

EDITORIAL

Plant allergens on pauci-micronic airborne particles

The clinical significance of small particles carrying plant allergens has generated considerable debate recently. The existence of these allergenic pauci-micronic particles has been demonstrated clearly over the last decade for several plant groups including *Ambrosia* [1], *Alternaria* [2], *Quercus* [3], *Gramineae* [4,5] and *Betula* [6]. They can be detected prior to the pollen season and can persist long after pollen is no longer present in the air but they can also make a notable contribution to the total plant allergen load in the air during pollen seasons. For example, it has also been shown that during the peak *Ambrosia* season almost 50% of the total allergen can be associated with particles of less than pollen grain dimensions [2]. Allergenic particles and aerosols have been detected as small as $0.1\ \mu\text{m}$ in diameter and may originate directly from plant material or may form in combination or reaction with air pollutants. They are of respirable dimensions so they may penetrate deep into the lung and could be involved in the provocation of asthma attacks. The allergens on the particles are also important as agents in causing pollinosis. However, little is known about the prevalence of these particles in the ambient atmosphere, their geographical and temporal variation and their detailed chemical composition. Similarly their exact origins are unknown but it is likely that several mechanisms are operating and that the relative importance of the processes varies according to climate, habitat, pollution and season.

Sources and significance

One possible source is from plant material other than pollen. Fernandez-Caldas *et al.* [3] suggested that red oak pollen allergens in small particles originated from decaying leaves, male catkins and other plant material. More recently, Knox [7] noted that the orbicules (spherical particles $0.02\ \mu\text{m}$ in diameter) on the anther linings of some plant groups, including ragweed and grass, may become airborne. These bodies consist of sporopollenin, the same biopolymer as pollen. Evidence suggests that some allergens may become airborne as aerosols from sap, as for example, when lawns are cut. Several workers including, Ledford *et al.* [8], Morrow-Brown [9] and Fernandez-Caldas [10] have demonstrated that grass allergens are present in the leaves of lawn grasses. This supports the idea that allergen aerosolized from leaves contributes to the symptoms of allergic individuals as suggested by Rowe *et al.* [11].

From the work done so far, it is apparent that pauci-

micronic allergen bearing particles are much more abundant during the pollen seasons than in the rest of the growing season. Consequently, many workers accept that the most important contribution comes from pollen grains. Solomon [1] suggested that one possible source is from fragments formed by the slow, physical degradation of pollen grains in the environment but it is unlikely that this would make a substantial contribution because pollen grains are generally robust and resistant to abrasion in dry air. In the time taken for a pollen grain to break into fragments in this way, the proteins in the wall would probably be leached out by moisture. However, the surface proteins could be transferred to small particles in the air through physical contact or by leaching. This mechanism could result in combinations of allergens on pollution particles. Clearly there is a potential for interaction with air pollutants by binding with suspended particulate matter (SPM) or diesel exhaust particles (DEP). This may increase the potential of the allergens to sensitive individuals but insufficient research has been conducted to date to determine the abundance and chemical characteristics of this fraction.

Mature pollen grains of some species can rupture through osmotic shock if they are wetted when fresh. As they burst, starch granules inside the grain are released into the airflow. It is interesting to note that this phenomenon was first observed by Blackley [12] but its significance was not recognized until recently in the context of asthma episodes associated with thunderstorms [7]. For instance, several epidemics of asthma have occurred after thunderstorms in Melbourne [13,14]. Each epidemic featured during November suggesting that a seasonal factor was responsible. Air pollution was not implicated since concentrations of O_3 , NO_2 , and SO_2 were no higher than on non-epidemic days but patients admitted during the epidemic all suffered from hay fever and were allergic to *Lolium perenne* (Rye grass) pollen. The asthmatics who suffered attacks during these storms had significantly larger skin-prick reactions to this allergen than asthmatic patients who were not affected on these occasions. The immunodominant component of rye grass, *Lol pI* is located mainly on the exine, the surface covering of the grain, but a newly identified allergen, *Lol pIX* [15] is located mainly in the starch granules within the pollen grain. Knox [7] points out that a single mature rye grass pollen grain contains about 700 starch granules in the size range $0.6\text{--}2.5\ \mu\text{m}$. Four patients with thunderstorm-associated asthma had marked bronchoconstriction after experimental

inhalation of these starch granules [16]. It is suggested that the Melbourne asthma outbreaks were probably caused by allergic reactions to pauci-micronic particles released from rye grass pollen. Certainly, the mechanism of starch granule release could result in a sudden surge of respirable sized particles carrying grass allergen.

A similar major asthma epidemic occurred in south east and central UK on 24/25th June 1994 [7] after thunderstorms. This episode happened just days after the peak of the grass pollen season. Preliminary data suggest that most accident and emergency departments experienced a 10-fold increase in expected attendances for asthma treatment over the 24–36 h following the thunderstorms. Many of the patients were already known to have asthma but others were patients with hay fever experiencing their first attack of asthma. The exact geographical range and the features of the episode are currently being assessed by an interdisciplinary study group. This group plans to conduct three research projects, namely a descriptive study, a case control study and a historical study to examine relationships between previous asthma episodes, thunderstorms, aero-allergens and air pollution in the UK. The coincidence of peak concentrations of grass pollen, the thunderstorm and the asthma episode of June 1994 share features with the Melbourne outbreak. It seems likely that small particles bearing grass allergens played a role in this incident but further research is necessary before this can be clarified in relation to other possible contributing factors.

