

Atmospheric pollen and spores in relation to allergy. I

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The multitude of seeds produced by an Agaric or a Boletus is innumerable! It is astonishing! Yet not one in ten thousand answers the purpose of propagation. Is not the air we breathe charged with them all the declining part of the year? Do we not receive them into our lungs with every breath we draw? Whence proceed the quinsies, coughs and other complaints which prevail in Autumn?

JAMES BOLTON: *An History of British Ferns*
(Huddersfield, 1790)

1. Introduction. Thommen's postulates re-stated

Of all the causes of inhalant allergy, known or suspected, none lend themselves more readily to scientific investigation than do pollen grains and fungus spores.

Plant spores in the widest sense are found in all divisions of the plant kingdom but only those formed by the flowering plants (and known as pollen grains) and the fungi contribute significantly to the aerospora; other phyla produce airborne spores, but these are numerically of minor importance.* (Bacteria, except for the Actinomycetes, are outside our present terms of reference.) In an effort to focus attention on those flowering plants which are the proper concern of allergists Thommen (1931) proposed five postulates all of which he said should be known to apply to a plant before it should be regarded as a probable cause of epidemic or widespread hay fever. These postulates have been widely accepted: they were, however, put forward when the use of aerobiological methods in the service of allergy had only just begun and when the very existence of fungus spore allergy had been barely hinted at. They are now due to be revised to meet modern requirements.

The following re-statement is proposed:

- (1) The pollen or other spore type must contain an excitant of hay fever or asthma.

* Spores of a clubmoss (*Lycopodium clavatum*) are allergenic (Salen, 1951) as are those of bracken fern (*Pteridium aquilinum*). Algal cells (especially those of *Gloeocapsa*) are often observed in the air in Great Britain (Gregory & Hamilton, 1955) and have been trapped in large numbers in dust clouds in the U.S.A. (McGovern, McElhenney & Brown, 1965b; McGovern, Haywood & McElhenney, 1965a), they are most frequent in Cardiff in spring (Adams & Hyde, unpublished).

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It would be tempting here merely to say 'must contain an antigen' but it seems better at this stage to insist on effects produced in the human subject, not only in the lower animals. If, as seems at least possible, any kind of pollen or plant spore may cause hay fever or asthma (and pine, though often cited, is no exception; Newmark & Itkin, 1967) then this postulate is unnecessary, but certainly some kinds do not evoke epidemics and it seems reasonable to postulate either a more potent excitant or a larger quantity of a general excitant in those that do.

All anemophilous and some entomophilous pollens would be included here. Anemophilous, i.e. wind-pollinated,* flowering plants possess inconspicuous, non-scented flowers lacking nectar, and release into the air large quantities of loose (non-sticky), smooth pollen grains. Entomophilous, i.e. insect-pollinated, plants produce very limited quantities of sticky, often rough, pollen grains from brightly coloured, scented flowers provided with nectar. Wodehouse has characterized as amphiphilous those plants, such as common ling, *Calluna vulgaris* and some species of maple and willow, the flowers of which are normally insect-pollinated but whose pollen is liable to become airborne in quantity. Entomophilous plants greatly outnumber in species anemophilous plants, but *per contra* these latter occupy large areas of the earth's surface and probably make up the major part of world vegetation. Many, perhaps the majority, of the fungi form spores which are adapted to wind dispersal. The various mechanisms concerned to promote a successful take-off have been described by Hirst (1959) and Ingold (1960, 1965). Thus many ascomycetous fungi discharge their spores to a height which carries them outside the laminar layer (see below); and the larger toadstools release their spores (ballistospores) from raised sporophores. In many groups spores are liberated passively: dry-spored forms including moulds such as *Botrytis* and *Cladosporium* being dispersed by wind, slime-spored forms by water, e.g. *Fusarium*, or insects. Slime spores scattered by rain splash may so become airborne.

(2) The spore must be produced in sufficiently large quantities.

Probably the most useful measure of pollen production is the number of grains released per annum from unit area occupied by plants of the species in question, e.g. for rye (*Secale cereale*) 1272 million and for false oat-grass (*Arrhenatherum elatius*) 560 million pollen grains per square metre (Pohl, 1937). Similarly about 125 million smut spores may be liberated from every square metre of a field of maize only moderately infected with corn smut, *Ustilago zeae* (Christensen, 1942); but such data are relatively few. In practice heavy production of a particular kind of spore is usually inferred from high atmospheric concentrations observed by modern methods of trapping (see sections 3 and 6 below). It has more than once been observed (e.g. Stier, 1955) that patients who have become sensitized to a particular pollen by long residence in areas subject to high atmospheric concentrations evince symptoms when they remove to areas where concentrations of the same pollen are too low to induce sensitization in the native population.

Recent work suggests that after 'large quantities' (above) should be added the words 'regularly every year and not only in certain years'.

(3) The spores must be sufficiently buoyant to become airborne.

Plant spores (like all particles of a similar size, say 1–100 μ in diameter) liberated

* Pollinate (verb, transitive): besprinkle with pollen, shed pollen upon (O.E.D.). The use of the verb in an intransitive sense, meaning to flower or to liberate pollen, is not recognized by either botanists or grammarians.

into still air sink with a constant terminal velocity which is characteristic for each species; in general larger spores fall faster than smaller ones: small smooth spheres of a given density fall with a velocity proportional to the squares of their radii. For spheres having a density of 1 g/cm^3 falling in air, Stokes's Law governing the rate of fall of such particles may be expressed in the form $V = 1.2 \times 10^{-2} r^2 \text{ cm/sec}$, where V = terminal velocity in cm/sec and r = radius (not diameter) of sphere in cm (Gregory, 1961). Few spores are perfectly smooth or perfectly spherical and their densities vary somewhat (though most are probably around 1 g/cm^3) so that observed terminal velocities seldom agree exactly with their theoretical values (e.g. Dyakowska, 1937). To judge from spores actually caught in numbers from the atmosphere the upper limit of size which normally allows of their becoming airborne is about 50μ ; the pollen grains of pine and certain other conifers are much larger but their buoyancy is enhanced by air bladders. Buoyancy (as the word is used in the present context) might perhaps best be defined as the reciprocal of the terminal velocity as determined by experiment; so that if terminal velocity is measured in cm/sec then buoyancy will be expressed by the number of seconds taken to fall 1 cm. Thus cocksfoot pollen (*Dactylis glomerata* 34μ diam., terminal velocity 2.77 cm/sec ; Durham, 1946) has a buoyancy of 0.36 sec/cm , silver birch pollen (*Betula verrucosa*, 24μ diam., 2.94 cm/sec ; Dyakowska, 1937) one of 0.34 sec/cm , short ragweed pollen (*Ambrosia artemisiaefolia*, 20.0μ , 0.89 cm/sec ; Durham, 1946) 1.12 sec/cm , the spores of the common mushroom (*Agaricus campestris*, $9.7 \times 5.8 \mu$, 0.161 cm/sec ; Buller, 1909) 6.22 sec/cm and those of loose smut of wheat (*Ustilago tritici*, 6.4μ , 0.07 cm/sec ; Stepanov, 1935) 14.3 sec/cm .

Outdoors, comparative buoyancies are relatively unimportant because in the atmosphere (apart from the laminar layer, which covers the ground and the surfaces of objects thereon and which varies in thickness from less than a millimetre in the day-time to several metres—though only exceptionally—at night; Gregory, 1961) the air is in a constant state of movement, and airborne spores are borne up and down, hither and thither by air currents and eddies having in general a much greater velocity than their terminal velocities (Schrodter, 1960). Size (and therefore buoyancy) affects the distance to which spores penetrate into and are retained in various portions of the human respiratory tract. Pollen grains (relatively few of which are smaller than 20μ aerodynamic diameter) and other large spores are largely removed by impaction in the upper respiratory tract (hence perhaps the characteristically nasal symptoms they produce—'hay fever') while a large proportion of the fungus spores inhaled (the great majority of which fall into the aerodynamic size group $5\text{--}20 \mu$ diam.) reach the respiratory portion of the system and are then deposited by sedimentation (Hatch & Gross, 1964; Austwick, 1966; Druett, 1967).

(4) The plant species concerned must be widely and abundantly distributed. The importance of this is almost self-evident.

No species, however productive or easily airborne its pollen may be, can affect many people if it occurs only here and there as isolated individual plants.

If these postulates are satisfied by a given kind of spore then it will at times be present in effective concentration over a wide area and in a certain length of time will bring about sensitization in an averagely susceptible subject. No estimate of an effective concentration in this sense seems to have been made. Once the subject has become sensitized a much lower concentration will serve to produce symptoms. Residents in central Cardiff are exposed regularly every year to grass pollen concentrations of over $1000/\text{m}^3$: of those who in consequence develop hay fever 1% may

evinced symptoms (some of them severe symptoms) when the 24 hr mean grass pollen concentration exceeds 1 grain/m³ and 10% are likely to have severe symptoms when the concentration exceeds 10/m³. Immigrants to Canada from Europe who are actually or potentially allergic patients are likely to develop hypersensitivity to ragweed pollen in 2 or 3 years; at Hamilton, Ontario, where these observations were made, ragweed pollen counts range up to 400–600 grains/cm² of exposed slide in 24 hr (Hughes, 1959).

