

**PALYNOLOGICAL CONTRIBUTIONS TO
AEROBIOLOGICAL STUDIES IN
SOUTH-EAST SCOTLAND**

by

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CONTAINS

PULLOUT

DEDICATION

This thesis is dedicated to my wife Helen, but for whose constant encouragement, interest and enthusiastic support over the years to which it relates, it might not have been written.

DECLARATION

Of thirteen relevant publications, nine have been selected for submission in this thesis. Each is based on investigations and/or contributory data compilation undertaken personally by the author.

Eric Caulton

August 2002

ACKNOWLEDGEMENTS

The Pollen Centre's existence, organisation and development owes much to the encouragement and active participation of a number of academic and volunteer scientific colleagues since starting operations in 1987.

It is a pleasure to make grateful acknowledgements to the following:

Professors Charlie Bryce and Paul Read for their support and encouragement; Dr Mavis Tullet and Mr Ian Will, who helped to set up the Centre in the first few years; to the members of the Pollen Centre's team: Mrs Gina Angus, Mr Robin Carmichael, Dr John Ford, Dr Brian Flannigan, Mrs Sue Keddie, Mrs Jill Sales and Mrs Linda Wood for their active contribution to the work of the Centre; to the numerous young people – Job Creation Volunteers and students: Higher National Diploma and French *stage*, all of whom through their involvement in the daily pollen count and project work, have played an important role in maintaining the Centre's programme. Last, and by no means least, grateful thanks are tendered to Mrs Miriam Wallace for her patience in undertaking the task of typing the manuscript, to Miss Marina Mocogni for her technical advice and assistance and to Dr Paul Tett and my daughters Sue and Gill for reading and commenting on the text.

ABSTRACT

Airborne pollens and, more recently, spores, which occur in the rooftop airstream over Edinburgh have been monitored and the data analysed, since 1988. The thesis describes the developing organisation, methodologies and resources which underpin the daily pollen count, data of which are transmitted daily to the UK National Pollen Research Unit at Worcester for the British Aerobiology Federation's database. The data of birch (*Betula*) and grass (POACEÆ), two highly allergenic components of the pollen circulating, are forwarded daily to the European Aeroallergy Network's database in Vienna.

Each of the nine selected publications presented are of significance both nationally and internationally. Both the birch and grass pollen studies revealed problems associated with geographical location, varying heights of trapping sites and determination of start dates for pollen seasons. Likewise, the factors involved in asthma mortality within Scotland, the effect on human health that may be passed by high concentrations of bracken spores and the impact of Dutch Elm disease on the elm population, all highlighted problems in determining which environmental factors are significant and, possibly, causative. The paper which dealt with the possible use of pollen rain analysis on vegetation surfaces, was a response to an hypothesis, which could have proved useful had it been positive. The two papers on the technique of pollen analysis of animal faeces has proved to be of value in determining preferred diet, habitat and cause of pollinosis. Lastly, the biographical paper on Dr Cunningham once again underlined the importance of an individual's contribution to the gradual development of techniques for measuring, evaluating and understanding of the roles of various parameters and their interaction in the discharge, aerial movement and impact of bioparticles.

The thesis concludes with a description and evaluation of the author's contribution to the science of Aerobiology.

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1. INTRODUCTION

The air we breathe and what it contains has prompted the interest and curiosity of man for a very long time. The association of inhalation and wind-bearing noxious substances was believed by Hippocrates and Lucretius, both of whom also held views which associated moving particles in air with disease.

Palynology, which is the principal aspect of aerobiology with which this thesis is concerned is, *sensu strictu*, the study of pollen and spores, and includes not only present-day but also fossil material (Palaeobotany). As with other aspects of rapidly developing and intensively studied disciplines (e.g. modern Taxonomy and Nomenclature), a plethora of terms results, particularly when the studies are international in their scope. Inevitably a point is reached, if the discipline is to be credible and useful, when researchers have to get together to bring some order to a potentially chaotic state of terminology. In Palynology and Palaeobotany, this point was reached in 1994 with the publication by the Dutch Laboratory of Palaeobotany & Palynology Foundation (LPP) of an agreed Glossary of Pollen and Spore Terminology (Punt et al, 1994).

Palynology is the study of pollen and spores, which constitutes not only a discipline in its own right but also a subdiscipline within Pollen Analysis and Aerobiology.

Pollen analysis had its origins at the beginning of the 20th century with Swedish studies of peat deposits (L.von Post, 1916). These early studies became very much the province of the Scandinavian palynologists and only later became more widespread in Europe.

Pollen grains are characterised by distinctive morphological features recognised at specific, generic and familial levels. The virtual indestructibility of the pollen grain's outer coat (exine) due to the complex substance – sporopollenin – of which it is composed, allows the preservation of grains for long periods of time, depending on the nature of the substrate in which they occur (Dimbleby, 1957). The taxonomic specificity of pollen allows the construction of plant communities, of which pollen grains are the representatives (Godwin, 1975, Pennington, 1969).

the light of subsequent developments in the field in which he was interested and worked.

AEROBIOLOGIA publishes from time to time biographical accounts of past eminent scientists, distinguished for their researches, which firmly place them as forerunners of today's aerobiologists. One such, was the Scottish-born medical doctor, a graduate of the Medical Faculty of Edinburgh University, David Douglas Cunningham (1843-1914). Early on in his career he joined the Indian Medical Service having passed with distinction the course at the Army Medical School. In the same year as Charles Blackley published his researches (Blackley, *op.cit*) Cunningham produced a monumental treatise entitled "Microscopic Examinations of Air". Cunningham used an "aeroconiscope" (see p.104) for his observations on the air-spore (Maddox, 1870, 1871). An illustration of this appears as Figure 2 in Chanda & Caulton (1999 see paper pp.96-99).

