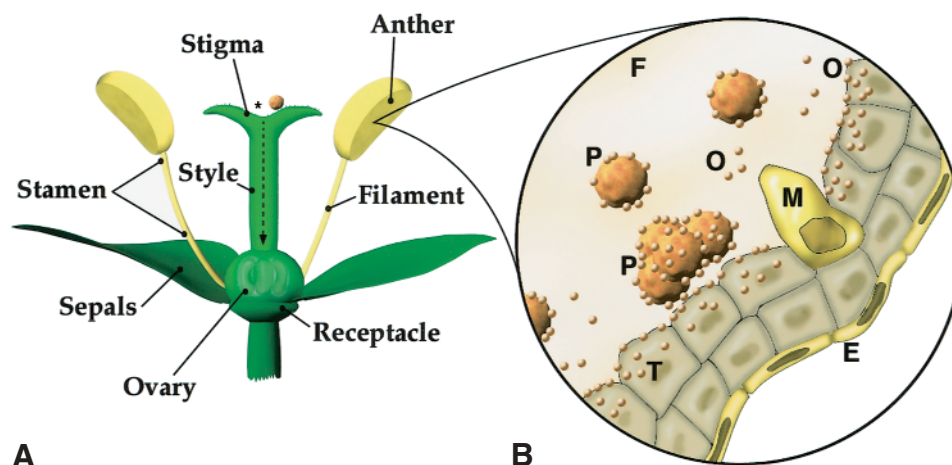


FIG 1. **A**, Schematic diagram shows a “perfect” (ie, bisexual) anemophilous flower. The *asterisk* denotes a contact point for incoming pollen; the *dashed line* indicates the route of pollen tube growth (if compatible). Note the lack of petals and nectaries. A stamen comprises a suspending filament and anther, usually containing 4 cavities (sacs). **B**, Schematic diagram shows segment of an anther sac wall and cavity. Pollen grains in tetrad and monad stages are suggested. *T*, Secretory tapetum; *M*, pollen mother (progenitor) cell; *F*, tapetal fluid; *O*, orbicules of sporopollenin; *E*, epidermis. Orbicules derive from the tapetal endoplasmic reticulum and occupy cell surface sites until released.

sive, a function of gravity, anther splitting (dehiscence) when dry, and agitation of the source plant by air currents. Acute “catapulting” (eg, in mulberries and nettles), producing visible “puffs” of pollen, is otherwise rare. Pollen grains of many species develop in tetrads and later separate (as monads), but the pollen of a few species (eg, wood rushes, broad-leaved cattail, and heath family members) is released in stable groups of 4 or in larger “polyads.” Adaptations that facilitate transport of wind-borne pollen as single grains include relatively low sculpturing of the exine and a thin layer of surface lipid (termed *pollenkitt* or *tryphine*), which minimizes cohesion. Decreasing relative humidity is an almost universal signal for particle release at maturity, promoting anther wall cracking and partial drying of discrete grains while reducing the risk of washout of released grains by rainfall. When it occurs, rain scavenging varies closely with its duration; brief showers might in fact refloat more previously deposited grains than are captured by falling drops.⁴

WANDERINGS

Although most windborne grains fall out within 100 m of their source, measurable transport over hundreds of miles can occur.^{4,5} Health effects of resulting remote pollen levels or those of smaller associated allergen-bearing units are largely speculative. However, well-documented late-summer ragweed pollinosis in Swiss subjects appears to reflect transalpine particle transport from now extensive French sources in the upper Rhone valley.⁵

Pollen prevalence (in grains per cubic meter) at a point reflects (plant) source strength and location as well as the dynamics of the intervening atmosphere. Although val-

ues fall exponentially with distance from a (point) source,⁴ most clinical offenders form extensive area sources of hyperexposure that confound simple models. In addition, weather factors interact so complexly—affecting both pollen release and transport—that any single parameter (eg, wind speed) will predict prevalence with limited confidence. Rather, determinants center on the volume of air in which entrained pollen is diluted and how particles move within that “mixing volume.” The advent of a morning overcast or temperature inversion aloft, for example, can “blunt” anthesis but also greatly limit the depth of the mixing volume, concentrating already released bioaerosols near the ground.⁴ Much (perhaps most) windborne pollen is shed diurnally, though small secondary peaks can be recorded after dusk as cooling, pollen-laden air falls toward the surface. Such movement (termed *subsidence*), incidentally, increases as wind speeds fall (unpublished data).

