AIR SPORA BAGHDAD

by

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ABSTRACT

A quantitative investigation into the fungal spore and pollen content of the air of Baghdad is reported. Air sampling methods and equipment are discussed. A Burkard, 7 day volumetric airborne spore sampler was operated in Baghdad from March, 1982 until June, 1983. Drums bearing sticky tapes exposed in the trap in Baghdad were returned to London in air-tight containers. After training in Mycology and the visual identification of fungal spores and pollen grains, the tapes were mounted for microscopic examination. Deposits were scanned and the mean daily concentrations obtained for various fungal spore types e.g. *Alternaria*, *Cladosporium*, *Helminthosporium*, *Stemphyllium*, Ascospores, and Basidiospores including rusts and smuts. *Cladosporium*, the Basidiospores and *Alternaria* represented 46, 25 and 12.5 percent of the total spores trapped. A simple pollen calendar which shows the months of the year when various pollens occur in the air together with the months of peak concentrations has been produced for Baghdad. The most common airborne pollens as a percentage of the total pollen catch were *Juniperus* 29, *Gramineae* 17, *Chenopodiaceae* 14, *Urticaceae* 12, *Ziziphus* 11, *Oleaceae* (*Olea* and *Fraxinus*) 6. There is little plant growth in the hot dry Summer, and in July and August apart from chenopod pollens few grains were trapped.

Fungal spores occurred in the air throughout the year, the highest monthly concentrations being obtained in March, April and June, the lowest in August and September. A study of mould fungi cultured from house dust samples collected each month is described. The importance of the results in relation to inhalant allergies are discussed, and the literature on the allergen content of the air of other countries with geographical features similar to Iraq and Baghdad is reviewed. Attention is drawn to the need for aerobiological surveys to be undertaken in the different climatological and topographical areas of Iraq. The need for diagnostic pollen antigens from plants indigenous to the Middle East and not available elsewhere is noted.

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INTRODUCTION

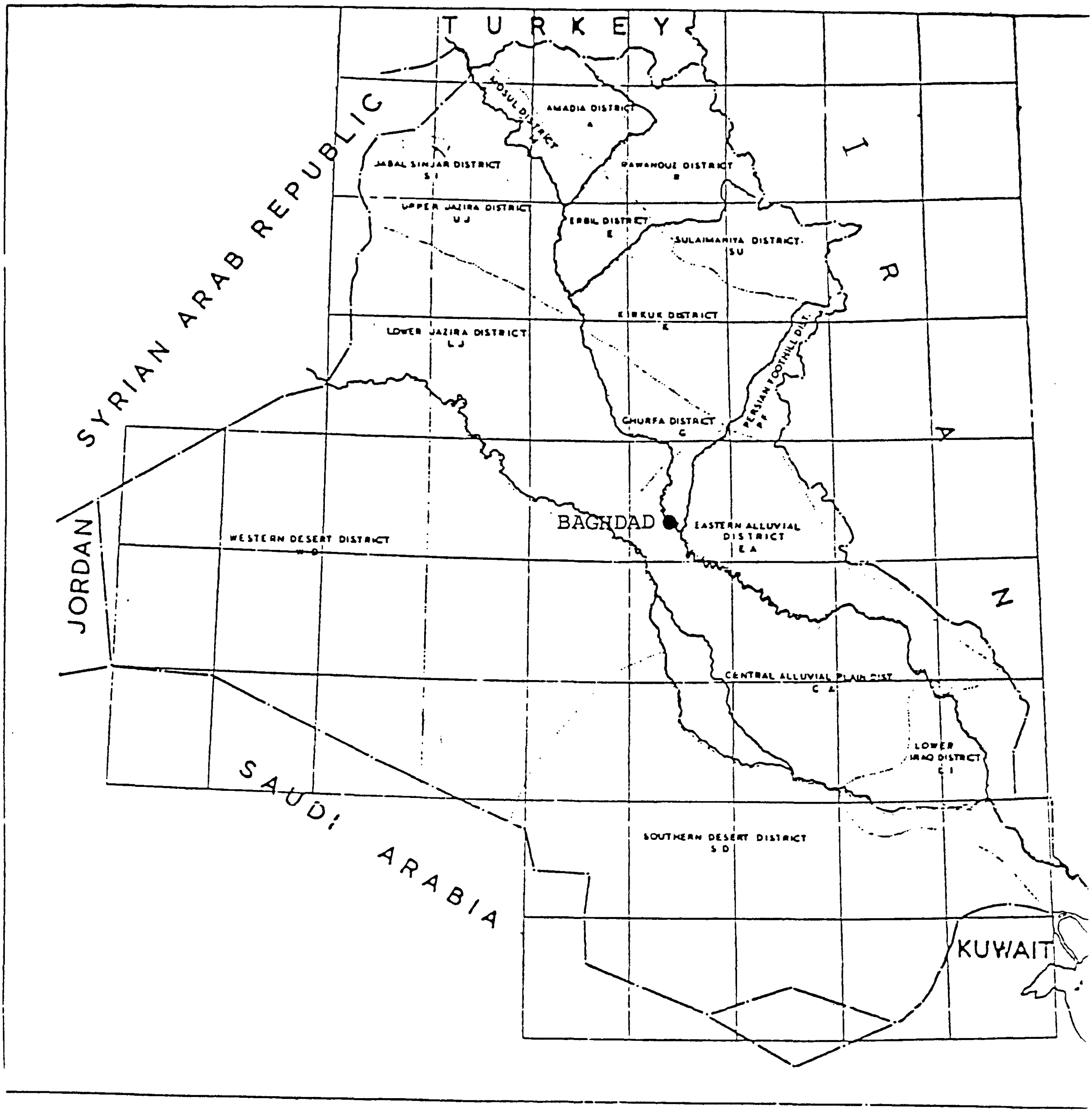
The term "air spora" was coined by Gregory (1961) to describe the fungal spores and pollen content of the air, the pollen grain of the flowering plant being known botanically as a microspore. Credit for the first study on the content of the air in relation to allergic disease must be given to C.H. Blackley (1873) a family physician in Manchester, who by experiments upon himself proved that grass pollen was the cause of the Summer conjunctivitis and rhinitis commonly termed hay fever. Blackley exposed sticky faced microscope slides to the air and related the severity of his own symptoms to the numbers of pollen grains trapped. The year Blackley's monograph was published in England, Cunningham (1873), in an attempt to discover the cause of cholera, reported on the content of the air in two Calcutta goals and in so doing produced the first monograph on the fungal spore content of the air. Miquel (1883) analysed the bacteria and moulds in the air of Paris and appears to have been the first worker to estimate the number of microbes in a measured volume of air. Since these pioneer publications appeared in England, France and India, more than a century elapsed before any study on the aeroallergens of Iraq was undertaken.

In the first report on the airborne pollen and moulds of Baghdad, Al-Tikriti et al (1980) pointed out that there are few known or accurate pollen surveys for the various climates of the Middle East. The only published surveys were primitive, and based on searching for the families of other nations' hay fever plants within their boundaries. They concluded "no sophisticated studies exist".

The purpose of this thesis is to report a quantitative investigation into the content of the air of Baghdad in relation to allergic disease and to produce pollen and spore calendars for Baghdad.

Pollenosis has been the subject of research at St. Mary's Hospital since Noon (1911) first practiced therapeutic inoculation with aqueous extracts of grass pollen. Under the directionship of Freeman, a department of allergy was established in 1920, and in 1935 a pollen farm was commenced to provide pure pollen in the kilogramme quantities needed for hyposensitization and research in the clinic. Freeman (1950) described hay fever as the key to the allergic disorders and Frankland & Davies cited by Davies & Smith (1973a) showed that when the grass pollen concentrations over central London reached 50 grains per cubic metre, all the clinically sensitive patients experienced symptoms. Having in mind the experience of aerobiology and its relationship to allergic disease obtained at St. Mary's, a brief comparison will be made between the content of the air of Baghdad and that recorded at St. Mary's, in central London.

Fig.1 PHYSIOGRAPHIC REGIONS OF IRAQ



GEOGRAPHICAL FEATURES OF IRAQ

GEOGRAPHICAL LIMITS

The territory of the Republic of Iraq - roughly the lower portion of the Mesopotamia of history, lies between longitudes 38^{0} 42E, and 48^{0} 23E and between latitudes 29^{0} 27N and 37^{0} 23N (Guest, 1966): the physiographic regions are shown in Fig 1.

The longest axis of Iraq is nearly 1000 km long and runs N.N.W. to S.S.E. from the Turkish frontier above Zakho to the shore of the Arabian Gulf at Fao. Its greatest width, runs N.E. to S.W. from the border, north of Rawanduz to the junction of the Saudia Arabia, Kuwait and Iraqi frontiers and is nearly 750 km.

The total land area is compiled as nearly 444, 500 sq.km of which rather less than a half is desert.

TOPOGRAPHY

Topographically Iraq has four main regions:-

1. The Mountain region This is bounded by the N. and N.E. frontiers of Iraq with Turkey and Iran respectively, from W. of Zakho to S.E. of Halabja.
2. The Upper Plain and Foothills region These comprise the out lying district of the region in the N.W., and the margin of the lower plain at the foot of the Jabal Hamrin. There is no sharp topographic feature to mark

the lower margin of the Upper Plains and Foothills region, and here most of the land is hilly.

3. Desert Plateau region This is bounded to the N. and N.W. by the lower boundary of the Upper Plains and Foothills region as far as the R. Diyala and in the E. and S.E. mainly by the right bank of the R. Euphrates until it reaches the southern marshes near Nasiriya. Most of the area in this region is flat and comprises the western desert bounded by Syria and Jordan, Saudia Arabia and Kuwait.
4. Lower Mesopotamian region The great alluvial plain comprises the remainder of the territory of Iraq. It is bounded by the desert plateau region to the N.W. and W. and S. and by the lower margin of the Upper Plain and Foothills region and the Persian frontier to the N.E. and E. Baghdad lies in this district.

Baghdad is located in the central alluvial plain district (C.A) Fig 1, the main central Mesopotamian Plain which lies between the River Tigris and the outer Hindya channel of the Euphrates. It is bounded to the East and South-East by the Southern marshes. This fertile area is chiefly devoted to arable farming and was the grannary of ancient Mesopotamia.

EFFECT OF CLIMATE ON THE VEGETATION OF IRAQ

Zohary (1950) has briefly discussed the effect of the climate of Iraq on its vegetation and concluded that the climate of the country as a whole, lies between the typical Central Asian (Irano-Turanian) and Saharo-Sindian climates. The former is characterized by the occurrence of three seasons; cold Winter, a mild growing period of Spring and a hot dry Summer, and the latter by the occurrence of two alternating seasons, a growing period and a hot dry Summer with no plant growth. Apart from the high mountains, there is no part of Iraq where the mean monthly temperature drops below 0^{o}C, which is a common feature of the main Irano-Turanian territory. On the other hand there is only one district in Iraq where the mean monthly temperature of January is appreciably above 10^{o}C (50^{o}F), as commonly found in the Saharo-Sindian countries. Zohary considered the temperature of 10^{o}C to be near the critical point for plant life in Iraq. Where the mean January temperature is not materially above 10^{o}C the development of the Winter flora is delayed until Spring. Thus, despite a sufficient annual rainfall, the growth period of vegetation in most parts of Iraq occurs in Spring and not in Winter. He concludes that in this respect the climate of Iraq as a whole approaches much more to the typical Irano-Turanian climate than the Saharo-Sindian and Baghdad has this type of climate.

Zohary (1950) recognizes four main variants from the climate of most of Iraq.

1. The Southern Desert has a climate with very low rainfall (50-150mm) and a mean January temperature not below 10°C. The season for rain generally occurs between November and March. There are only two pronounced seasons; Winter and the "dead" Summer season starting at the end of April.
2. The Western Desert, Lower Jazira and parts of the Lower Alluvial Plain have a climate with an annual rainfall between 100-200mm, but the mean January temperature drops below 10°C (50°F). Although January is a month of comparatively heavy rain there is a sharp interruption in plant development because of the prevailing low temperature, and the main growing season is in the period, March to April.
3. The Steppe Zone of the Upper Plains and Foothills, has an annual rainfall of from 300-600mm. Although the mean January temperature here is 7°C (45°F), with absolute minima below -15°C, low temperatures also prevail in February. The temperature rises rapidly in March and this is accompanied by sufficient rain during the late Spring of March and May for the development of a rich perenial herbaceous flora.
4. The Mountain Region has a climate with a higher annual rainfall from 700 to 1200mm or more, distributed over a longer period of months and an even more severe Winter. The mountains above, about 1800m, are snow-

bound for several months and snow often falls in the valleys. The vegetation here does not begin to develop much before the end of April and the main growing period is from May to June onwards.

THE CLIMATE OF BAGHDAD

Baghdad is 34m above m.s.l. The mean annual rainfall of 58 seasons based on observations at the British Embassy from 1888 to 1914 and 1918 to 1922, the British Royal Air Force 1923 - 1936 and the Iraqi Meteorlogical Service 1936 - 1948, was 149mm.

The relative humidity at 3 p.m. during the Summer months of June, July, August, September and October averaged 16%.

The atmospheric humidity is therefore low. In relation to plant growth and development it should be noted that the rate of evaporation has been calculated to exceed the annual rainfall by a factor of 20-40. The rainfall is extremely variable, both from year to year and in its distribution during any given Winter season. In 1908-1909 only 50mm (2 ins.) of rain was recorded at Baghdad, whilst in an exceptionally wet year 1889 - 1890, 420mm (17ins.) was recorded.

Very little rain falls during the Summer and rarely any at all during the months of June, July and August. The coldest month of the year in all parts of Iraq is January, when the mean daily maximum and minimum temperatures at

Baghdad range from 2^{o} - 15^{o}C (Fig 2) with recorded absolute minima of -8^{o}C.

The hottest months of the year are July and August (Fig 2) when the daily maximum and minimum temperatures range from 43^{o}C - 25^{o}C with record absolute shade maxima of about 51^{o}C.

The prevailing winds throughout the year are N.W., but during the Winter months, depressions pass from Eastwards across the country from the Eastern Mediterranean. The depressions result in unsettled weather with strong S.E. easterly winds, often of gale force bringing in rain and dust-storms.

Since the creation of the Thirthar Lake in the Jazira, dust storms are of less frequent occurrence, and are mainly encountered in the hot season of June, July and August.

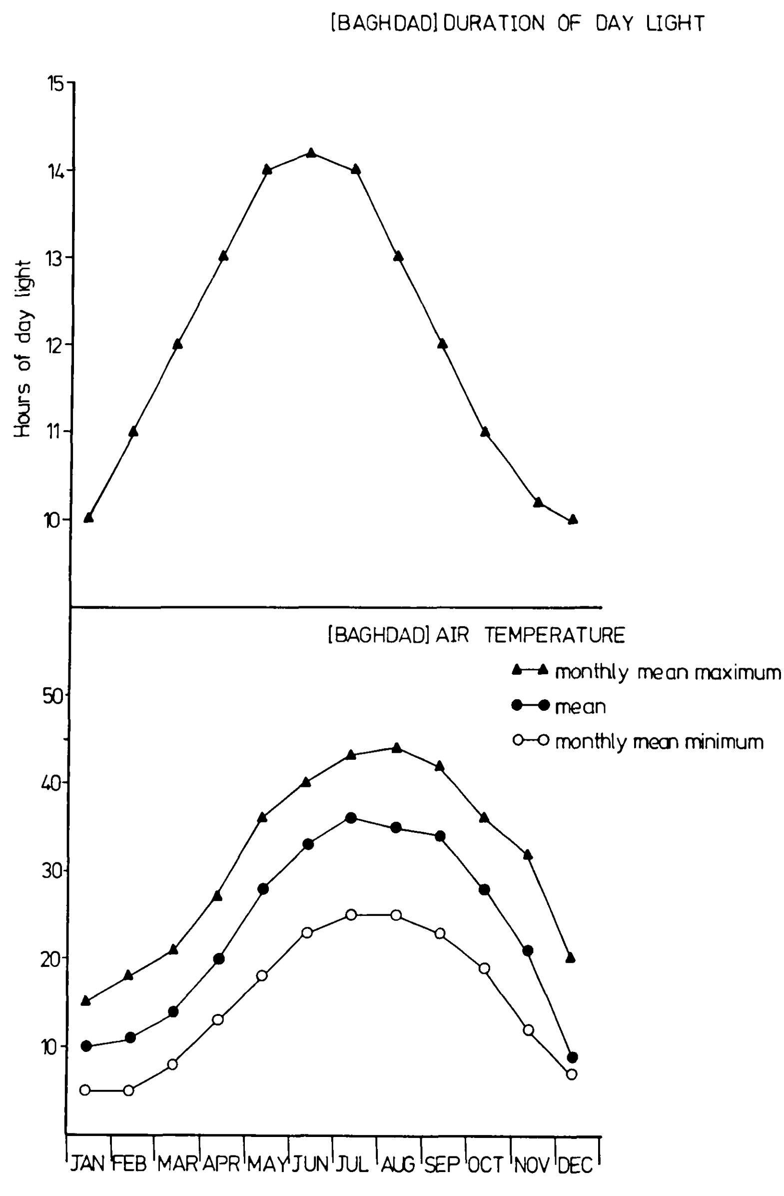


Fig 2: Mean monthly air temperatures and duration of daylight in Baghdad (Drawn from data in Guest & Al-Rawi 1966.)

SECTION I

METHODS IN AEROBIOLOGY

SUMMARY OF LITERATURE ON THE CONTENT OF THE AIR WITH SPECIAL RELEVANCE FOR BAGHDAD AND THE MIDDLE EAST AND THE METHODS EMPLOYED

METHODS IN AEROBIOLOGY

FALL OUT OR SEDIMENTATION METHODS

The Gravity Slide

In the past, much useful information on the pollen content of the atmosphere and the aerial occurrence of such fungal spores as *Alternaria* have been obtained with this simple method. Such pioneers as Charles Harrison Blackley (1873), Wodehouse (1935), Durham (1946) and Hyde and Williams (1944) exposed microscope slides horizontally, sticky side upper-most, in a shelter which although open to the wind, protected the slide from rain. A major criticism is that this method is based in favour of the larger particles, large pollen grains settle more quickly than smaller ones. If the air were still and the particles settled according to their terminal velocities, it would be possible to calculate aerial concentrations from the numbers which settled on the slide in a given time. The air outdoors, however, is never still, and Durham (1944) showed the term "gravity slide" to be a misnomer when he greased both surfaces of a slide and after exposing it horizontally found the lower surface caught as many as 50% of the number of ragweed pollen grains which was trapped on the upper surface. In wind tunnel experiments, Gregory and Stedman (1953) have shown that deposition on a horizontal slide does not only depend on the simple resultants of gravity and wind, but is a complex process. They found that trapping efficiency varied in different parts, it is subject to edge effects which can lead to underestimation by, from 5 to 500 times at even moderate wind speeds, turbulent deposition occurs at high wind speeds and the highest collection efficiency for Lycopodium spores (approximately the same size as grass pollen grains) was only 4%.

The horizontally exposed sticky slide is inefficient but cheap; it may show which pollens were in the air during the exposure period, but since it cannot be used to measure concentrations it should be abandoned in favour of volumetric methods. Hyde (1959) cited Davies (1962) found that after three years experience with one of these (the Hirst trap) the relative importance of Urtica in the atmosphere had been seriously underestimated during a thirteen year study with gravity slides.

Another drawback to use of the settle slide is that particles are distributed over a wide area of slide. This makes scanning tedious since a larger area has to be scanned than is the case with a narrow deposit impacted from a volume of air. However, although the numbers of particles trapped cannot be used to obtain a volumetric estimate, in developing countries where there is no information, the method may be used to obtain purely qualitative information on the pollen content of the air.

Studies with this method of air sampling are listed in Table 1.

The Settle Plate

In the "settle plate" method, a petri dish of agar medium is horizontally exposed to the air for a standard interval of time. Following incubation the colonies which develop are counted and identified. It has been widely used for studying the fungal content of the air outdoors, and the concentration of bacteria in the air indoors. It has been criticized by Du Buy et al (1945) and Bourdillon and Lidwell (1948) who, from theoretical considerations, state that petri dishes used alone are of little value for particles smaller than 30μ in dia. It has been further criticized by Gregory (1952) who reports that surface traps are highly selective and favour the heavier spores. Gregory and Stedman (1953) using Lycopodium spores in wind tunnel investigations found that both horizontally exposed slides and petri dishes had low trapping efficiencies, except at wind speeds of between 1.1 and 1.7 meters/sec.

The use of the horizontally exposed petri dishes should be restricted to situations in which the free falling velocity of the particle to be sampled is known.

Studies on the fungal content of the air outdoors with settle plates were undertaken by Ambler and Vernon (1951), Van de Werff (1958) and Ordman (1963b). Investigations with special references for Iraq are listed in Table 2.

Indoors the method was used to trap fungi by Richards (1954). In addition to the low trapping efficiency, the settle plate only records the spores which germinate and grow to form an identifiable colony on the agar medium employed. There are always fungi which form colonies with no spores and which cannot be identified.

The method does not record basidospores and the spores of obligate plant pathogens.

Airborne ascospores can be recorded as an imperfect state. Dead spores can be allergenic. Further the rapidly growing colonies tend to inhibit those which are slow to develop.

VOLUMETRIC AIR SAMPLING

Impactors

These are devices which sample airborne particles by impaction onto a solid surface.

General Principles

Davies (1971) reports that apparatus for use outdoors should have a feather edged intake nozzle and be freely pivoted so that the intake aperture faces the wind. If the air velocity in the intake tube is too low large particles are blown into the orifice and too many large particles occur in the sample. When the suction rate is too high, although small particles follow the streamlines converging into the orifice, large particles with significant mass and inertia fail to follow the curvature of the streamlines and are underestimated in the sample. Ideally, particles should be sucked into the sampling orifice at the same speed as the wind speed, a condition which was described by Druett (1942, cited Davies 1971) as isokinetic, but which is impracticable other than under controlled conditions of lamina flow in a wind tunnel.

A compromise solution for this problem was devised by May (1945) in his design of the "Cascade Impactor" and later adopted by Hirst (1952) in the "Automatic Volumetric Spore Trap". In use outdoors, both are directed into wind by a vane and have air velocities in the intake tubes which approximate to the mean wind speed encountered in the British Isles. With such apparatus unless the sampling is for a very brief period, anisokinetic sampling errors tend to be averaged as the wind speed fluctuates above and below the mean. Anisokinetic sampling errors can be of significance when studying the airborne concentrations of large particles such as pollen grains ranging from 12 to 80μ in diameter and "rafts" upon which microbes are carried, but the greater the size of the intake orifice the smaller are these errors likely to be.

May (1967) contrasts the high efficiency of sampling in still air with the variable errors of aniso-kinetic sampling in an unsteady air stream and suggests that "if moving aerosols could be brought nearly to the rest about the sampling point, then high efficiency sampling over a wide range of air speed and direction might be achieved by an orifice sucking at a constant rate".

In May's concept of "Stagnation point sampling" a baffle of suitable shape and size is fixed behind the sampling nozzle so that air approaching the baffle is brought nearly to rest by a cushion of air in the baffle. May and Druett (1953) in tests with the pre-impinger, found that with 30μ dia. particles in a 6 m.p.h. wind, the intake of the bulb was increased from 58 to 80% if the sample was taken through the centre of a 4 in. diameter baffle normal to the wind direction. Similarly, Edwards (cited May, 1966) found the intake efficiency of the multi-stage liquid impinger (May, 1966) for 15μ dia. particles in a wind of 10 m.p.h. was raised from 9.6 to 99% with a 6 in. square concave baffle. Since isokinetic sampling outdoors is usually impossible further experimental evaluation of what May has termed "Stagnation point sampling" is highly desirable. However, whilst this principle may be used for spot sampling (sampling over a short time interval) equipment for continuous sampling in variable wind conditions has yet to be designed and evaluated.

Davies (1971) observed that apparatus for sampling airborne microbes must not only be free from collection errors but also have high retention efficiencies. There should be no sharp bends so that the deposition of large particles on walls is minimized. The surface upon which particles are impacted should be highly retentive and air velocity, jet dimensions and distance from the sampling surface are particularly important variables. Since these features are easily evaluated, the retentivities of the volumetric air samplers in common use are well known.

The Slit Sampler

The slit sampler described by Bourdillon et al (1941), employs the principle that air is sucked at high velocity through a narrow slit and the entrained particles impacted onto the surface of the agar medium in a petri dish. During sampling the petri dish is rotated on a turntable so that the particles are distributed over a wide band. After suitable incubation, the numbers of colonies which develop are counted, and from a knowledge of the sampling rate which is constant and the time of exposure which may be varied, the numbers of viable microbes per unit volume of air can be calculated. Since the speed at which the dish is rotated is also known, the time when particular microbes were present in the air above the sampling intake can be determined. An improved slit sampler with accurate timing was described by Bourdillon et al (1948b) and a large slit sampler for bacteriologically clean air by Bourdillon et al (1948c).

The collection efficiency of the slit sampler is reported to be 96% for an aerosol of Staph-albus sprayed from distilled water as single cocci (Bourdillon et al 1941). From a formula given by May (1945), Bourdillon and Lidwell (1948) calculated that the impaction efficiencies for the 1 mm and 0.3 mm slit widths respectively were 90 and 98% for spheres of 1μ diameter and unit density. This apparatus is simple to operate and has the convenience of sampling microbes directly onto the surface of the agar medium in a petri dish. Use of the slit sampler has contributed much to our knowledge of air hygiene and cross infection in hospitals as illustrated in the work of Bourdillon and Colebrook (1946) Bourdillon et al (1948 b,c), Duguid and Wallace (1948), Drummond and Hamlin (1952), Blowers et al (1955), Williams et al (1956) and Williams et al (1962). Examples of its use in mycological studies of the air outdoors are to be found in the work of Pady and Kapica (1953), Maunsell (1954), Davies (1957) and Noble and Clayton (1963).

The apparatus was designed for sampling under relatively still air conditions indoors and the intake orifice faces vertically upwards. Outdoors it cannot be operated isokinetically and sampling errors are likely to become increasingly significant as particle diameters become greater than 5μ.