To the four postulates stated above, which now more than cover the implications of Thommen's original postulates, should be added one other, viz: in order that any type of spore shall be regarded as the causative agent in a particular case of hay fever or asthma it must be shown that he experienced his symptoms at a time or times when the spores concerned were present in large quantities in the air.

2. The principal sources of airborne pollen and other spores

All phyla of the plant kingdom contribute to the aerosopora but above all the flowering plants and the fungi. Of recent years the interest of allergists has tended to shift from pollen to fungus spores which accordingly are treated here in greater detail.

A. *Spermatophyta*. *Seed plants*

Erdtman's textbook (1957) covers the field of descriptive palynology. For an account of hay fever plants (though with special reference to North America) Wodehouse's textbook (Wodehouse, 1945) may be referred to. The traditional grouping into trees, grasses and weeds (i.e. other herbaceous plants) (e.g. Duchaine, 1959) is one mainly of convenience. Trees as a group are taxonomically and therefore presumably antigenically heterogeneous, and so are 'weeds'. If all the known pollen allergens are listed in a natural sequence (e.g. that of Bentham and Hooker's *Genera Plantarum* 1862–93), which brings related and similar plants together, it is immediately apparent that allergenicity is closely related to taxonomy. Out of 333 families in the world flora only about thirty contain even a single species which is of primary importance either as a cause of epidemic hay fever or because its pollen is especially abundant in the atmosphere. This 'hard core' of the world's hay fever flora comprises representatives of just over one hundred genera, almost half of which belong to one or other of two families, viz: Gramineae (grasses, forty such genera) and Compositae (six).

The grasses are a remarkably natural and uniform family: over sixty genera are reported as allergens or suspects and probably all are at least potentially so in proportion to the abundance of their pollen. In temperate regions those mainly concerned are the grasses which are cultivated on a large scale for fodder and which have so become cosmopolitan. Among the Compositae 'all the members of the (wind pollinated) tribe Ambrosieae (ragweeds) cross-react almost like a single species and in turn cross-react with members of the (insect pollinated) tribe Heliantheae to which they are most closely related and from which they may well have been derived; but they do not react with the more distantly related tribe Anthemideae (which includes the (allergenic) Artemisias). The families Amaranthaceae (with two allergenic genera) and Chenopodiaceae (with five) are very closely related (they adjoin in the natural sequence) their pollen grains are identical and their antigens cross-react as if they were all in the same family' (Wodehouse, 1957, 1963). Two other alliances containing numerous important allergens or potential allergens may be mentioned. The Moraceae (mulberry family, with two genera of primary importance), Cannabaceae (hemp family, with two),

Ulmaceae (elm family, three) and Urticaceae (nettle family, three), are so closely related that the older taxonomists include them all in the same family; and finally Betulaceae (birch family, four) and Fagaceae (oak family, three) have only been separated relatively recently.

B. *Fungi*

For a general account reference may be made to Ingold (1961). The fungi differ fundamentally not only from the flowering plants but also from all the other phyla of the plant kingdom in that they all lack chlorophyll and must live either as parasites on living plants (or rarely animals) or as saprophytes on decaying organic matter; except in the yeasts (see below) the plant body is composed purely of fine hair-like tubes (hyphae) which in the microscopic forms constitute a mycelium or in the higher types are massed together to form relatively large reproductive bodies; and all, with very few exceptions, reproduce themselves by means of microscopic spores of one kind or another, the vast majority of which are adapted to wind dispersal. Certain groups are represented mainly in the aerospora by asexual spores (conidia) rather than by their characteristic 'sex' spores. Fungus spores by and large are vastly more abundant numerically (though not in total bulk) than any other kinds of spore found in the atmosphere, pollen grains included. Fungal spores in general (though with some exceptions, such as *Alternaria*) are much smaller than most pollen grains and therefore are likely to be carried deeper into the respiratory tract (Austwick, 1966); this may be the reason why asthma rather than hay fever is 'almost diagnostic' of fungus as opposed to pollen allergy (Hyde, Richards & Williams, 1956). The Eumycetes, or fungi proper (excluding the bacteria and the slime fungi), comprise four classes, all of them as wide apart from one another as are the flowering plants from the other major groups of the Plant Kingdom. The salient features of these four classes and of their principal subclasses or orders are set out below, together with the names of genera known or believed to be of importance as allergens, either singly or as part of a 'reactive pattern'. Not all supposedly allergenic genera have been reported as prominent anywhere in the aerospora, 'or have had their status as allergens confirmed by inhalation test, by passive transfer or by a corresponding history: further work is needed to establish the antigenicity of many of these groups beyond doubt' (Williams, 1965; Wittich, 1939; Morrow & Low, 1943; Feinberg, 1946; Frankland & Hay, 1951; Prince, 1951; Prince & Morrow, 1954; Maunsell, 1954; Hughes & Hamilton, 1958; Volterrani & Tosco, 1957; Van der Werff, 1958, 1959; Bruce, 1963; Evans, 1963, 1965; Prince & Morrow, 1963, 1967; Herxheimer, Hyde & Williams, 1966; Austwick, 1965, 1966; Targow, 1966; Ripe, 1966; Ross, Reid & Speirs, 1966; Medical Mycology Committee, 1967).

1. *Phycomycetes. Lower fungi.* Microscopic forms, not unlike the filamentous algae except for the absence of chlorophyll (some produce motile zoospores). 265* genera, of which about thirteen (say 5%) have been recognized in the form of spores caught from the air (or colonies derived therefrom). Many are obligate parasites causing destructive diseases (the so-called 'false' or downy mildews) on higher plants: these include *Peronospora*, spores of which are caught regularly from the air; some affect insects, e.g. *Empusa* (also a common aeromicrobe) or other animals; others are saprophytic, notably most of the *Mucorales* (pin-moulds) three genera of which are re-

* Numbers of genera quoted (by permission) from Ainsworth & Bisby (1963).

ported as inhalant allergens, viz: *Absidia*, *Mucor* (in particular *M. mucedo*) and *Rhizopus* (*R. nigricans*).

2. *Ascomycetes. Sac fungi*. Characterized by producing spores (ascospores) usually eight together, in a closed sac (the ascus, pl. asci). 1820 genera, of which about thirty (less than 2%) have been recognized in the aerospora.

Many occur also in an imperfect state (see Fungi Imperfecti below) producing asexual spores.

Discomycetes (cup-fungi). Asci forming a distinct layer and bursting (often simultaneously: 'puffing') to shoot-out ascospores as far as 50 cm up into the air (Walker & Harvey, 1966; Ingold, 1967). Mostly saprophytes, but some parasites.

Pyrenomycetes (flask-fungi). Asci contained in flask-shaped perithecia, and typically emerging singly through the neck of the flask. Include parasites and saprophytes. Reported as allergens: *Chaetomium* (*C. olivaceum*), *Claviceps* (*C. purpurea*) and *Xylaria* (*X. polymorpha*).

Erysiphales ('true' or powdery mildews). Asci in enclosed cleistothecia, bursting when discharged therefrom; 'repeating spores' (conidia) formed and released in large numbers in summer; mycelium external to host plant. Parasitic on higher plants, e.g. cereals. Reported as allergens: *Microsphaera* (*M. alni*), *Erysiphe* (*E. graminis* f. *tritici* and f. *hordei*, wheat and barley mildews respectively).

Eurotiales. Cleistothecia known in certain species only; conidia in distinctive heads. Saprophytic moulds, here treated for convenience as Fungi Imperfecti (see below). Reported as an allergen: *Eurotium* (*E. herbariorum* = *Aspergillus glaucus*).

Saccharomycetaceae ('true' yeast). Essentially unicellular, living on surfaces of fruits, in aqueous sugary solutions reproducing indefinitely by budding, but under certain conditions forming asci. Reported as an allergen: *Saccharomyces* (*S. cerevisiae*, brewers' 'top' yeast).

3. *Basidiomycetes*. Characterized by possession of basidia, microscopic spore-bearing organs from each of which (usually) four minute basidiospores are budded off externally. 550 genera, of which about twenty-five (say 4%) have been recognized in the aerospora. Except for the rusts (114 genera) and smuts (forty-two genera) (q.v.) the great majority are saprophytes growing on wood or on the ground.

Hymenomycetes. The plant is an open fruit-body with an exposed spore-bearing layer (the hymenium), each basidium in which when mature shoots off its four basidiospores (ballistospores) in rapid succession, and which is so formed that the spores when so ejected do not impinge on other surfaces of the fruit body. Wherever the aerospora has been assessed at all completely, basidiospores derived from Hymenomycetes have been found to be very abundant and therefore probable allergens (cf. Gregory & Hirst, 1952).

Agaricaceae (Toadstools). Hymenium on vertical plates called 'gills'. Reported as allergens: *Agaricus* (*A. bisporus* = *Psalliota hortensis*, cultivated mushroom), *Armillaria* (*A. mellea*, honey fungus), *Coprinus* (*C. micaeaeus*, an 'inky-cap'), (*H. fasciculare*, 'sulphur tuft').