EYES ON THE PRIZE

Despite its human health and other incidental effects, the unitary adaptive function of pollen is to reach a receptive stigma and there, by a complex and “obstacle-strewn” pathway, to deliver 2 haploid nuclei (“sperm”) to the recipient ovary. Success promotes species survival and, if self-fertilization is prevented, broad gene flow. However, the “window of opportunity” for achieving this can be as short as 1 hour or less (as in common grasses), after which pollen function will not occur. In other species, the release of relatively dry pollen might permit true dormant periods, extending the grain’s physiologic life span.⁶

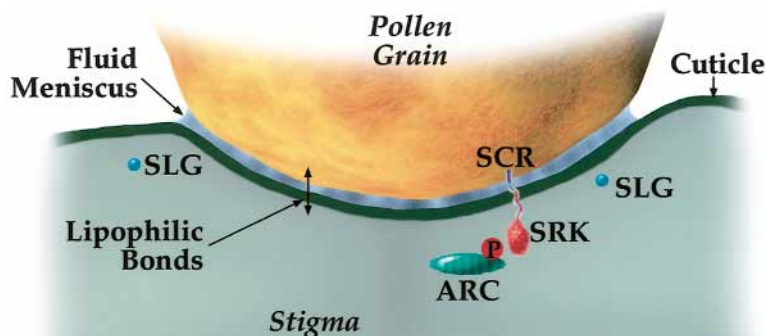


FIG 2. Participants in pollen-stigma surface interactions. Commonly, 1 or both members secrete fluid, forming a meniscus. SCR is the S-linked polymorphic pollen determinant, SRK that of the stigma. SLG is an agent modulating their interaction, perhaps as an SRK cofactor or ligand for SCR transport. Phosphorylation of ARC initiates a “rejection” response.

REJECTION AND REDEMPTION

The arrival of pollen grains on any surface appears to be entirely a chance event. However, species-specific adhesion can be fostered by unique proteins or shape complementarity between the grain and the fissures of the appropriate stigma surface. Functionally viable grains on “receptive” stigmas (see below) begin growth (ie, germinate) if hydrated by active secretion of stigmatic fluid, provided that other factors are favorable. Failure to secrete might be sufficient to abrogate further pollen activity, though high relative humidity can override this barrier,¹ excessive moisture, at times, even prompting germination within closed anther sacs.⁷ Hydrated grains can be seen to protrude a pollen “tube” within as short a period as 90 seconds after contacting a suitable stigma in some species. This outgrowth of the protoplast carries some surface material from the intine and grows rapidly through the extracellular matrix of the stigma and its supporting style (Fig 1). Germination and growth provoke florid RNA synthesis and ongoing elaboration and restructuring of the actin cytoskeleton⁶ of grain and pollen tube. Where they are present, the pollen tube will follow preformed channels; in other species, active lysis of stylar matrix accompanies its advance. In all cases, necrosis of lining cells follows passage of the pollen tube—a fact that suggests enzymatic action (inasmuch as acid hydrolases are prominently released) and/or induced apoptosis.⁸ The growth of genetically “unwelcome” grains that escape rejection at the stigma surface can still be thwarted during pollen tube development by the infliction of direct (often osmotic) lethal damage or the blocking of growth by rapid deposition of a sclerotic tissue (callose).

Ideally, the pollen tube, on reaching an ovary, penetrates its entrance (the micropyle) in a highly directed fashion; however, homing signals remain obscure. Discharge of sperm follows, leading to (1) a zygotic precursor of the seed embryo and (2) a nutritive “endosperm” to serve the seed as a metabolic fuel.

Of particular interest is the capacity of stigma and/or

style to “reject” 2 de facto groups of incompatible grains: those of other taxa and, in most plant species, those of the same individual (hermaphroditic) plant*—ie, rejection of self (!). Self-incompatibility has received the greater amount of study, the precise basis of interspecific barriers being still largely obscure, though homospecific pollen-stigma binding by lipophilic interactions is critical early.⁹ Although the details of self-incompatibility vary among studied species, a general paradigm has emerged. The presence of a surface group of pollen-coat proteins (PCPs) had been described early, derived variably (among species) from tapetum (sporophyte) and/or developing pollen grains. Interposition of specific PCPs between a grain and a stigma can reverse preexisting compatibility or promote acceptance of otherwise naturally incompatible grains. PCP specificity among conspecific individuals is determined at a highly polymorphic S-locus† that also controls critical stigmatic recognition components. The resulting pollen wall gene products, now purified and cloned, comprise a group of small, cysteine-rich, strongly charged proteins designated *SCR*.¹⁰ In their initial “conversation,” PCPs interact with S-locus-determined and related stigma cell surface components (viz, SLG, SLR, and SRK; Fig 2). In the few species examined, the most critical of these is a transmembrane receptor kinase (SRK).¹¹ When activated either directly by SCR or through intermediates (eg, SLG) via its extracellular portion, SRK rapidly phosphorylates a cytoplasmic substrate termed *ARC*, leading to rejection by steps still under investigation. ARC has been cloned, and its importance has been underscored by blocking it with antisense DNA, leading to a breakdown of otherwise predictable rejection.¹² Additional S-