The 'subsequent developments' above mentioned, embrace the development of research into air-borne particles – the physics (and mathematics) of their dispersal; the chemistry/biochemistry of their contents and reactions and the contribution of the biological components: bacteria, spores of fungi, bryophytes, pteridophytes and the pollen grains of gymnosperms and angiosperms, to the aerial dimension of environmental pollution and health hazard.

The study of the aerial transport of biological particles has, during the last eighty years, come to form the basis of what is now the interdisciplinary science of aerobiology. Originally, when first conceived, the term 'aerobiology' was restricted to the study of living organisms in the air, ranging in size from viruses, through spores and seeds, to insects and birds (Pedgley, 1980). The journal AEROBIOLOGIA include the following aspects in the published 'Aims and Scope' of the publication: 'publication in the interdisciplinary field of aerobiology. Subjects covered include: bioaerosols, transport mechanisms, biometeorology, climatology, biological pollution, microbiology, aeromycology, aeropalynology, arthropod dispersal, and subjects linked to aerobiology (Kluwer, 2000).

The term 'bioaerosols' has come into common usage during the past decade as covering more or less the concept put forward by Pedgley in 1980 (*op.cit.*). The distinguished Indian aerobiologist Shripad Agashe describes bioaerosols as affecting

every aspect of our lives. 'The air, both out of doors and indoors, is almost always laden with microorganisms, pollens, insects and mites, whose numbers and types change with the time of day, weather, season, geographical location and the proximity of local sources'. (Agashe, 1994). Thus the study of aerobiology embraces those aspects of airborne organisms which include sources, take off, dispersal and deposition. The effects of these aspects on other organisms generated the term "aerobiological pathways" (Edmonds, 1979). In his keynote address to the 5th International Conference on Aerobiology held in Bangalore, India in 1994, the late John Lacey stated, 'Aerobiology is a discipline in its own right and it is also a tool used in many other disciplines'. He defined aerobiology as the study of bioaerosols, of airborne particles of biological origin; of their sources, liberation, dispersal, deposition and impact on other living organisms; and of the effects of environmental conditions on each of these processes (cf Edmonds 1979 *op.cit.*). Lacey considered aerobiology to have emerged as 'a single discipline over many years through a synthesis of elements from a wide range of different sources, (Lacey, 1994). During the evolution of this synthesis referred to by Lacey (*op.cit.*) a twin concept pertained to the newly emerging science of aerobiology. In Gregory's 2nd edition "Microbiology of the Atmosphere" (Gregory, 1973), reference is made to this twin concept put forward by Moulton in a publication of the American Association for the Advancement of Science (Moulton, 1942). Aerobiology was at that time conceived as being "extramural" on the one hand and "intramural" on the other. The former related to Plant Pathology whereas the latter was the concern of the hygienist. This approach contrasts with the concept some thirty years on where a binary separation of the discipline more related to the natural dispersion and transport of well adapted organisms as opposed to the planned experimental dispersion or incidental transport of organisms. If a binary division exists today it might well be 'external' (or outdoor) as opposed to 'internal' (or indoor) aerobiology.

2. THE RESEARCH PROGRAMME

2.1 The Pollen Count: Organisation

The Scottish Centre for Pollen Studies was established by the author in November 1987, located in a small laboratory (Plate 1a) and first produced pollen counts for 1988 (Fig. 3). In the earliest years, the workforce comprised JOB CREATION volunteers, who were enthusiastic workers even when no scientific knowledge was evident. In two cases, the training given by the author resulted, at the end of their year allowed at the Centre, in obtaining employment as palynologists *via* the Royal Scottish Museum. The co-author of the French paper (Caulton & Gibson, 1988), was the first Job Creation worker. In 1990 the Centre was re-located to its current site at Redwood House, one of the University campuses, and occupied a part (one wing) of a substantially built greenhouse (Plates 1b&2). Opportunities for expanding resources and equipment were forthcoming and the present laboratory houses a range of equipment, data files, exposed slides collection (now numbering some 4,000 slides), type slide collection (numbering circa 600-700 slides), a working seminar type library of books, journals and offprints – over 500 of which are catalogued. The journals currently taken are AEROBIOLOGIA and GRANA, the two principal internationally listed refereed publications in Palynology. Through the author's personal contacts with French, Spanish and Hungarian colleagues PALYNOSCIENCES, Association des Palynologues de Langue Française (APLF), POLEN (formerly ANALES DE LA ASOCIACION DE PALINOLOGOS DE LENGUA ESPAÑOLA (APLE) published at the Universidad de Cordoba 1984 – present) and PLANT CELL BIOLOGY AND DEVELOPMENT at the University of Szeged respectively, are received. Brief accounts of the Pollen Centre's development have been published from time to time (Caulton, 1994, 1996 and 2001).

An important component of the centre's library, built up over the years by the author, is the collection of keys for the identification of pollen and spores (Hyde and Adams, 1958; Smith, 1984, 1986; Moore et al, 1991; Reille, 1992). Additional works (atlases) which give pollen calendars for various national or international regions have been very useful to date (Charpin et al, 1974;



Plate 1a: Pollen Centre - Original Location (1987-1990)