Polyporaceae (Polypores). Hymenium lining vertical tubes; fruit body frequently bracket-shaped, woody or membranous. Reported as allergens: *Serpula* (*S. lacrimans* syn. *Merulius*, dry-rot fungus), *Ganoderma* (*G. applanatum*), *Polyporus* (*P. squamosus*, 'dryad's saddle').

Boletaceae. Hymenium lining tubes; fruit body fleshy, circular.

Hydnaceae. Hymenium on projections of the fruit-body called 'teeth'.

Gasteromycetes: 'plant' a closed fruit body within which the basidiospores accumulate en masse, being later dispersed passively by wind and rain. Include Lycoperdaceae (puff balls) and Podaxales.

Uredinales (*Rust fungi*). Obligate parasites often epidemic on cereal crops. Repeating spores (conidia called uredospores) produced in vast numbers. Reported as allergens: *Puccinia*: (*P. graminis*, f. *tritici* and f. *hordei*, black rust of wheat and barley respectively; *P. glumarum*, f. *tritici* and f. *hordei*, yellow rust of wheat and barley respectively; and *P. hordei*, barley rust).

Ustilaginales (*smut fungi*). Almost all obligate parasites affecting especially grasses and sedges. Spores in some genera produced in vast numbers in 'spore masses'. Reported as allergens: *Sphacelotheca* (*S. cruenta*, and *S. sorghi*, Johnson-grass smut), *Tilletia* (*T. caries* and *T. foetida*, bunts or stinking smuts of wheat), *Urocystis*, *Ustilago* (especially cereal smuts, viz: *U. avenae* and *U. kollerii* [on oats], *U. tritici* [wheat], *U. hordei* [barley] and *U. maydis* [maize]).

4. *Fungi Imperfecti* (Smith, 1954; Barnett, 1960; Gilman, 1957). So-called because life histories are imperfectly known; most are probably Ascomycetes which, though they form conidia, have not been observed to form ascospores; their classification is one of convenience rather than natural relationships. 1425 genera, of which over 100 (or say 8%) have been recognized in the aerospora. For a comprehensive review of the literature on atmospheric moulds see Van der Werff (1958).

Cryptococcaceae. Yeasts, reproducing mostly by budding and forming neither ascospores nor ballistospores. Reported as allergens: *Candida*, *Pityrosporum* (*P. ovale*, human dandruff), *Rhodotorula* (*R. glutinus*).

Sporobolomycetaceae. *Mirror yeasts*. Live on leaf surfaces, reproducing by budding but also forming 'ballistospores'. Reported as allergens; *Sporobolomyces* (which may include some *Rhodotorula* spp. (see below) (Last & Deighton, 1965); *Tilletiopsis*.

Sphaeropsidales. Conidia produced in flask-shaped 'pycnidia'. Include parasites and saprophytes. Reported as an allergen: *Phoma* (*P. conidiogena*).

Hyphomycetales (incl. *Moniliales*). Conidia produced on more or less branched hyphae (conidiophores). About 650 genera, nearly 100 of which have been recognized in the aerospora. Almost all are saprophytes with a distinct mycelium or spore mass (the moulds properly so called) but some are parasites. Probably because the fungus aerospora has heretofore been studied largely by culture methods, to which the moulds in general are amenable, members of the *Hyphomycetales* are mentioned as allergens much more frequently than are those of any other group of fungi: twenty-four out of the fifty-odd fungus genera so far reported as allergens belong here.

Xerosporae: with dry spores, normally wind dispersed (Mason, 1937). Reported as allergens: *Alternaria* (*A. tenuis*), *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*), *Botrytis* (*B. cinerea*), *Cladosporium* (*C. fulvum*—tomato leaf mould, *C. herbarum* and other species) (De Vries, 1952; Hendy, 1964; Harvey, 1967). *Epicoccum* (*E. nigrum*, *E. purpurescens*), *Helminthosporium*, *Hormodendrum*, *Monilia* (*M. sitophila*), *Paecilomyces*, *Penicillium* (*P. crysogenum*, *P. digitatum*, *P. notatum*), *Sporotrichum*, *Stemphylium* (*S. botryosum*), *Trichothecium* (*T. domesticum*, *T. roseum*).

Gloiosporae: with slimy spores, normally dispersed by water. Reported as allergens: *Cephalosporium*, *Fusarium*, *Gliocladium*, *Aureobasidium*, *Trichoderma* (*T. lignorum*).

Indeterminatae (cf. Wakefield & Bisby, 1941). Reported as allergens: *Conios-*

porium, *Dicoccum* (*D. asperum*), *Epidermophyton*, *Nigrospora*, *Torula* and *Trichophyton*.

Actinomycetes ('ray fungi'). Mould-like forms of bacteria having true branching and in some a well-marked mycelium with hyphae about $1\ \mu$ in diameter. Recorded as allergens: *Micropolyspora*, *Thermomonospora*, *Thermoactinomyces*.

Note on materials used for making extracts

Evidence was adduced many years ago (Pratt & Crossman, 1942; Feinberg, 1946) that the characteristic antigens of certain moulds resided in their spores. However, the non-sporulating mycelium of at least one mould (*Alternaria*) has been found to evoke positive skin reactions (Prince, Kaplan & Morrow, 1956).

Prince and his co-authors working with six moulds grown in synthetic media concluded that the culture broth affords the highest source of allergen content (Prince, 1961). Bonilla-Soto (1969) similarly concluded that best recoveries (of antigens) are obtained by extracting from the whole growth and medium together. Furthermore, the fungus *Aureobasidium* (*Pullularia*), which has been reported as an allergen, is trapped on atmospheric slides and counted in the form of clumps of dark brown thick-walled cells (not as conidia) (Hamilton, 1959): and it has been shown that mycelial fragments of other fungi are abundant in the lower atmosphere (Hamilton, 1959a; Pady & Gregory, 1963): do they too act as allergens? Evidently the whole subject requires further investigation, and not with reference to the moulds only: if it could be shown that antigens identical with those present in the spores of the larger fungi (and in particular those of the Ascomycetes) were also contained in other parts of their fruit bodies, an important advance would have been made.

Meantime the parts of plants to which patients are mainly exposed to inhaling are the spores, and extracts are normally made either from mature spores or from cultures containing such spores, which it may for the present be supposed are the main seat of the exciting substance(s) if any. With pollen this is no difficulty, though great care is necessary to avoid impurities (Ellis & Dahl, 1947; Voorhorst, 1965); but among the fungi the situation varies. The spores of rusts, smuts and puff-balls are all easily separable; hymenomycetous basidiospores are released, and must be collected, as soon as they are formed: the fruit body does not at any given moment contain quantities of spores ready to be separated; relatively few of the Ascomycetes (except the Erysiphaceae) can be made to yield spores in quantity; many culturable moulds produce conidia which in some instances, e.g. *Cladosporium*, can be sucked off the cultures; some other moulds, however, including the yeasts, merely vegetate in ordinary culture: a growth of *Sporobolomyces* in a Roux bottle probably contains only a relatively small proportion of the ballistospores which at certain times teem in the air. It would perhaps not be surprising if extracts made from the complete mould felt or pellicle did not always evoke the expected response.

3. Pollen and spore surveys. Technical methods

For the most part the aerospora of a given region is derived from local sources. A rapid botanical survey would therefore suggest which kinds of plants make up the local hay fever flora, and further what kinds of fungi might contribute to the aerospora, but such a purely qualitative assessment would obviously be insufficient: the physician needs to know far more: he wants to know the kinds of spore actually present in the air in significant numbers at any time of the year, and if possible their actual concentra-

tions, in spores per unit volume of air inhaled, from day to day and if possible from hour to hour. In order to obtain the desired information pollen grains and spores must be trapped from the atmosphere, identified and counted.

The choice of situation for a regional spore observatory is of some importance. Both theory and commonsense would suggest, and actual observation has confirmed, that it is advisable to avoid the vicinity of a large source of spores of any kind, as being certain to give locally high, and therefore atypical, concentrations of the spore concerned. Thus, during a 14 day summer run with a spore trap at 2 m a.g.l. on a salt marsh in southern England (Gregory & Sreeramulu, 1958) concentrations of *Sporobolomyces ballistosporus*, doubtless derived from the surrounding vegetation, rose at times to the unusually high level of 1 million per cubic metre. Again, observations made simultaneously inside growing crops and a few feet above them showed a very rapid falling off at only a short vertical distance in the atmospheric concentration of the spores of fungi parasitic on the crops (Last, 1955a, b, c). Somewhat similar results were obtained when a comparative survey of the summer aerospora at 0.5 m a.g.l. was made at two stations in rural Berkshire, the one in a little valley and near important sources of both pollen grains and fungus spores, the other only 450 m away on a breezy hillock and surrounded by closely cut grass: over the whole season there were 2.6 times as many fungus spores and 3.5 times as pollen grains at the first site as at the second (Lacey, 1962). It would seem that in order to obtain a reasonably typical sample of the aerospora of the region concerned the observatory should ideally be placed at a height well up into the turbulent air layer (say 50 ft a.g.l.) and well away from trees. Ground-level concentrations due to local sources are always liable to differ from roof-level figures general to the area concerned (Davies, 1964a, b, c); but on the other hand in a town area of tall buildings and narrow streets, concentrations at roof level (due it may be supposed to spores borne from a distance) may be much higher than those near the ground (and due mainly to fall-out from winds passing above). (Davies, Denny & Newton, 1963). More recently the relation between the concentration produced by a local source has been studied in relation to background concentration due to multiple distant sources (Raynor, Ogden & Hayes, 1966). There is evidently room for further investigation of the problem.