*This relates, of course, only to plants that are monoecious—ie, that either bear both stamens and ovaries in separate florets on a single individual or have bisexual (ie, perfect) flowers. Dioecious species have wholly “male” and wholly “female” individuals (eg, common poplar, willow mulberry, ash species, and box elder).

†More than 1 S-locus is now recognized in certain species with well-defined hierarchies of dominance.

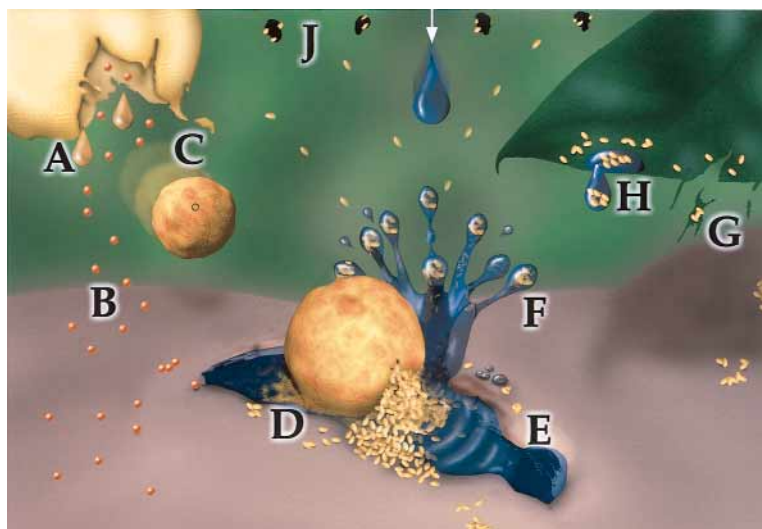


FIG 3. Potential mechanisms for dispersion of pollen allergens as microaerosols: direct liberation of tapetal fluid (A), orbicules (B), and wall fragments (C) from ruptured anthers; leaching of allergen from shed pollen (D) and discharge of eluate and starch granules (E) on moist surfaces, with liberation by wind and outwash (F) from impacting droplets; dry dispersion of vegetative fragments and plant hairs ("indument") sharing pollen allergens (G) and elution of shared allergen from wet surfaces (H); absorption of bioaerosols to other (often inorganic) particles, including products of fuel combustion (J).

locus-linked "histocompatibility" factors modulate the response to the pivotal interaction of SCR with SRK. Both of these S-linked entities are polymorphic, and where no S-component is shared between them, a "compatible" scenario proceeds; however, a shared factor leads to SRK activation and pollen grain rejection. The scale of this surveillance is submicroscopic, as shown by the divergent behavior of adjacent grains—one compatible, another not—in contact on a stigmatic surface.¹³ Although it is not clear how (or whether) recognized allergens correspond to biochemically defined, soluble pollen components, the multiplicity of the latter is intriguing. Furthermore, some of both groups are rapidly released, as befits histocompatibility factors that must determine a response in minutes or less.^{6,14} Species that are self-compatible could, by inbreeding, establish local clones that are genetically distinctive, with potentially different pollen allergen content—a possibility that calls to mind at least 1 previous report of site-to-site differences in ragweed pollen activity.¹⁵

THE LEGACY

Regardless of past disposition, shed pollen remains in the environment, subject to radiational, chemical, and microbial degradation with leaching of soluble components.¹⁶ How rapidly allergen activity is lost to these factors is a matter of pure speculation, but the refloating of effete, intact grains by wind and snow scouring has garnered passing interest.⁴ The possibility of carriage of pollen components in aerosols of a few micra (ie, paucimicronic) or smaller was generally dismissed until recently. The report of Busse et al¹⁷ (regarding ragweed), reinforced by data pertaining to the use of absolute filtra-

tion,¹⁸ reawakened interest; subsequently, small-particle carriage was reported for several major pollen allergen categories, including grass, oak, birch, mountain cedar, "sugi" cypress, and *Parietaria*.¹⁹ Most data describe microaerosols during periods of source plant anthesis,¹⁹ but reports of extraseasonal occurrence also exist.²⁰ However, it is still not clear how total allergen carriage is partitioned among aerosol size fractions²¹; diurnal patterns and many weather-related effects remain equally speculative, though rainy-day peaking of microparticulate grass pollen allergen is now an accepted event.²¹