In a study of the naturally occurring clouds of Cladosporium Davies (1957) obtained values with a slit sampler which were similar to those obtained with cascade impactor. Since 70% of the spores were trapped as single conidia and less than 5% as clumps of 5 or more, and Noble et al (1963) reported the "median equivalent diameter" for Cladosporium spores to be 4.9μ, clearly with this apparatus, anisokinetic sampling errors are unimportant with spores as small as Cladosporium.

The Cascade Impactor

This apparatus was described by May (1945) as an instrument for sampling coarse aerosols and comprises a system of four air-jets and sampling slides in series. When used outdoors the apparatus is freely suspended and a vane directs the feather edged intake orifice into the wind. For sampling in still air a bell mouth adaptor is fitted to the intake orifice which must then be faced upwards to avoid a deficiency of large particles in the sample.

The sampling rate of 17.5 litres a minute that is usually employed gives air velocities through the four jets of 2.2, 10.2, 27.5 and 77 metres per second respectively. Their performance when sampling drops of unit density is such that the maximum drop size found in the deposits behind the second, third and fourth jets have diameters of 20, 7 and 2.5μ, each being near the minimum size impacted with 100% efficiency by the previous orifice. Impaction velocities are such that 50% of particles with diameters of 12, 4, 1.5 and 0.4μ penetrate the respective jets. The instrument not only gives good retention for particles down to 0.5μ diameter but traps droplets as large as 50μ diameter and aggregates such as clumps of fungal spores without shattering them. May's design of the cascade impactor is particularly important since it traps particles according to size in a similar manner to the human respiratory tract. Particles larger than spheres of 7μ dia. and unit density are deposited in the upper respiratory tract, 50% of those 4μ dia. reach the alveoli of the lung (C.N. Davies, 1963; Druett, 1967). Since the first and second stages of the cascade impactor have a 100% retention for 7.1μ dia. spheres of unit density, it simulates the upper respiratory tract in which the naturally inhaled pollens and larger fungal spores are deposited, and which in the allergic subject produce such symptoms as rhinitis, and asthma. The third and fourth stages trap such particles and spores as may be inhaled and retained in the deepest parts of the lung. Maximum retention in the lung alveoli is 50 - 60% for particles 1.5μ to 2 μ dia. (C.N. Davies, 1952) and 50% of of particles 1.5 μ dia. are trapped at the third stage of the cascade impactor.

The cascade impactor represents a milestone in air sampling technique and it is the reference instrument by which the efficiencies of other air sampling devices are compared (Davies, 1971). The size grading slit sampler (Lidwell, 1959) portable spore trap (Gregory, 1954) and Hirst (1952) trap are based upon it. In microbiology it has been used to study the influence of particle size on infection with anthrax spores (Druett et al 1953) and with Pasteurella pestis (Druett et al 1956) and to study the spores liberated from mouldy hay associated with farmers lung disease (Gregory and Lacey, 1963).

Slides or cover glasses may be made adhesive for microbiological sampling by either smearing with petroleum jelly which is best applied hot to get an even layer, or a strip of clear plastic may be stuck on and made sticky with glycerol jelly as used by Gregory and Lacey (1963). For use in hot climates, Davies (1969c) used silicone grease. The sticky surface must not be applied too sparingly since with too thin a layer retentivity may be impaired.

The automatic volumetric spore-trap (Hirst trap)

Hirst (1952) in experiments in the field and in a wind tunnel found most fungal spores were trapped on the slide behind the second jet of the cascade impactor and devised a continuously recording sampler based upon it.

In the Hirst trap air is drawn at 10 l/min. through a feather edged 14 x 2mm orifice which is directed into the wind by a vane. The particles in the air stream are directed onto a sticky surfaced microscope slide which is moved 2mm an hour behind the inner edge of the orifice. On a slide exposed for 24hr the trapped particles occur in a trace measuring 14 x 48mm. After suitably mounting in either glycerine jelly or lactophenol solvar (Hirst 1953) the trace may be scanned longitudinally to obtain a daily mean or transversely, at 4mm intervals, to trace two hourly changes in concentration.

When operated continuously this apparatus provides a complete record of the diurnal and seasonal changes in the air-spora. Under field conditions in Britain, anisokinetic sampling errors are unlikely to be large and Gregory and Hirst (1957) report a mean trapping efficiency of 80%, but which varies with wind speed and particle size.

Identification is visual under the microscope and whilst the spores of plant pathogenic fungi such as rusts, smuts and powdery mildews which are difficult to grow in artificial culture are recorded, the conidia of such common moulds as Aspergillus and Penicillium are indistinguishable and underestimated because the impaction velocity is sufficient to trap only about 50% of particles of 4μ diameter. Noble et al (1963) reported that in Aspergillus fumigatus the spores have a median equivalent diameter to 4μ and that in Summer the spores have smaller equivalent diameters than in Winter. Davies (1971) observes that when airborne the spore is subjected to dessication and the dimensions quoted from direct measurement are usually for fully turgid spores.

The Hirst trap is robust and reliable. Davies (1962) reports that the Hirst trap is the apparatus of choice for surveys of airborne spores and pollens in relation to inhalant allergy. It samples continuously and overcomes the problem that the collection of air samples at a particular time or times each day can miss peak concentrations of important allergens since their diurnal periodicities are affected by the vagaries of the weather.

In this instrument the spores and pollens are impacted in a dense deposit which makes scanning less tedious and time consuming than searching an area of sticky slide upon which particles have been trapped by sedimentation and impaction by eddy diffusion. It seriously underestimates small spores such as those of Sporobolomyces which Evans (1965) considered an important allergen and the fitting of a narrower sampling orifice whould increase impaction. However, in the polluted atmospheres of cities the environment for most of the allergic population, increasing the air speed in the orifice in addition to an increase in anisokinetic sampling errors would lead to a greatly increased deposition of soot, and ash, and in the Middle East sand on the slides. This makes visual identification and counting difficult, and the amount of sand in the air of a desert region as reported by Davies (1969c) can so overload the adhesive surface as to reduce its retentivity.

The use of this method of air sampling is illustrated in general accounts of the fungal air-spora by Hirst (1953), Gregory and Hirst (1957), Davies (1969 a,b,c) and for grass pollen Davies and Smith (1973a). Studies undertaken with the Hirst trap with relevance for Iraq are also listed in Table 3.

The Burkard Volumetric Trap

This comprises a compact unit with a built-in vacuum pump and is based on the specification of Hirst (1952) with the difference that it samples airborne particles continuously for periods of up to seven days without attention. It is shown in (Fig 3) p50.

The particles are not impacted on to a sticky faced microscope slide but on to an adhesive-coated transparent plastic tape supported on a clockwork-driven drum. The drum circumference is 345mm. In use it is moved at 2mm/h and this allows a 9mm gap or an approximately 4h interval before the spore catches become superimposed. The standard model has a 2 x 14 mm orifice as in the Hirst trap but interchangeable orifices are available to improve the trapping efficiency for particles in the range 1 to 10µ in diameter.

Preparation of the trapping surface

1. The drum is placed on a special bracket and locking nut tightened so that the drum can be revolved (Fig 4) p51
2. Across the drum over the two black indicator marks a piece of double sided adhesive tape is fixed.
3. A length of Melinex plastic strip is cut and placed on the drum in such a way that the joint coincides with the black marks and the two ends of the Melinex strip are held in place by the adhesive tape. The ends of the Melinex tape should not overlap. The tape is then made sticky with petroleum jelly, or a mixture of petroleum jelly and paraffin wax. For use in hot countries silicone grease has been employed. Silicone grease is dissolved in chloroform. A camel hair brush is dipped into the mixture of the silicone grease and chloroform, and then held lightly against the Melinex strip while the drum is revolved. With care, a uniform coat of silicone grease can be applied to the Melinex. Too thick a film could foul the inside of the orifice, too thin a film affects retentivity.

Use of silicone grease as a sticky surface upon which spores and pollens could be impacted and retained was essential because of the high temperatures encountered in Baghdad during the Summer time. Shade temperatures of 45^{o}C occur and the apparatus is sited on a roof-top where it has to be freely exposed to the air streams and is therefore in the direct heat of the sun. Under these conditions the petroleum jelly used by Hirst (1952) and Davies et al (1963) simply melts and runs off the tape.

The Rotorod

This instrument described by Perkins (1957) relies upon the efficiency with which small air-borne particles are deposited on narrow cylinders orientated at right angles to high velocity winds (Gregory, 1951).

It consists of a pair of thin brass rods of square cross section which are whirled at a constant speed by a battery operated motor. Particles are trapped from an annulus of air. The leading edge of each rod carries a strip of sellotape smeared with either petroleum jelly, glycerine jelly or silicone grease. After exposure the strip is removed, cut into several lengths and mounted in glycerine jelly beneath a cover glass on a slide.

The volume of air sampled is calculated from that swept by the rod and the number of revolutions made by the motor during the sampling interval. Harrington et al (1959) compared a modified rotorod with membrane filters and reported its minimum collection efficiency to be 85% for particles having an equivalent diameter of 12μ. Carter (1960), however, tested an English model against an iso-kinetically operated Cascade impactor in a wind tunnel and found with Lycopodium spores (30μ dia.) trapping efficiency varied between 50 and 90% according to wind speed. When compared with a slit-sampler under the relatively still air conditions of a laboratory, Davies (1971), reported its efficiency for Cladosporium was less than 50%. Since the Rotorod samples a large volume of air for use in surveys a number would have to be run in sequence, especially in cities with a high level of pollution. The amount of labour required in preparing sampling surfaces and subsequent counting renders this method uneconomic. As a portable pollen trap however, the Rotorod is particularly useful since it can be carried in an open basket through public places. Its use is illustrated in the publications of Harrington et al (1959), Davies et al (1963), Gregory and Lacey (1964) and Raynor et al (1961).

Roto-slide

This method used by Ogden (personal communication R.R. Davies) employes the principle of the rotorod, with the difference that a pair of microscope slides are spun, with the edges directed forwards into the swept annulus. The edge of the slide is made sticky and the slides mounted vertically in a rack for microscopic examination.

Impingers

These are exemplified by the Porton impinger and multi-stage liquid impinger of May (1966), and details are given by Davies (1971).

Impingers sample particles from air by impingement onto a liquid. Whilst impingers could be used to sample air for allergens, and samples examined both microscopically and by cultural methods, in general the time and labour employed renders the method uneconomical. Whereas the multi stage liquid impinger (May, 1966) is the apparatus of choice for sampling air for such allergens as rat urine proteins in an experimental animal house, or the airborne allergens causing humidifier disease, the Hirst trap remains the apparatus of first choice for surveys of airborne spores and pollens since it has the outstanding advantages that it is not only continuous, but enables the time when a pollen or spore was trapped to be determined. This is important in air sampling outdoors as the time when a pollen or spore cloud becomes airborne can vary with the weather.

SUMMARY OF LITERATURE ON THE FUNGAL SPORE AND POLLEN CONTENT OF THE AIR OF COUNTRIES WITH RELEVANCE FOR BAGHDAD AND THE MIDDLE EAST

A detailed world wide review on the content of the air in all the various countries of the world from which studies have been published would require more time than that available for the present project. Climate and topography determine the vegetative cover of an area and the weeks or months of the year when such seasonal diseases as pollenosis and fungal spore allergies occur. To a large extent the climate of an area is determined by latitude. Emphasis has therefore been given to studies from places lying in similar latitudes to Iraq, or having climatic or similar vegetative features, or being important as pioneer studies. For ease of reference the reports selected as having relevance for studies in Baghdad and Iraq is presented in Tables 1, 2 and 3.

Table 1: "Gravity slide" Based Studies of Airborne Allergens

	Author	Year	Country and Place
(1)	Bieberdorf,F.W. Hampton,S.F.	1946	U.S.A. Texas, San Antonio (fungal spores)
(2)	Burtness,H.I. & Allen, Soniae.	1950	U.S.A. California, Santa Barbara
(3)	Kessler,A.	1953	Palestine, Tel Aviv (fungal spores and pollens)
(4)	Kessler,A.	1954	Palestine, Jaffa, Central of Tel Aviv (fungal spores and pollens)
(5)	Kessler,A.	1958	Palestine, Jaffa, Tel Aviv
(6)	Shapiro,R.S. Eisenberg,B.C. & Binder,W.	1965	U.S.A. California, Huntington Park and Los Angeles (Fungal spores and pollens)
(7)	Baruah,H.K. & Chetina,M.	1966	India, Gauhati (Fungal spores and pollens)
(8)	Agarwal ,M.K. Shivpuri,D.N. & Mukerji,K.G.	1969	India, Delhi (Fungal spores)
(9)	Amin,R. & Bokhari,M.H.	1979	Iran, Shiraz (Fungal spores)
(10)	Sneller,M.R., Hayes, H.D. & Pinnas,J.L.	1981	U.S.A. Tucson, Arizona (Alternaria spores)

Table 2: "Settle Plate" Studies on the Fungal Spore Content of the Air

	Author	Year	Country and Place
(1)	Feinberg,S.M. & Little,H.T.	1936	U.S.A. Chicago Important as a pioneer study
(2)	Bieberdorf,F.W. & Hampton,S.F.	1946	U.S.A. Texas, San Antonio
(3)	Deamer,W.G. & Graham,H.W.	1947	U.S.A. California, San Francisco
(4)	Passarelli,N., De Maranda,M.P. & De Castro,G.	1949	Brazil, Rio de Janeiro
(5)	Toro,R.A.	1950	Puerto Rico
(6)	Burtness,H.I. & Allen (Soniae)	1950	U.S.A. California, Santa Barbara
(7)	Blackaller,A.	1950	Mexico,Guadalajara
(8)	Mendes,E. & Lacaz,C. Das	1952	Brazil, Sao paulo
(9)	Collier,T.W. & Ferguson, Betty,A.	1953	U.S.A. Georgia
(10)	Rooks, Roland & Shapiro Richard S.	1958	U.S.A. Iowa
(11)	Gueva,J., Tellez,G. Ortiz,L.,& Castillo Yolanda	1958	Mexico, Mexico City
(12)	Barkai-Golan,R.	1958	Palestine, Tel Aviv
(13)	Barkai-Golan,R. & Glazer, I.	1962	Palestine, Eilat and Tel-Hashomer

Continued/....

Table 2/Continued

	Author	Year	Country and Place
(14)	Lima,A.O., Seabra, O., Tavares Franca, A. & Cukier,J.	1963	Brazil, Rio de Janeiro
(15)	Barat, Roma & Das, A.C.	1963	India, Calcutta
(16)	Sandhu,D.K. Shivpuri,D.N. & Sandhu,R.S.	1964	India, Delhi
(17)	Liebeskind,A.	1965	Palestine, Haifa
(18)	Yousef,Y.A. & El-Tarabishy,N.M.	1966	Egypt, Cairo
(19)	Baruah,H.K. & Chetina,M.	1966	India, Gauhati
(20)	Dworin,M.	1966	U.S.A. Arizona, Tucson
(21)	Goodman,D.H., Northey,W.T., Leathers,C.R. & Savage,T.H.	1966	U.S.A. Arizona, Phoenix
(22)	Al-Doory,Y.	1967	U.S.A. Texas, San Antonio
(23)	Dupont, Eileen,M., Field,R.C. Leathers,C.R. & Northey,W.T.	1967	U.S.A. New Mexico, Metropolitan Area
(24)	Brunini,J.R. & Correa Celia De L.H.	1967	Brazil, Sao Paulo
(25)	Chabert,J. Nicot,Jacqueline	1968	Rabat, Morocco
(26)	Agarwal,M.K. Shivpuri,D.N. & Mukerji,K.G.	1969	India, Delhi

Continued/...

Table 2/Continued

	Author	Year	Country and Place
(27)	Ozkaragoz,K.	1969	Turkey, Ankara
(28)	Mishra,R.R. & Kamal	1971	India, Gorakhpur
(29)	Stalker,W.W. & Moore,P.M.	1972	U.S.A. Alabama
(30)	Chadli,A. & Hugues,J.N.	1973	Tunisia, Tunis
(31)	Papavassiliou,J.T. Bartzokas,C.A.	1975	Greece, Athens
(32)	Moustafa,A.F. & Kamel,S.M.	1976	Kuwait, Kuwait
(33)	Okuyan,M., Aksoz,N. & Varan,A.	1976	Turkey, Ankara
(34)	Yulug,N. & Kustimur,S.	1977	Turkey, Ankara
(35)	Barkai-Golan,R., Frank,M., Kantor,D., Karadavid,R. & Toshner,D.	1977	Palestine, Arad Desert Town
(36)	Hariri,A.R., Chahary,A., Naderinasab,M. & Kimberlin,C.	1978	Iran, Ahwaz
(37)	Al-Tikriti,S.K. Al-Salihi,M. & Gaillard,G.E.	1980	Iraq,Baghdad

Table 3: Investigations into the Fungal Spore and Pollen Content of the Air by Volumetric Methods

	Author	Year	Country & Place	Method
(1)	Rooks,R. & Shapiro,R.S.	1958	U.S.A. Iowa	Combination of electro-static precipitation with impingements
(2)	Rooks,R. Shapiro,R.S. Horman,E.C.	1960	U.S.A. Iowa	Volumetric sampler (impingement onto moving sticky tape)
(3)	Chabert,J. & Nicot,J.	1966	Morocco Rabat	Hirst Trap
(4)	Pady,S.M. Kramer,C.L. & Clary,R.	1967	U.S.A. Kansas	Slit sampler
(5)	Chabert,J. & Nicot,J.	1968	Morocco Rabat	Hirst Trap
(6)	Davies,R.R.	1969c	Kuwait Ahmadi	Hirst Trap
(7)	Al-Doory,Y.	1970	U.S.A. Texas San Antonio	Anderson Sampler
(8)	Salvaggio,J. Seabury,J.	1971	U.S.A. New Orleans	Roto slide sampler of Ogden & Raynor
(9)	Stalker,W.W. & Moore,P.M.	1972	U.S.A. Alabama Birmingham	Flag Sampler and Roto slide
(10)	Street,D.H. & Hamburger, R.N.	1976	U.S.A. California San Diego	Intermittent retractable arm rotorod

Continued/...

Table 3/Continued

	Author	Year	Country & Place	Method
(11)	Barkai-Golan, R., Frank,M., Kantor,D. Karadavid,R. & Toshner,D.	1977	Palestine Arad (desert town)	Casella-slit sampler
(12)	Calvo Torras, M.A., Guarro Artigas,J. Vicente,E. & Suarez Fernandez,G.	1978	Spain Barcelona	Slit-sampler
(13)	D'Amato,G. Stanziola,A. Liccardi,G. Loschia,M. & Melillo,G. Vo.	1982	Italy Naples	Burkard volumetric spore trap

SECTION II

AIR SPORA BAGHDAD

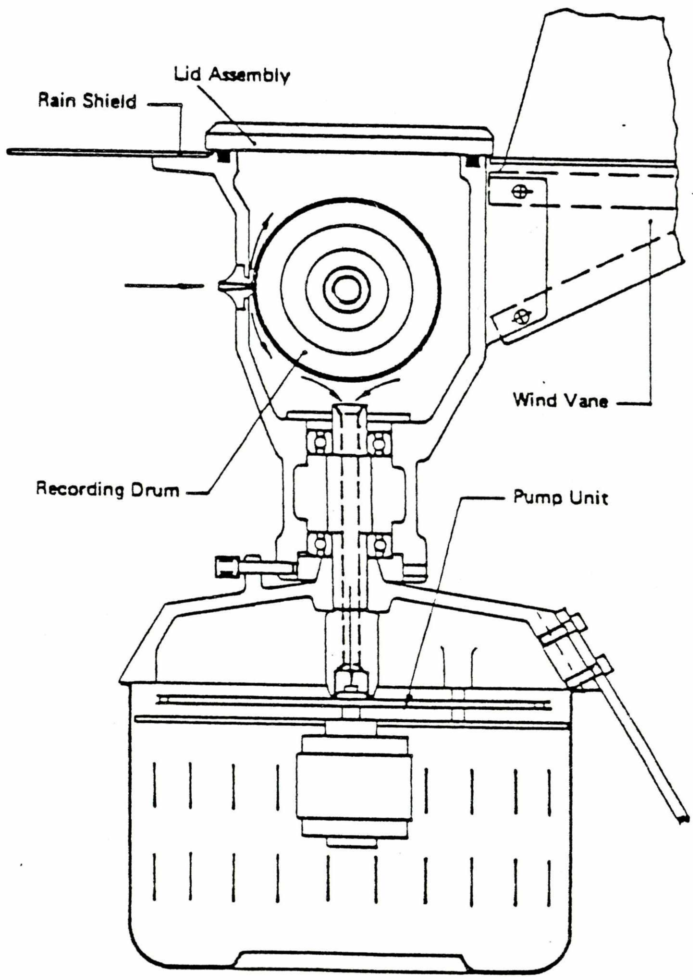


Fig 3. Burkard Seven-day Volumetric Spore Trap. Above on the roof of the Central Allergy Hospital Alwiah, Baghdad. Below - sectional drawing.

Fig 4: Containers used to transport drums bearing sticky faced tapes for exposure in the Burkard trap in Baghdad and their subsequent return to London. The bracket with a handle holds a drum carrying Melinex tape. The handle is rotated when a brush loaded with silicone/chloroform is applied so that a uniform layer of the grease is obtained. The clear plastic block inscribed at 48mm intervals was used to cut the exposed tapes into lengths appropriate to each day of exposure in the trap.

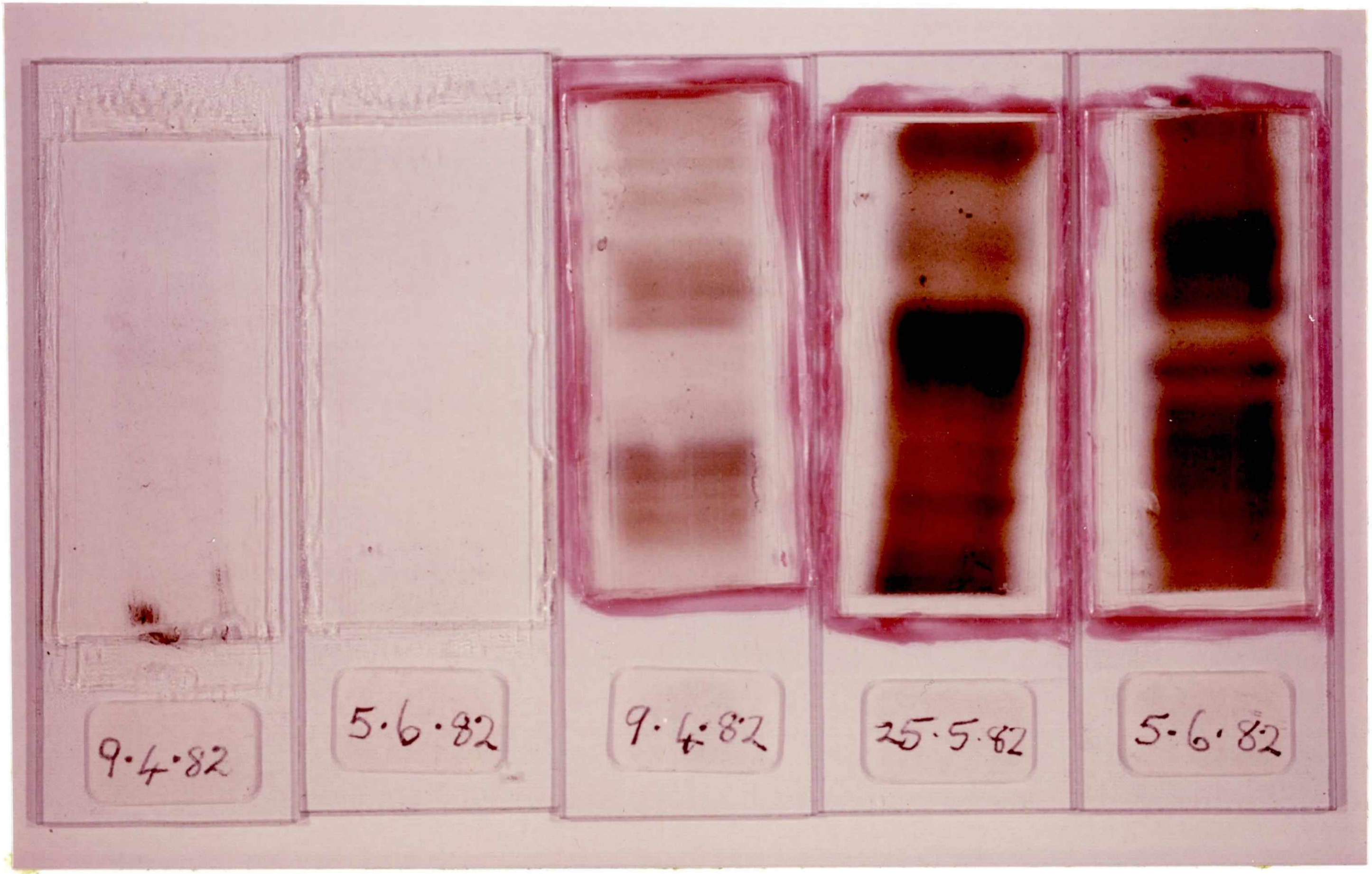


Fig 5: Appearance of deposits impacted from the air. The three slides to the right show deposits trapped from the air of Baghdad, the two to the left are from London. In consequence of a Clean Air Act (1956), the air of London now contains very little soot and ash. In contrast the air of Baghdad is polluted with soot and fine wind blown sand which on some days made the examination of slides for pollen and spores difficult.

AIR SPORA BAGHDAD

MATERIALS AND METHODS

The Burkard trap has been described (pp 38). Silicone grease was employed for the retentive surface, because it has outstanding heat stability over the range of 40°C to 200°C. Drums with the tape already prepared for exposure in the trap were packed in special airtight (Fig 4) cans and sent to Baghdad through the London office of the Medical Attaché, Embassy of the Republic of Iraq. After exposure in the trap, the drums were repacked in the special cans and returned to the office of the Iraqi Medical Attaché in London, from which they were collected. In the laboratory, the sticky faced Melinex tape was carefully removed from the drum, and lain on a perspex block with the exposed surface upwards. The block was inscribed at 48 mm intervals to facilitate the cutting of the tape into 48mm lengths. Each length of tape was carefully placed on a clean microscope slide and mounted beneath a 22 x 50 mm coverslip in gelvatol mounting fluid. Each slide was labelled with the date of the day of exposure in the trap and the 14 x 48 mm area of deposit was that impacted from the air on that day. When the gelvatol was set, the slide was ready for scanning under the microscope.