The site having been chosen, the next desideratum is a suitable spore trap: this should trap efficiently, irrespective of the wind velocity, all spores within the range 3–50 μ and should be capable of yielding a continuous volumetric record of the atmospheric spore content; it should also be simply constructed, easily maintained and capable of long-term continuous operation; and finally the ideal trap would be independent of a power supply. Air sampling techniques are described by P. H. Gregory, and discussed in the light of his own fundamental researches, in his book *The Microbiology of the Atmosphere* (1961). Methods of sampling have also been summarized by Wolf *et al.* (1959). The germ of more than one kind of spore trap is to be found in Blackley's *Researches* (1873), which is still worth reading today.

Only a few of the many types of apparatus can even be mentioned here: they are grouped on lines of convenience rather than aerodynamic principle.

(A) 'Gravity' traps. Spores are allowed to settle on the trapping surface (supposedly) under gravity.

(i) The horizontal so-called gravity slide, which has been so widely used in both Europe and America (e.g. Durham, 1946; Hyde, 1950a) is simple and admirably suited to continuous operation, but deposition on the slide is greatly influenced not

only by gravity but also by atmospheric turbulence; its efficiency as a trap varies widely according to the thickness of the slide and its orientation in relation to the wind, the wind velocity obtaining from time to time, and the size of the spore concerned, large particles being greatly favoured. All in all the horizontal slide inevitably gives a distorted picture of the aerospora, but in practice it can be improved in two ways; first, to ensure that the wind always blows in the same direction relative to the long axis of the slide, it can be mounted on a rotating stand provided with a wind vane; second, to reduce 'edge shadow' the slide can be made of thin glass and with its leading edge bevelled (Gregory & Stedman, 1953). The performance of such an improved gravity slide trap should be tested against that of a suction trap of known efficiency; and the results should always be stated in the first instance in terms of the numbers of spores trapped on unit area.

(ii) The 'gravity' Petri dish, poured with a suitable, slightly acid medium (e.g. Sabouraud's) has been widely used for assessing culturable moulds: in practice it can be exposed for short periods only, so that catches are unlikely to be truly representative of 24 hr mean values. As ordinarily operated the rim reduces the efficiency of the trap greatly below the expected values, especially at moderate wind speeds, an effect which can be largely eliminated by sinking the dish to a suitable depth below the level of its surrounding platform (Gregory & Stedman, 1953).

(B) *Impaction traps*. Particles are impacted by air currents on to the trapping surface. The simplest of these traps (iii-v below) depend on wind movement against a trapping surface which remains stationary in relation to the wind; in a small second group (vi-vii) the surface is made to move more or less rapidly relative to the wind velocity; in the remainder (vii-xi) air is drawn by suction on to the surface.

(iii) Vertical or inclined slides. Several workers (e.g. Hyde & Williams, 1945a, b; Kennedy, 1953) have made use of a vertical slide protected by a shelter and mounted on a pivot provided with an air-vane. The numbers of spores caught per hour by this type of trap from a spore cloud of a given concentration are very small indeed when the wind speed is low, but increase out of all proportion to wind speed when this rises. Other workers have used slides inclined to the vertical at various angles (e.g. Swinney, Swinney & Hicks, 1968).

(iv) Rempe (1937) used a vertical sticky cylinder (14 mm diam.): pollen grains were trapped all round the surface according to the wind direction; on the other hand:

(v) In the flag sampler (Harrington, Gill & Warr, 1959) one end and the same side of the cylindrical surface (that of a 2 in. pin mounted vertically in a $\frac{1}{16}$ in. glass-tube bearing) is kept turned toward the wind by means of a paper flag gummed to the pin.

(vi) Durham's airwhip (Durham, 1947) is a 36 in. aluminium rod which is made to swing a sticky slide in a circle.

(vii) The rotorod sampler (Perkins, 1957) depends in principle upon the high efficiency with which small airborne particles are deposited on narrow cylinders oriented at right angles to high velocity winds (Gregory, 1951). As used at Rothamsted, a small constant-speed motor whirls two thin sticky-coated vertical brass rods ($\frac{1}{16}$ in. square cross section, each 6 cm long, 8 cm apart) about its vertical axis at a constant high speed (Gregory, 1962). The sampling efficiency of the original model is said to be unaffected by wind speeds up to 30 miles/hr. Continuous sampling for 24 hr may produce a dense deposit impossible to count; but a rotorod can be employed with advantage for 'spot testing' (Gregory & Lacey, 1964; Davies, 1964a). An intermittent type has been described which enables the rotorod to rotate only so many minutes of

each hour out of the 24 (Raynor *et al.*, 1961) and also a sequential type which brings twelve rotorods in succession into operation each for an hour (Perkins, 1957). The flyshield roto-bar (Solomon, Stohrer & Gilliam, 1968) is a simplified impaction sampler with motion-regulated shielding. The roto-slide sampler depends on the same principle as the rotorod (Ogden & Raynor, 1967).

(viii) Filters (cf. Blackley, 1873, p. 118). (a) Erdtman drew air by means of a vacuum cleaner at an observed rate through a paper filter; the filter exposed each day was afterwards dissolved to liberate the pollen grains which had been trapped by it and which resisted the reagents employed. The apparatus was used by its inventor to trap airborne pollen at a low level over the Atlantic (Erdtman, 1938) and later by Andrup (1945) for a pollen census at Oslo. A similar method was followed by Williams (1964). (b) Richards (1955) described an alginate filter, to be used with a suction pump. (c) The molecular filter membrane is a synthetic paper-thin filter, 1 in. diam., containing five by ten pores per square centimetre; air passing through it at the rate of 9 l/min deposits all airborne particles greater than 0.2μ ; the filter is afterwards removed, mounted and scanned (Gurney & Cryst, 1955). (d) In the Andersen sampler, air is drawn through a series of six multiperforate disks of progressively decreasing pore size (0.0465 down to 0.0100 in. diam.): airborne particles are thereby automatically separated into six aerodynamic sizes and collected on plates placed on below each of the six disks (Andersen, 1958). It is useful *inter alia* for sampling volumetrically organisms requiring cultural determination. (e) The respirator spore trap (Maunsell, 1962a, b) incorporates a tightly-fitting face mask, fitted with in and out valves, and a filter holder lined with acetate fibre wadding (Febroceta); air is breathed in through the filter by the operator (whose breathing rate and tidal volume must be known) and the wadding is later dissolved in acetone, thus allowing the spores caught to be counted by means of a haemocytometer.

(ix) Slit samplers all work on the principle of allowing air to be drawn in rapidly through a narrow orifice and to discharge its load of spores by impact on to a sticky surface placed immediately inside behind the slit; by observing the rate of suction and counting the spores caught it is then possible to calculate the average concentration of the spore cloud concerned. (a) The original slit sampler (Bourdillon, Lidwell & Thomas, 1941) was designed for indoor studies with bacteria. (b) The Pady-Rittis sampler, a modification for outdoor aerobiological survey (Pady, 1954), has a flow of $1.2\text{--}2.1 \text{ ft}^3/\text{min}$; it has been operated for 30 min or an hour daily (Kramer *et al.*, 1959). (c) In the Marx Impinger (Marx, Spiegelman & Blumstein, 1959; Blumstein & Spiegelman, 1959) a clock-faced sticky disk, centred at a point on the long axis of the slit, rotates beneath it so that a zone-like trace is formed in the course of the 24 hr; it is operated at a flow of 10 l/min. (d) The cascade impactor (May, 1945; Lippman, 1961) utilizes the principle of isokinetic sampling, viz: that the numbers of airborne particles can be estimated ideally by means of a sampling tube provided that its orifice faces upstream with suction applied at such a rate that the air velocities in the tube and the main stream are equal. A version with moving slides is also available (May, 1956) and consists in effect of four slit samplers in series, with jets of decreasing width and therefore collecting progressively finer samples. (e) The Hirst Automatic Volumetric Spore Trap samples with equal efficiency all airborne spores from 3 to 100μ in diameter, though in practice sampling is only approximately isokinetic for ordinary wind speeds, but the counts obtained are conservative. Operation is continuous at a sampling rate of 10 l/min, the sticky surface—a 3×1 slide which passes in front of the

slit at a uniform rate—being renewed daily. Most pollen and spore surveys in Great Britain since 1954 have been carried out with the Hirst trap. Its disadvantages are that it is not portable, it requires a source of power, it demands skilled maintenance when used continuously over long periods and the catch cannot easily be cultured. A later type employs a sticky tape and runs for 7 days without attention (Hirst, 1965; cf. (g) below). Gregory's portable volumetric spore-trap is of impactor type and hand operated; it is useful for short period and 'spot' testing (Gregory, 1954). (f) Pady's continuous spore sampler (Pady, 1959) resembles a Hirst trap except that the slide is elevated a short distance once every hour and so receives twenty-four short 1 hr bands daily; at 11.3 l/min it is claimed to have an efficiency of 50%. Kramer & Pady (1968) have described another intermittent slit sampler giving 2 mm bands hourly, 2-hourly or 4-hourly during the day. (g) In Stenborg and Hall's volumetric sampler (Hall *et al.*, 1963), spores drawn in through a slit are impacted on to a moving sticky tape (cf. Hirst, 1965, above); the apparatus has been used to determine diurnal variation in ragweed pollen (Smith & Rooks, 1954). A somewhat similar trap has been described by Nair & Kaul (1963). (g) Schenck's trap is essentially a home-made version of Hirst's AVST run on a car battery (Schenck, 1964).