Plate 1b: Present Laboratory

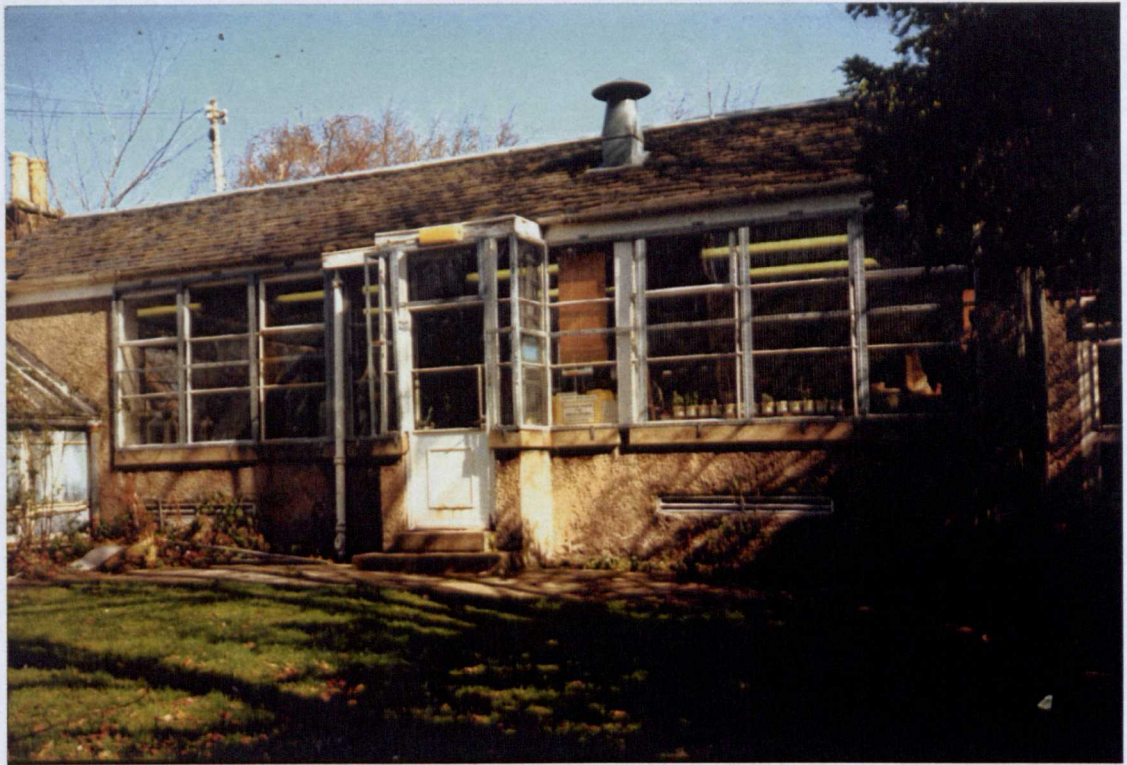


Plate 2: External View of Pollen Centre

Ciampolini & Cresti, 1981; Dominguez-Vilches et al, 1984; Boros et al, 1993).

Resulting from direct personal contacts by the author in 1993 a collaborative programme involving students attending the Universities of Toulon and Montpellier began. The students were in the second year of their courses based respectively in the Institute Universitaire de Technologie (IUT) and the Institute Universitaire de Pharmacie (IUP). In 2000, one student came from the IUT of Bordeaux University at Perigeux. To date 29 French students have spent between six and eight weeks stage (placement) in the pollen centre. Each year students receive training in operating the Burkard Trap (q.v. 2.2 below) and the subsequent operations in producing the pollen count. In addition each student is allocated a project which, for the students from the IUP, involves work with allergenic pollens data, or for those with IUT background, assistance with the research programme not related directly to the pollen count. The reason for all students being involved in the daily pollen count service provided, is that it is a community project which, since the Centre's inception, has been more or less the *raison d'être* of the organisation, and complete familiarity with the aim and objective of the service is regarded as of prime importance. Apart from the expression of interest in working with pollen made at the time of their applications, students usually also express a wish to improve their English language competence. Only in cases of difficulty in understanding processes given in English, is French resorted to. The report at the end of the stage is written in either French or English, which may involve a study of one taxon over a number of years or a total pollen count calendar correlating temperature and rainfall (e.g. Figs. 3 & 4).

The Centre has received workers during the past few years from Greece, Spain and the Netherlands, undertaking pollen studies and research during a sabbatical, fulfilment of requirements for a European doctorate or undergraduate placement, respectively.

The permanent scientific team at the Pollen Centre currently comprises eight persons each involved in some aspect of aerobiology or undertaking data handling or overseeing the finances.

2.2 The Pollen Count: Methodology – Sampling

Pollen trapping and counting has been undertaken for just over a century, using a range of sampling devices designed to sample the pollen component of the air (Tomás et al, 1997). Gravitation, suction and adhesion and direct impact and adhesion have been the principal sampling methods (Gregory, 1973). The most widely used samplers are the Burkard (Plate 3a) and the Rotorod (Fig. 1). The former type is a development of the original Hirst Impact Sampler (Hirst, 1952) and is now used world-wide. The Rotorod sampler (Fig. 1), used principally in the United States and Canada but occasionally, also, elsewhere (Khandelwal, 2001), was developed by W A Perkins (Perkins, 1957) and was subsequently modified (Harrington, et al, 1959). The principle of the Rotorod sampler (Fig. 1) is based on a rotating U-shaped holder which contains two vaseline or silicone greased adhesive surfaces. The apparatus rotates at 2400 rpm sweeping out 120 l air per minute. Particles ranging in size from 1-100µm are collected. There are modifications of the above Rotorod sampler, where microscope slides replace the rod holder. The slide edges, when greased and inserted, act as the sampling surface. Collecting is efficient within wind speeds of 1-7 m/s. The optimum sampling time lies between 5 and 30 minutes (Perkins, 1957). The apparatus can be linked to a timer for sampling at desired times and intervals. The rods or slides are examined directly microscopically, when set in a suitable holder. The Rotobar modification involves metallic strips which carry adhesive strips. These can be removed and mounted on microscope slides for examination. The Rotorod, Rotoslide and Metalbar samplers are widely used in the United States. A brief description of the time consuming methodology involved in the use of the Rotorod sampler was published by Frenz and co-workers (Frenz et al, 1996). The third type of sampler – Cour Girouette (Cour, 1974) (Plate 3b) - is also widely used in western Europe. It is of particular value and use in France for predicting the grape harvest as well as allergenic pollens and spores (Plate 3b). In 1978, a simplified volumetric spore sampler for monitoring airborne pollen and spores was devised by Dr Morrow Brown for his aerobiological studies at the Midlands Asthma and Allergy Association (MAARA) Centre in Derby (Morrow Brown and Jackson, 1978) (Fig. 2a&b). A fourth type of sampler – the Morrow Brown trap (Fig. 2a&b) is based on the Hirst spore sampler – the precursor of the widely used Burkard Volumetric Spore Sampler. Where the Burkard samplers have the trapping surface