Visual identification of fungal spores and pollens

Specimen slides

A collection of pollen grains from identified plants was available in the Mycology Laboratory, St. Mary's Hospital Medical School, where an interest in aerobiology in relation to allergic disease has been maintained for many years. Specimen slides of fungal spores were prepared from a culture collection of common mould fungi. Slides exposed in a Hirst trap over a period of more than 20 years were available for study. Scanning was undertaken with a Leitz Laborlux microscope using a x40 objective and x10 eyepieces. When necessary fine details of spores and pollens were examined with a x54 oil immersion objective.

Reference works

When necessary during scanning reference was made to the following works with information on the identification of fungal spores and pollen grains.

- An Atlas of AIRBORNE POLLEN GRAINS, H.A. Hyde and K.F. Adams, (1958). MacMillan & Co. Ltd., London, New York, pp 112.
- THE MICROBIOLOGY OF THE ATMOSPHERE, P.H. Gregory (1961). Plant Science Monographs, Leonard Hill Books, London, pp 252.
- Air Sampling for Fungi, Pollens and Bacteria, R.R. Davies (1971) Chapter XIII in METHODS IN MICROBIOLOGY, Vol 4. Edited by C. Booth, Academic Press, London and New York, pp 795.

- A MANUAL OF CLINICAL ALLERGY, J. Sheldon, R. Lovell and K. Mathews (1953). W.B. Saunders Company, Philadelphia and London, pp 413.
- ATLAS OF EUROPEAN ALLERGENIC POLLEN (Atlas Européen des pollens allergisants). J. Charpin, R. Suriniyach, A.W. Frankland (1974). Sandoz, Editions, pp 229.
- ATLANTE DEI PRINCIPALI Pollini allergenici presenti in Italia. F. Ciampolini, N. Cresti (1981). Universita di Siena, pp 190.

Photographs of Vegetation

Plants growing in the vicinity of the trapping site in Baghdad were photographed and as necessary reference made to:-

- Flora of Iraq, Vol. 1. Edited by Evan Guest (1966) Baghdad Min. Agric. Rep. Iraq.
- Wild Plants of Iraq with their Distribution. Ali Al-Rawi (1964) Baghdad Government Press.

Pollen Collections from Baghdad

Specimens were obtained from plants flowering in Iraq at different times, and the pollen obtained by crushing the anthers in lactophenol on a microscope slide. Specimens of pollen were obtained from plant collections in the Herbarium of the Royal Botanical

Gardens, Kew. this facility was invaluable and enabled the pollen of Ziziphus trapped from the air to be compared with those of the three species of Ziziphus.

Drawings of pollens in the deposits and from herbarium specimens were made by means of a camera lucida to obtain the correct proportions and size of the pollen.

Scanning and Calculations

During a 24h period of exposure in the trap, with an air flow of 10 l/min, a total of $14.4 m^3$ of air is sampled. The particulate matter from this volume is impacted into a deposit which measures 48 x 14.4 mm. The deposit is wider than the orifice as there is a slight scattering of the particles due to the distance between the sticky surface and the inner edge of the orifice. Scanning was undertaken with a mechanical stage.

The width of the microscope field scanned parallel to the length of the deposit to obtain the mean daily concentrations was 0.36 mm.

Total volume of air sampled in 24h = $14.4 m^3$
Width of deposit = 14.4 mm
Multiplication of the number of spores or pollen grains counted along the traverse

gives the number per M3 of air

0.36 x 3 = 10.8 mm

The trap is approximately 80% efficient and with this in mind the concentrations given tend to be underestimates.

Changes in the diurnal concentrations of *Juniperus*, *Ziziphus*, *Grass* and *Urtica* were calculated at 2h intervals as follows. As the suction rate (10 l/min), the cross sectional dimensions of the orifice (2 x 14 mm) and the rate of movement of the drum (2 mm/hr) are known, the number of spores or pollens counted in a 360 micron traverse can be converted into an estimated number per cubic metre of air.

Suction rate = 10 l min

= 600 l in 1 hour

2 mm trace deposited from 600 l air

0.36 mm trace deposited from 108 l air

1 spore or pollen grain in 108 l is taken as 9 per cubic metre. As the trap is never 100% efficient this figure tended to underestimate the actual number present and in practice a count of one spore in the deposit was taken as equivalent to 10 spores per cubic metre of air.

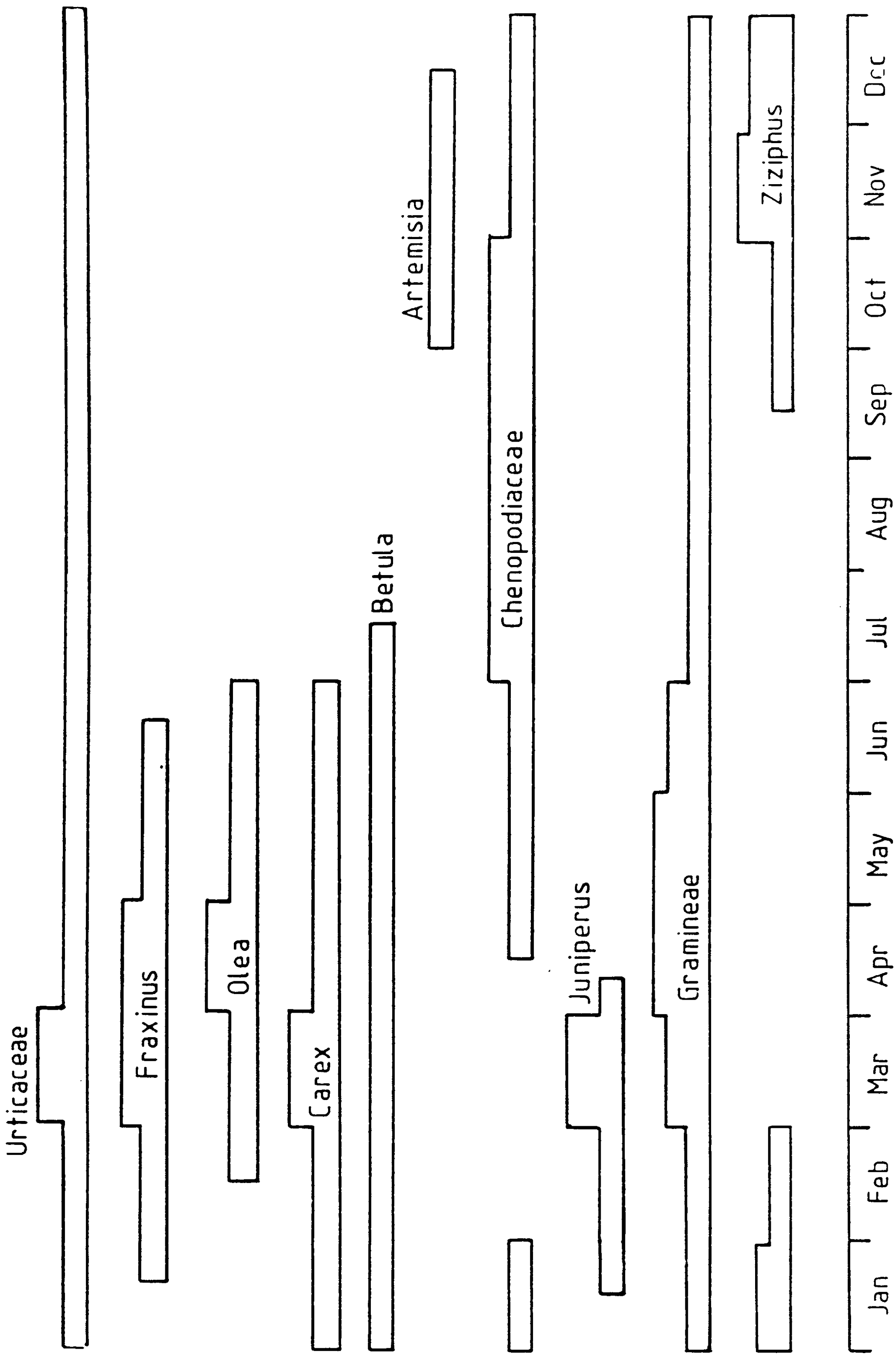


Fig 6: The Baghdad pollen calendar, showing peak months and months of the year when the genera of pollen were recorded.

RESULTS

The Pollen Calendar

The pollen content of the air of Baghdad is summarized as a pollen calendar in (Fig 6) which shows the months of the year in which the genera were trapped from the air, together with the seasonal increase. The calendar is in no sense quantitative and the mean daily concentrations for the different form genera are given below and discussed separately in relation to both numerical and allergenic importance.

The calendar (Fig 6) shows that pollen from the Gramineae and the Urticaceae were trapped from the air in all the months of the year. Pollen from the Chenopodiaceae was only absent from the air in February and March. This pollen was unique in that the concentrations rose in July and August (Fig 10) when the Summer temperatures were at their highest. Pollen from tree pollens of the genera Betula and Fraxinus were recorded in the air between January and June, Juniperus - February until April and Olea - February to June. Carex was recorded from January to June, Artemisea from October until December, and Ziziphus from September until February.

In temperate countries, sources of pollen allergens are generally attributed to Catkin bearing trees in the Spring, the grasses in the Summer and herbaceous plants and "weeds" in the Autumn. This pattern of pollen production in Iraq is complicated by the intense heat of the Summer months, which is inimical to floral development. It is noteworthy that whilst Artemisia has its season in the Autumn, the peak for the Urticaceae is in the Spring, and Ziziphus flowers in the Autumn and Winter.

The importance of these pollens as a cause of allergic disease in Iraq must be considered in relation to Thommen's (1931) Postulates.

1. The pollen must contain an excitant of hay-fever.
2. The pollen must be anemophilous or wind borne as regards its mode of pollination.
3. The pollen must be produced in sufficiently large quantities.
4. The pollen must be sufficiently buoyant to be carried considerable distances.
5. The plant producing the pollen must be widely and abundantly distributed.

As the present study is based on pollen grains trapped from the air, postulates 2, 3 and 4 are met. Postulates 1 and 5 are considered in the detailed discussions below.

POLLEN

Gramineae - Grasses

Results

In Baghdad, the peak season for grass pollen commenced in early March and lasted until the end of June, in both 1982 and 1983 (Figs 7,8).

In 1982 the highest mean daily concentration of 48 grains/cubic metre was recorded on April 6th, in 1983 the highest concentration was 36 grains/cubic metre on April 15th. The mean daily concentrations given in (Figs 7,8) show two peaks of flowering, an April peak which is the highest followed by a second peak in May. In both 1982 and 1983,this second peak was on May 16th.

In the very hot months (Fig 6) in July grass pollen was trapped from the air on 11 days and in August on 2 days. In September, as it became cooler, grass pollen was trapped on 21 days, in October on 20 days and November, 22 days. In the Winter months grass pollen was recorded on 4 days in December, 18 days in January and 10 days in February. 64% of the annual grass pollen catch was obtained during the 4 month season, and 36% during the other 8 months. Grass pollen comprised 17% of all the pollens trapped from the air of Baghdad and its proportion in the total catch is shown in (Fig 9).

Discussion

Two peaks of pollen production consequent upon an

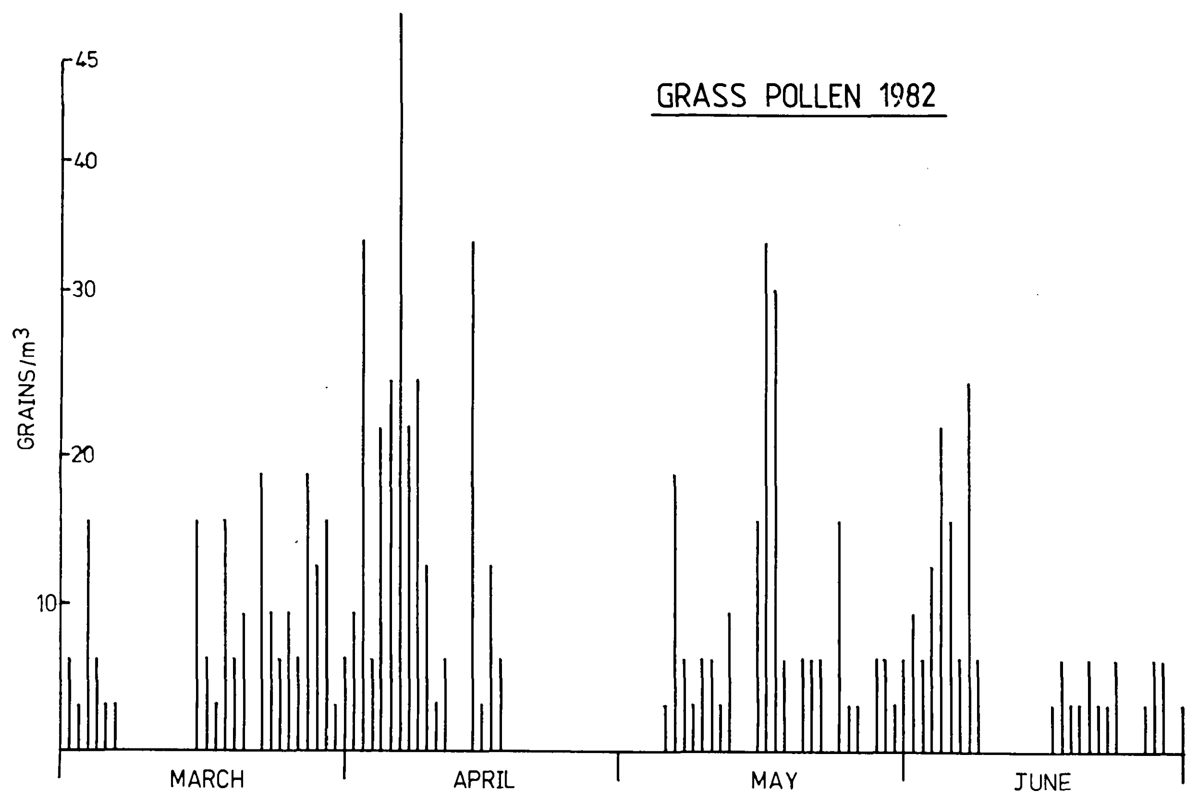


Fig 7: Mean daily grass concentration in the air over Baghdad in 1982.

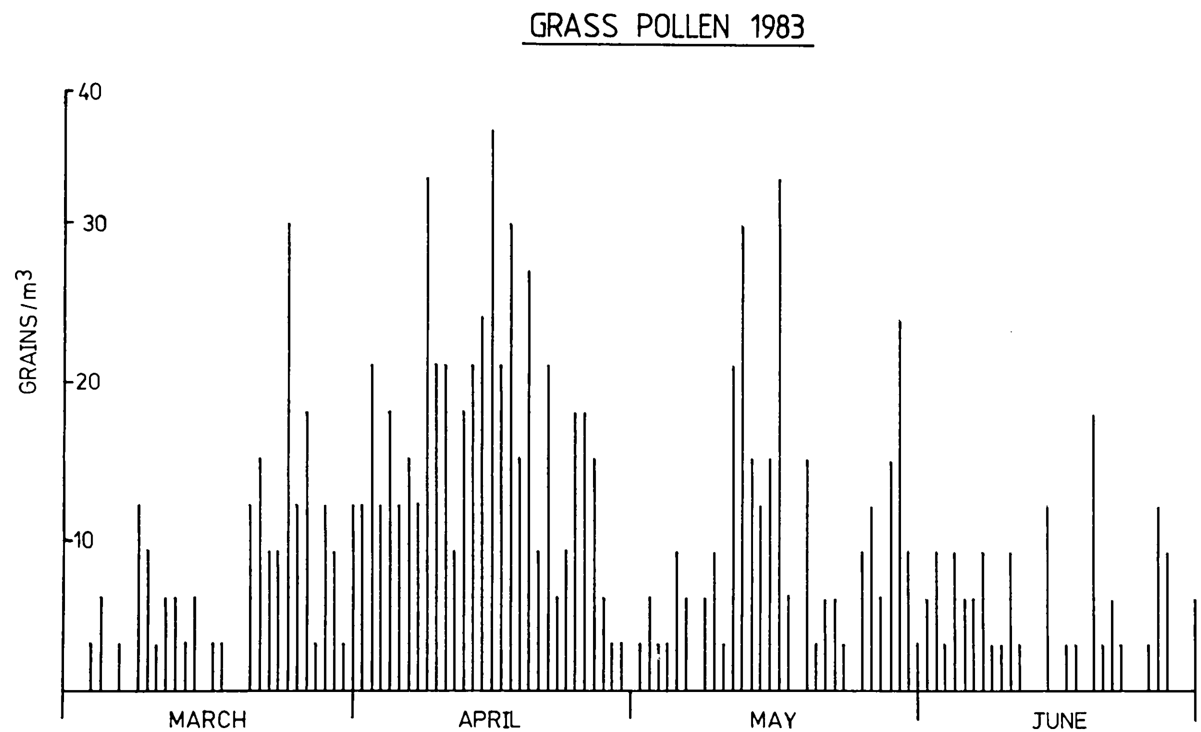
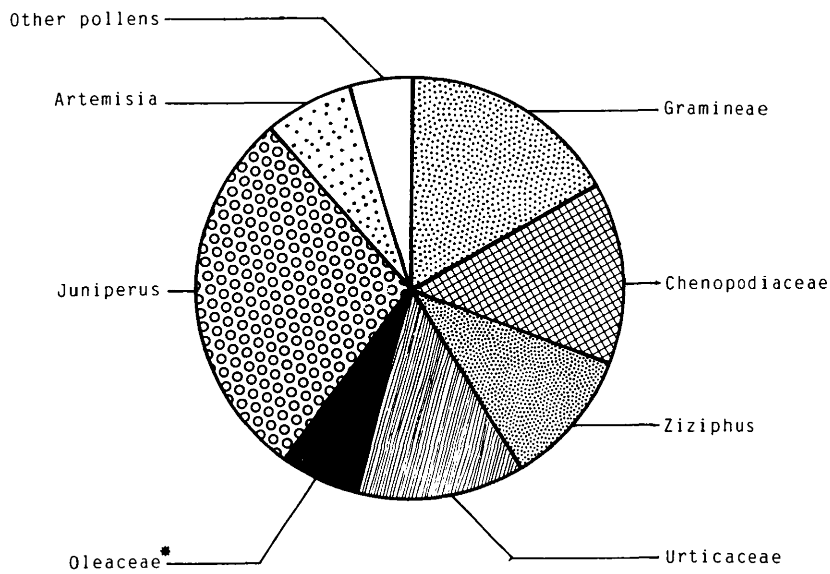


Fig 8: Mean daily grass pollen concentrations in the air over Baghdad 1983.



*Oleaceae includes both Olea & Fraxinus.

Fig 9: Principal pollens as a proportion of the total pollen catch from the air of Baghdad.

early and late peak of flowering is consistent with the results obtained by Bagni et al (1976) in a study of the grass pollen content of the air over European cities. In 1973 at Bologna (geographical position 44, 31°N) there was an April and May peak, in Brussels (50, 48°N) and London (51, 29°N) June and July peaks. Baghdad (32, 20°N) lies further South than Bologna and the grass pollen season clearly commenced in March, whereas in Bologna in 1973 on only one day was grass pollen recorded during the first fortnight of April. Davies and Smith (1973a) report that when the sum of the mean air temperatures for April and May at four stations around London reached 21°C, the grass pollen season commenced on June 4th. In Southern England, when the sum of the mean of the maximum and minimum air temperatures for April and May reaches 21°C, there has been an exceptionally warm Spring, and this ensures an early start to the grass pollen season in London. In Baghdad, the sum of the mean air temperatures for January and February (Fig 2) averaged over a 60 year period was 21°C.

The warm temperature of the very early Spring in Baghdad facilitates an early onset of grass pollen production in March. On a basis of the 2 years studied the grass pollen season in Baghdad extends over 12 weeks. It rarely extends for more than 6 weeks in London (Davies & Smith 1973b). The grass pollen concentrations recorded in Baghdad are very much lower than those recorded in the European cities (Bagni et al 1976, Davies & Smith 1973b). The lower concentrations in

Baghdad should not be taken to imply that grass-pollenosis is less of a problem than in European cities.

There is a distinct season, and the fact that the pollen concentrations are much lower than those experienced in European cities must also mean that the degree of hypo-sensitisation which the sufferer obtains from the natural challenge may be lower than that experienced in the pollen sensitive populations to the North. The pollen allergic population of Iraq is probably highly sensitive in March and April and since Davies (1973) reports an apparent decline in sensitivity during the season, the Iraqi patients are probably less sensitive by the end of June. Clearly a clinical investigation of this is necessary.

Another feature is that apart from the hottest month, grass pollen was likely to be encountered at any time. (Cynodon dactylon) Bermuda grass is common and this grass species is adapted to growth at high temperature. Although a poor pollen producer it is an important allergen and may be responsible for the almost constant presence of grass pollen in very low concentrations throughout the year in Baghdad (Fig 6). The pollen of Cynodon dactylon is known to give positive immediate responses in the skin-prick test in Iraqi patients (Personal Communication Dr. Basil Behnam). The grasses which produce the pollen clouds in the March to June season show early and late flowering peaks similar to that seen with the Pooid grass species in Northern

Europe.

In London the early June peak is exemplified by the copious pollen production of Dactylis glomerata, and a later peak typified by Phleum pratense.

The wheat and other cereals grown in the central alluvial plain and marsh areas of Iraq are unlikely to be important in causing allergic disease. Wheat, oat, barley and rice are normally self pollinated, the major part of the pollen produced by a floret remains within the floret, fertilization often occurring by the direct extension of a pollen tube through the wall of the anther sac to the stigma (Thommen 1931).

The grasslands of England and Wales comprise the major source of airborne grass pollen in the U.K. and Davies & Smith (1973b) from a study of airborne grass pollen concentrations, recorded in central London between 1961 and 1970, related the catch to wind direction and the percentages of land under grass in the "home counties" to the NW, NE, SE and SW. The prevalance of hay fever differs in different parts of the British Isles. (Morbidity statistics for general practices, 1962) and this has been related to climate topography and land useage (Davies 1969b). In areas of rough grazing and mountainous areas, less pollen is produced and the climatic features of the valleys ensure high convection with dilution of pollen concentrations through a greater depth of air than occurs from comparable sources on a plain (Davies 1969b). In England a series of grassland surveys have shown that most of the grasses in the swards are native to Britain. About 6 percent of the grassland and enclosed rough grazing area of England and Wales is reseeded annually (Hopkins, 1979). The grasses sown can be determined from Ministry of Agriculture Fisheries and Food statistics. Comparable statistics are not yet available for Iraq, and it has not yet been possible to determine the species of grass sown for pasture in Iraq. Hopkins (1979) reports that over 80 percent of the grass seed currently sown in British agriculture consists of rye grasses, two thirds *Lolium perenne* (Perennial rye grass) and one third *Lolium multiflora* (Italian rye grass) and hybrid rye grasses. Of the other grass species *Phleum pratense* has now surpassed *Dactylis glomerata* in relative importance and its use at about 5-7 percent by weight of the total grass seed used has remained fairly constant.

The capacity of the individual average plant to produce pollen was estimated by Thommen (1931) who gave *Ambrosia trifida* (giant ragweed) the most productive hay fever plant in the USA a score of 100. In comparison, *Dactylis glomerata* was given a value of 80, *Phleum pratense* 60, *Poa pratensis* 50 and *Agrostis alba* 45. Although accurate data is not available, it is unlikely that *Lolium* species have a greater pollen production than *Agrostis alba* (personal communication R.R. Davies).

In SE England the hay fever challenge is characterised by two peaks of pollen production, and in any season, although the onset of the pollen challenge is determined by the weather, Dactylis glomerata always sheds its pollen before anthesis commence in Phleum pratense. In Iraq Dactylis glomerata is common only in the upper forest zone, with penetration into the lower margin of the thorn cushion zone.

The grasses sown for fodder in the British Isles are all classified in the Pooideae of Clayton (1981), and since the pioneer studies of Dunbar (1903) and Noon (1911) employed pollen extracts from Pooid species, research into the allergenicity of grass pollen has been mainly confined to common Pooid species with the noteable exception of Cynodon dactylon of the Chlorideae. The fodder grasses of Iraq are listed in Table (4) P75. The three species described as very commonly grown for fodder by Bohr and Guest (1968) together with Aegilops speltoides, Avena fatua, Lolium rigidum, Phalaris minor and Secale montanum are all classified in the Pooideae. It is likely that these grasses will be found to be antigenically similar to those causing hay fever in the British Isles and N.Europe, but clearly an experimental investigation is desirable. The observation that hay fever is a seasonal disease in Iraq is to some extent indicative of the importance of these Pooid grasses since Cynodon dactylon probably pollinates throughout much of the year. _Avena fatua_ occurs as a common weed in British agriculture, _Lolium rigidum_ is a native of the Mediterranean and has been observed in Britain by Hubbard (1968) who also reports _Phalaris minor_ as being of casual occurrence on waste land. The contributions of these three species to the total grass pollen challenge in England is likely to be extremely small whereas in Iraq they are probably important. _Cynodon dactylon_ is of rare occurrence in coastal sandy areas in S.England where it is unlikely to be of allergic significance.

Evidence of a common antigenicity which extends throughout the grasses can be found in reports where patients have been challenged with extracts from a wide variety of grasses, and from serological studies. Noon (1911) tested the pollen of _Phleum pratense_, _Poa trivialis_, _Holcus lanatus_ and _Agropyrum caninum_ with the opthalmo-reaction of Dunbar (1903). Although all reacted in the conjunctival sac of patients who suffered from hay fever, it was pollen from _Phleum pratense_, which gave the most active extract. Freeman (1911) tested pollen from rushes, sedges and grasses and concluded none gave so strong an opthalmic reaction as _Phleum_. He observed that the ratio between the strength of an extract of _Phleum pratense_ pollen and that of _Alopecuris pratensis_ was 4:1. Furthermore, "a patient inoculated with Alopecuris pollen vaccine is found to become immune to Phleum pollen extract as tested by the opthalmo-reaction. Apparently, therefore, we need not select different types of pollen for treating different patients".