(x) The Wells air centrifuge (Committee on Apparatus in Aerobiology, 1941) consists essentially of a glass cylinder into which 30–50 litres of air are drawn per minute and which rotates at 3500–4000 rev/min so that suspended particles are impacted by centrifugal force on to the wall of the cylinder. For pollen trapping the cylinder is lined with an ordinary adhesive, for moulds or bacteria spores with nutrient agar. The difficulty of scanning particles on a curved surface is obvious. The apparatus was used for a mould spore survey in Spain (Jimenez-Diaz *et al.*, 1960).

(xi) In Panzer, Tullis and van Arsdell's 24 hr slide spore collector, twenty-four slides are mounted on the outside of a rotating drum, so that each in turn receives the spore load drawn in through the (rounded) orifice in an otherwise closed cover. It has been used for comparing day and night air loads (Panzer, Tullis & van Arsdell, 1957). Powell & Morf (1967) described an improved form of this apparatus. Voisey & Bassett's continuous pollen sampler (Voisey & Bassett, 1961) is very similar.

(C) *Other methods*

(xii) Bubbling devices. A known volume of air is bubbled through water and the grains thereby left in suspension in an aliquot portion of the water are counted. A simple form of such a device was used to sample airborne pollen at Columbus, Ohio (Gilbert, 1950).

(xiii) The General Electric electrostatic air sampler makes use of the movement of charged particles in an electrostatic field (Luckiesh, Taylor & Holladay, 1946): it was used to sample the aerospora over Montreal (Kelly & Pady, 1954). Ogden, Hayes & Raynor (1968) described an 'automatic grab sampler' which traps a known volume of air into an evacuated container.

4. Atmospheric pollen and spores in Great Britain

(i) *The vegetation as a source of pollen and spores*

The vegetation of Great Britain, apart from the mountain tops and parts of the maritime fringe, is almost entirely determined by man and his animals. Four-fifths of the total land area of the country is given over to agricultural uses, rather more than one-

twentieth to forestry, and the rest to urban uses including roads. The agricultural area in England and Wales is predominantly 'good' grassland and arable in the proportion 4:3, with a relatively low area of so-called 'rough grazings' (mountain and other 'waste' land): in Scotland rough grazings predominate and the areas of good grassland and arable (except in the lowlands) are relatively small. The area of 'good' grassland includes both meadows (which are grown for hay and are highly floriferous and therefore highly productive of grass pollen and to a lesser degree of plantain and *Rumex* pollen) and 'good' grazings (which are pastured and therefore less floriferous but doubtless still important sources of the same kinds of pollen). The principal kinds of grasses in the lowland areas (both meadows and pastures), all of which produce a large quantity of pollen from each head of flowers, are sweet vernal (*Anthoxanthum odoratum*), meadow foxtail (*Alopecurus pratensis*), timothy (*Phleum pratense*), bents (*Agrostis tenuis* and *A. stolonifera*), Yorkshire fog (*Holcus lanatus*), false oatgrass (*Arrhenatherum elatius*), cocksfoot (*Dactylis glomerata*), meadow-grasses (smooth, *Poa pratensis* and rough, *P. trivialis*), tall fescue (*Festuca elatior*), meadow fescue (*F. pratensis*) and perennial rye-grass (*Lolium perenne*). The grasses which dominate the great area of upland and mountain in Wales and Scotland do not form dense stands of flower heads and, though their acreage is large, their importance for hay fever is probably relatively small. The grasslands are an important source also of mould spores derived from fungi growing either (like *Cladosporium*) as weak parasites on grasses and other higher plants (Webster, 1957; Hudson & Webster, 1958) or as saprophytes on dead and decaying vegetation: large numbers of *Cladosporium* conidia have been observed in the air as the result of mowing grass (Sreeramulu, 1958). Grasslands are also the home of very many of the saprophytic Basidiomycetes and Ascomycetes and doubtless they are an important source also of the mirror yeasts (Sporobolomycetaceae) which, however, as inhabitants of the phyllosphere, grow on the leaves of many kinds of plants other than grasses (Last & Deighton, 1965). Arable fields, while doubtless contributing their quota of the spores of saprophytic moulds (including perhaps most of the *Alternaria*; Hyde & Williams, 1946b), supply a large proportion of the pollen grains of certain late summer weeds (including those of *Chenopodiaceae*) and also the spores not only of plant pathogens such as powdery mildews (e.g. *Erysiphe*), rusts (e.g. *Puccinia* uredospores), and smuts (*Ustilago*), 'Phoma' and *Botrytis*, but also of non-parasitic fungal epiphytes.

More than half of the forest area in Britain today is made up of coniferous plantations; but pollen from conifers—apart possibly from that of the cypresses (*Chamaecyparis* spp.) which are occasionally planted on a commercial scale—seems to be of little importance. The principal broadleaved forest-tree species in the order of the area they respectively occupy in woodlands are oak (*Quercus robur* and *Q. petraea*), ash (*Fraxinus excelsior*), birch (*Betula verrucosa* and *B. pubescens*), beech (*Fagus sylvatica*), sycamore (*Acer pseudopatanus*), elm (*Ulmus* spp.) and Spanish chestnut (*Castanea sativa*), the pollens of all of which have been regarded as antigenic. The leaf surfaces of broadleaved trees are inhabited by *Sporobolomyces*, *Pullularia* and other fungi, and woodland floors are the homes not only of many of the larger fungi (mostly Basidiomycetes) but also of many microscopic and submicroscopic forms. Broad-leaved trees are also widely scattered in the form of small woods and hedgerow specimens throughout England. Hedgerows and roadside verges everywhere except in towns are an important and ubiquitous source of grass and weed pollens (notably of *Urtica*) and of the kinds of fungus spores associated with grassland. Urbanized areas

are not necessarily sterile: much pollen is released by trees grown in towns for ornament, including lime (*Tillia* spp.), horse chestnut (*Aesculus hippocastanum*), and (especially in London) plane (*Platanus hybrida*). Finally, towns as such are specially productive of *Penicillium* spores (Richards, 1954b; Hamilton, 1955).

(ii) *The aerospora*

In what follows, an account is given first of the aerospora at Cardiff, since the records of the pollen and spore census there are the longest and fullest available to the writer (Adams, 1964; Harvey, 1967; Hyde, 1949, 1950a, b, c, 1952a, b, c, 1955, 1956, 1959a, b, 1960a, b, c, 1963; Hyde & Adams, 1958, 1960a, b; Hyde *et al.*, 1956; Hyde & Williams,

Table 1. Fungus spores and pollen grains at Cardiff: average daily mean concentrations in spores per cubic metre, 1 January 1958 to 31 December 1961 (fungus spores as in Adams, 1964)

Fungi:			Spermaphyta:	
1. Phycometes		2.0	1. Gramineae (grasses)	16.86
2. Ascomycetes			2. Trees	
Erysiphe	4.2		(a) <i>Ulmus</i> (elm)	5.05
Ascospores (coloured)	76.1		(b) <i>Betula</i> (birch)	2.53
Ascospores (hyaline)	468.0	548.3	(c) <i>Fraxinus</i> (ash)	2.06
3. Basidiomycetes			(d) <i>Quercus</i> (oak)	1.58
(a) Agaricales (coloured)	224.4		(e) <i>Taxus</i> (yew)	1.11
Agaricales (hyaline)	539.4		(f) <i>Alnus</i> (alder)	0.95
(b) Lycoperdales (puff balls)	0.5		(g) <i>Corylus</i> (hazel)	0.63
(c) Sporobolomyceteae			(h) <i>Castanea</i> (chestnut)	0.62
Sporobolomyces	271.2		(i) <i>Pinus</i> (pine)	0.61
Tilletiopsis	18.5		(j) Others	1.14
(d) Uredinales (rusts)	6.6		Total trees	16.28
(e) Ustilaginales (smuts)	85.0	1145.6	3. Miscellaneous herbs (weeds)	
4. Fungi Imperfecti			(a) <i>Urtica</i> (nettle)	8.41
(a) <i>Alternaria</i>	8.0		(b) <i>Plantago</i> (plantain)	0.92
(b) <i>Botrytis</i>	29.2		(c) <i>Rumex</i> (sorrel and dock)	0.70
(c) <i>Cladosporium</i>	1204.2		(d) <i>Artemisia</i> (mugwort)	0.25
(d) <i>Epicoccum</i>	15.4		(e) Compositae (excl. <i>Artemisia</i>)	0.12
(e) Aspergillaceae	49.8		(f) <i>Chenopodiaceae</i>	0.09
(f) <i>Polythrincium</i>	7.6		(g) Others	0.12
(g) <i>Pullularia</i>	27.5		Total weeds	10.61
(h) <i>Torula</i>	8.7		4. Other pollens (determinate)	0.77
(i) Others	11.0	1361.4	Other pollens (unknown)	3.52
5. Undetermined		24.7	Total pollen grains	48.04
Total fungus spores		3082.0		