Plate 3a: Burkard Volumetric Spore Sampler (in situ)



Plate 3b: Cour Girouette

vertically aligned, the Morrow Brown trapping surface is horizontal, with the orifice (2 x 14mm) directed upwards. There is no wind vane. The apparatus can be raised 1m above the ground. A coated microscope slide lies immediately underneath the orifice and moves horizontally 2 mm/hr by means of a clock-operated pulley. A rain and insect-proof shield overlies the orifice. The rate of air flow is 10 l/min.

A mobile form of the Morrow Brown sampler is an auto sampler (Fig. 2c) which can be attached to the bonnet of a motor car and can be used to sample pollen and spores when travelling along the road network. A decade later he designed a mobile slit sampler for sampling pollen and spores whilst driving along motorways (Morrow Brown, 1991) (Fig. 2). The Morrow Brown Trap is still in use at the Derby site.

The third sampler, the Cour Girouette (Plate 3b), consists of two vertically held gauze filters raised to 3m height. Nearby is a horizontal filter stand, 1m above ground. The gauze surface area exposed in each case is 200 cm², the vertical filters held in a blue acrylic frame and the horizontal filter in a green frame. Vertical filters are 5 layers of muslin and the horizontal filters are 8 layers thick. The vertical filter stand has a large wind vane which ensures the exposed trapping surfaces faces into the wind. Treatment of the filters is a lengthy process (Cour 1974), but produces high quality slides.

The Pollen Centre obtained its first Volumetric Spore Sampler (Burkard) through the generosity of ALLERGON AB of Sweden. The site chosen for sampling airborne pollens was the south-east corner of the roof of the general teaching block at Merchiston (now the Merchiston Campus). It is some 21m above ground level, is the highest trapping site in the UK (Caulton, 2001), is operated electrically and air is sucked in at a rate of 10 l per minute through an orifice 14mm x 2mm. The air and any airborne particles contained impact upon a vaselined-coated tape fastened to a rotating drum operated by a 7-day clock. The drum tape can be changed daily during the height of the season or be allowed to run a full seven days out of season (i.e. when birch and grass pollens are not being shed on to the wind). The orifice always faces the oncoming wind by virtue of the whole body above the motor being freely rotating and directed by a large rear wind vane. If the drum head (Plate 4b)

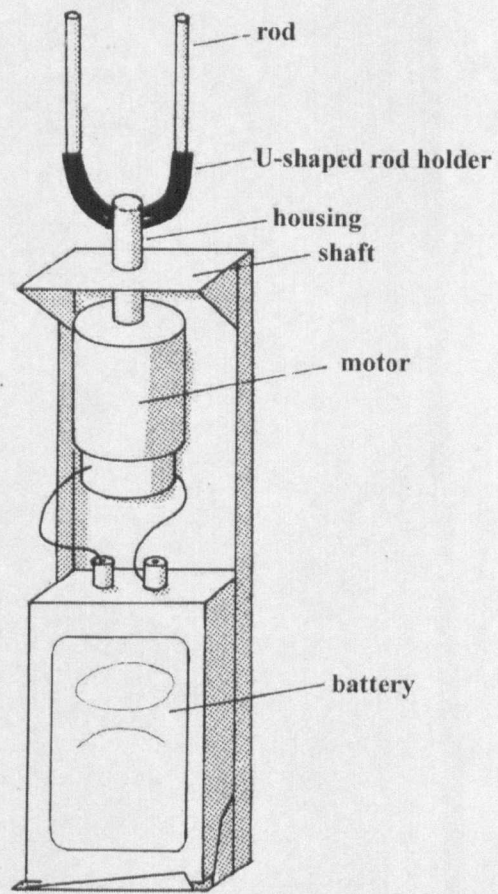


Figure 1a: The Rotorod Sampler

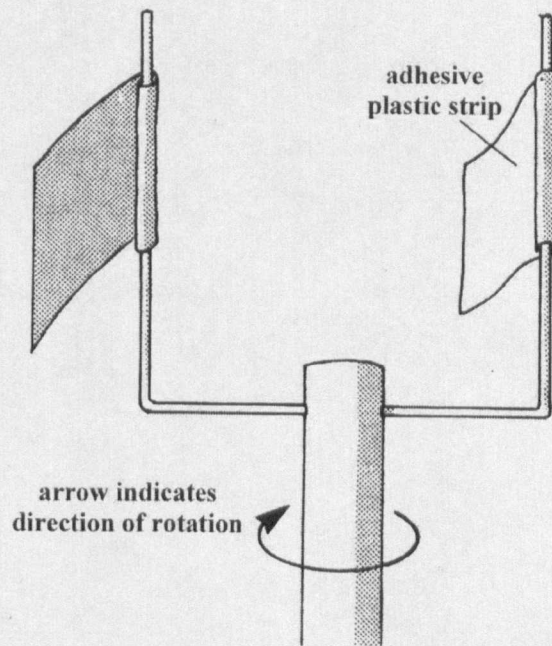
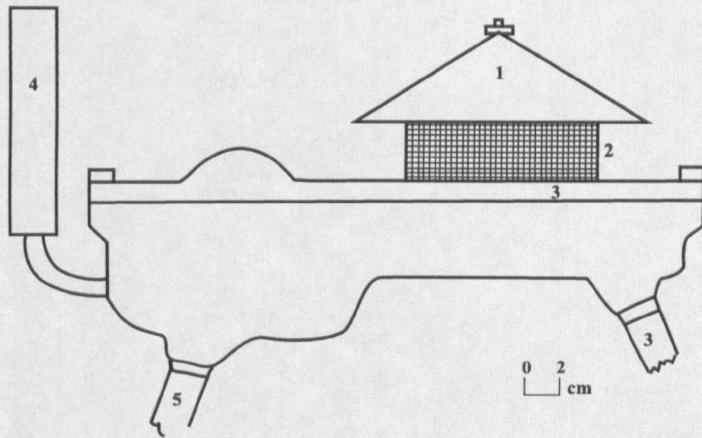