In a later study in which a skin test was used to examine the spectrum of antigenicity in the grasses, Thommen (1931) employed grass pollen extracts from 81 species representing 50 genera. These species may be classified as Pooideae 49, Panicoideae 19, Chlorideae 6, Arundinoideae 4, Cynodonteae 2, and Bambusoideae 1. With 30 patients clinically sensitive to grass pollen, in each instance a positive reaction of varying degree was obtained whilst in 8 normal subjects all the tests were negative. It was found by Scheppegrell (1923) cited Thommen (1931) that with a prolonged series of tests with over a hundred varieties of grass pollens, all the grasses had a similar hay fever reaction. The similarity differed in degree, but there was no patient sensitive to one of the grass pollens who did not react to the other grass pollen extracts with which he was tested. Both Thommen and Scheppegrell employed an intracutaneous test. They considered that absorption by the cutaneous method makes the result less dependable. It is another view that the intracutaneous test gives a greater number of non-specific reactions. Moreover, Thommen's results are open to the criticism that his tests were performed "prior to the usual pre-seasonal course of treatment".

In the likely event his 30 patients had previously been injected with grass pollen extracts their skin test responses could have been modified.

Apart from Cynodon dactylon, none of the fodder grasses listed as common or very common in Iraq are to be found in the list of 81 species from which pollen was obtained and tested by Thommen (1931). Weeke & Lowenstein (1973) have shown the presence of six antigens in the pollen of Phleum pratense which migrate towards the anode and bind serum IgE from sensitised persons. Watson & Knox (1976) in a study on the cross-reactivity of 46 grasses to a rabbit antiserum against Lolium perenne found the six precipitin lines detected for L.Perenne were also obtained with Phleum pratense, and only one of the lines of identity was not found in an extract of pollen from Dactylis glomerata.

Thus, the Phleum and Dactylis extracts of Freeman (1950) contained all the common antigens detected in precipitin tests with a rabbit antiserum to Lolium perenne, and L.multiflorum and hybrid rye grasses are the most widely grown pasture grasses in the British Isles.

The Pooid grasses tested by Watson & Knox (1976) included 26 species from 17 genera, and all showed at least one reaction of identity and one of partial identity with the pollen of L.perenne. Watson & Knox also examined 5 genera and species in the Chloridoideae, Cynodon dactylon showed 2 reactions of identity and 2 of partial identity. Four genera and species were tested in the Arundenoideae, Stipa falcata showed 1 line of identity and 3 of partial identity Cortarderia sellanoa showed 2 lines of identity and 2 of partial identity whereas Phragmites australis showed 2 of identity and 1 of partial identity. Danthonia eriantha gave no precipitin response against the Lolium antiserum. Pollen from Stipagrostis plumosa the only common fodder grass in Iraq which belongs to the subfamily Arundenoideae has neither been tested in this type of serological study nor does it appear to have been employed in a skin-prick test.

Eleven genera and species of the Panicoideae were tested and showed fewer shared antigens with Lolium. An antigen present in all the Pooid grasses tested by Watson & Knox (1976), present in 3 of the Chloridoid and 3 of the Arundinoid grasses was notably absent in the Panicoideae.

The Panicoid grasses of Iraq are second only in importance to the Pooid species and the common fodder grasses, Echinochloa Crus-galli, Chrysopogon gryllus, Hyparrhena hirta and Imperata cylindrica belong to the Panicoideae. The pollen of not one of these common Iraq grasses has been tested serologically in the manner of

Watson & Knox (1976).

Moreover, apart from Cynodon dactylon pollen extracts from the grass species commonly used as fodder in Iraq (Table 4) are not commercially available, with the result that even the responses of Iraqi patients to their own native grass pollens in the skin-prick test is unknown.

Table 4: Fodder Grasses of Iraq (From Bohr & Guest, 1968)

VERY COMMON

Aegilops kotschyi Boiss, Aegilops triuncalis L., Stipa capensis Thumb.

COMMON

Aegilops speltoides Tausch., Avena fatua L., Chrysopogon gryllus L. Trin., Cynodon dactylon (L.) Pers. Echinochloa crus-galli (L.) P. Beauv., Hyparrhena hirta (L.) Stapf. Imperata cylindrica (L.) P. Beauv., Lolium rigidum Gaud., Phalaris minor Retz., Stipa barbata Desf., Stipagrostis plumosa (L.), Munro ex T.anders.

Clayton (1981) has classified the Gramineae into 6 sub-families, the Arundinoideae, Bambusoideae, Centothecoideae, Chloridoideae, Panicoideae and Pooideae. Those listed belong to the Pooideae, with the exception that Stipagrostis plumosa is classified in the Arundinoideae, Cynodon dactylon in the Chloridoideae, Echinochloa crus-galli, Chrysopogon gryllus, Hyparrhena hirta and Imperata cylindrica are classified in the Panicoideae.

Chenopodium

Results

In Baghdad pollen from the genus *Chenopodium* was recorded in the air from the middle of April until the end of the year (Fig 6). In January 1983 Chenopod pollen was trapped on only 4 days, this pollen was not found in either February or March (Fig 6).

This pollen comprised 14% of the total annual pollen catch in Baghdad (Fig 9), and showed a seasonal occurrence in the air which extended from July until the first week of November (Fig 10). The peak month in 1982 was September, when the highest concentration of 48 grains/m^3 was recorded on the 14th (Fig 10). 77% of the Chenopod pollen was trapped in the peak season and clearly the *Chenopodiaceae* are an important source of atmospheric pollen in Baghdad. The aerobiological evidence shows the *Chenopodiaceae* are well adapted to withstand the hot summer. It should be noted that there is a gap in the records from August 11th to 17th, the electricity supply to the equipment was disrupted by the war.

Discussion

Baghdad lies on the banks of the Tigris in the central alluvial plain and although much of the pollen trapped may be local in origin, wind borne pollens may have their origins in the Eastern Alluvial District, Gurfa

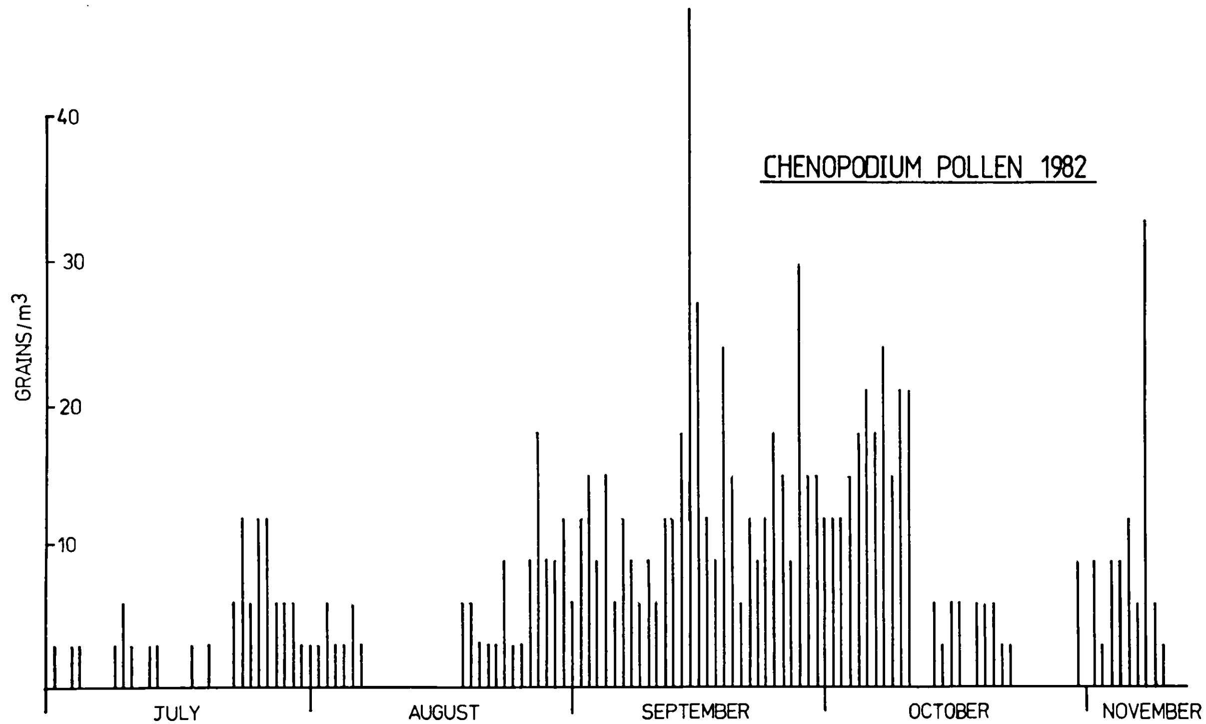


Fig 10: Mean daily concentrations of chenopod pollen in the air of Baghdad in 1982.

or lower Jazira District, or the Western Desert. As a percentage of the total pollen catch, the 14% recorded in Baghdad may be contrasted with the report of Davies (1969c) that pollen from the Chenopodiaceae comprised 66% of the catch at Ahmadi, a new town in the desert in Kuwait. Davies (1969c) associated the Chenopod pollen in the air of Kuwait with Salsola baryosma (Roem et Schult), Dandy and a Thorny Shrub Cornulaca leucacantha Charif et Aellen. Both these plants are common weeds in the desert and had been seeded in the vicinity of Ahmadi to bind the sand. Guest and Al-Rawi (1966) report the commonest plant association in the deserts of Iraq to be Haloxyletum salicornici. This covers vast tracts of the Southern and Western Deserts and is wide spread in the lower Jazira. It is therefore probable that Haloxylon salicornicum (Moq.) Burge is an important source of the chenopod pollen which occurs in the air of Iraq, and it is recommended that extracts of this pollen for skin-prick testing should be prepared for use in a survey of pollen sensitivities among the Iraqi people.

A local source of chenopod pollen could be the varieties of cultivated Beta maritima which are abundantly grown in the gardens and small farms in the area of Baghdad.

Al-Ani and Haddad (1975) in the list of the Herbarium, Department of Plant Protection, College of Agriculture, University of Baghdad, list 22 genera and 69 species, whereas Al-Rawi (1964) lists 25 genera and 82 species in the Chenopodiaceae of Iraq.

Since pollen of the Chenopodiaceae is likely to be an important cause of allergic disease throughout the Middle East, Al-Rawi's check list of this family is given in the appendix, together with details of the Districts of Iraq where the plants were found; these districts are shown in Fig 1.

The pollen grains of the Amarantaceae are morphologically similar to those of the Chenopodiaceae. Since the genera Amaranthus and Alternanthera are not mentioned in the introduction of the Flora of Iraq (Guest and Al-Rawi, 1966) it may be concluded that they are of little importance in the comparison with the Chenopodiaceae. However, a list of the genera and species in the Amarantaceae is included in the Appendix.

Allergenicity of Chenopod pollens

Immediate positive skin-prick tests to the pollen of Chenopodium album are common in patients from the Middle East (A.W. Frankland, personal communication). Selles et al (1982) related the aerial occurrence of chenopod pollen to the clinical sensitivity of 10 patients with pollenosis in Spain. Skin-prick tests with a pollen extract of C.album were positive. Selles et al (1983) studied the allergenicity of the pollen extract from 4 species of chenopod (C.album, Salsola kali, Beta vulgaris, and Atriplex latifolia) and one species of the Amarantaceae

(Amaranthus retroflexus) by skin-prick tests on patients sensitive to C. album, RAST inhibition tests using discs sensitized with the C. album extract and a serum pool from Chenopodium sensitive patients, CIE and CRIE techniques using a rabbit anti-serum to Chenopodium. With slight differences in activity all the pollen extracts gave positive skin responses. RAST inhibitions were reported to be in agreement with the skin-prick tests. All the extracts were able to inhibit the binding of specific IgE to Chenopodium sensitized discs suggesting that common allergen(s) are present in the 5 species tested. CIE techniques showed the presence of common antigens. Incubation of the CIE immunoplates with the human serum pool used in the RAST experiment and ^{125}I-anti IgE (CRIE) showed a few allergens were shared by the 4 chenopod and 1 amaranth tested. It was concluded that there were common allergenic determinants among some species of Chenopodiaceae and Amarantaceae and the possibility of specific allergenic determinants cannot be ruled out.

Gupta et al (1970) investigated the atopic relationships amongst 3 species of Chenopodium, C.album, C.murale, C.album(Tetraploid) and C.ambrosioides and 2 species of Amaranthus, A.spinosus and A.hybridus. The pollen extracts were prepared in a 1:50 W/V concentration, buffered saline was used as a control, and the pollen extracts were tested in healthy volunteers. Positive intradermal skin reactions were confirmed by repeating the tests the same day.

The patients tested suffered from bronchial asthma and/or allergic rhinitis. 100 patients were tested with the pollen extracts of the *Chenopodiaceae* and 103 with those from the *Amarantaceae*. 31.5% of the patients reacted to species of chenopod pollen, 27% to *C.album*, 41% to *C.murale* in contrast with 7.2% in healthy volunteers. 28.2% of the patients gave positive skin responses to the 2 species of *Amaranthus* in contrast with 9.2% of healthy volunteers. The fact that 9.2% and 7.2% of healthy volunteers gave positive skin responses may be held to support the view that the intradermal skin test gives non-specific responses. It was concluded that species specific allergens occurred in the *Chenopodiaceae* but the evidence was insufficient to draw any conclusions concerning the *Amarantaceae*.

The cross reactivity among weeds of the Amaranth and Chenopod families has also been investigated by Weber et al (1978). However, this study is difficult to understand since a number of the plants are referred to by non-specific names such as smotherweed, greaseweed, allscale, and it was found impossible to determine the precise identity of some of these species.

Thommen (1931) reported 4 species of *Amaranthus* as causing hay fever in the U.S.A., *A.retroflexus*, A.spinosus, A.graecious and A.palneri. Not one of the species listed by Thommen (1931) occurs in Iraq. Apart from A.hybridus which was studied by Gupta et al (1970) the Amaranthus species in Iraq have never been investigated as a cause of allergic disease.

Ziziphus

Results

Ziziphus pollen was trapped from September until February (Fig 6) the main season occurring in the months of November, December and January. The seasonal mean daily peak concentration of 69 grains/m^3 of the air was recorded on Nevember 15th (Fig 11) the Ziziphus pollen comprised 10.5% of the total annual pollen catch in Baghdad (Fig 9), 85% of this pollen was trapped during the November, December and January season. Ziziphus pollen was first trapped on the 18th of September and was found on only 3 days in this month. The pollen was recorded on 11 days in October and 12 days in February.

Discussion

When it was first observed this pollen (Fig 13) could not be identified. It is not illustrated in the reference works listed on page 54. As an aid to identification the pollen in the deposits trapped from the air was measured and drawn with a camera lucida (Fig 13), and photographed. The silicone grease used as a retentive surface together with the amounts of soot and fine sand

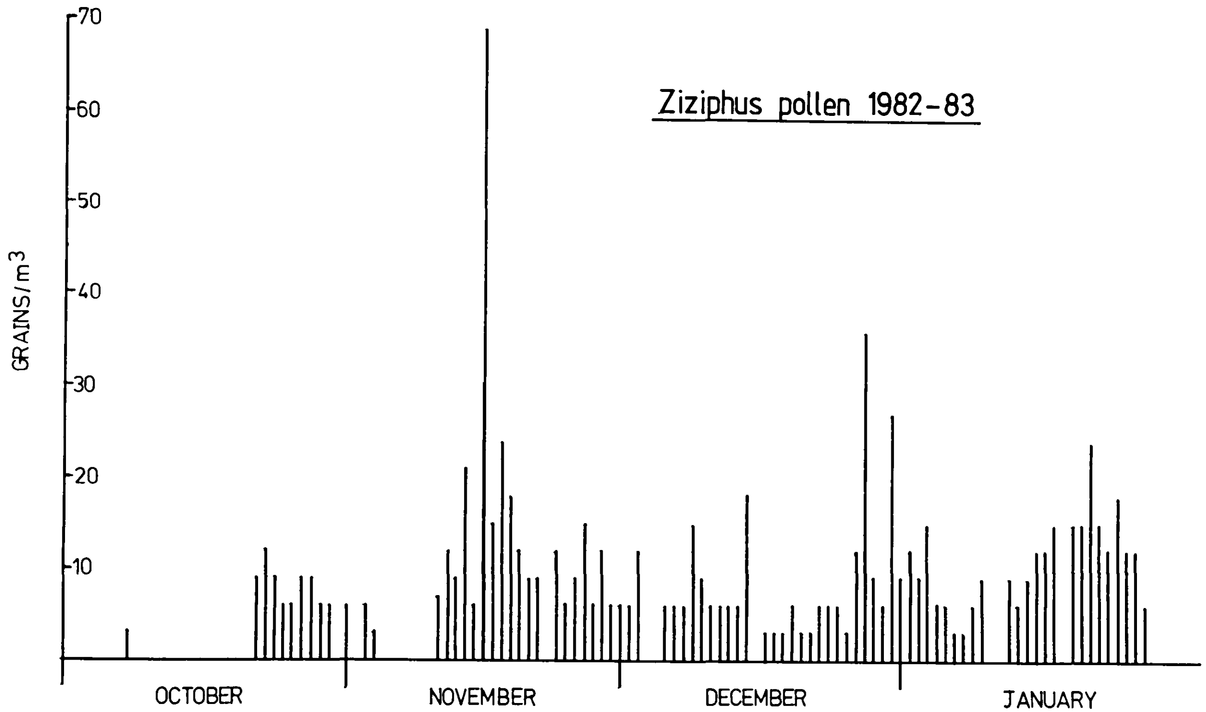
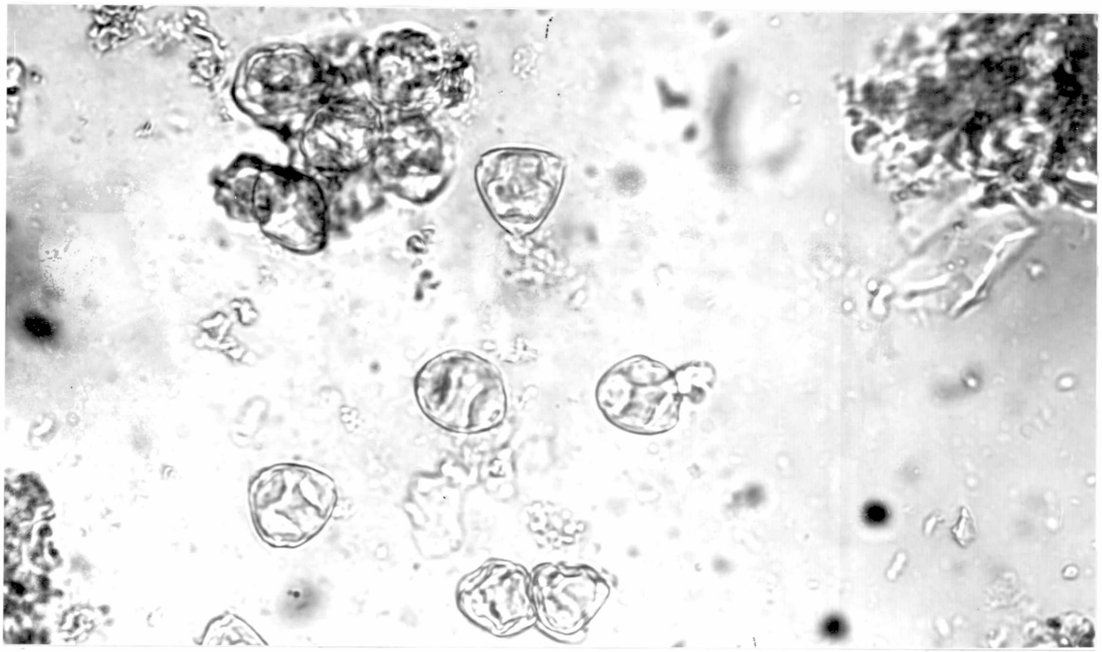
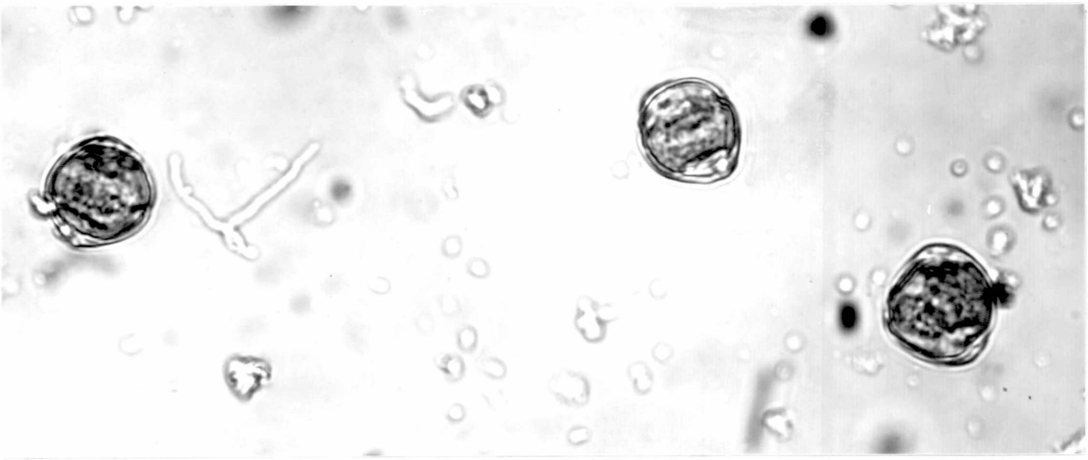


Fig 11: Mean daily concentrations of <u>Ziziphus</u> pollen in the air of Baghdad between October 1982 and January 1983.



Ziziphus jujuba

Ziziphus nummularia



Ziziphus spina-christi

Fig 12: Pollen grains from 3 species of Ziziphus

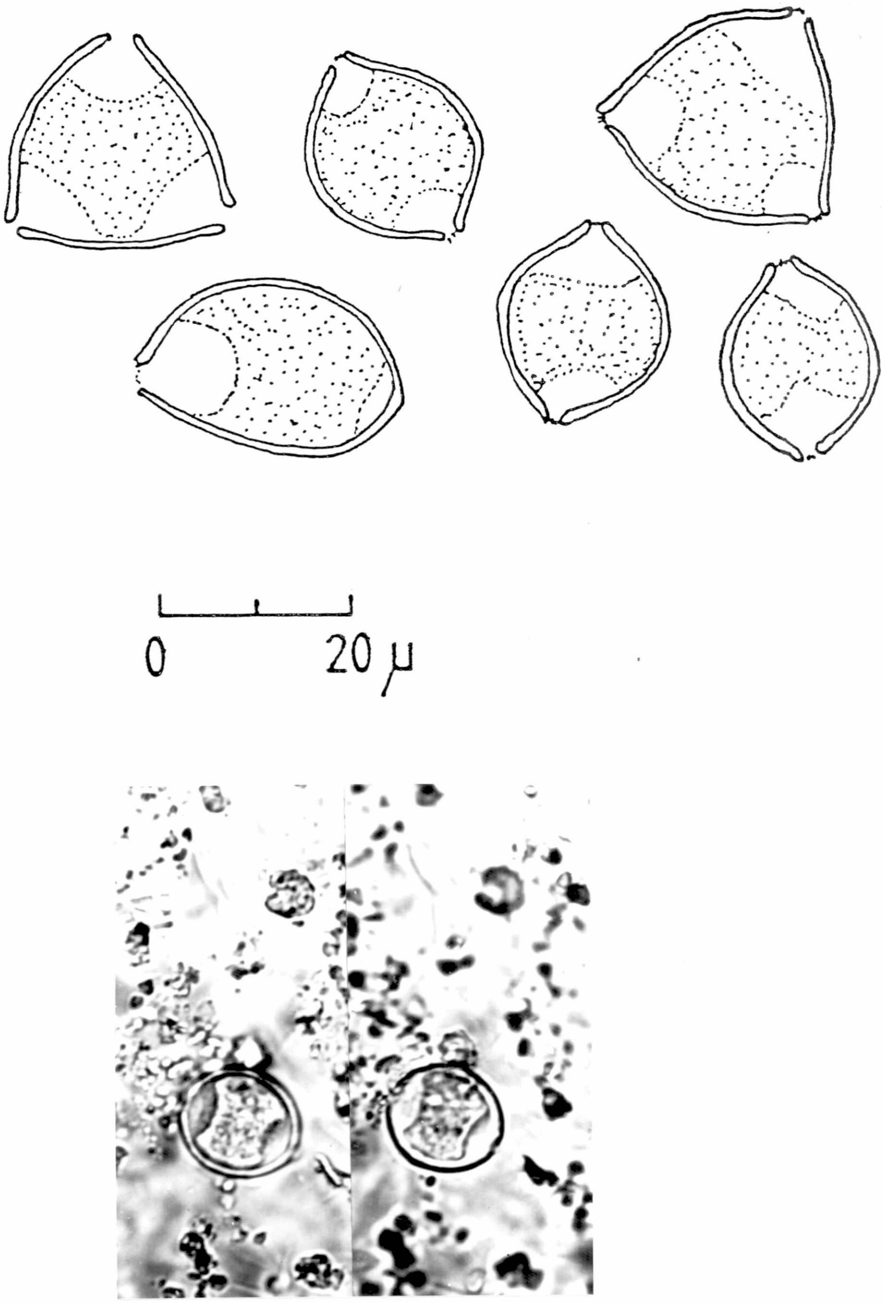


Fig 13: Above camera Lucida drawing of Ziziphus jujuba. Below appearance of Ziziphus pollen grains in deposit trapped from air and provisionally identified as Ziziphus jujuba.

Fig 14: _Ziziphus_ trees in gardens and in streets of Baghdad

on the slides rendered photography difficult as many of the grains were partially occluded. Photomicrographs of Ziziphus pollen grains trapped from the air are shown in (Fig 13). Photomicrographs of the pollen grains of Ziziphus jujuba, Ziziphus nummularia and Ziziphus spina-christi from material supplied by Dr. I.K. Ferguson, Herbarium, Royal Botanic Gardens, Kew, may be seen in (Fig 12).