1943, 1944, 1946b, 1949, 1953, 1959, 1961; Richards, 1953a, b, c, 1954a, 1956). Pollen and spores have been trapped at Cardiff without a break from late in 1941, first by means of gravity slides and petri plates and from 1954 onwards also with a Hirst trap (Hirst, 1952). The spore observatory (47 ft a.g.l. at 50 ft O.D. 2 miles from the Bristol Channel) is in an area which, apart from open and parklike grounds with many trees especially elms (*Ulmus*) immediately to the West, is closely built up for about 1½ miles around, suburbanized for a similar distance beyond, and beyond that again surrounded to landward with agricultural land from which the aerospora of central

Cardiff is doubtless in part derived. The general situation could be matched in almost any county in Southern Britain.

(a) *Average composition of the aerospora* (Table 1)

The average mean 24 hr numerical concentration of fungus spores over the 4 years 1958–61 was $3082.0/\text{m}^3$, that of pollen grains $48.04/\text{m}^3$, i.e. fungus spores on average outnumbered pollen grains by over 60 to 1. The mould *Cladosporium* (the principal type of fungus spore) (with $1204.2/\text{m}^3$) outnumbered grass pollen (the principal pollen type) (with $16.9/\text{m}^3$) by 70 to 1. 'Basidiospores' as a group (764), ascospores (544), *Sporobolomyces* (271), Smuts (*Ustilago*, etc.) (85), *Aspergillaceae* (50) and four individual generic types of fungus spore (*Botrytis* 29, *Pullularia* 27, *Ganoderma* 19 and *Tilletiopsis* 18), each exceeded grass pollen in numbers. No other single type either of pollen or of fungus spore exceeded $16/\text{m}^3$. Average mean 24 hr numerical concentrations of fungal spores during a further 5 year period 1962–66 did not in general differ very widely from those for 1958–61: they included fungus spores $3208.2/\text{m}^3$, basidiospores $933.5/\text{m}^3$, ascospores $814.0/\text{m}^3$, *Sporobolomyces* $181.4/\text{m}^3$, *Aspergillaceae* $139.0/\text{m}^3$, *Botrytis* $25.6/\text{m}^3$, *Pullularia* $24.3/\text{m}^3$, *Tilletiopsis* $83.4/\text{m}^3$ (Hyde, unpublished); pollen grains averaged $52.14/\text{m}^3$, including grasses. All the concentrations quoted are very low in comparison with the daily concentrations to which patients may be actually exposed at the various seasonal peaks or even at other times during their respective seasons: thus at various times *Cladosporium* reached $31,500/\text{m}^3$ (mean 24 hr value), hyaline basidiospores 8400, hyaline ascospores 7600 and *Sporobolomyces* 25,000 but grass pollen only 782.

Since, when all the principal kinds of plant spore, including both pollen grains and fungus spores, are arranged in order of numerical abundance, grass pollen, admittedly the most important plant allergen in western Europe, comes so far down the list, it is evident that numbers of spores alone are misleading as guides to the relative importance of atmospheric allergens. But if the numerical frequencies of the various kinds of spore are weighted according to their respective individual volumes, their abundance can then be expressed on a common basis of cubic microns of spore substance per cubic metre. Thus an average pollen grain of timothy grass *Phleum pratense* (which may be taken as a typical 'wild' grass) has a diameter of $32\ \mu$ and therefore a volume of $17,164\ \mu^3$: hence the average 24 hr mean bulk concentration of grass pollen over the 4 years may be taken as 16.9 (the average 24 hr mean numerical concentration in pollen grains per cubic metre) $\times 17,164 = 289.4 \times 10^3\ \mu^3/\text{m}^3$. When this has been done for each of the pollen and spore types concerned they fall into a different and more rational sequence (Hyde & Adams, 1960a, and unpublished). The principal components of the aerospora in Cardiff are dealt with below in the order so arrived at.

1. Grass pollen average 24 hr mean numerical concentration 1958–61 16.86 grains/ m^3 ; average 24 hr mean bulk concentration 1958–61, $289.4 \times 10^3\ \mu^3/\text{m}^3$; highest 24 hr mean numerical frequency 909 grains/ m^3 . The pollen grains of grasses are all morphologically very similar. Grains of the cereal type can be distinguished from the smaller 'wild-grass' type (Rowley, 1960), but in the aerospora of Cardiff the first-named is negligible. Grains of the wild grasses cannot in general be distinguished even generically one from another (but see Liem, 1967); though not all antigenically completely identical the various species seem as hay fever plants to be important in proportion to their abundance.

2. *Cladosporium* average mean 24 hr numerical concentration 1958–61, 1204.2

spores/m³; average mean 24 hr bulk concentration 1958–61, $191.5 \times 10^3 \mu^3/\text{m}^3$; highest mean 24 hr numerical count 1958–66, 31,387 spores/m³ has long been known in the U.S.A. as an important allergen and it is a proven cause of asthma in Britain (Hyde *et al.*, 1956); at least six saprophytic species, including presumably the kerosene fungus (*C. resinae*), contribute to the local aerospora (Richards, 1953b; Harvey, 1960, 1967; Hendey, 1964).

3. Ascospores average 24 hr mean bulk concentration 1958–61, $102 \mu^3/\text{m}^3$ including hyaline $73.0 \times 10^3 \mu^3/\text{m}^3$ and coloured $29.9 \times 10^3 \mu^2/\text{m}^3$; average 24 hr numerical counts 1958–61: hyaline 468.0 spores/m³, coloured 76.1 spores/m³; highest 24 hr numerical counts 1958–66: hyaline 11,943 spores/m³, coloured 1700 spores/m³. The ascomycetes are morphologically and taxonomically very diverse. About half the coloured ascospores counted can be referred to particular genera (Adams, 1964); other ascospores are at present determined under various form headings. The conidia (not ascospores) of *Erysiphe*, an ascomycete and a recently recognized allergen, are listed separately (see p. 158).

4. 'Basidiospores' (coloured $48.9 \times 10^3 \mu^3/\text{m}^3$ and hyaline $44.5 \times 10^3 \mu^3/\text{m}^3$). In the sense employed here basidiospores are derived from the higher Basidiomycetes (the Hymenomycetes) only, a group comprising in Britain nearly 100 genera. A small proportion of the basidiospores caught from the atmosphere may be discriminated generically (Adams, 1964) but it is usual to group all except the very characteristic and common spores of the bracket fungus *Ganoderma* ($6.6 \times 10^3 \mu^3/\text{m}^3$) as either coloured ($48.9 \times 10^3 \mu^3/\text{m}^3$) or hyaline (clear and colourless ($44.2 \times 10^3 \mu^3/\text{m}^3$)) basidiospores. Gregory & Hirst (1952), who first demonstrated the abundance of basidiospores in the air, thought they should be investigated in connection with seasonal asthma; certainly the Agaricaceae (gilled fungi) are a very natural family and like the grasses might reasonably be expected to be antigenically uniform. In 1952 the only kind of basidiospores which had been shown to meet the requirements of Thommen's postulates were those of one of the polypores, viz: *Serpula* (*Merulius*) *lacrymans*, the dry rot fungus (Frankland & Hay, 1951) which, however, invariably grows indoors. More recently, basidiospores of four species of toadstool (Agaricaceae) and two further polypores (Polyporaceae) have been shown individually to cause symptoms of asthma when inhaled (Herxheimer, Hyde & Williams, 1966, 1969).

Only five types other than Nos. 1–4 above have a bulk incidence of around 10% that of grass pollen. Of these elm (*Ulmus*) pollen is greatly over-represented in central Cardiff. Gravity slide counts at a rural station $2\frac{1}{2}$ miles away in 1943 were only one-tenth those in central Cardiff (Hyde, 1952a). The uredospores of rusts (Uredinales) though relatively few in number rank high in bulk because of their size; they come from several genera but mainly *Puccinia* (cereal rusts, etc.) (Adams, 1964); allergy to cereal-rust spores has been affirmed by some clinicians (though not in Europe) but disputed by others. Pine (*Pinus*) pollen is generally though not quite universally regarded as non-allergenic. *Pullularia* has long been known to rank high as a culturable mould at Cardiff (Hyde & Williams, 1949) as has *Epicoccum* (Richards, 1954b): positive results to skin tests have been obtained with both.