Figure 1b: Metalbar

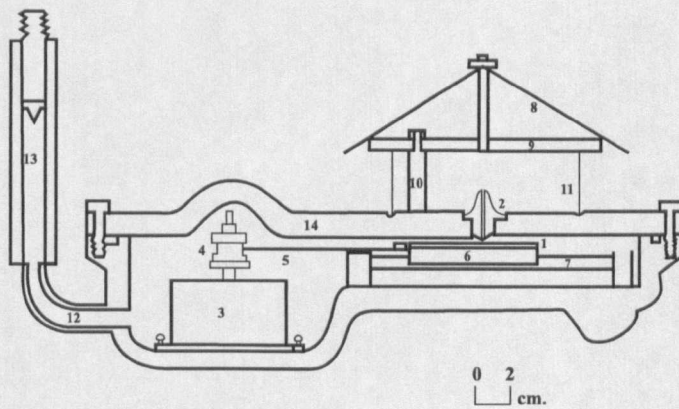
Figure 2 Morrow Brown Spore Trap



KEY

1. Rain shield
2. Wire gauze
3. Lid
4. Flow meter
5. Legs (length 80 cm).

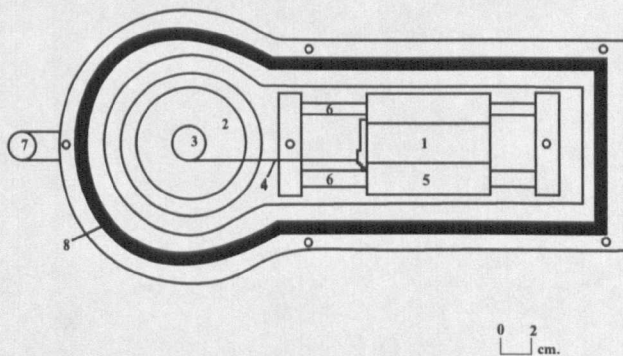
Figure a: M.B. Spore Trap, side view.



KEY

1. Coated slide
2. Orifice - tapering to 0.5 mm. at inner edge
3. Clock
4. Clock spindle - diam. 15mm.
5. Nylon cord
6. Slide carrier
7. Slide carrier runners
8. Rain shield
9. Supporting plate for rain shield
10. Supporting pillar
11. Wire gauze cylinder
12. Air exit pipe
13. Flow meter
14. Lid.

Figure b: M.B. Spore Trap (24 h sampler). Longitudinal section.