The anthers were teased out of the dried flowers and crushed in lactophenol. The appearance of the grains in squash preparations differs from that when the pollen is impacted onto a sticky surface from the air. In its aerial transmission the grain adapts an in flight attitude with the result that on impaction most of the grains in the deposit show two pores. The grains have three pores and when squashed from anthers the greatest number show three pores. The grains are triporate, suboblate or oblate and small. In optical section the exine is thin with thickening around the pores, the external surface of the exine is smooth and the intine beneath the pores appears to be granular. The pollen grains of Z.jujuba are colourless, those of Z.nummularia and Z.spina-christi are yellow. The Ziziphus grains seen on the slide are colourless. The measurements of pollen grains from the three species are Z.jujuba, 17-21μ x 18-22μ, Z.nummularia 21-23μ x 21-23μ and Z.spina-christi 19-21 x 21-23μ. The appearance of

Ziziphus trees is shown in (Fig 14) and the species illustrated is Z.jujuba. There are very many of these trees in Baghdad, some are self sown, others planted by the local authority. Many are more than 5 metres tall and these are examples in close proximity to the trapping station at the Central Allergy Hospital, Alwiah.

Since Z.jujuba appear to be the common species in Baghdad and the pollen grains are colourless and the smallest of the 3 species, it is concluded that the Ziziphus grains trapped from the air belong to this species.

Dr. Behnam considers it likely that the pollen of Ziziphus is an important allergen in Baghdad. However, until a pollen extract suitable for use in allergen testing has been prepared, this can neither be confirmed or refuted. It is recommended that pollen be collected from Z.jujuba and extracted in buffered saline for clinical use.

Guest & Al-Rawi in (1966) reports Zizyphetum nummulariae as a plant association on flood plains and in wadi beds in the desert. Z.nummularia is reported between Basra and Ur, between Baghdad and Takrit although Zohary (1940) considered this species to be limited to the eastern part of the Syrian desert. It is rare to find any extensive stands of this species of Ziziphus in its natural state in Iraq, although small thickets are reported in the southern desert and lower Jazira, (Guest & Al-Rawi 1966).

The survival of _Z.nummularia_ in the desert is attributed to the fact that the busn is extremely thorny and there is a widespread belief that _Ziziphus_ is sacred. In an area of the S.Desert, _Z.spina-christi_, is the dominant species (Guest & Al-Rawi 1966). The specific epithet is derived from the legend that the thorns of this bush were used to crown Christ.

Juniperus

Results

Juniperus pollen was recorded as early as the middle of January and persisted in the air until the middle of April (Fig 6). This pollen had a distinct season with very high concentrations occurring in March. The results for 1982 are given in Table 5 and on March 4th a peak concentration of 135 grains/m^3 was recorded. In 1983 (Fig 15) the highest concentration of 855 grains/m^3 was found on March 8th and on the following day the concentration was 537 grains/m^3.

Pollen from the genus _Juniperus_ comprised 29% of the total annual pollen catch in Baghdad (Fig 9). 82% of the annual catch of _Juniperus_ pollen was trapped during the month of March. In January 1983, _Juniperus_ pollen was recorded in a concentration of 6 grains/m^3 on only two days; it was trapped on 9 days in April. In April 1982 no _Juniperus_ pollen was recorded.

Discussion

Juniperus pollen occurred in a higher con-

Table 5 Mean Daily Concentrations of *Juniperus* Pollen in March, 1982

Day	*Juniperus* grains/m^3	Day	*Juniperus* grains/m^3
1	30	17	66
2	48	18	39
3	90	19	12
4	135	20	33
5	63	21	0
6	57	22	72
7	42	23	27
8	No record due to failure of electricity supply	24	42
9		25	63
10		26	15
11		27	12
12		28	27
13		29	45
14		30	39
15	30	31	60
16	45		

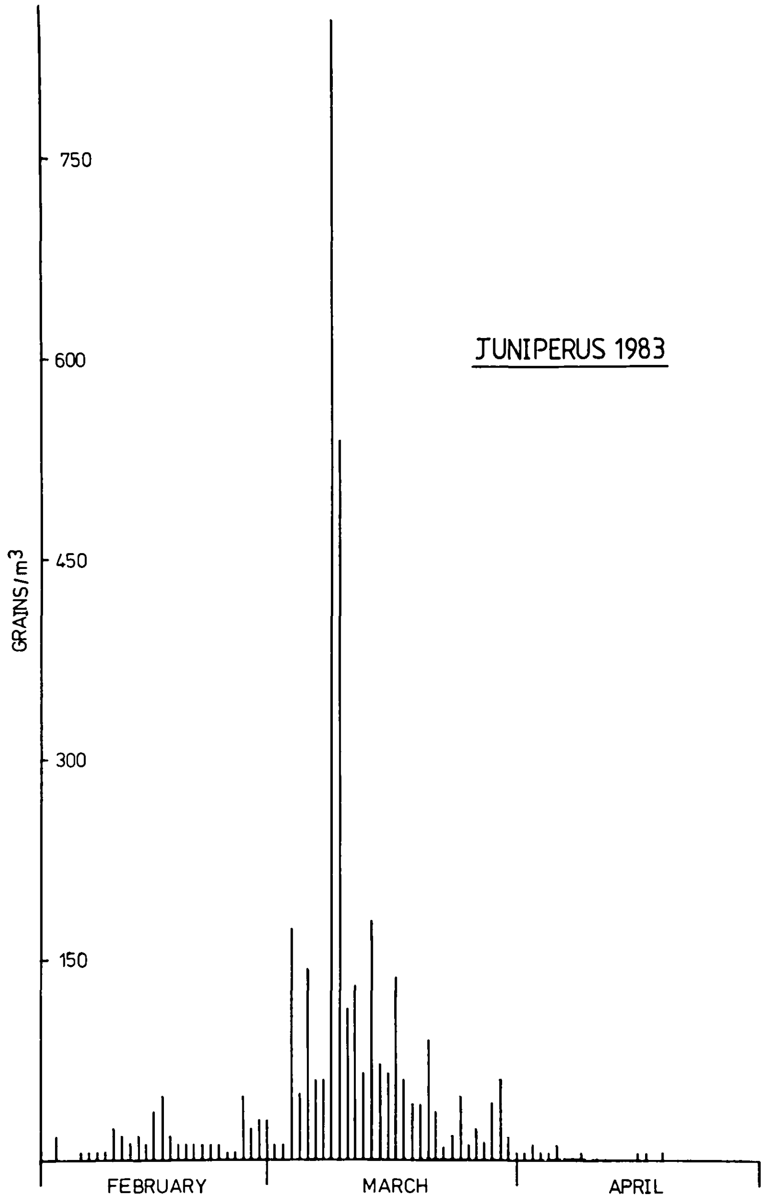


Fig 15: Mean daily concentrations of <u>Juniperus</u> pollen in the air over Baghdad.

centration than was observed for any other genus. The season was short and mainly confined to the cool month of March. The only two species recorded in Iraq by Al-Rawi (1964) are J.oxycedrus L. and J. polycarpos Boiss (macropoda Boiss). The former is distributed in A, R, Su, the latter in Su (Fig 1) and it must be concluded that the trees occur in the northern forest. This implies long distance transport to Baghdad and there is a possibility that the pollen of the form genus Juniperus may be from another source. However, since Juniperus is the only genus in the Cuppressaceae which has been recorded in Iraq this is unlikely.

Key (1918) has reported the pollen of J.salinoides as an important cause of hay fever in Central and Eastern Texas. The pollen season was from about December 20th until the end of February, and the trees are recognized as producing extraordinarily large quantities of buoyant pollen. The Gymnospermae contain few genera of allergenic significance although Pinus and Picea produce massive quantities of pollen. Hesselman (1919) calculated that the forests of Picea in South and Central Sweden produced about 75,000 tons of pollen per annum. Cupressus and Juniperus have been reported to produce highly allergenic pollen; the Cupressaceae including Chamaecyparis, Cupressus, Juniperus, Libocedrus and Thuja by Yoo et al (1974); species of Juniperus by Black (1929), French (1930), Kahn & ˊ ,ɔı), Sellers (1935) and Lewis & Imber (1975b)

Although no species of _Cupressus_ is reported to grow in Iraq, pollen attributed to _Cupressus_ was trapped on 5 days in March, 1983. It is possible that this pollen, trapped in a maximum concentration of 18 grains/m^3 was from a source exogenous to Iraq and an example of long distance pollen transport.

Allergy to _Juniperus_ pollen in March is worthy of investigation.

Urticaceae

Results

The pollen of this family was found in all the months of the year (Fig 6) with the exception that although the pollen was trapped from the air in 4 days in June 1982, it was not recorded in June 1983.

Pollens of the _Urticaceae_ comprised 12% of the pollens trapped from the air in Baghdad (Fig 9). Mean daily pollen concentrations are given in (Figs 16,17), which clearly shows a March and April season in both 1982 and 1983.

In 1982, the highest concentration recorded was 99 grains/m^3 on April 3rd, in 1983 a peak concentration of 159 grains/m^3 was recorded on March 24th.

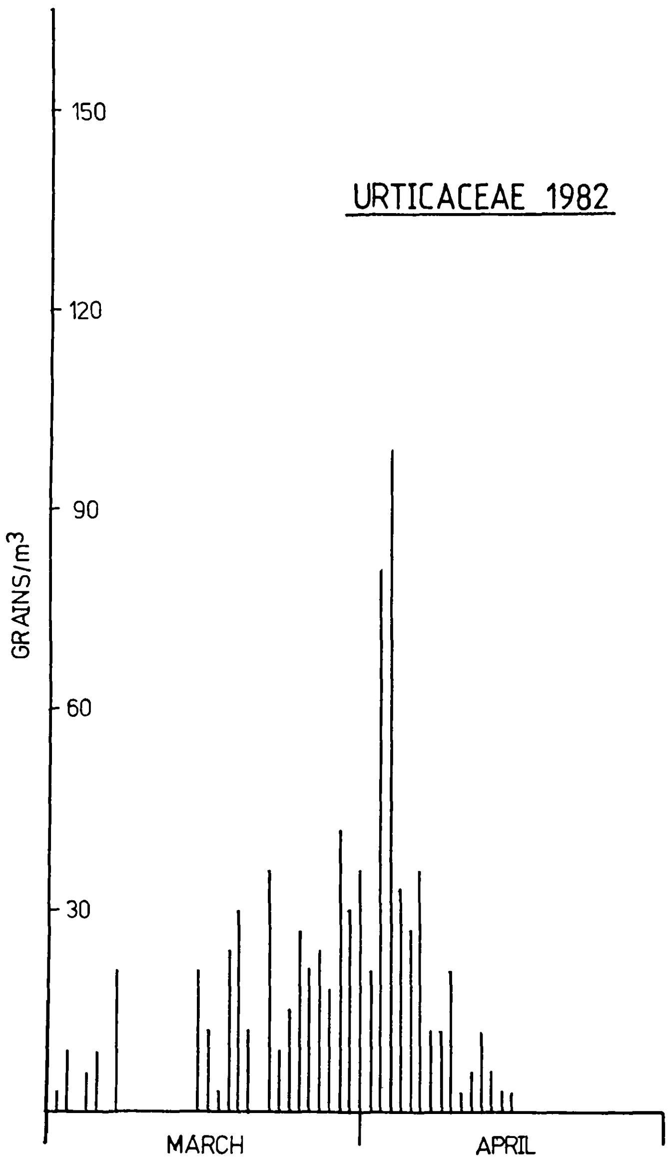


Fig 16: Mean daily concentrations of pollen from the Urticaceae in the air over Baghdad 1982

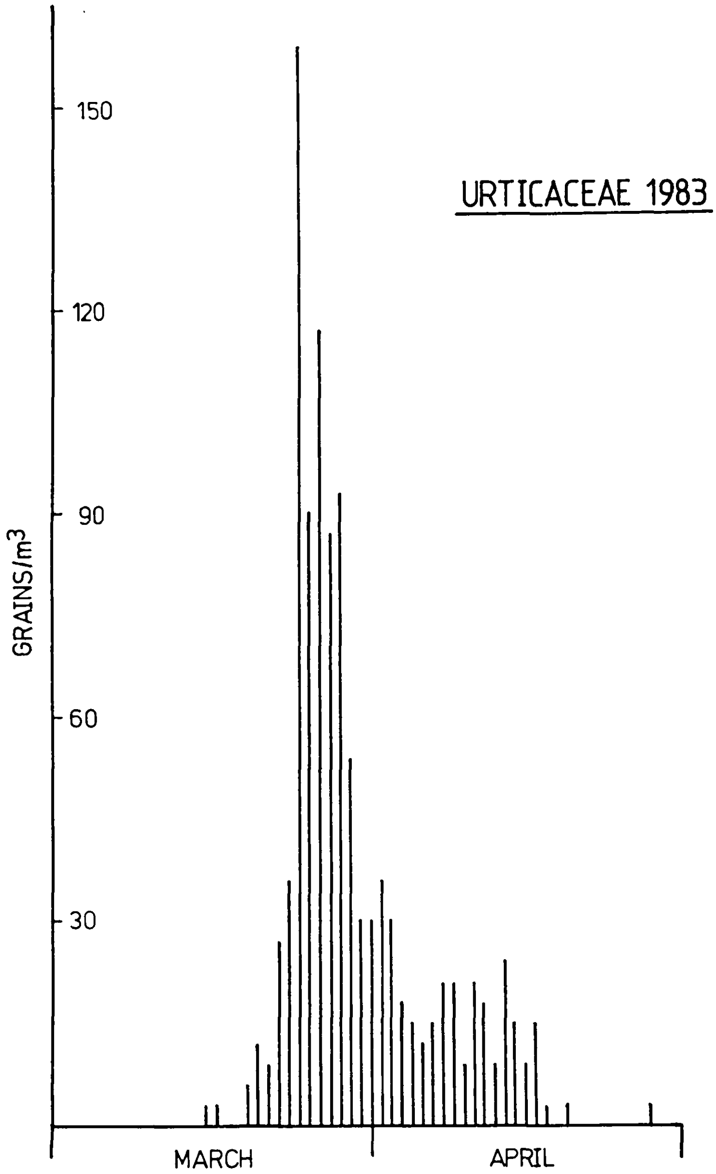


Fig 17: Mean daily concentrations of pollen from the Urticaceae in the air over Baghdad in 1983.

Discussion

Al-Rawi (1964) lists the Urticaceae of Iraq as follows:-

Parietaria alsinefolia Del

Distri: LJ, E, PF (Fig 1)

P.judaica L

Distri: A, R and P.lusitanica L.

Distri: A, R, E, Su

Urtica dioica L.

Distri: LJ, WD, CA, EA, A, R

U.pilulifera L.

Distri: E, UJ, R and U.urens L.

Distri: Baghdad, Abughraib, Amara

In the British Isles Urtica dioica, the common stinging nettle, is the probable source of most of the airborne pollen from the Urticaceae. In Britain, numerically, Urtica pollen is second in importance to grass and surprisingly its importance as an allergen has not been well evaluated. It occurs in the air from May onwards and frequently reflects two peaks of flowering, one during the June and July season of grass pollenosis, and one in September, October period (Davies 1962) and unpublished aerobiological records Mycology Laboratory, Dept. Medical Microbiology, St.Mary's Hospital, London). D'Amato et al (1983) in a study on the allergenic pollen content of the air of Naples considered the most important pollen to be that of Parietaria which occurs in the air from March to November. In this study a Burkard 7 day recording spore trap was employed, and the peaks _Parietaria_ concentrations for 1979, 1980 and 1981, were 100 grains/m^3, 400 grains/m^3 and about 350 grains/m^3 in these years respectively. There were two peaks of pollen production, one in late May and June, the second in mid August and September.

Naples has a dry Summer, where average monthly temperatures do not fall below 20^{o}C. _Parietaria_ is the most important hay-fever provoking plant in Naples and Southern Italy (Serafini 1957; D'Amato, Cocco & Melillo, 1979). The plant grows on walls under trees and bushes and is favoured by the climate, and by tufaceous, volcanic rocks on which the city of Naples is built.

In a study of all allergens, both seasonal and perennial, by means of skin-prick tests. D'Amato et al (1979) found a frequency of sensitization to _Parietaria_ of 17% in the paediatric age group, and 31% in adults. D'Amato et al (1983) observed that days with _Parietaria_ pollen counts of 50 or more may be described as 100% symptom days for patients sensitive to this pollen.

The season for pollen of the _Urticaceae_ is reported to lie between late March and early August in Barcelona, and from late April until the end of June in Istanbul. (Charpin, Atlas of European Allergenic Pollens, Sandoz Editions).

It has been difficult to evaluate the importance of the Urticaceae as hay fever plants in the British Isles because the seasonal occurrence in June and July coincides with the season for grass pollenosis. Parietaria officinalis is rare in comparison with Urtica dioica in the British Isles. The pollen extracts from both these species are commercially available prior to obtaining extracts of pollen from the Parietaria species indigenous to Iraq, a preliminary study based on skin-prick tests with U.dioica and P.officinalis would seem worthwhile.

Artemisia

Results

Artemisia pollen was found in the air from the first week of October until the beginning of December (Fig 6). Its season is October and November, and the highest mean daily concentration recorded was 51 grains/m^3 on October 24th, 1982 (Fig 18). Artemisia pollen comprised 7% of the total pollen catch in Baghdad (Fig 9) and 71% of this was trapped in October.

In December, the pollen was trapped from the air on only 6 days (Fig 18).

Discussion

Artemisia is classified in the Compositae and most of the genera and species in this family are insect pollinated.

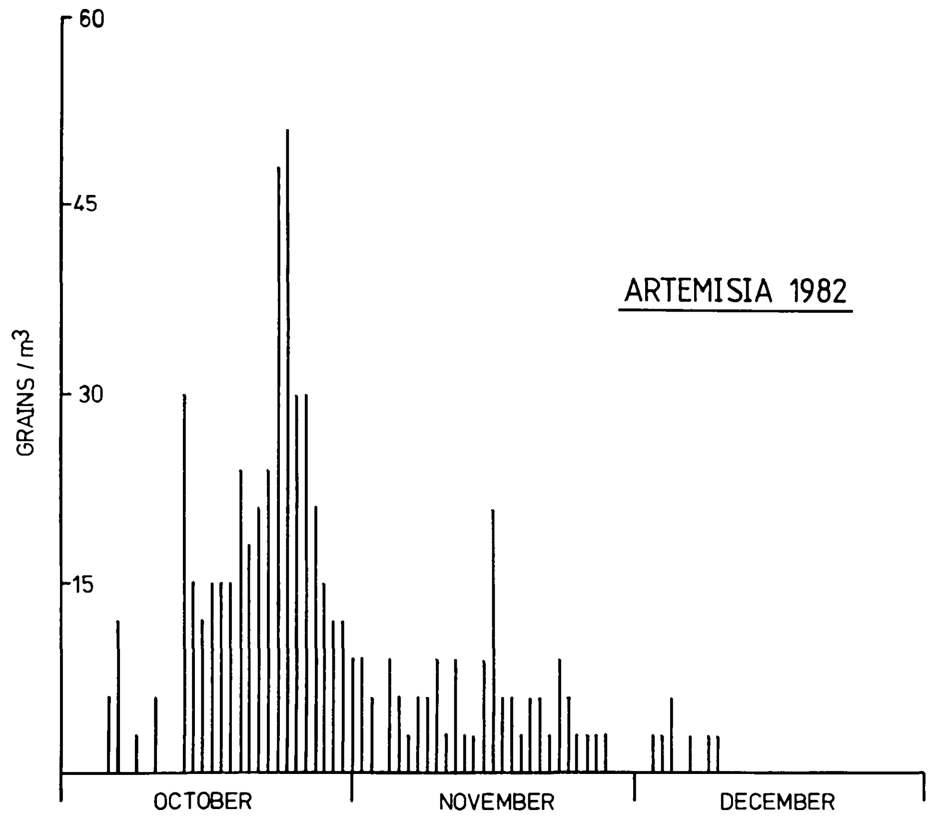


Fig 18: Mean daily concentrations of _Artemisia_ pollen in the air over Baghdad in 1982.

The RAGWEED family Ambrosiaceae is considered to be closely related to the Compositae and it is considered that Artemisia pollen causes symptoms when inhaled by ragweed sensitive persons.

In Iraq, Rawi (1964), has listed five species of the genus Artemisia and these species with the districts in which they occur are listed below:-

A.Campestris L.
A.haussknechtii Boiss
A.herba alba Asso
A.Scoparia W. et K.
A.Splendens Willd.

A.herba alba and A.scoparia are found in the Central Alluvial Plain, Eastern Alluvial Plain, Lower Jazira and the Western Desert.

Artemisia vulgaris is of occasional occurrence in the air of London during the Summer months. However, the use of selective weed-killers in agriculture appears to have led to a reduction in the occurrence of this pollen in the air of London, (unpublished records, Mycology Laboratory, Dept. Medical Microbiology, St.Mary's Hospital Medical School, London). D'Amato et al (1983) found Artemisia vulgaris to be the third most important herbaceous allergenic pollen in the air of Naples. This species is very important cause of hay fever in California, Colorado and New Mexico which include the hottest parts of the U.S.A. A.californica and A.tridentata are also important as causes of hay fever in the U.S.A. (Thommen, 1931) Sheldon et al (1953) list A.frigida, A.filfolia and A.annua as three other species of Artemisia causing hay fever in parts of the U.S.A.

The role of the Artemisia species of Iraq as hay fever plants is clearly worthy of investigation. Allergenic extracts of the pollen of eight species of Artemisia are commercially available, but not one of these species occurs in Iraq. The possibility of a common antigenicity justifies testing Iraqi patients with extracts from the species which do not grow in Iraq until extracts of pollens from species of Artemisia which are common in Iraq have been prepared.

Oleaceae

The pollen of two members of this family, Olea europea and Fraxinus ornus are included in (Fig 9).

Olea europea

Results

In 1983 Olea pollen was trapped on 6 days in February, 7 days in March, 17 days in April and 7 days in May. Concentrations as high as 42 grains/m^3 and 63 grains/m^3 were recorded on March 20th and April 16th, respectively, (Fig 19).

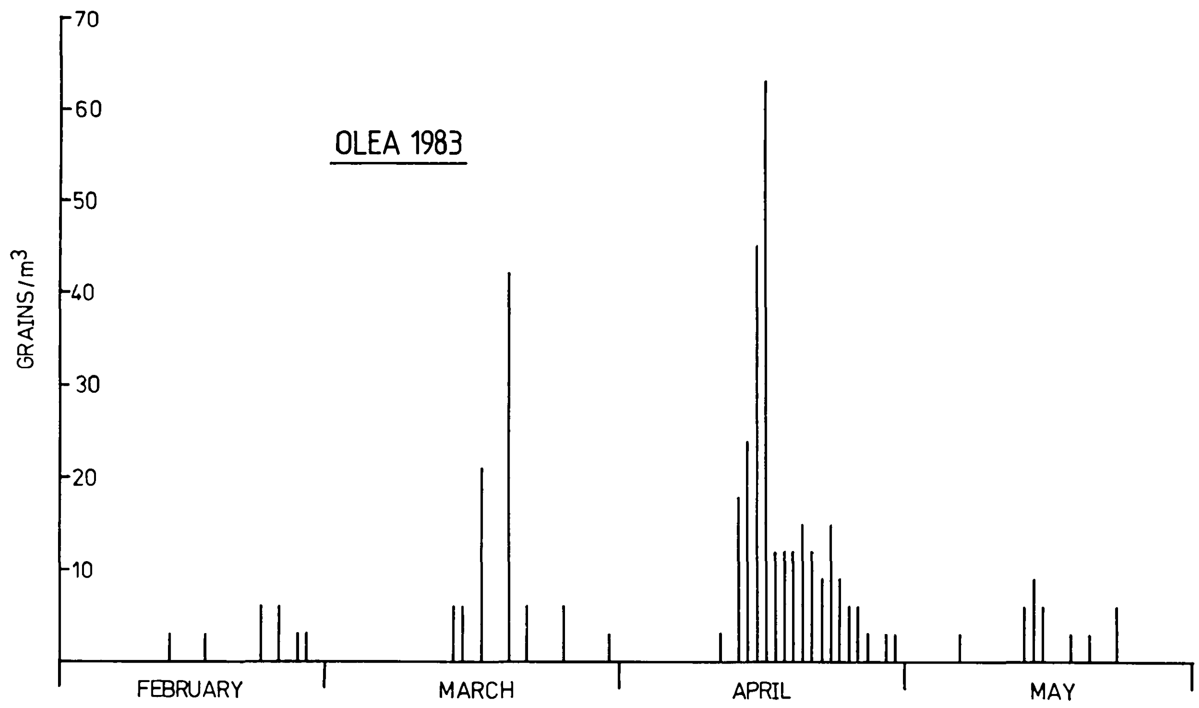


Fig 19: Mean daily concentrations of <u>Olea</u> pollen in the air over Baghdad in 1983.

Discussion

Olea europea requires a warm Winter and thrives in a hot dry Summer. It has been cultivated in the Mediterranean region from early ages (Willis, 1908) and is of widespread occurrence in the Middle East. An Iraqi law requires every household to grow at least one tree, with the result that very many gardens have an olive tree. The olive is an evergreen and in recent years it has been planted along the roadways in Baghdad. The tree is grown in plantations in close proximity to Baghdad. Gutman (1950), considered that *Olea europea* is not wind pollinated, but that the pollen was light enough to become airborne and cause hay fever. Kessler (1952) following tests on exposure concluded that there were many cases of hay fever due to the pollen of *Olea europea* in Palestine. D'Amato et al (1983) considered *Olea europea* to be the only tree of allergenic importance in Naples and that *Olea* pollen was as important in areas to the south of Naples such as *Calabria* and *Puglia*. It is possible that the Burkard trap underestimates the importance of olive pollen in the air of Baghdad. Some tree pollens e.g. *Prosopis spicigera* produce sticky grains which clump. The clumps settle quickly to the ground in the vicinity of the trees, and although uncommon in Hirst trap deposits, the pollen is an important cause of allergic disease as in Kuwait, (Davies 1969c). Whether or not the pollen of *Olea europea* is produced in clumps, or occurs in high concentrations in the vicinity of the trees does not appear to have been investigated. It is possible that Olea europea is an important cause of pollenosis in Baghdad and further investigation is required.

Extracts of this pollen are commercially available. Pacini & Cresti (1977) have reported viral particles in developing pollen grains of Olea europea. Whether or not the presence of the virus has any effect on the allergenicity of the pollen appears to be unknown.

Fraxinus

Results

Pollen of the genus Fraxinus occurred in the air from mid January until mid June (Fig 6). The highest mean daily concentrations of 54 grains/m^3 were recorded on April 15th and 16th, 1982 (Fig 20). The peak month was April and 65% of the Fraxinus pollen was trapped in this month. The pollen was trapped on only 2 days in January, 3 in February and on just one day in June, 1983.