The bulk incidence of each of the remaining individual types in the pollen and spore calendar (Fig. 1) ranges from 7% down to just over 1% that of grass pollen. Several known allergens, both pollens (*Quercus*, *Betula*, *Fraxinus*: all trees) and fungus spores (*Alternaria*, *Smuts*, *Erysiphe* (mildew) and *Botrytis*, grey mould) come fairly high. *Urtica* ranks highest among the 'weeds' though, since its grains are small, not as

high as its numerical incidence would suggest. The tail of the list includes *inter alia* Sporobolomyces (in spite of its very high numerical standing), Composite pollen and Aspergillaceous mould spores (*Penicillium* and *Aspergillus*). Consideration of the data on bulk incidence as a whole suggests that ascospores and, perhaps even more, basidiospores seem best to merit further investigation by allergists.

(b) *Year-to-year variation in spore abundance*

Most types of pollen grains and spores vary more or less widely in their abundance from year to year. Grass pollen, which is relatively steady in this respect (Hyde, 1952c, 1960a), was twice as high in 1955 and 1957 as in 1956; and patients' symptoms varied correspondingly (cf. Freeman, 1950). Plantain (*Plantago*) pollen varied between 63% of its 7 year average in 1961 and 132% in 1956 while nettle (*Urtica*) varied between 67% in 1957 and 134% in 1953 (cf. Hyde, 1959a). The principal tree-pollens varied much more widely (cf. Hyde, 1956); indeed some types in some years almost dropped out of the calendar: oak pollen ranged from 28 to 225% of average, birch 13–205%, elm 30–175% and ash 1–365%. In general, year-to-year variation is somewhat less marked among fungus spores than among tree pollens. Nevertheless, during the 4 years (1958–61) for which the completest figures are available, at the time of writing, basidiospores were twice as prevalent in 1960 as 1959, *Ustilago* spores twice as prevalent in 1959 as in 1958, and *Epicoccum* five times as prevalent in 1959 as in 1960; *Cladosporium*, for which figures are available from 1954 onwards, was 6.4 times as prevalent in the summer of 1957 as in 1954 (Adams, 1964).

These variations are attributable only in part to concurrent weather. Grass crops grow (and flower) most abundantly in response to spring rains, though the amount of pollen which becomes airborne may be limited by dull wet weather in June and July. Nettle pollen is usually produced in abundance in the first half of June but if there is a drought the pollen output falls off steadily and then, given rain, may rise again to a high (perhaps its highest) level in August. On the other hand, the abundance of flowers formed by spring-flowering trees may be correlated in part at least with the weather obtaining at the time the flower initials are being laid down, i.e. during the late summer of the previous year, hot dry weather being more conducive to this process (other things being equal) than is cool damp weather.

As was first demonstrated in detail by Hirst most types of fungus spore respond more or less immediately to favourable or unfavourable weather (Hirst, 1953). The mould *Cladosporium* is most prolific at times when the mean temperature is above 60°F (Hyde & Williams, 1953); such periods may occur during every month from June to September inclusive; the seasonal incidence of *Cladosporium* spores is therefore highest in hot dry summers (Hyde & Adams, 1960a). Ascospores in general are most abundant at Cardiff during damp summer weather (Adams, 1964). Hyaline basidiospores respond rapidly to rain (presumably because their fructifications, already present, only spore when damp). Coloured basidiospores are at their highest a few days to a week after rain (presumably because the fructifications of many species take about that time to develop). Both hyaline and coloured basidiospores are very much more abundant in wet autumns (Adams, 1964). *Epicoccum* is favoured by hot, dry weather, as are *Alternaria* and *Torula*. Smut spores (at Cardiff almost exclusively *Ustilago avenae*, together with *U. nuda* and *U. hordei*) are at their highest on dry hot days in June, *Erysiphe* conidia (at Cardiff largely *E. graminis*, wheat mildew) under similar conditions during early summer (cf. Sreeramulu, 1964). Botrytis spores

(presumably *B. cinerea*, grey mould of fruits) are most numerous on warm humid days after rain. *Sporobolomyces* is specially sensitive to changes in humidity, at times registering a 200-fold rise in its mean 24 hr concentration in the course of 2 days, as the result of rain and increased R.H. (Adams, 1964; cf. Gregory & Sreeramulu, 1958) and *Tilletiopsis* responds to increased R.H. even in the absence of rain. Although a rise in the 'Penicillium' type (which is due mainly if not entirely to an increase in the numbers of *Aspergillus*) takes place in winter, it is not favoured by unusually cold weather such as was experienced in January 1963. The relation between spore incidence and concurrent weather 1962-66 has been further studied by the author (Hyde, unpublished). The effects of weather on spore incidence are further referred to in a later paper.

If one of the conditions conducive to sensitization to a particular allergen is repeated exposure over several years to high concentrations then, other things being equal, the relatively steady types, such as grass pollen and basidiospores, are perhaps more likely to affect large numbers of people than are those types whose incidence fluctuates very violently from year to year, such as most of the tree pollens and (among the fungus spores) *Cladosporium* and *Epicoccum*. Variable abundance from year to year adds to the difficulty both of diagnosis and of assessing the effects of treatment (Hyde, 1956). The effect on symptoms of even such fluctuations as those of grass pollen has been alluded to above. A consistently cool summer favours sufferers from *Cladosporium*-asthma (Williams, 1964) even in the absence of treatment.

(c) *Pollen and spore phenology* (Fig. 1)

Each season of the year possesses a more-or-less distinctive spore spectrum characterized by the dominance of a particular type or types. Thus the pollen band of the spectrum in February of an average year is dominated by elm (*Ulmus*) and hazel (*Corylus*), which are joined in March also by yew (*Taxus*) and alder (*Alnus*); in April the band is dominated by elm, yew, ash (*Fraxinus*), poplar (*Populus*) and birch (*Betula*), in May by birch, oak (*Quercus*), beech (*Fagus*), maples (*Acer*), horse chestnut (*Aesculus*), plantain (*Plantago*) and pine (*Pinus*); in June by grasses, plantain, sorrel and dock (*Rumex*), and nettle (*Urtica*); in July by grasses, plantain, dock, nettle, lime (*Tilia*) and sweet chestnut (*Castanea*); in August by grasses, nettle and mugwort (*Artemisia*); and in September the pollen band becomes virtually negligible.

Pollen activity is largely confined to the first half of the year, the fungus spore band is significant all the year round, especially from late spring until November. *Cladosporium* and certain other moulds, ascospores, basidiospores, and mirror yeasts (*Sporobolomycetaceae*) are present in every month. In January, *Alternaria* and 'Penicillium' are also present and *Aspergillus* (as indicated by plate exposures) may be at its maximum phase; in February, *Botrytis* appears, to be joined soon after by *Pullularia* and *Torula*; in March, *Cladosporium* becomes more prominent; in April *Epicoccum* comes in, but *Aspergillus* virtually disappears; in May rust uredospores and *Erysiphe* conidia appear; in June, *Cladosporium* may reach its climax (though this may be deferred until July or August), *Erysiphe* and *Ustilago* pass their short-lived peaks, *Sporobolomyces* may rush up to its maximum (or this may come later, in July or August), basidiospores increase markedly and *Polythrincium* comes in; in July the number and variety of spores are likely to be very high; *Alternaria* reaches its peak in August; ascospores, rusts and *Botrytis* are usually at their highest in September when basidiospores enter on a prolonged phase of abundance which lasts until cut