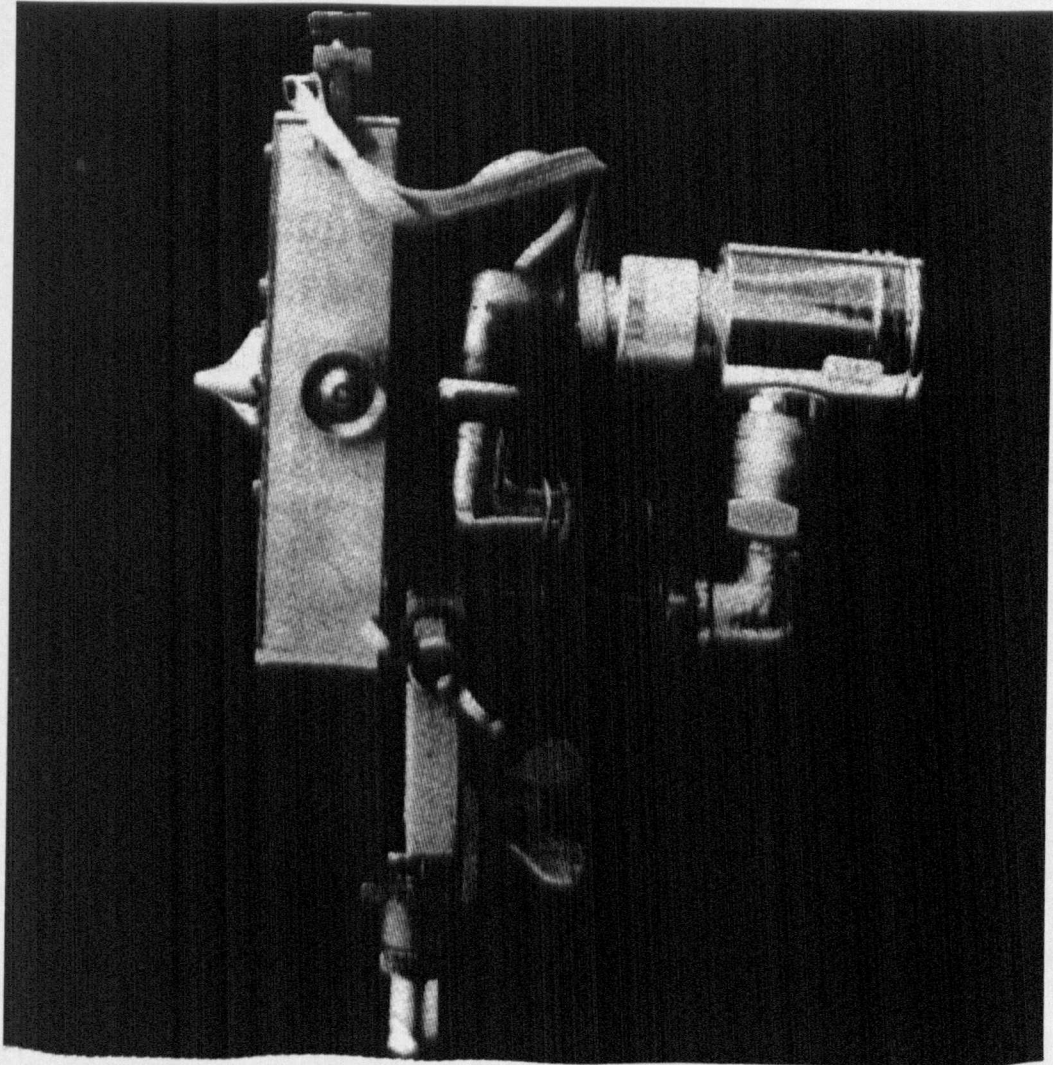


KEY

1. Coated slide
2. Clock
3. Clock spindle
4. Nylon cord
5. Slide carrier
6. Slide carrier runners
7. Air exit pipe
8. Rubber seal

Figure c: M.B. Spore Trap (24 h sampler). Top view, lid removed.

Figure 2d : The Morrow Brown Auto Sampler



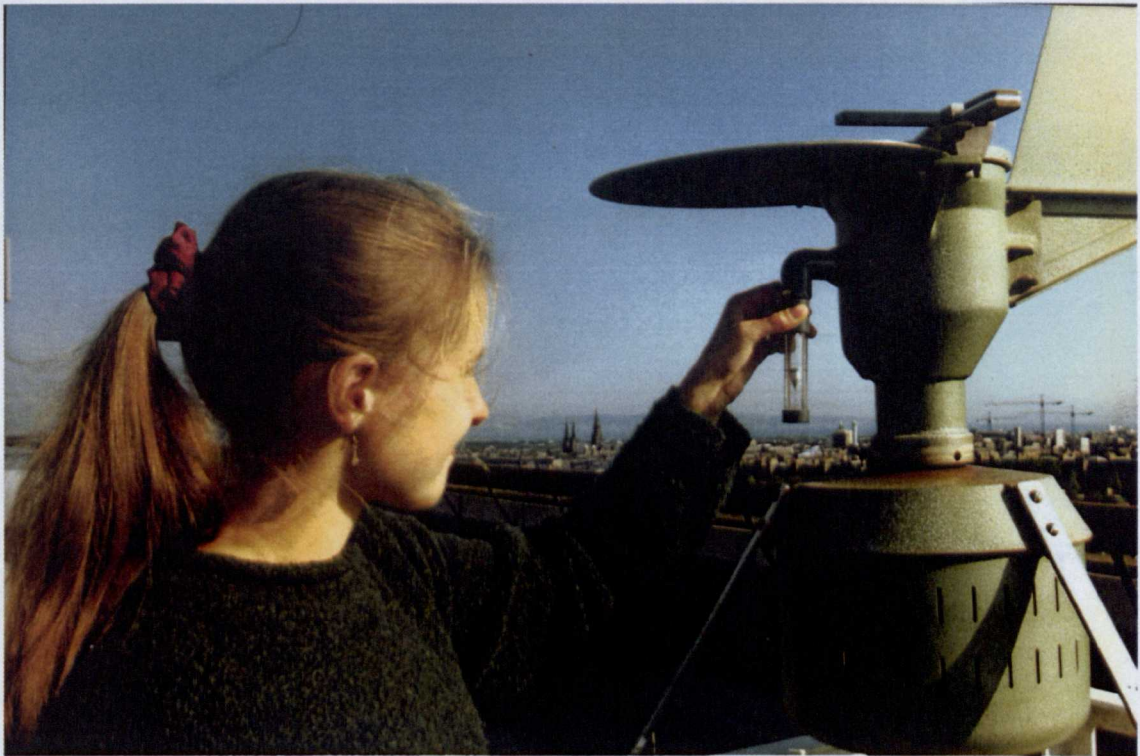


Plate 4a: Testing the Rate of Air Flow in the Burkard



Plate 4b: The Drum Head with 7-Day Tape

(i.e. the apparatus including the clock and rotating drum *in situ*) is used regularly as the principal means of sampling, then once a week only need the drum be changed and the clock rewound. However, a second type of sampling mechanism, the slide head, also has a seven day clock, but instead of carrying a drum, there is a moveable gantry which holds a standard 3" (7.5mm) x 1" (2.5mm) slide. As with the drum tape the slide is vaselined. The slide is set to move vertically over a period of 24 hours (Caulton et al, 1995a). The rate of air flow through the orifice is checked weekly by an air flow meter to ensure the movement of air neither exceeds nor is less than the standardised 10 l per minute (Plate 4a).