The dramatic power of thunderstorms to worsen or incite grass pollen-induced asthma,^{21,22} coupled with the concurrent finding of defined allergen in respirable particle fractions,²¹ provides the only recognized clinical scenario implicating such pollen-related microaerosols. Grass pollen grains are released substantially hydrated and imbibe additional moisture readily. Osmotic forces can then prompt the release of many hundreds (mean, 500-700) of tiny starch granules (amyloplasts),⁷ primarily via the germinal pore. These particles are readily dispersed and carry grass allergens—especially group V allergens (eg, Lol p 5 and Phl p 5)—on their surfaces. Amyloplasts, once entrained after wind or splash dispersal, can deliver allergen to the lower airway and have been noted to increase 10-fold during convective showers; at these times,²¹ numbers of emergency room visits by distressed allergic asthmatics have risen comparably, reaching "epidemic" magnitude.^{23,24} Both the "blooms" of implicated microparticulate allergenic aerosols and the onset of worsening asthma are synchronized closely, though extended symptoms can follow brief showers.

Other bioaerosols might contribute to storm-related asthma outbreaks^{4,25}; however, a scenario matching the grass

pollen experience has not yet been reported. Neither birch nor ragweed grains exhibit osmotic lysis/discharge at pH 7, though birch will germinate on wet surfaces, the pollen tube thereafter releasing potent allergens.²⁶ To understand how allergenic microaerosols arise in additional species is a potentially accessible and rewarding research goal.

Fig 3 suggests candidate sources of paucimicronic or smaller aerosols with pollen allergen activity. For any plant species, more than 1 mechanism might operate as environmental forces and life-cycle phases change. A possible role for “Übish” bodies (orbicules) has been advocated strongly, because they are tapetum-derived and allergen-bearing in some (but not all) studies.³ (However, it is noteworthy that orbicules are absent from some clinically important *Artemisia* [sage] and *Ambrosia* [ragweed] species.³)

Plant fragments, bearing or sharing pollen allergens, are an especially controversial source of allergenic microaerosols. Effete tapetal tissue and fluid might be dispersed after comminution in nature; furthermore, vegetative tissues might carry shared structural and metabolic determinants initially described as pollen allergens.^{27,28} Symptoms during turf work by grass pollen-sensitive subjects was ascribed to this, though other exposure factors might have contributed.²⁹ Elution of allergen from any of these materials and drying of the leachate with later dispersion as fine dusts or mists are not easily excluded. Recovery of ragweed allergen in the condensate from outdoor air, rendered free of micronic particles by absolute prefiltration, is consistent with this scenario.³⁰ Furthermore, such agents as wind, animal movements, bursting bubbles, and impacting droplets of rain or dew splash could provide the needed dispersive energy. Stable associations of unlike aerosol particles in ambient air have been well described, affording additional potential³¹ vectors for allergen. Microaerosols bearing grass pollen components have been reported to bind to diesel exhaust particles, forming relatively stable complexes. The aerosols produced might foster increased deposition of allergen in smaller airways as well as serve to “steer” the immune response toward a T_H2 pathway.³²

EPILOGUE

The demographic extent of pollinosis and its cost in monetary terms and human resources are those of a major (and increasing) public health problem. Despite our natural focus on this impact, pollen, including windborne pollen, can be considered in an even larger context: that of flowering plant evolution and development. The chemical complexity of pollen, including a host of diverse allergens, reflects its dual tissue origin and the demands of the complex reproductive mission that it subserves. Whether definable allergens function in the variable and complex recognition process at stigmatic surfaces is unclear; however, the very rapid release of factors from all 3 wall layers makes them candidate participants in these “negotiations.” Pollen-stigma interactions and their sequelae provide an intriguing counterpoint to human transplantation mechanisms, here

with astonishingly rapid recognition and response. Finally, evaluation of the sources and comparative impact of pollen allergen in variously sized aerosols deserves ongoing attention. Insight might help explain additional events, as exemplified by convective showers during grass anthesis. More broadly, such information should allow increasingly realistic dose-response constructs for both the upper airway and the lower airway. Better definition of real-time exposure levels promises opportunities to evaluate host and additional environmental determinants of allergic morbidity, to validate competing prevalence indicators, and to realistically integrate exposure into evaluations of therapy and the clinical progress of individuals.

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