Allergenic particles and pollen counts

Some basic relationships between pollen concentrations and allergen on other particles have been determined but many aspects remain to be investigated in detail. Work has demonstrated the presence of allergens on particles of different size ranges and has achieved some quantification [18]. It has revealed some basic relationships with meteorological factors [6] especially rainfall, and a few projects have looked at variations through the year. However, little work has been done on interactions with pollution particles, on spatial and temporal variations or on detailed relationships with pollen concentrations. This aspect is important because it is well known that the onset and the severity of pollinosis symptoms do not always relate closely to the magnitude of daily pollen counts or indeed the course of pollen seasons [19]. Pollen counts provide valuable information but they do not always give a good indication of the allergen load in the atmosphere. Proteins may be leached from grains and pollen may be re-suspended after deposition especially in urban environments. In these cases the grains may not

invoke allergic reactions. Similarly, the allergen on amorphous particles, aerosols and water droplets would not be taken into consideration.

The paper by Spieksma, Nikkels and Dijkman in this issue [20] makes a notable contribution to this field by investigating the relationship between traditional grass pollen count data and the incidence of grass allergens on smaller particles. The sampling for the research extended to the times before and after the pollen season but in contrast to some other studies little airborne allergen was detected outside the pollination period. The results demonstrate that the traditional pollen count is a good indicator of the grass allergen load of the atmosphere on dry days but the sampling was not conducted on wet days because of the problems of water impaction on the filters. Unfortunately this leaves one of the most crucial aspects of the debate unresolved. More needs to be known about the allergen load on wet days when large particles such as pollen would be washed out of the atmosphere quickly, or may burst because of osmotic shock releasing starch granules. On these occasions the grass allergen content of the air may be high, especially on small particles or as aerosols, but the pollen count itself would be low.

Sampling methods

In common with most researchers working in the field of sampling atmospheric allergens, Spieksma *et al.* have used high volume samplers (HVS) mainly because of the need to collect sufficient allergen for quantification. These are usually combined with cascade impactors to fractionate particles by size classes. HVS achieves high capture efficiency for particles of a diameter of $0.3\text{ }\mu\text{m}$ and above typically reaching 96–99%. If the approach is used with RAST it can be very sensitive but there are several major disadvantages. The sampling is non-isokinetic and is usually non-directional. The air flow rates range up to $1.2\text{ m}^3/\text{min}$ (for comparison, the flow rate of the Burkard volumetric spore sampler used for the pollen count is $0.635\text{ m}^3/\text{h}$) and do not mimic human breathing. The fast flow may rupture pollen grains producing bias in the samples. Opinion on this is divided with some workers [5] finding no evidence of grain damage in their HVS systems but others [6] are convinced that this occurs. One of the main problems with these systems is the use of large filters which usually need to be sub-sampled, require long elution times, and yield samples subject to considerable dilution.

Low Volume Samplers (LVS) have been used less frequently in this context but offer several advantages. The results give a better representation of the allergen challenge to people and may be compared with the usual volumetric pollen counts more easily. This aspect is

important because the volumetric pollen counts are likely to be the standard source of information for some time. If the main relationships between allergenic particles and the pollen concentrations can be established then pollen count information could be supplemented on the basis of extrapolation between those sites which do use immunochemical techniques. LVSs are less likely to damage pollen grains and so generally produce a better representation of the size ranges of particles in the atmosphere. LVS has been used in conjunction with cascade impactors and RAST or ELISA techniques to produce quantification of allergens. Like the HVS, the LVS systems mainly use filters for particle collection. The main problem with these is the time taken for elution, which may be up to 24 h, and the large volumes of solvent needed. The bioaerosol sampler developed by the Finnish Aerobiology group [21] is a major advance in LVS particularly in that it has a bifurcated flow system to overcome some of the fractionation and slippage problems inherent in other samplers. However, it still has the disadvantage of large filters which require extensive elution times. A recent advance in this field is the development of a modified directional cyclone sampler which collects particles directly into an Eppendorf tube for immunological analysis or for particle counting [22]. This is a LVS capable of operating in all weather conditions.

There is a clear need for more basic research in sampling small particles from the atmosphere and in immunochemical techniques of quantifying aeroallergens, particularly for the development of a quick and reliable method that can be applied routinely to supplement information from pollen counting. This is especially important on wet days when the pollen count may not give a good indication of the allergen content of the air-flow. Further research on this topic could lead to methods of forecasting conditions, such as those associated with certain thunderstorms, which are potentially hazardous for asthmatics. In the longer term immunological techniques are likely to replace the traditional pollen count for allergological applications but pollen counting will still have a role for other aspects such as monitoring pollen seasons for horticulture, botany and climatic changes.

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