Discussion

Sheldon et al (1953) considered Fraxinus to be the only wind pollenated genus in the Oleaceae of importance in the U.S.A. Allergic response of Fraxinus pollen is rare although all species shed large quantities of pollen. Fraxinus ornus is of widespread occurrence in Italy, where it flowers from April to June (Charpin et al 1974).

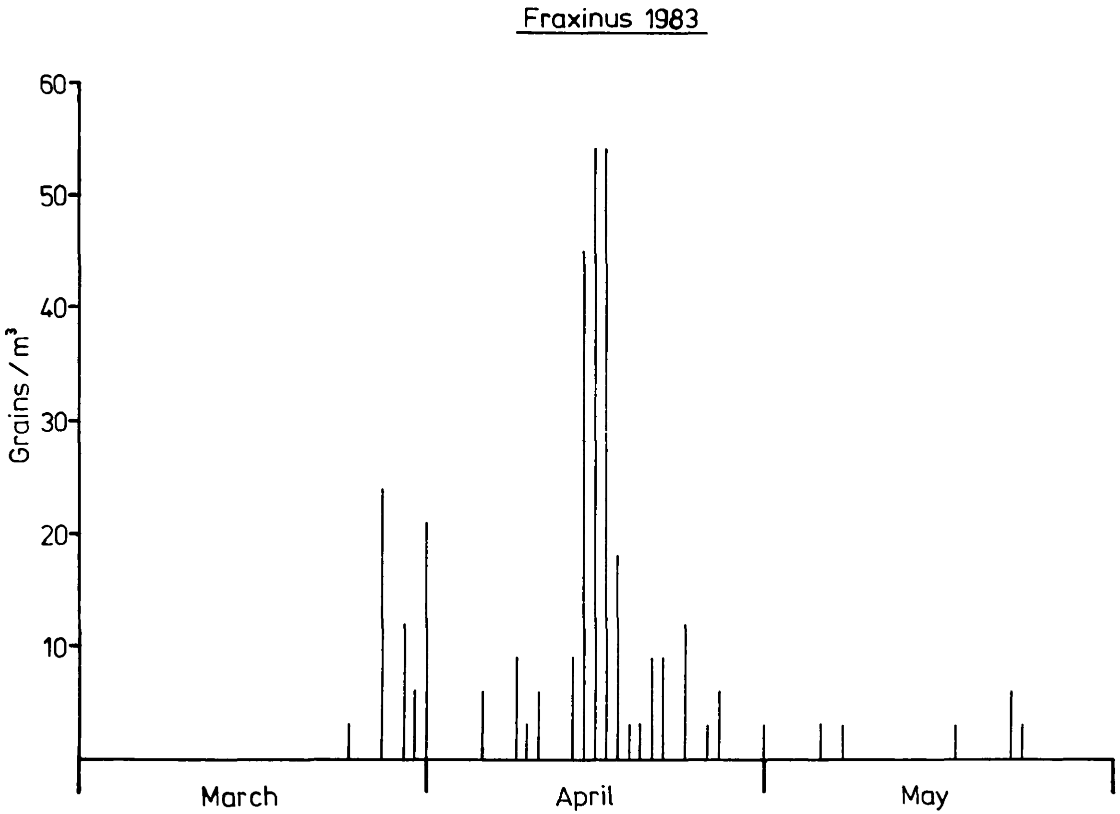


Fig 20: Mean daily concentrations of Fraxinus pollen in the air over Baghdad in 1983.

Fraxinus ornus occurs in the Northern forest of Iraq, where it is used as a source of manna. The tree is commonly known as the manna ash.

The pollen grains of three genera in the *Oleaceae*, *Olea*, *Fraxinus* and *Ligustrum*, are reported to occur in the air. Yet, Gutman (1950), considered *Olea* was not wind pollinated. Furthermore, Sheldon et al (1953) considered the privet *Ligustrum* to be normally insect pollinated, although the pollen could be a cause of hay fever for persons in close contact with this shrub.

Ligustrum pollen is commonly trapped from the air over central London (unpublished records Dept. of Medical Microbiology, St. Mary's Hospital Medical School, London). *Ligustrum* does not occur in Iraq. However, from these studies, it must be concluded that pollen grains of the *Oleaceae* do occur in the air, and should be considered as possible allergens in the investigation of allergic patients in Iraq.

Pollen recorded, but infrequent in occurrence

Trees

Alnus pollen was trapped on one day, July 26th 1982 in a mean daily concentration of 6 grains/m^3.

Betula was trapped intermittently between the beginning of January and mid-July. Highest mean daily concentration was 12 grains/m^3 on January 17th and March 23rd, 1983.

Platanus was recorded in a mean daily concentration of 3 grains/m^3 on varying occasions during February, March and April.

Pinus, found between the end of March and the end of June. Highest mean daily concentration was 21 grains/m^3 and was recorded on June 21st 1982. This pollen, produced in massive quantities in the forests of Scandinavia, has never been shown to cause allergic disease.

Quercus pollen was occasionally found in mid January and April, 1983. The highest mean daily concentration of 12 grains/m^3 was recorded on April 15th 1983.

Morus, a few grains were seen in the deposits during the second week of January and in February.

Mean daily concentrations of 6 grains/m^3 were trapped on February 18th and 28th, 1982.

Discussion

Betula pollen is an important allergen in the Scandinavian countries, and causes symptoms in suburban areas where the trees are cultivated in Britain.

London plane is wind pollinated and its pollen can occur in the air in very high concentrations, a mean daily concentration of over 2000 grains/m^3 having been reported in Central London (unpublished). It is a cause of pollenosis in some London patients. Both Betula and Platanus should be considered as sources of allergenic pollen in Iraq in the places where these trees occur. Platanus occidentalis, Betula alba and the following Quercus species occur in the Northern forest zone in Iraq. The Quercus species are Q. aegilops, Q. infectoria, Q.hibani, Q. macrocantha and Q. castanaefolia. It is unlikely that these species investigated as a cause of allergic disease, Q. castanaefolia occur in the Gurfa district which is fairly close to Baghdad. Alnus is not recorded as occurring in Iraq by either Guest & Al-Rawi (1966) or Al-Rawi (1964). It is therefore probable that the occurrence of these tree pollen grains in the air of Baghdad reflects long distance transport, with the possibility that some of the grains e.g. Alnus were from a source exogenous to Iraq.

Herbaceous Plants (Weeds)

Carex was found between the middle of January and the end of June, and the highest mean daily concentration, 12 grains/m^3 on May 16th.

Asteraceae (compositae) - pollens attributed to this family were trapped from March 19th until the end of the first week in April. Highest mean daily concentration 12 grains/m^3 on March 28th 1983.

The grains trapped could not be assigned to a genus. Artemesia is a member of this family but having morphologically distinct grains the pollen has been dealt with separately. In the U.S.A. Ambrosia (ragweed) is a very important allergenic plant in the Autumn but it is not listed as occurring in Iraq by Al-Rawi (1964). Since the family contains important sources of allergenic pollens in other countries, and 99 genera are listed as occurring in Iraq it is likely that members of this family may be important in places. Rhantorium epapposum is a common and conspicuous feature of Zubair (Guest and Al-Rawi, 1966). In the Rhanterium epapposi desert association Plantago ciliata is recorded by Guest and Al-Rawi (1966). Plantago lanceolata (English plantain) is reputed to cause more pollinosis than any other Plantago species (Solomon and Durham, 1967). However, it is possible that P. ciliata has never been investigated and pollen which could be assigned to this genus with certainty was not found in the Burkard trap deposits.

Anthriscus, a few grains were seen towards the end of July and on one day in August. 6 grains/m^3 were recorded for July 22nd, 1982. *Anthriscus vulgaris* Bernh is recorded as occurring in Singar. This member of the *Umbelliferae* is a common weed of agriculture, the source of the pollen is unknown, and it is not known to cause allergic disease.

The Diurnal Periodicity of Airborne Pollen Concentrations in Baghdad

The mean daily concentration of a pollen grain in the air is a useful parameter for Clinicians seeking to correlate the aerial concentration of the pollen with patients symptoms. Davies and Smith (1973) state that when the mean daily concentration for grass pollen over central London reaches 50 grains/m^3 all patients who are clinically sensitive to the pollen have symptoms.

D'Amato et al (1983) have published similar findings based on their experience in Naples. However, the concentration of pollen and other spores varies during the day. Grass pollen grains may not occur in the air throughout the 24 hours of the day, but may be present in the air for 12 or 8 hours.

In order to calculate the concentrations every 2 hours, the slides were scanned at right angles to the length of the deposit at 4mm intervals (Method p57).

Results

Two hourly concentrations for Ziziphus are given in (Fig 21), Juniperus in (Fig 22) and for Urticaceae in both Baghdad and London is given in (Fig 23). The two hourly changes in grass pollen concentration on June 19th, 1984 in London is shown in (Fig 24).

There was no Ziziphus pollen in the air between 1600 hours and 0200 hours the next day. Maximum concentration was reached by 1000 hours. Similarly, in Baghdad, the maximum concentration of Juniperus pollen was recorded at 1000 hours on March 9th and 1200 hours on March 8th, maximum concentration for pollen from the Urticaceae was 1200 hours in Baghdad, and 2000 hours in London. The maximum concentration for grass pollen on the day shown (Fig 24) in London was at 18 ,00 hours.

Discussion

Pollen becomes airborne earlier in the day and attains its maximum concentration for the day by 1200 hours in Baghdad, whereas in London the peak concentrations are rarely attained before 1800 hours.

It is dessication of the anther which leads to the bursting of the pollen sacs and liberation of the dried pollen grains. This process is known as

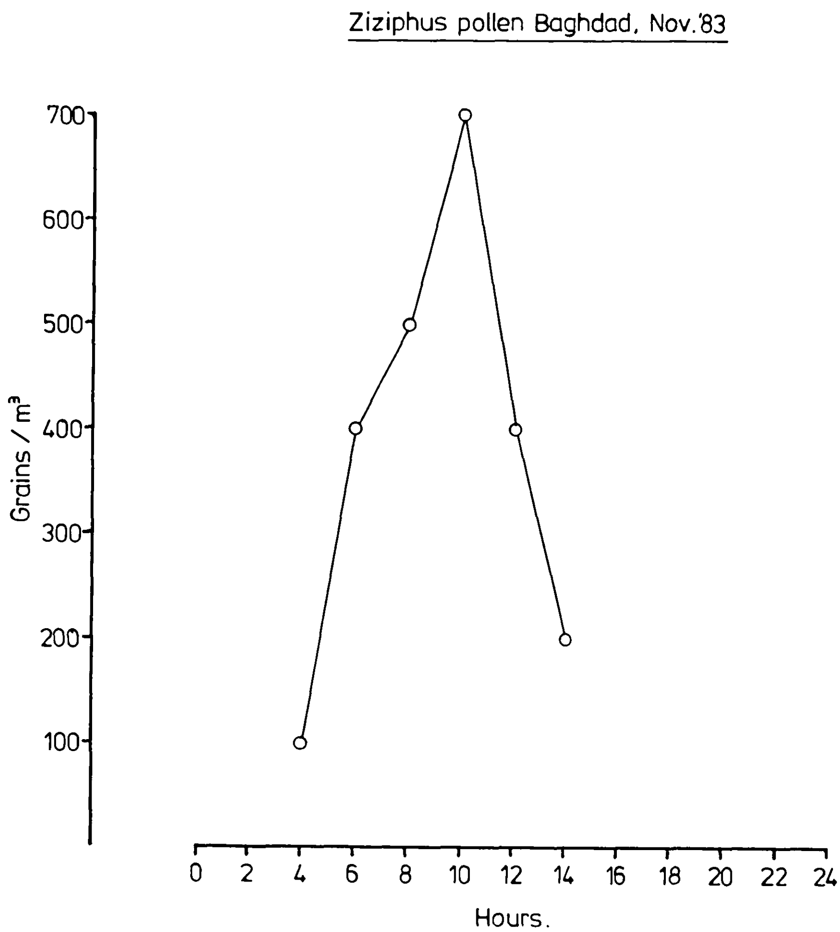


Fig 21: Concentrations of Ziziphus pollen at two hourly intervals, November 1983 showing diurnal periodicity. With peak concentrations at 10 a.m., which was before the maximum temperature of the day was reached.

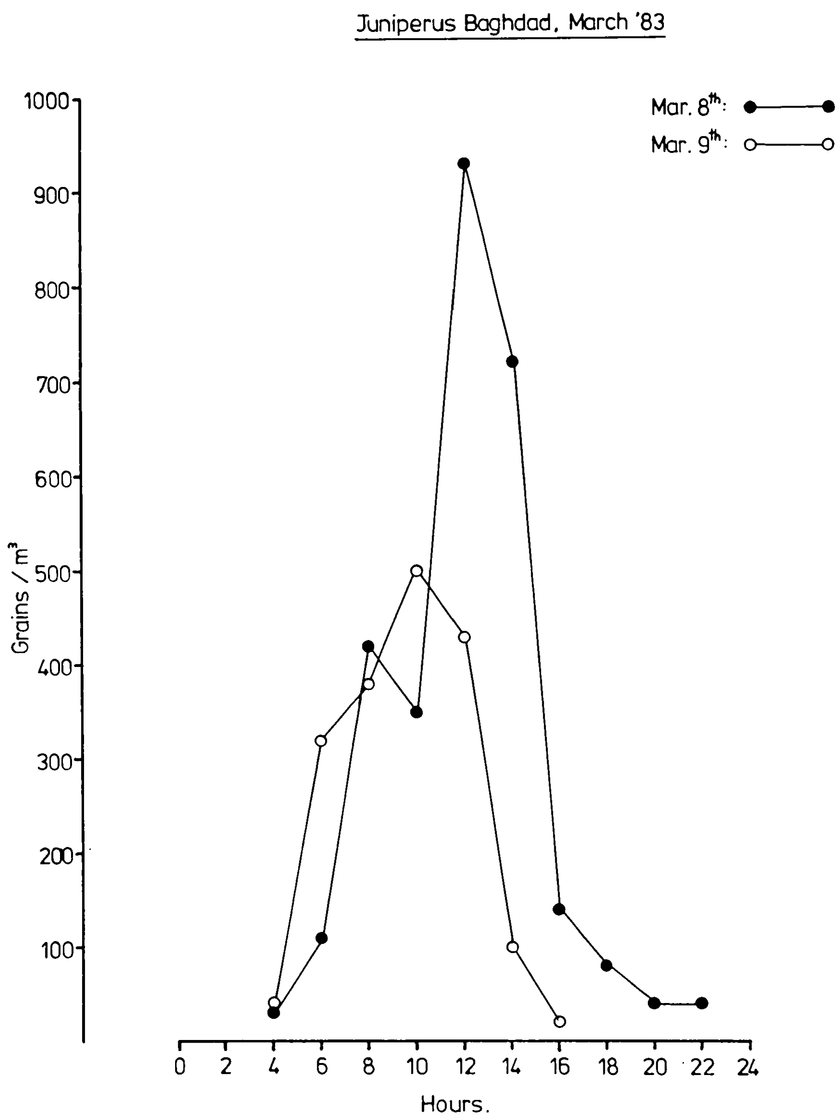


Fig 22: Concentrations of Juniperus pollen at 2 hourly intervals in the air over Baghdad. On March 8th the peak concentration was recorded at 12,00. On March 9th at 10,00. On both days the diurnal peak concentration was obtained before the maximum temperature for the day was reached.

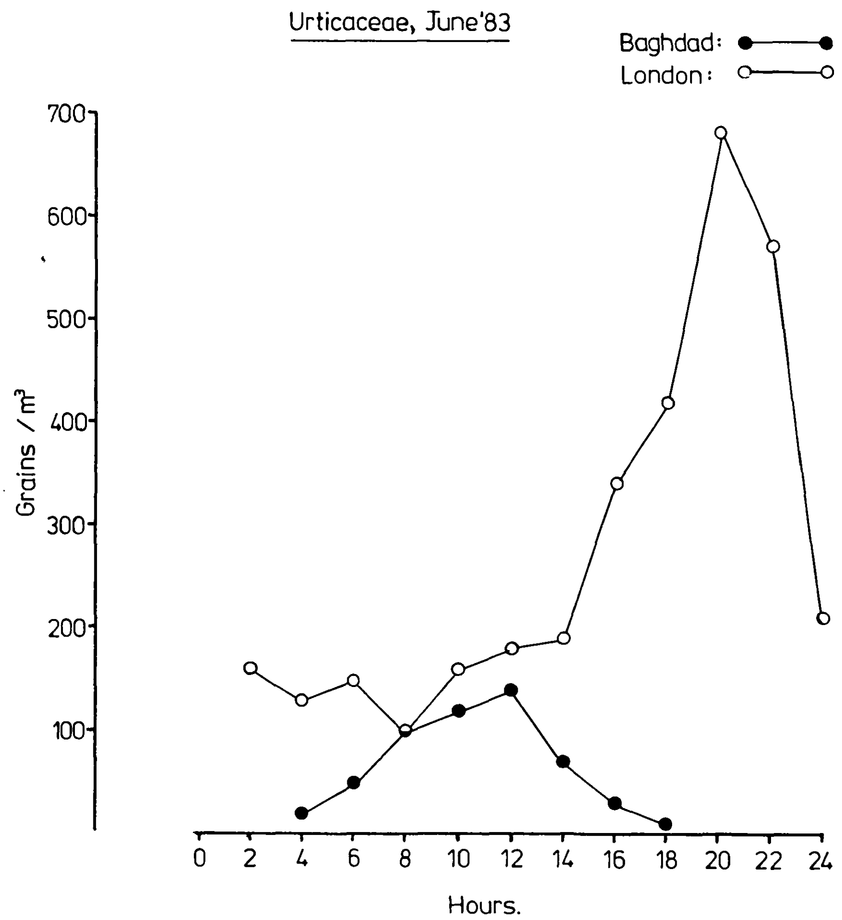


Fig 23: Two hourly concentrations of Urtica pollen in the air over Baghdad and London, the diurnal peak concentrations occur at 12,00 hrs in Baghdad and 20,00 hrs in London.

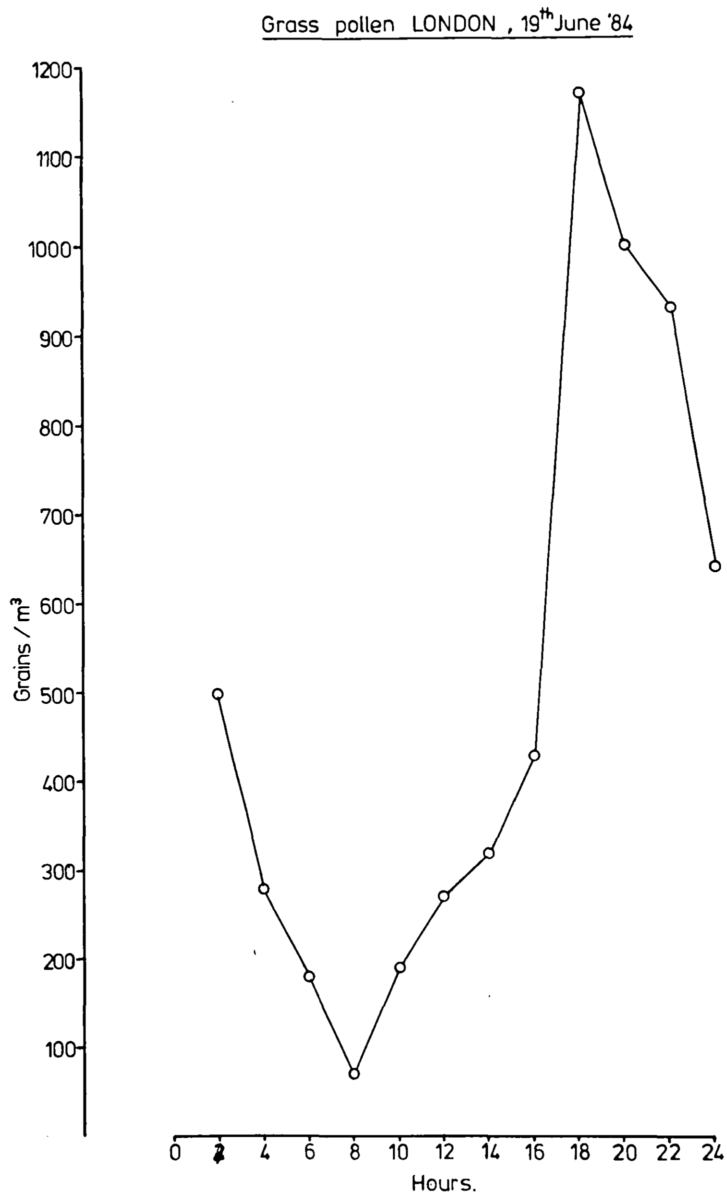


Fig 24: Concentrations of grass pollen at 2 hourly intervals in London. On 19th June 1984. The diurnal peak concentration is attained much later in the day than has been observed for airborne pollen in Baghdad.

dehiscence. Hours of sunshine in Baghdad are given in (Fig 2). It is light by 0400 hours in the Spring whilst in London sunrise is never before about 0500 hours.

In S.E. England, in the morning, the vegetation is frequently wet with dew. Temperature inversions occur near the ground and until heat of the sun ensures the inversion breakdown the air is still. Air movement is necessary to shake the pollen grains out of the anthers.

In Baghdad, the Spring and Summer weather conditions are constant, the air is dry. An earlier sunrise and rapid increase in insolation leads to an earlier dessication of the anthers, an earlier development of convectional air movement and an earlier rise in pollen concentration in Baghdad than is found in London. In Baghdad, the pollen concentrations are at their maximum before the temperature rises to its maximum for the day at about 1400 hours, whereas in London, the peak pollen concentrations occur several hours after the maximum temperature has been reached. Davies and Smith (1973) found that between 14^{o} and 22C^{o}, the pollen concentrations increased for every degree centigrade, whereas above 22^{o}C, the slope of the curve increased by 1.5^{o}C for every degree to a maximum of 30^{o}C. In June daily maximum temperatures of 30^{o} are rarely attained in the British Isles, in Baghdad, temperatures of 40^{o} are common. The effect of these high temperatures on pollen concentration requires further investigation.

Other possible reasons for the development of earlier daily maximum pollen concentrations in Baghdad may reflect greater amounts of the pollen having its origin in local sources.

In the London area, most of the pollen trapped from the air has its origin outside the conurbation, and concentrations go on building up until the temperature begins to fall. In the Summer Britain enjoys a period of dusk each day. In the Middle East, it gets dark very quickly. In Britain there is brilliant sunshine at 2000 hours, whereas it is dark in Baghdad at 1800 hours. However, since Baghdad experiences maximum pollen concentrations before the maximum temperature for the day, it is the rate at which the temperature rises coupled with high isolation which is probably of greatest importance, and further studies are needed to determine the effect of temperature and sunshine on pollen concentration in Baghdad.

FUNGAL SPORES

Hirst (1953) and Gregory (1961) distinguished a day time "dry air" spora and a characteristic "damp air" spora. The dry air spora is characterized by such spore types as Alternaria and Cladosporium, whereas the damp air spora is characterized by Ascospores, Basidio spores and the spores of Tilletiopsis and the mirror yeast Sporobolomyces. The Ascospores of some species are discharged when the ground is wet with dew, and the spores of these fungi appear as part of the nocturnal air-spora. Hirst (1955) found high concentrations of the ascospores of Venturia inaequalis in the air after 2 and 3 hours of rain. Basidiospores require high humidity for spore discharge. Even the spores of Cladosporium and the Aspergillaceae are affected by changes in humidity which can lead to the hygroscopic disruption of spore chains and aid take off. Since Baghdad has a very dry climate with low relative humidities (page 22), no dew formation in the Summer months, and negligible rain (page 22), the fungal air spora was clearly worthy of investigation.

The flora of an area varies with the climate and topography, and the genera and species of higher green plants in Iraq comprise an assemblage which differs from that growing in temperate areas. Whether the genera and species of fungi encountered from the air of Baghdad differed from those trapped from the air of temperate regions required investigation.

Results

Cladosporium

The distinctive conidia of this genus were trapped throughout the period studied (Fig 25). The mean monthly concentrations were highest in November, January, February and April, lowest in the months of May, June, July, August and September, which are the hottest dry months of the year.

Cladosporium was the predominant genus of spore trapped and comprised 46% of the total fungal spores recorded (Fig 26).

The highest mean daily concentration recorded for the spores of this genus each month is given in Table 6 .

Alternaria

Alternaria spores were trapped throughout the year, and the average monthly concentrations are shown in (Fig 25). Again there is a trend for concentrations to be lowest in the hot dry months of June, July, August and September.

The highest mean daily concentration recorded for the spores of this genus each month is given in

Average Monthly Concentration for all Fungal Spores, Cladosporium and Alternaria

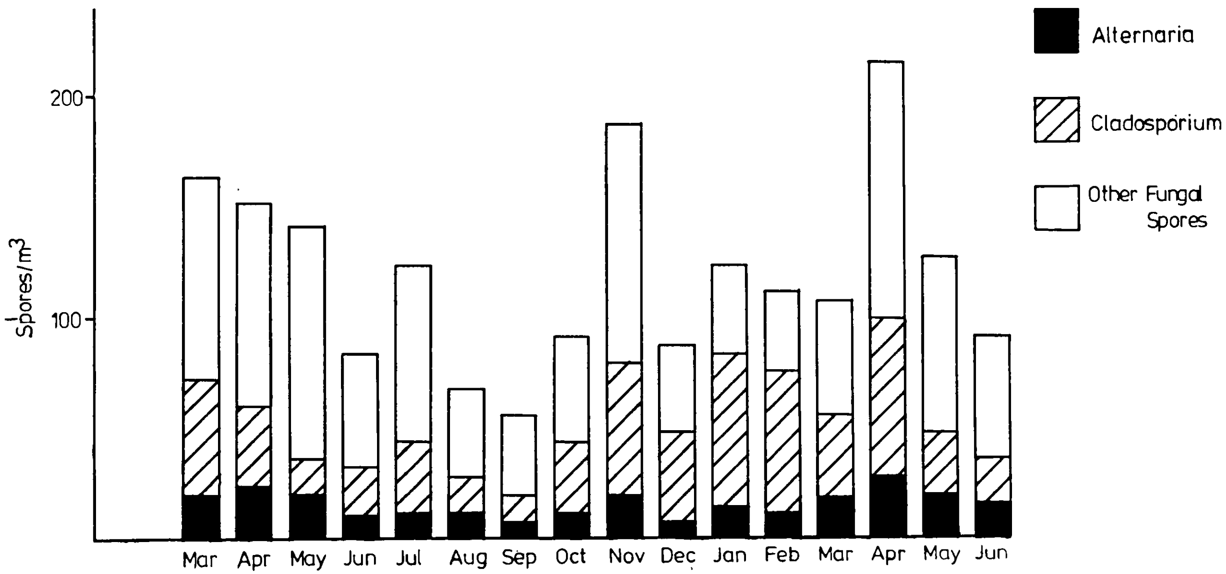


Fig 25: Average monthly concentrations for <u>Cladosporium</u>, <u>Alternaria</u> and all other fungal spores in the air over Baghdad.