2.2.1 The Pollen Count: Methodology – Slide Preparation

When the drum head (or the slide head) is removed and taken to the laboratory, each is contained during its transit in a metal box or slide box to avoid damage to the vaselined surface or contamination by airborne dust or particles not trapped during sampling. The exposed tape or portion thereof is transferred to lie along the length of a plastic block which is evenly scored at 24hours intervals. The tape is cut into daily segments arranged chronologically, each placed on an appropriate dated slide. At this juncture and beyond, treatment of exposed tape (on slide) and exposed slide are the same (Caulton et al, 1995a). Special long coverslips (65mm x 2.5mm) are used, onto which an appropriate amount of medium – phenolysed glycerol glycerine - to which has been added 30 drops of basic fuchsin from a Pasteur pipette into 15 mls of the medium. The slide is then ready to be read.

2.2.2 The Pollen Count: Methodology – Counting

The slide is placed under the microscope – a Leitz Biomed – and the position of the edge of the tape determined on the horizontal vernier - the mm reading against the moveable zero. The slide is then moved 2mm along the vernier to set the position of the first transect. Transects are read across the width of the slide. When the first transect is complete, the slide is moved along 4mm. The 4mm interval separating each transect is maintained until the last, No.12, has been reached, which represents 24 hrs completed. All pollen grains are identified and counted (x400 magnification) and recorded on a five score

notation on a data recording sheet. Damaged grains and unrecognised pollens are recorded as “unidentified pollens”. Where and when pollen grains are very numerous, i.e. part of a very high count, tally counters are used for each taxon, the totals reached recorded at the end of each transect. When the identification and count are completed, the slide is sealed with commercial colourless nail varnish, and filed in a dated slot in a 100-slide box container (q.v. 2.1 above).

2.2.3 The Pollen Count: Methodology – Determining the Count

The pollen count is expressed as pollen grains *per* cubic meter of air ($\text{pg}/\text{m}^3\text{air}$). To express the total number of pollen grains as $\text{pg}/\text{m}^3\text{air}$ a conversion factor is required to be calculated (Caulton et al, 1995a). This varies according to the make of microscope and to the diameter of the field under x400 magnification. The correction calculated and used with the Leitz Biomed is x0.56. The number of pollen grains per taxon in each of the twelve transects is recorded horizontally on the second data sheet. The totals per 2 hour period from 0900h when first exposed to 0900h the following day, 24 hrs later, are recorded at the bases of the vertical columns. From these totals, the diurnal periodicity of total (and individual taxa) pollens can be seen. The horizontal totals for each taxon can be added to cross check with the sum of the vertical totals. Each taxon's total pollen count per transect (12 in all) is multiplied by the conversion factor (0.56) to obtain the pollen count in $\text{pg}/\text{m}^3\text{air}$. The overall total number of pollen grains of all taxa recorded for the 24 hour exposure period is also converted. Whilst the final count per m^3air may be to two places of decimals, recorded as such, the total is rounded up or down to the nearest whole number for the purposes of publication. The total pollen counts for 1988 and 2000 are correlated with temperature and rainfall as shown in Figures 3&4. Counting methods may differ from country to country. For example Spanish aerobiologist pollen and spore counters read along 3 longitudinal transects. Comparison between the two methods viz. Spain and UK have recently been made using Hirst type (Burkard) volumetric trap (Cariñanos et al, 2000).

2.2.4 The Pollen Count: Methodology – Transmission of Data

The daily count sheet, when completed, serves as the Centre's official

Daily Pollen Count for Edinburgh – 1988

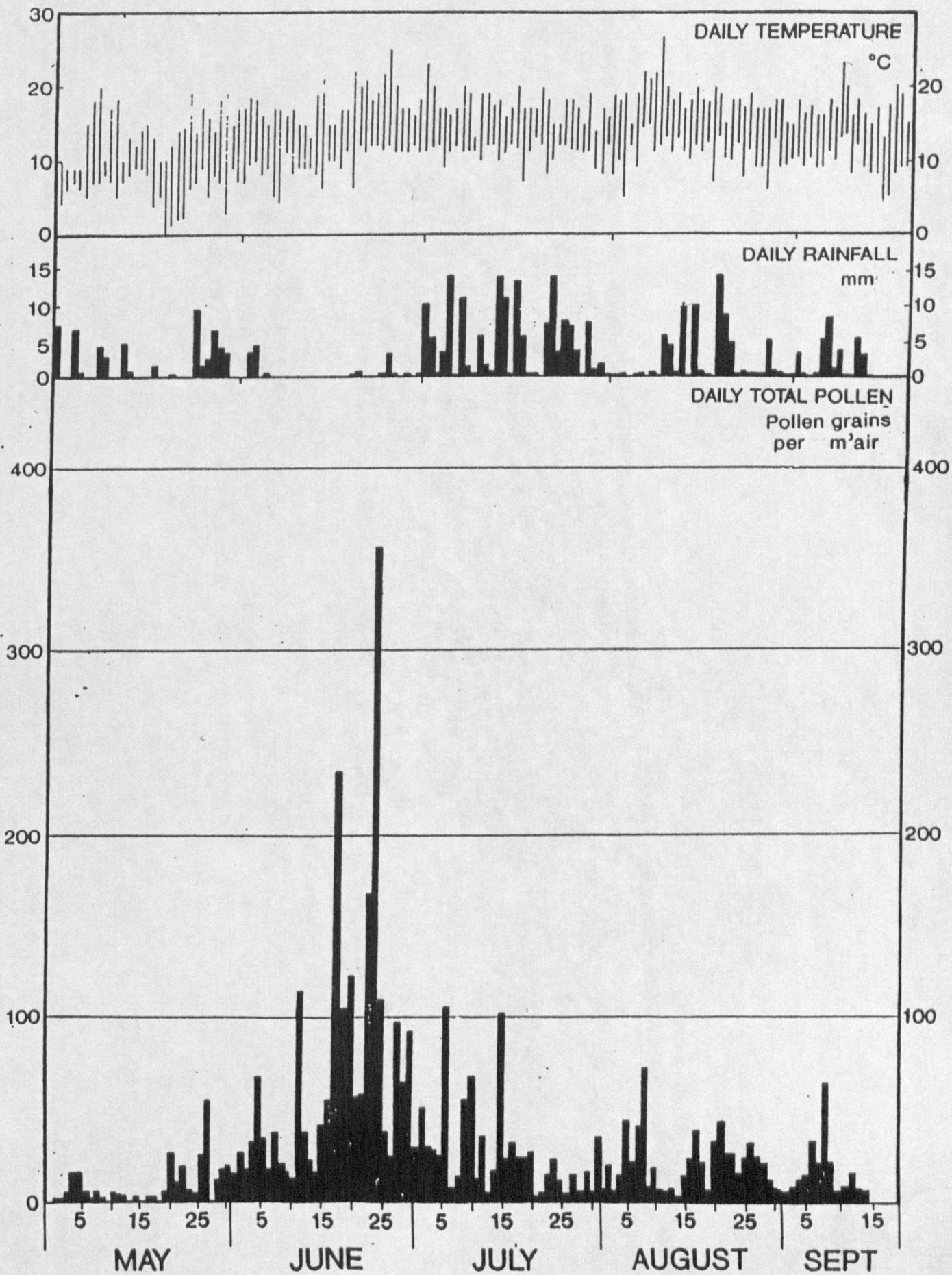
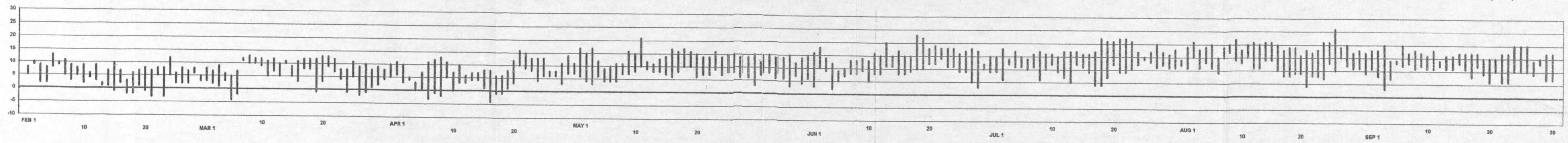