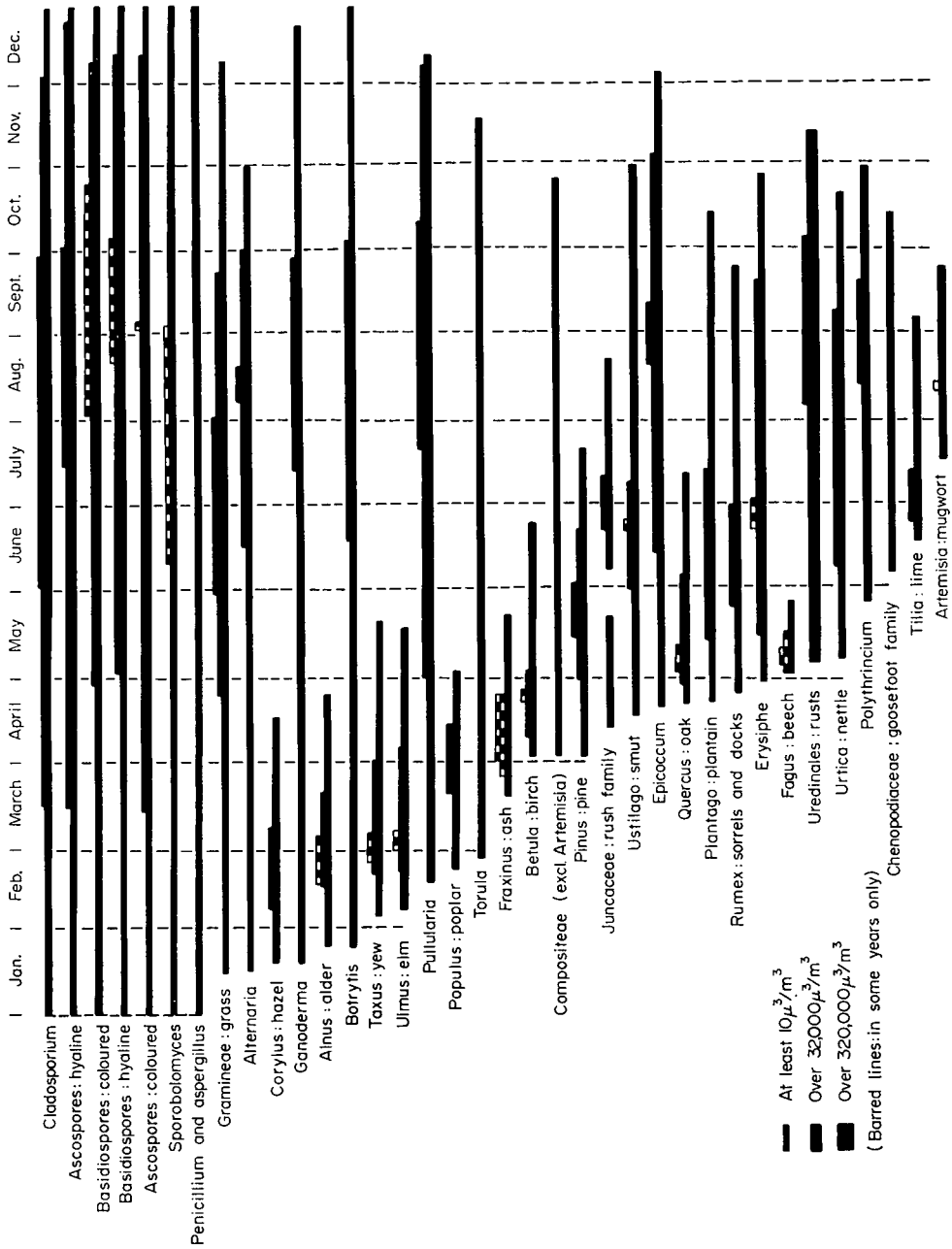


Fig. 1. Pollen and spore calendar, Cardiff.

short by the early frosts. *Pullularia* is at a maximum in September and *Epicoccum* may be; but at the end of this month *Cladosporium* usually declines markedly; in October there comes a general diminution, though the spore band nevertheless remains varied; but in November several types (*Erysiphe*, *Ustilago*, *Alternaria*, uredospores and *Polythrincium*) drop out and by December the spectrum has resumed its winter aspect.

The succession of phases in the pollen and spore seasons sketched above is likely to occur in the same order year after year but it is liable to be longer drawn out in certain years and more closely telescoped in others according to weather. The earliest and latest dates of effective onset of the principal types of tree and 'weed' pollen may vary in different years to the extent of 5 or 6 weeks, though most pollen types there is a 'constant period' during which pollen of a particular kind is present (though not necessarily abundant) in the air every year. Phenological variation is most marked in those pollen types which flower or begin to flower in spring and least so in those which flower at high summer. The constant period is relatively short in most trees, but long in the principal 'weed' pollens. The prevernal and vernal trees flower in their almost invariable order in response to a sustained rise in temperature, and as soon as their (preformed) flower-buds have all opened their pollen season ends. The effective onset of the grass pollen season (given a necessary minimum temperature) is determined by length of day, as is its duration: it is therefore closely tied to the calendar (Fig. 1). *Artemisia* behaves very similarly. In some other herbs, crops of flowers continue to be formed given sufficient moisture and warmth all through the spring and summer: hence the long pollen seasons in plantain (*Plantago*) and nettle (*Urtica*).

Phenology in the fungi is as a rule less clear-cut than in flowering plants. The life histories of the parasitic types (*Erysiphe*, *Ustilago*, rusts), and therefore their sporing periods, are bound up with the annual cycles of their respective hosts: thus the peak of the smut spore season in England coincides with or slightly anticipates the flowering of the oat crop (Sreeramulu, 1964). Some of the saprophytes, e.g. *Epicoccum* and *Botrytis*, are strictly confined to definite seasons but others, as stated above, are perennial.

(d) *Variation in the aerospora from place to place*

The pollen and spore content of the air at various places in the South of England has been assessed by a number of workers using the Hirst trap, from 1952 onwards (Gregory, 1952; Hirst, 1953; Gregory & Hirst, 1957; Gregory & Sreeramulu, 1958; Hamilton, 1959a, b; Last, 1955b, c; Lacey, 1962). A continuous census of the London aerospora, begun on 1 January 1958 at the Wright-Fleming Institute, is still in progress (Hamilton & Denny, 1960; Davies, 1962; Hamilton, 1963; Davies, 1965; Davies, Denny & Newton, 1963a, b). The aerospora of Liverpool and Glasgow has been compared with that of London (Davies *et al.*, 1963a; Davies, 1965, and unpublished). Observations have also been made at Nottingham (Pawsey, 1964; Pawsey & Heath, 1964). An outstanding feature of the work of Gregory and his associates has been that on diurnal variation in the air spora and its relation to meteorological factors (see section 6). Earlier, mostly between 1943 and 1950, the Cardiff gravity slide census had been extended for periods of up to 8 years to thirteen other stations, situated in a wide variety of habitat from remote islands to mountain tops from Cornwall to Aberdeen (Hyde, 1950a, b, 1952a, and unpublished); a petri-plate mould census was extended to seven other centres in 1951 only (Richards, 1956); a Hirst trap was operated by E. H. Chater at Aberystwyth as a Cardiff out-station during spring and summer 1961.

Petri-plate counts were made of *Cladosporium* in the London region by Ainsworth (1952).

These various surveys have shown that the pollen and spore types indicated in the calendar (Fig. 1) are found throughout Southern Britain; and their general phenology agrees with that at Cardiff. Concentrations vary from place to place. The pollens of certain trees which have been planted in large numbers in towns, e.g. elms at Cardiff and plane in London (Hyde, 1950a) are present there in unusually high concentration. Grass pollen is almost equally as prevalent in central London as at Cardiff; Rumex pollen is rather more so and nettle pollen—in some years at least—much more so (Hamilton & Denny, 1960). Certain kinds of fungus spore including smuts (three years out of four, 1958–61). *Alternaria* (three years out of four) and *Erysiphe*, are consistently more abundant over London than over Cardiff: presumably because their hosts (the cereal crops) are grown more extensively in the South-East of England than in South Wales; conversely ascospores are more, and basidiospores much more, abundant at Cardiff than in London, presumably again because the climate of South Wales is much more humid than that of South-East England, though, since both these kinds of spores are more abundant in woodlands, the preponderance over Cardiff as compared with central London may be due at least in part to closer proximity thereto (Adams, Hyde and Williams, unpublished 1967). In 1952 *Cladosporium* was more abundant in rural areas than in large towns (Richards, 1956) and during 1961 it was 4.9 times as abundant at a rural site outside Cardiff as in a built up area inside the city (Harvey, 1967).

Cladosporium was more prevalent in London than in Cardiff in 1958 (when counts were low at both stations), about equally prevalent in 1959 and 1961 (counts high at both) and rather less so in 1960 (a 'medium' season): and the successive phases of the several seasons corresponded very closely throughout (Hamilton & Denny, 1960). In 1954 *Cladosporium* was much more abundant in Hertfordshire (23 miles out of London) than in central London (Hamilton, 1959a); during the summer of 1961 (June–August) it was much more prevalent in London than in Liverpool but thereafter (September–October) it was much the same at both (Davies *et al.*, 1963a). As already noted *Aspergillaceae* (which include *Penicillium* and *Aspergillus*) are more abundant in large towns than in rural areas (Richards, 1956; Hamilton, 1955).

Nearness to the sea (with the consequent occurrence at certain times of on-shore winds) has a marked effect. Places right on the seashore and therefore subject at times to on-shore winds might be expected to experience low average spore concentrations; notable periods of onshore winds with low pollen catches were in fact observed at a station situated 60 ft above ground level on the sea front at Aberystwyth in April and July 1943 (Hyde, 1950a). Steady offshore winds at the same station produced high catches, and wind changes occasioned marked oscillations in pollen catch. Observations made in 1961 on Penglais Hill, 1 mile inland from Aberystwyth, gave, on the whole, similar results. Here the average concentrations of six principal pollen types were all lower or much lower than at Cardiff, and the same applied to most but not all important types of fungus spore. The average weekly concentrations of *Cladosporium* spores at Cardiff and Aberystwyth rose and fell in unison throughout the season (Hyde & Adams, unpublished).

The essential uniformity in behaviour of the aerospora in Wales and Southern England doubtless reflects the basic similarity in conditions throughout. In Scotland the various pollen seasons set in later than Southern Britain (Hyde, 1950a; Davies,

1964b, c, 1965). The differences observed may be attributed to a variety of causes including climate (especially whether relatively dry or humid), land use (e.g. preponderance of grassland or arable in the region concerned) and of course local situation (especially nearness to the sea and the proximity or otherwise of extensive sources of spores). Much further study is required in order to evaluate the effects of all the factors possibly concerned.

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