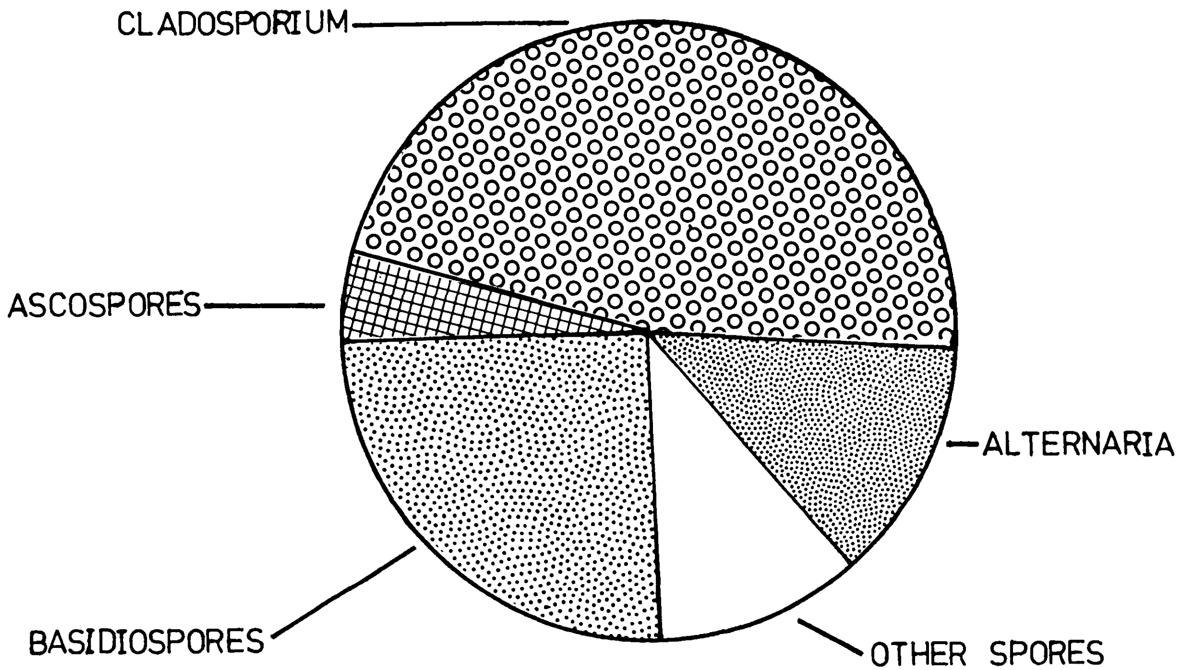


Fig 26: Cladosporium, Alternaria, Ascospores and Basidiospores as a as a proportion of the total spore catch from the air over Baghdad.

Table 6 Days with the highest mean concentration of *Cladosporium* and *Alternaria* at Baghdad in 1982 and 1983

Month	Day	*Cladosporium* spores/m^3	Day	*Alternaria* spores/m^3
Jan.	15 - 1983	435	15 - 1983	39
Feb.	4 - 1983	180	20 - 1983	45
March	22 - 1982	135	17, 29 1982	36
	13 - 1983	225	13 1983	96
April	9 - 1982	108	4 - 1982	45
	9 - 1983	249	12, 14, 22 1983	42
May	9 - 1982	78	15 - 1982	63
	9, 16, 25 - 1983	75	12 - 1983	42
June	28 - 1982	105	4 - 1982	42
	27 - 1983	78	16 - 1983	39
July	14 - 1982	96	12 - 1982	42
Aug.	2, 3 - 1982	51	4 - 1982	24
Sept.	14 - 1982	42	14 - 1982	27
Oct.	29 - 1982	138	25 - 1982	39
Nov.	9 - 1982	180	7 - 1982	45
Dec.	2 - 1982	108	30 - 1982	33

Table 6 . Alternaria comprised 12.5% of the total spores trapped from the air of Baghdad(Fig 26).

Discussion

Under the mesophytic conditions of temperate climates, Cladosporium and Alternaria are characteristic of the day time dry-air spora. Both are ubiquitous mould fungi. Cladosporium has been recorded from the air over the Atlantic Ocean (Pady and Kapica, 1955), in the air of the Arctic (Pady and Kapica, 1953), in the air of an Alpine Valley (Davies,1969a) various situations in America (Feinberg,1949; Bieberdorff and Hampton,1946; Burtness and Allen,1950; Collier and Ferguson,1953; Rooks and Shapiro,1958; Dworin,1966; Al-Doory,1967; Stalker and Moore,1972), Brazil (Passarelli, DeMaranda and DeCastro, 1949; Lima et al,1963); Puerto-Rico (Toro,1950), Palestine (Kessler 1953,1954; Golan Barkai 1958,1962), India (Barat, Roma and Das,1963; Sandhu, Shivpuri and Sandhu,1964; Baruah and Chetina,1966; Agarwal, Shivpuri and Mukerji,1969), Kuwait (Davies,1969c), Rabat (Chabert and Nicot,1968); Turkey (Ozkargoz,1969; Okuyan, Aksoz and Varan,1976; Yulug and Kustimur, 1977), Iran (Hariri et al 1978) and Iraq (Al-Tikriti, Al-Salihi, Gaillard, G.E., 1980). Whenever, and wherever, the ambient atmosphere has been sampled Cladosporium has occurred in the fungal air spora. It is noteworthy that in a number of early reports from the U.S.A., the term Hormodendrum was erroneously used to describe Cladosporium conidia which were septate.

The same species C.herbarum, C.sphaerospermum and C.macrocarpum have been recorded when the content of the air has been studied with cultural methods, and many of the conidia trapped from the air can be assigned to these very commonly occurring species.

The assemblages of Cladosporium and Alternaria spores in the Burkard deposits from the air of Baghdad are not distinguishable from those seen in similar deposits trapped from the air over Central London. Reports on the occurrence of fungi on vegetation from Iraq appear to be rare, although Mathur (1969) has reported C.macrocarpum Press, C.sphaerospermum Penzig, Alternaria tabacina (Ell and Ev.) A.tenuis Ness and A.tenuissina (Ness ex Fries) Wiltshire on plants in N. Iraq. On the evidence so far available it is reasonable to assume that in allergic investigations A.tenuis, C.sphaerospermum, C.herbarum and C.macrocarpum would seem to provide reasonable sources for the preparation of allergic extracts for use in Iraq.

Credit for the early recognition of mould fungal allergy is generally ascribed to Cadham in Canada (1924), Van Leeuwen in the Netherlands (1924) and Bernton (1930) and Hopkins, Benham and Kesten (1930) in the U.S.A., although as early as 1873, Blackley had described his own allergy to the spores of Penicillium and Chaetomium. The literature on atmospheric mould spores has been reviewed by Feinberg (1949), Maunsell (1954), Hyde (1972, 1973) and reports which may have relevance for Iraq and Baghdad are listed in Tables 1, 2 and 3.

Allergic sensitivity to Cladosporium was first described by Cobe (1932) and to Alternaria by Hopkins, Benham and Kesten (1930). Although Alternaria and Cladosporium are well recognized as fungal allergens, there appears to be little epidemiological or clinical information on atmospheric concentrations in relation to the development of patient symptoms. Hyde, Richards and Williams (1956), who tested 627 patients by injecting 0.02 ml of a 1/100 extract of Cladosporium, reported 8% gave positive reactions and that 4% were clinically sensitive to the mould because their seasonal asthma occurred in the Summer when the spores are most abundant in the air of Cardiff. Frankland and Davies (1965) report that in patients in Greater London and S.E. England, with hypersensitivity to this spore, symptoms are precipitated when concentrations in Central London rise to 3000 or more/m^3. Many patients with allergic bronchial asthma are relieved of their symptoms in high mountains and Davies (1969a, b) related the freedom from asthma experienced by British children in the Alpine resort of Davos to low concentrations of fungal spores. In Davos in 1966, concentrations of 3000 Cladosporium spores/m^3 were recorded on 6 days, whereas in London concentrations of $3000/m^3$ were recorded on 40 days. In 1967, Cladosporium concentrations of 3000 spores/m^3 or more were recorded on 2 days in Davos and on 25 days in London. Moreover, very much higher Cladosporium con-concentrations were recorded in London. For British children with an experience of the seasonal allergen challenge in Great Britain, the challenge in the Alpine Valley was very low and there was a clinical benefit gained from temporary residence in the valley.

The mean daily concentrations for Cladosporium in Baghdad were all less than $3000/m^3$ and similar results were reported from Kuwait by Davies (1969c). Europeans with a history of Summer asthma might benefit from staying in Baghdad when the dry air-spore challenge was at its height in the third quarter of the year in Britain. However, it must not be assumed that Cladosporium concentrations in Baghdad are too low to cause allergic disease in Iraqi patients. Allergic bronchial asthma occurs in patients native to the Alpine valleys, and the clinical sensitivity of a population must depend on its experience of exposure over many seasons. The patient with a seasonal allergic disease is free from symptoms until the seasonal challenge occurs. At the begining of the season, the patient appears to be more sensitive to an inhalant allergen such as grass pollen than he is towards the end of the season, (Davies,1973).

The patient's sensitivity declines, through natural exposure and the development of a degree of hyposensitization during the season.

In the cereal growing areas of Europe where high concentrations of Cladosporium occur in the Summer months, the allergic population may be less sensitive to this spore allergen than the comparable population of Baghdad, where the concentrations in the ambient atmosphere are low. On the other hand it may be the case that in such countries as Iraq, where the relative humidity of the ambient atmosphere is very low, Cladosporium spore allergy is rare, or perhaps occurs only in the marsh area. Further investigations are required and test reagents for allergy to the common species of Cladosporium and Alternaria are readily available suitable from commercial sources.

Sensitivity to Alternaria is frequently present in patients sensitive to Cladosporium and Frankland and Davies (1965) reported its clinical importance in Britain to be equal to that of the Penicillia and second only to Cladosporium as a mould allergen. Frankland and Davies (1965) considered mean daily concentrations of 50 Alternaria spores/m^3 to be clinically significant, and higher concentrations than this were recorded in Baghdad on March 13th, 1983 and May 15th, 1982 (Table 6).

Alternaria and *Cladosporium* occur in the air at the same time and under the same conditions. They are the most important constituents of the dry air spora and it must be appreciated that the ambient atmosphere never contains a pure suspension of only one spore type. The allergenic aerosol is a mixture of allergenic particles and both *Cladosporium* and *Alternaria* are associated with cereal crops, with aerial concentrations rising at harvest time in areas of arable farming (Davies, personal communication). The air immediately downwind from a combine harvester contains many millions of these spores per cubic metre.

Baghdad lies in the area of historical Mesopotamia, a cereal growing area, and the low concentrations recorded for *Alternaria* and *Cladosporium* must be attributed to a very dry micro-climate within the crops. These fungi commonly occur on the phylloplane and stems of grasses in temperate regions (Hudson and Webster, 1958).

Ascospores

The Ascomycetes comprise the largest group in the fungi and are characterised by the production of ascospores in special cells termed asci. Since the spores are formed following cytoplasmic cleavage within the ascus, the spores lack such points of attachment as the apiculus in the basidiospore or collar as seen in many conidia. In most, the ascospores are explosively discharged, "squirted out" from the ascus by a mechanism described by Ingold (1967). In Great Britain, highest airborne concentrations of ascospores are recorded during damp weather in the Summer and Autumn (Gregory and Hirst 1957; Hamilton, 1959; Davies et al 1963; Davies 1969a). Details of genera with morphologically distinct ascospores are given by Hamilton (1959) and Davies et al (1963). These include Chaetomium, Leptosphaeria, Pleospora, Sordaria and Venturia. Hamilton (1959) classified unidentified ascospores into Filiform Fat, Filiform Thin, Fusiform and miscellaneous ascospores.

The species of Leptosphaeria are "leaf spot fungi" which in the British Isles commonly occur on plant leaves and stems in the Autumn. The ascospores of Leptosphaeria are multiseptate fusiform yellow spores which may or may not have one markedly swollen cell (Hamilton, 1959). She restricted Leptosphaeria to include only ascospores with one swollen cell. However, Davies et al (1963) included hyaline, yellow to brown, cylindrical or fusiform ascospores with transverse septae. The ascospores of the L.eustoma group do not have a swollen cell. Last (1955) recorded ascospores of the L.eustoma group in concentrations of 65,000 spores/m^3 in the air of a barley crop.

Results

The ascospore types recorded in the air of Baghdad were hyaline once septate, brown ovoid and septate, but as concentrations were low they were simply grouped as Ascospores. The proportion of Ascospores to other spore types is shown in Fig 26, they comprised 4.2% of all the fungal spores trapped. Highest concentrations of ascospores were recorded in April, May and June (Table 8,9).

Discussion

The role of ascospores in the aetiology of seasonal allergies has been scarcely investigated. Ascospores recognised in deposits trapped from the air have either not been grown in artificial culture, or if developing in artificial culture are attributed to well known genera of the fungi imperfecti whose relationships to perfect states have yet to be established. For example, _Leptosphaeria acuta_ (Hoffm. ex Fries) Karst grows on the lower stem regions of the common stinging nettle _Urtica dioica_ Linn and produces yellow/brown ascospores which are fusiform and septate and characterise the perfect form of the fungus. When portions of nettle stem are planted in agar media, the fungus that develops is the imperfect form _Phoma acuta_ which produces simple hyaline spores in flask shaped pycnidia. Ganderton (1968) obtained positive skin-prick tests with an extract of _Phoma_ in patients with a clinical history

Table 7 Days with the highest mean concentration of <u>Basidiospores</u> and <u>Ascospores</u> at Baghdad in 1982 and 1983

Month	Day	Basidiospores spores/m^3	Day	Ascospores spores/m^3
Jan.	15 29-1983	51	15-1983	30
Feb.	3-1983	84	3 4 5-1983 9 20	9
March	27-1982	102	27-1982	30
	31-1983	105	9-1983	12
April	1-1982	75	7-1982	42
	1-1983	270	15-1983	15
May	8-1982	117	17-1982	30
	10-1983	60	20-1983	27
June	7-1982	81	4-1982	24
	23-1983	60	15-1983	21
July	25-1982	90	2-1982	12
Aug.	3-1982	51	2 3-1982	9
Sept.	9-1982	42	9-1982	12
Oct.	22-1982	60	25-1982	15
Nov.	11-1982	147	3 10-1982	18
Dec.	3-1982	39	14-1982	12

Table 8 Mean monthly spore concentrations in the air of Baghdad from March to December, 1982

Spore Type	March	April	May	June	July	August	September	October	November	December
Ascospores	61	128	112	43	14	20	36	46	54	40
Basidiospores	351	229	321	200	503	180	147	188	570	153
Curvularia	4						15	13		
Epicoccum	8	2							1	
Helminthosporium	20	11	19	12	13	18	18	17	25	11
Stemphyllium	42	9	80	20	49	11	12	16	52	23
Torula	1	1	14	2	6		2	4	2	
Myxomycetes		39	106	6	47	13	8	4	74	18
Ustilago	27	15	10	28	35	12	1	42	15	5
Uredospores	23	12	68	30	23	10	17	23	73	23
Teleutospores	13									4
indeterminate				3						

Table 9 Mean monthly spore concentrations in the air of Baghdad from January to June 1983

	January	February	March	April	May	June
Ascospores	29	26	22	33	82	104
Basidiospores	171	167	234	642	350	100
Curvularia		3	2	12		
Epicoccum	3	1	12	6	4	
Helminthosporium	11	9	17	46	32	14
Stemphyllium	16	5	31	32	29	22
Torula	5		6	1	1	1
Myxomycetes	2	1	3	22	28	10
Ustilago	10	5	16	87	32	41
Uredospores	20	13	29	39	41	110
Teleutospores	3	9		3	10	5
indeterminate				2	1	

of seasonal asthma when Leptosphaeria spores occurred in the air. Whether or not the antigenicity of the ascospore is the same as the pycnidiospore is unknown since Leptosphaeria ascospores have yet to be produced in a pure, weighable quantity. This applies to most of the ascospore genera recognized as occurring in the air. It is probable that the spores of Erysiphe are allergenic but material to challenge a patient with an appropriate history of allergic sensitivity to this ascomycete has never been available. There is thus scope for research into ascospores and the ascomycetes as inhalent allergens.

Ascospores are unlikely to be important fungal allergens in Baghdad. the microclimate over the surfaces of vegetation is probably too dry to facilitate colonisation and ascospore discharge by these fungi. However, elsewhere in Iraq, in the marshes, these fungi could occur locally in high concentrations. The following ascomycetes have been listed by Abdullah (1982) and were found mainly in sheep or donkey dung in the Southern Desert:-

Ascobolus crenulatus Kebat

Ascobolus immersus Pers ex Fries

Ascodesiuis microscopica (Grouhan) Seaver

Ascodesiuis porcina Seaver

Ascopharus lacteus (Cooke and Phill) Sacc

Chaetomium bostrychodes Zopf

Chaetomium circinatum Chirers

Chaetomium globosum Kuze ex Fries

Chaetomium murorum Corda

Chaetomium siprale Zopf

Coprotus dulpus Kimbrough, Luck-Allen and Cain

Idophanus carneus (Pep ex Pers) Korf

Idophanus sp.

Kernia nitida (Sacc) Neuil

Lasiobolus sp.

Lophotrichus brevirostratus Awes

Podospora communis (Spg) Niessel

Podospora inaequalis (Cain) Cain

Podospora longicaudata (Griff) Cain

Podospora prethopodialis Cain

Saccobolus depauperatus (Berk and Broome) Ex Hansen

Saccobolus minimus Vel

Sordaria fimicola (Rab) Ces and De Not

Sordaria humana (Fuckel) Winter

Sparormiella austrolis (Speg.) Ahmed and Cain

Sporormiella commutata (Nieisel) Ahmed and Cain

Sporormiella intermedia (Auersw) Ahmed and Cain

Sporormiella minima (Auersw) Ahmed and Cain

Sporormiella pulchella (Hansen) Ahmed and Cain

Sporormiella teretispora Ahmed and Cain

Tripterospora erostrate (Griff) Cain

Zygopleurage faigumensis Lung

Since these coprophilous fungi most probably have their origin in the fodder fed to the animals, they must occur on vegetation in Iraq. Evidence of ascomycetes growing on the surface of vegetation in the North of Iraq has been published by Mathur (1969) who records _Cynathodia trifolii_ on clover, Er_isiphe cuichoracearum_ or _Crepis spp_, _Leptosphaeria salvinii_ an _Oryzasatira_, _Phyllachara graminis_ on _Hordeum glaucum_ all from near Mosul. _Amphalospora melaveae_ was found on _Astragalus brugeiri_ and _Thielvaria brasicola_ on _Nicotiana tobacum_ from Sulaimaniya. _Didymosphaeria sinarogdina_ was reported by _Mathur_ (1969) as causing leaf spot on _Phoenix dactilifera_ near Basra in December. There is a possibility of ascopores occurring in high local concentrations in Iraq, but it is unlikely that ascospore sensitivity will be common. The scarcity of ascospores in the airspora of Baghdad may mean these fungi tend to occur in a mycelial form, which fails to sporulate and/or discharge ascospores under the very dry conditions within crops and vegetable litter in the vicinity of Baghdad.

Basidiospores

Basidiospores from toadstools, bracket fungi etc. are usually asymmetrical and characterized by a distinct apiculus. A few are sufficiently distinctive to be recognizeable and assigned to genera and even species in the _Agaricaceae_. In general, Basidiospores are classified as hyaline, yellow or brown. Hyaline basidiospores can generally be distinguished from the spores of the hemibasidiomycete Sporobolomyces. The toadstools occur in mycorrhizal association with the roots of forest trees and in consequence high concentrations of these spores may be encountered in forests.

Results

In the air of Baghdad, the total basidiospore catch comprised 25% of all the fungal spores trapped (Fig 26). The maximum monthly concentrations were recorded in July, November 1982 and April 1983 (Table 8,9) and the highest mean daily concentration of 270 spores/m^3 was obtained on April 1st 1983 (Table 7).

Discussion

In a paper entitled "Woodlands as a source of allergens" Adams, Hyde and Williams (1968) drew attention to the importance of basidiospores. the aerial occurrence of these spores in the air during late Summer and Autumn in the British Isles had been described by Gregory and Hirst (1952), Hamilton (1959), Hyde and Adams (1960) Davies (1962), Davies, Denny and Newton (1963), Davies (1965).

Frankland and Hay (1951) reported the spores of the dry rot fungus Merulius (Serpula) lacrymans which grows on timber indoors could be an allergen. Gregory and Hirst (1952) observed that basidiospores "obviously need investigation in connection with seasonal asthma". Then Bruce (1963) by means of intradermal skin testing and bronchial challenge showed that two different mixtures of basidiospores gave positive reactions (in 15 out of 20 patients skin tested with extracts 1:10 w/v and 12 out of 20 tested bronchially). Mixture 1 contained the spores of _Polyporus giganteus_, _Ganoderma applanatum_ and _Stereum hirsutum_: mixture 2 contained the spores of _Polyporus betulinus_, _Polystictus versicolor_, _Armillaria mellea_ and _Fomes annosus_. Herxheimer, Hyde and Williams (1966) by skin testing (intradermally with extracts 1:1,000 w/v) and by inhalation tests have shown that the spores of certain individual basidiomycetes viz: _Agaricus bisporus_, _Armillaria mellea_, _Coprinus micaceous_, _Ganoderma applanatum_, _Hyphaloma fasciculare_, _Polyporus squamosus_ and _Merulius (Serpula) lacrymans_ were allergenic in patients whose asthma was exacerbated in the late Summer and Autumn each year when airborne basidiospore concentrations are at their seasonal maxima. The challenge extracts were prepared from spores collected by laying the fructifications on glass under sheets of damp filter paper, and then scraping off the spores. This method is laborious and does not lend itself to the large scale manufacture of extracts in the pharmaceutical industry. It is probable that this reason accounts for the very few investigations into basidiospore sensitivity. Extraction of the entire macroscopic fungus is a possibility and Davies (unpublished) has compared pure spore extracts with whole fungus and dried pileus extracts and obtained similar results. Use of the entire fructification is complicated by the fact that the fungus may be infected by maggots without any outward sign.

Apart from the investigations on basidiospores as allergens which have been described from the British Isles, the only other studies appear to be those of Chabert and Nicot (1966, 1968) who employed a Hirst trap to study the content of the air at Rabat, Morocco. Chabert and Nicot (1966) appear to have been primarily concerned with Ustilago but record Coprinus, Ganoderma, and Boletus. In 1968 they record Pistillaria culmigena Mont. ex Fr. Botryobasidium sp and Sistotrema Aff brinkmanii (Bres.) J.Erikss. It is doubtful that spores in a deposit trapped from the air could be identified with the taxonomic precision given.

Little is known of the Basidiomycetes of Iraq. Rattan and Abdullah (1977) in a study of wood rotting fungi in Iraq listed Galinia cystidiata, sp nov. Gleocystidiellum luridum, Radulomyces submolaris, Athalia acrospora, Amphineum tomentellastrum, Sterum Sanguinolentum and Astreroma medium.

Rattan et al (1980) report the following basidiomycetes to be associated with the date palm: *Oliveonia pauxilla*, *Sistotrema brinkmanii*, *Trechispora farcinacea*, *Phanerochaeta cremea*, *Hyphoderma sambuci*, *Hyphoderma pubera*, *Hyphoderma pubera var dactylifera*, *Anerodia albida*. From Iran, Fallahyan (1975) has recorded the Gasteromycete *Cathrus cancellus*; the Hymenomycetes - *Amanita pantherina*, *Amanita phalloides*, *Amanita verna*, *Armillaria mellea*, *Boletus aereus*, *Boletus badius*, *Boletus edulis*, *Boletus luteus*, *Clitocybe geotropa*. *Collybia acervata*, *Collybia fusipes*, *Coprinus atramentarius*, *Coprinus cartinatus*, *Coprinus ephemeris*, *Coprinus miaceous*, *Hypholoma fasciculare*, *Hypholoma sublateritum*, *Lactarius vellereus*, *Lepiota helveola*, *Lepiota procera*, *Marasminus stipitarius*, *Mycena echinipes*, *Mycena inclinata*, *Paxillus atrotmentosus*, *Pleurotus ostreatus*, *Psalliota campestris*, *Russula ilacea*, *Russula sp*, *Volvaria iranica*, and in the Aphyllopharales - *Calocera viscose*, *Cantharellus ciborius*, *Cantharellus cinereius*, *Cantharellus tubaefaeasuis*, *Clavaria abietina*, *Clavaria vermicula*, *Clavaria spp*. *Hydnum (Sarcodon) repandum*, *Leptosporus adustus*. *Phoeolus alborubescens*, *Ungulina annosa*, *Unginula betulina*, *Xanthochonus hisbidus*.

It should be noted that in studies on the air spora by cultural methods, apart from *Trechispora brinkmanii* and the mirror yeasts *Sporobolomyces*, most basidispores, even if germinating on the medium employed, would only develop as sterile mycelium. Basidiospores are therefore, not recorded in settle plate surveys. Moreover, because they are small basidiospores are poorly impacted onto surface traps by eddy diffusion and tend not to be recorded on "gravity slides".

The concentrations of basidiospores trapped in Baghdad are very much lower than those recorded in the British Isles where they have been shown to be a cause of allergic asthma. However, the possibility of basidiospores causing asthma in local situations in the Northern forest areas of Iraq should be considered.

The patient living in the North of the country should be asked if his symptoms of bronchial asthma developed when he was in or in close proximity to woodlands and forests. Many of the toadstools occur in symbiotic association with forest trees. the trees act as a wind barrier with the result that in woodlands the air tends to be still, and a stable humid atmosphere develops which facilitates the production of high concentrations of basidiospores. The notes of Rattan, Abdul-Haffiz and Al-Dboon (1980) on the fungi associated with the date palm may be important for patients living in the area of Baghdad. In taking a clinical history it would be useful to ask patients if they suffered symptoms of asthma in the vicinity of date plams, or decaying fallen vegetation from date palms. In considering the diagnosis of basidiospore sensitivity, the allergist should appreciate that the small size of the basidiospores renders it unlikely that the patient will complain of eye symptoms as in pollinosis. Whereas the mass of inertia of a pollen grain >30u in diameter facilitates impaction of the grain on to the eye, at low wind velocities, small fungal spores are not impacted and retained. Studies on basidiospores as allergens in Baghdad and Iraq are worthy of further attention and should be based primarily on investigation of the environment of the allergic patient when symptoms were developed. Allergen challenge tests, and tests for specific IgE antibodies should be developed and in this the Clinical Allergist will require mycological assistance to produce the appropriate allergens.

The Aspergillaceae

The Burkard trap underestimates the aerial concentrations of both *Aspergillus* and *Penicillium*. Both produce hyaline conidia in chains, and morphologically it is not possible to distinguish between these two genera in a deposit trapped by impaction from the air. Because of the distance between Baghdad and London, and wartime complications, it was not practicable to undertake a cultural study by means of an impinger or impactor. A sample of house dust was collected each month, which should indicate some of the *Aspergillus* and *Penicillium* species occurring in the intramural environment in Baghdad (See below).

However, it must be noted that the conidia of both *Penicillium* and *Aspergillus* are important allergens. Frankland and Davies (1965) reported the genus *Penicillium* to be second in importance only to *Cladosporium* as a mould allergen. Ambler and Vernon (1951) considered the Penicillia to be particularly important in urban atmospheres, and on temperate climates the *Penicillia* are frequently found in household waste. Al-Doory, Y., Tolba, M.K. and Al-Ani, H. (1959-61) in a study of the fungal flora of soils in 5 areas of Iraq, Baquba, Faleuja, Hilla, Kufa and Mahmudiya, record the isolation of 23 species of *Penicillium* and 12 species of *Aspergillus*. However, the species of *Penicillia* occurred sporadically, and no species was isolated from all the samples. In contrast *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* were recovered from all 5 sampling sites. Over a period of many years, occasional soil and house dust samples from countries in the Middle East have been examined in the mycology laboratory at St. Mary's and *Aspergillus* species have been cultured more frequently than species of *Penicillium* (Davies, personal communication); the most common species being *Aspergillus niger* and species of *A.flavus-oryzae* series. The allergic patient in Baghdad may encounter high local concentrations of *Penicillium* spores because of its role in biodegradation but in the hot climate of the Middle East, *Aspergillus flavus* and *Aspergillus niger* are likely to be more important as inhalant allergens.

In temperate climates the most important species of *Aspergillus* is without question *Aspergillus fumigatus*. An extract of this fungus should be included in the range of skin-prick test reagents in every allergists clinic in Europe.

The importance of *Aspergillus fumigatus* as an allergen was first described by Storm van Leeuwen (1924). Without question, this species of *Aspergillus* is the most important in Europe since it is the cause of the Allergic Broncho-Pulmonary Aspergillosis syndrome (ABPA) described by Hinson,K.F.W. Moon and Plummer (1952). In ABPA, an asthmatic allergic to *Aspergillus fumigatus* suffers the complication of having asthma with a surfeit of excretion into the bronchial tree. A build-up of secretion in the lumen of the bronchioles results in a narrowing of the airways, an increased air velocity and impaction of inhaled spores into the secretion. Colonisation of the secretion can lead to the production of sputum plugs containing mycelium, and there is exacerbation of the allergic asthma.

A similar condition due to *Aspergillus flavus* has been observed in a patient from Pakistan (Davies, personal communication). It is probable that *Aspergillus flavus* and *Aspergillus niger* can cause ABPA in tropical areas. In Europe, an aqueous extract of *Aspergillus fumigatus* is an essential skin-test reagent for every allergy clinic. It is recommended that extracts of *Aspergillus flavus* and *Aspergillus niger* should be used in the routine skin-prick testing of asthmatic patients in Baghdad and tropical parts of Iraq.

Curvularia, Epicoccum,Helminthosporium, Stemphyllium, Torula, Myxomycetes, Rust and Smut Spores

Results

These spores are morphologically distinct and can be assigned to form genera. However, since they tended to occur sporodically, and in low concentrations in comparison with these recorded in London, the results are given as mean monthly concentrations in Tables 8 and 9.

Discussion

In this preliminary survey emphasis has been given to the months of the year when these spores attained their highest concentrations. Apart from the myxomycetes, all these fungi may be associated with cereal crops.

It is of interest that Ustilago was at its peak in April, 1983, Table 9 and October in 1982 Table 8. Ustilago tends to occur in the air in high concentration at the same time as grass pollen in Great Britain, and the peak month for grass pollen in Baghdad was April. the occurrence of the spores in the air in the Autumn may reflect a colonization of cereal straw. Ustilago sensitivity can occur in dock workers who unload grain, and in all probability 'silo' workers exposed to high concentrations. A diagnostic problem observed by Davies (personal communication) is that a patient who does not give a positive reaction with skin prick test to an aqueous extract of Ustilago, can react when the spores are applied to the skin, a drop of saline added and the skin pricked through the puddle. Sensitivity to Ustilago requires further investigation. Sensitivity to rust spores appear to have first been described by Cadham (1924) from a cereal growing area of the Canadian prairie. This sensitivity is not well established since in cereal growing areas, the weather facilitating high spore concentrations ensures the rust spores are present in a mixed suspension with Cladosporium and Alternaria.

The importance of Curvularia as a mould allergen appears not to have been investigated, probably because it is common in tropical countries and of rare occurrence in temperate areas.

The spores of Epicoccum, Stemphyllium, Helminthosporium and the Myxomycetes require further investigation as allergens in temperate countries where they occur in greater concentrations than in Baghdad. It seems unlikely that these spores will be individually important as airborne allergens for the population of Baghdad. However, they do add to the total amount of antigenic material in the atmosphere.

A high local spore concentration consequent upon a severe crop infestation or the composting of vegetation can be a cause of allergic sensitivity. In such cases it is the clinical history which indicates the possibility of a mycological investigation being worthwhile.

SECTION III

MOULD FUNGI IN HOUSE DUST

MOULD FUNGI IN HOUSE DUST

The Burkard trap has the trapping efficiency of the second stage of cascade impactor, which retains 50% of water droplets of 4μ diameter. Even when fully turgid the conidia of most members of the Aspergillaceae are smaller than this. Moreover, the Aspergillaceae contains the important genera *Aspergillus* and *Penicillium*, and although the conidia of the family can be identified in deposits impacted from the air, visual identification does not differentiate between the two genera. Since it was not practicable to take volumetric air-samples with either a slit sampler or a liquid impinger during the period of the survey, a pilot study of mould fungi in house dust was undertaken. The aim of this study was two-fold, first to provide information on mould fungi common in the environment in Baghdad, and secondly to gain an introduction to, and some experience of identifying common mould fungi in culture.

METHODS

Each month, a sample of house dust was collected in Baghdad and sent for examination. Samples of house dust were also collected from the houses of 8 allergic patients in February and March, 1982. A 10gm sample of dust was weighed and then added to 250ml of sterile distilled water, plus 0.1ml of Tween 80 in a 500ml conical flask. the flask was vigorously shaken and then 1ml transferred to 9ml of sterile water in a screw capped universal container. This was thoroughly shaken and similar decimal dilutions prepared. 0.1ml samples of the different dilutions were transferred to petri dishes of malt extract agar and spread with a glass spreader. Malt extract agar was used for all primary isolations and when necessary for identification purposes, subcultures were made on Czapek's agar. Details of the culture media are given in the appendix. From each dilution not less than four plates were cultured, with a minimum of two plates being incubated at 37°C and 2 at 25°C. Cultures incubated at 37°C were examined daily, those incubated at 25°C were examined after 5 days and then daily. A simple outline drawing of the colonies in each culture plate labelled with the names of the colonies was made for record purposes, and all colonies which could not be identified in primary culture were subcultured. All the Penicillia were grown on Czapek's agar for identification purposes.

Identification was based on the colony characteristics and microscopic details given in the following:-

(1) An Introduction to Industrial Mycology by George Smith. London, 1960. Edward Arnold (PUblishers) Ltd., pp 399.

(2) A Manual of the Aspergilli, by C.Thom and K.B. Raper. Baltimore. The Williams & Wilkins Company, 1945, pp 373.

(3) A Manual of the Penicillia by K.B. Raper, C.Thom & Dorothy J. Fennel. London Baillere, Tindall & Cox, 1949, pp 875.

Table 10 Fungi cultured from house dust samples collected each month from Baghdad

Fungi	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.	Dec.
Penicillium cyclopium Westling	$4x10^3$	$5x10^3$	$4x10^3$	$7x10^3$		$2x10^3$				$1x10^4$		$3x10^4$
Penicillium expansum Link							$1x10^4$	$5x10^3$			$4x10^4$	
Penicillium viridicatum Westling					$6x10^3$							
Aspergillus fumigatus Freserius	$5x10^3$										$2x10^4$	
Aspergillus niger van Tieghem		$6x10^4$				$5x10^3$	$2x10^4$	$25x10^3$	$2x10^4$	$3x10^4$		$2x10^3$
Aspergillus terreus Thom			$5x10^3$	$1x10^4$	$2x10^5$	$1x10^4$	$5x10^3$	$1x10^4$				
Alternaria tenuis Nees ex Wallroth	$6x10^4$									$15x10^3$		
Aureobasidium pullulans (DeBary and Low)			$1x10^4$	$5x10^4$								
Cladosporium herbarum Link ex Fries	$3x10^3$		$5x10^3$	$4x10^3$		$1x10^4$	$5x10^4$	$5x10^3$	$2x10^3$		$3x10^3$	$1x10^4$
Pestalotia gracilis de Notaris	$5x10^3$											
Sterile white mycelium	$5x10^3$	$5x10^3$								$1x10^4$		
Mucor racemosus Fresenius			$5x10^4$					$5x10^3$				
Rhizopus nigricans Ehrenberg		$5x10^3$			$5x10^4$	$2x10^4$	$5x10^3$					$5x10^3$
Yeasts												
Pink							$4x10^4$				$2x10^3$	
White									$3x10^4$			

Table 11: Numbers of colonies isolated per g of House Dust samples collected by patients in February and March, 1982.

Patient	Fungus	Temperature of Incubation 37C	25C
1	*Aspergillus niger*	4×10^3	1×10^3
	Penicillium spp		3×10^3
	White yeast	2×10^3	
2	*Aspergillus terreus*	3×10^3	1×10^3
	Penicillium spp	1.9×10^4	5×10^4
	White yeast		2.9×10^5
	Pink yeast		1.4×10^4
3	*Aspergillus niger*	3×10^3	1×10^3
	Penicillium viridicatum		1×10^3
	Cladosporium sphaerosperma		2×10^3
	Red yeast		1×10^3
4	*Penicillium cyclopium*		8×10^3
	Aspergillus niger	6×10^2	
	White yeast		1×10^3
	Cladosporium herbarum		6×10^3
5	*Aspergillus niger*	1×10^3	2×10^3
	Penicillium spp	1×10^3	
6	*Aspergillus niger*		3×10^3
	Penicillium spp		2.7×10^4
	Cladosporium herbarum		4×10^3
	White yeast	1×10^3	
	Pink yeast		1×10^3
7	*Penicillium spp*		2.3×10^5
	Paecilomyces varioti		1×10^4
	Cladosporium herbarum		4×10^4
	Sterile white mycelia		8×10^4
	Brown yeast	2×10^4	
8	*Aspergillus niger*	3×10^2	
	Penicillium spp		3×10^3
	Cladosporium herbarum		6×10^3
	White yeast	1×10^3	

Results

The results given in Table 10 show that 3 species of Penicillium, 3 species of Aspergillus, Alternaria tenuis, Aureobasidium pullulans, Cladosporium herbarum, Pestalotia gracilis, Mucor racemosus, Rhizopus nigricans, and both pink and white yeasts. With the technique employed, the lowest concentration found was 10^3 per gram, the highest 10^5 per gram. The pink yeasts were examined for the production of ballistospores, and as there was no evidence of this, it may be concluded that they belonged to the genus Rhodotorula. No further tests were made, and no attempt at identification of the white yeast colonies was undertaken. The fungi isolated from patients dust are shown in Table 11.

Discussion

In a study of mould colonies isolated from house dust samples collected in different parts of Britain, Davies (1960) reports that out of a total of 1,679 colonies only 2 were Cladosporium, moreover, in further investigations of samples of house dust from other countries including India, Mouritins, Canary Isles and Switzerland, Cladosporium was rarely isolated. If Cladosporium was left out of consideration, the mould genera most commonly recovered from house dust by Davies (1960) viz Penicillium. Aureobasidium (Pullularia), Phoma mycelia sterilia and Aspergillus, were those which Hyde & Williams (1949) and Richards (1956) found to be most frequently deposited from the air outdoors. All the species isolated by Davies had been recorded by Richards as present in the British atmosphere. It is therefore reasonable to conclude that the mould fungi recorded from dust samples collected in Baghdad reflect the content of the air in that city. However, in this study, Clad osporium herbarum Link ex Fries was recovered in a concentration of 10^3 and 10^4 per gram from 9 of the 12 samples examined (Table 10). Lactic acid has been successfully employed to examine British dust samples for the house dust mite. When lactic acid was added to house dust from Baghdad, the violent efferevescence that occurred prohibited the use of this technique, and showed a mineral content of "limestone" origin. It is possible that the alkaline content of the dust facilitated the survival of Cladosporium spores. The species recovered from the Baghdad dust were all recorded in the British study with the exception of Aspergillus terreus and Pestalotia gracilis. It is noteworthy that Aspergillus fumigatus was recovered less frequently than A.niger and A.terreus. The month of the year in which the dust samples were obtained appears to have no effect on the fungi grown. Although the mould content of the house dust is not a substitute for a cultural survey on the content of the air of Baghdad, it is reasonable to recommend that skin-test extracts of Penicillium cyclopium, Cladosporium herbarum, Aspergillus terreus. A.niger and A. fumigatus should be employed in the investigation of fungal allergy in Baghdad. Use of extracts of three species of Cladosporium and Alternaria tenuis has already been recommended for diagnostic use in Iraq.

The mould fungi isolated from dust samples collected in the homes of patients (Table 11), was combined with an attempt to record the presence or absence of the house dust mite. No mites were found. This study was undertaken prior to examination of the mould flora month by month, and the species of Penicillium were not determined. The assemblage of fungi in house dust from the patients homes did not appear to differ substantially from that given in Table 10. More species developed with incubation at 25^{o}C, but at 37^{o}C there was a greater recovery of Aspergillus spp.

SECTION IV

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APPENDIX

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APPENDIX

Collection of Pollen for allergen extraction

Preparation of Allergenic Extracts

List of genera and species of the Chenopodiaceae recorded in Iraq

Culture Media

Collection of pollen for allergen extraction

The method is based on that of Freeman (1951). Pick the "grass-heads" directly the anthers begin to form. Cut the stems to a uniform length, of about 30cms and carry them indoors so that they may be placed in bunches of 50 in a suitable vessel filled with water. The vessels are then placed on greaseproof covered tables in a sunny room. The anthers will continue to develop, and when anthesis commences the pollen will appear on the anthers coating them a bright yellow. When the "heads" are then gently patted the pollen is dislodged and settles onto the greaseproof paper, which facilitates its collection.

Freeman reported that when a table was placed in front of a S. facing domestic window, and then covered with jars containing the "Grass-heads", more was produced from the row of jars next to the window pane than from the jars on the rest of the table put together. To obtain grass pollen in kilogramme quantities, Freeman designed a special building, which he termed a Pollenarium. This was necessary because in S.England the weather of June and July is variable. In wet or cloudy weather little or no pollen develops. Freeman's pollenarium was a glass house fitted with overhead electric heaters. Since the weather conditions in Baghdad are constant when the grasses are in flower, it is likely that in any building used to ripen grass for pollen production indoors, the problem will be overheating and venti lation may be necessary. The air in the building must be relatively still to facilitate pollen collection.

Freeman describes a process be termed "milking". When a good crop of the yellow pollen has formed on the anthers of the grass-heads, all the windows and doors are rigorously closed, and the bunches of grass-heads in the jars are gently patted with hand. The pollen thus detached, produces a cloud which hangs suspended above the tables in the still air. After all the bunches of grass-heads have been thus "milked" the milker steals quietly out and locks the door.

After the pollen has settled onto the grease-proof paper after an hour or two, the jars with the grass-heads are removed, and taking great care not to spill a drop of water, placed on a neighbouring empty table which has already been prepared with greaseproof paper.

The uncovered sheets of paper, with all that has fallen on them from the milked flower-heads, are manipulated into a pile in the center of each sheet of waxed paper by lifting the corners or sides of the paper and gently tapping it. It is noticeable in this and the following manoeuvers with the pollen that, once it has settled in a mass, it does not easily become a cloud again. There seems to be some agglutination or attraction between grain and grain so that it pours almost like a fluid. For storage purposes the pollen must be bone dry. Since the developing rooms have hundreds of jars of water in them, the air may be humid.

The pollen may be dried in a room with no vessels of water, a ventilated incubator or by simply placing it in a suitable dessicator. If the pollen is damp, it will not pour , and if stored damp it will be useless for allergenic extraction. Dry pollen was successfully stored for a number of years at St. Mary's, by keeping it in large screw capped metal jars containing a small container of silica gel.

The principles developed by Leonard Noon, his botanist sister Dorothy, and Freeman, to collect pollen have been successfully used to collect pollen from trees and "weeds". The paramount principle is that the flowers are collected before the anthers start to shed pollen and the best time for collection can only be judged from experience.

Preparation of Allergenic Extracts

(as used by Dr. A.W. Frankland, formerly Director of the Allergy Department, St.Mary's Hospital, London)

Moulds

Grow in Sabouraud's broth in Thompson bottles at room temperature for the appropriate time. Pour away the culture fluid. Wet the pellicles with methylated spirits. Dry mats at 56°C. Test for viability and if safe, comminute the mats in a coffee grinder and store under dry conditions until required for extraction.

Yeast and Yeast-like fungi

Grow on Sabouraud's agar for an appropriate time. 37°C
Wash off growth with sterile distilled water.
Pour the suspension into weighed pots and dry.
Store under dry conditions.

Preparation of Diluting and Extracting Fluid

Phosphate Buffer

NaH_2PO_4	0.37g
$Na_2H\ PO_4$	1.42g
NaCl	2.5 g
Phenol	4.0 g
*Distilled water	1 litre

*For desensitizing sets this must be glass distilled and autoclaved immediately after collection. Store in the dark.

Extracting Procedure

General

Prepare a 10% w/v (=100,000 Noon Units) solution in phosphate buffer.

Leave at 4^{o}C for 1 week with frequent mixing.

Buchner filter (Whatman No.4).

Seitz filter using HP/EKS pore size.

(Small quantities may be centrifuged then passed through a 0.22u Millipore filter).

Test sterility.

Grass pollen - (Pollaccine)

Weigh out 5 gms pollen and add 100ml sterile distilled water.

Shake for exactly 1 minute.

Buchner filter (Whatman No. 4).

Using a clean bottle and funnel scrape the pollen from the filter into the funnel and wash into the bottle with 100ml phosphate buffer. Combine equal volumes of Cocksfoot and Timothy for stock solution.

Test for sterility.

Other pollen

Prepare and test as in general procedure.

Foodstuffs

Milk	- No extraction. Separate and discard cream. Filter remaining milk and test sterility.
Egg	- Yolk, white and whole egg prepared. Add 10ml volume to 90ml phosphate buffer and extract.
Fruits & Vegs.	- Express juice and Seitz filter.

Nuts & Cheese	- Comminute. Defat over several days with acetone changes. Dry. Extract as for general procedure.
Meat & Fish	- Cut into pieces. Obtain dry weight by drying 30 gms at 56°C. Using some of the original (not dried) material extract at 10% w/v as for general procedure.

Some extracts, notably meat and fish, may throw a deposit on keeping. This is of no significance allergenically and the potency of the extract is unchanged.

All other dry materials, e.g. house dust, animal danders are extracted as in the general procedure.

Skin Testing Extracts

All material, except Pollaccine, is made up to 100,000 units/ml.

Pollaccine is made up with 100,000; 10,000; 1,000; 100 and 10 units/ml.

All skin testing solutions have Tween 80 1 in 10,000 added to each bottle.

Nasal Provocation Extracts

Dilution made as requested.

Extracts are dispatched in clean bijou bottles with a dropper attached.

Nasal Sensitization Extracts

Dilutions made as requested.

Diluent for Nasal Provocation and Sensitization Extracts

50% glycerol.

40% distilled water.

10% 10X conc. Extracting fluid.

Seitz filter before use.

Sterility Testing

Media

Aerobic		Anaerobic
L-cystine HCl	1.0g	
NaCl	2.5g	1000ml Aerobic base + Thioglycollic acid 0.5ml
Dextrose	5.0g	+ 1% Methylene blue 0.1ml
Yeast Extract	5.0g	
Tryptone	15.0g	pH to 7.4
Distilled H_2O	1 litre	Bottle in 20ml amounts in 1 oz bottles
Warm to dissolve		Autoclave
Filter (Whatman 40)		
pH to 7.4 and refilter.		
Dispense 25ml into 50ml bottles.		

Method

Withdraw 0.4ml of fluid from the bottle of fluid to be tested.

Inject 0.25ml into the aerobic medium.

Inject 0.15ml into the anaerobic medium.

Incubate at 37^{O}C for 1 week.

General

Brown glass bottles should be used, washed thoroughly in distilled water, and sterilised by dry heat at 160^{O}C for 1 hr.

All bottles having contained extracts must be discarded. No. bottle must be used twice. 100,000 unit will keep indefinitely at 40^{O}C. Never freeze.

Extract Dilutions in 5ml bottles.

Chenopodiaceae - Genera and species of the area around Baghdad, Central Alluvial (CA), Eastern Alluvial District (EA), Western Desert (WD) and Lower Jazira (LJ). The district abbreviations are those used in (Fig 1).

Agathophora	alopecuroides(Del.) Bunge
	WD
Anabasis	setifera Moq.
	WD
Arthrocnmrum	fruticosum L.
	WD
Atriplex	
	flabellum Bunge (obione flabellum Bunge)
	CA
	hortensis L.
	CA
	lasiantha Boiss
	CA, EA, WD
	leucoclada Boiss
	subsp. turcomanica (Moq.) Aellen
	LJ, WD, EA
	tartarica L
	WD
Bassia (Kochia.)	
	eriophora (schrad.) Aschers
	CA, EA, WD, LJ
	hyssopifolia (Pall.) kuntze
	CA

Beta

ssp. maritima (L.) Thell

CA, EA

var. foliosa (Ehrenbg.) Aellen

CA, EA

var. glabra (Dell.) Aellen

CA, EA

Bienertia

cycloptera Bunge

LJ, WD, EA

Chenopodium

album L.

CA, EA

ficifolium Sm.

CA, EA

murale L.

CA, EA, WD

vulvaria L.

EA

Cornulaca

aucheri Moq.

CA, WD, LJ

leucantha Charif et Aellen

CA, LJ

monacantha Del.

WD

Girgensohnia

oppositiflora FAl

WD

Halocharis	sulphurea Moq. CA, EA, WD, LJ
Halocnemum	strobilaceum (Pall.) M. Bieb. CA, EA, WD, LJ
Halopeplis	pygmaea (Pall.) Bunge (Salicornia pygmaea Pall.) WD
Haloxylon	
	ammodendron (C.A.M.) Bge. (persicum Bunge) LJ
	articulatum (Cav) Bunge subsp. ramosissimum Eig EA, WD, LJ
	salicornicum (Moq.) Bunge EA, WD, LJ
Kochia	scoparia schrad. var. culta farwell EA
Noaea	mucronata (Forsk.) Asch. et Schw. WD, LJ
Panderia	pilosa F. et M. CA
Salicornia	europaea L var. harbacea L. WD

Salsola

autrani Post

WD

baryosma (Roem et Schultes) Dandy

WD

canescens (Moq.) Boiss. (Noaea

canescens Moq.)

WD, LJ

crassa M.B.

WD, LJ

cyclophylla Baker

WD

incanescus C.A.M.

CA, LJ

inermis Forsk.

WD, LJ

jordanicola Eig.

EA, WD, LJ

Lancifolia Boiss

WD

longifolia Forsk.

WD

pestifera A. Nels.

CA, EA

pseudonitraria Aellen

WD

rigida Pall.

WD

	rosmarinus (Ehr.) Solms-Laubach.
	(seidlitzia rosmarinus Bunge).
	WD
	subaphylla C.A. Mey.
	WD, LJ
	vermiculata L.
	subsp. villosa (Moq.) Eig
	CA, LJ
	volkensii schw. et Aschers.
	WD
Seidlitzia	rosmarinus (Ehrenb.) Bunge
	(salsola rosmarinus)
	(Ehrh.) Eig
	EA, WD, LJ
Suaeda	baccata Forsk.
	(schanginia baccata (Forsk.) Moq.)
	CA, EA, WD, LJ
	mesopotamica Eig.
	CA, EA
	vermiculata Forsk
	CA, EA, WD
Traganum	nudatum Del.
	WD
Amarantaceae	
Alternanthera	sessilis (L.) R.Br.
Amaranthus	albus L.
	blitoides S. Wats.

graecizans L. (angustifolius Lam.)

hybridus L.

retroflexus L.

tricolor L.

viridis L.

CULTURE MEDIA USED

Malt Extract Agar

Distilled water	1,000 cc
Malt extract	20 grams
Dextrose	20 grams
Agar	20 grams

Czapek's Solution Agar

Water	1,000 cc
$NaNo_3$	3.0 grams
K_2HPO_4	1.0 gram
$MgSO_4.7H_2O$	0.5 gram
KC1	0.5 gram
$FeSO_4.7H_2O$	0.01 gram
Sucrose (cube or other good commercial grade)	30.0 grams
Agar	15.0 grams