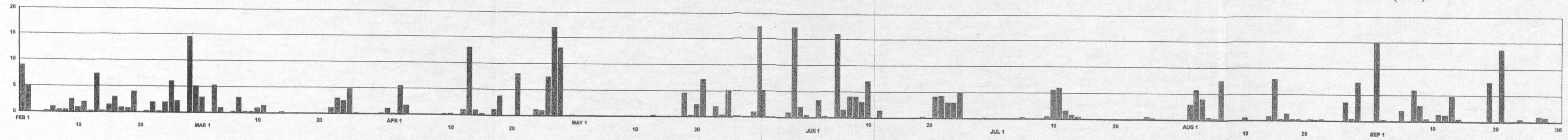
Figure 3

POLLEN AND WEATHER DATA 2000

MIN / MAX TEMPERATURE (° C)



RAINFALL (mm)



POLLEN GRAINS / m³ AIR

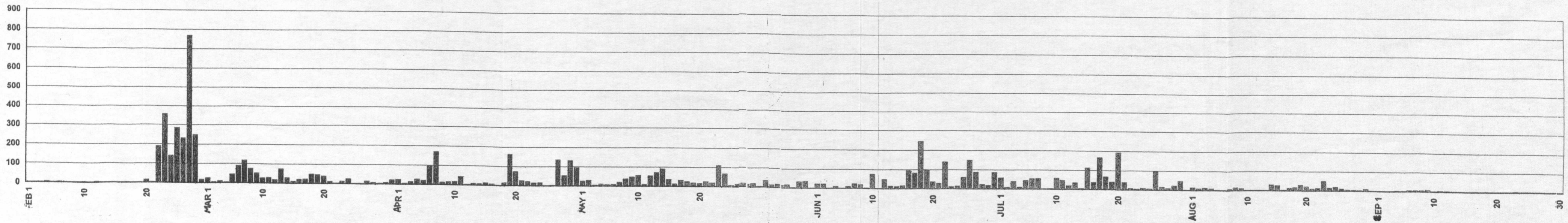


Figure 4

data record for transmission by fax to the National Pollen Research Unit at University College, Worcester, whose director, Professor Jean Emberlin, organises the BAF and EAN(UK) pollen data for the UK for daily transmission to the media – Press, Television, Sponsor’s Pollen Count ‘Phone line etc. and on to the European data base in Vienna, organised by Dr Siegfried Jäger. The daily routine required for the transmission of data to Worcester, imposes a strict time limit on the Centre’s operations. Time taken from the removal of the exposed drum tape or slide from the Burkard to faxing the pollen count is 1½ hours. The processes outlined in 2.2.1 – 2.2.3 above must be completed within the time scale as the total pollen count to the UK outlets and the birch/grass counts to Vienna involve computer time for a limited and set period. During the months of February, March, April, August, September and October, data are recorded on a weekly basis.

2.3 Presentation of Data

Pollen Calendars are now the conventional way of graphically expressing the total or individual taxon’s pollen season. The pollen season is expressed as the period when pollen is being liberated and dispersed. It is calculated in terms of start and finishing dates, by subtracting 1% (or 5%) from each end of the total pollen count recorded, noting in each case the coincidence of the percentage deducted number with the calendar date, noting also the number of days which have elapsed from 1st January until the calculated start date (Emberlin et al, 1994). The latter information is of value in determining trends in the start dates in relation to climate over a number of years.

The construction of pollen calendars has been of use in our studies on the incidence of airborne spores of bracken (Caulton et al, 1995b, 1999, 2000; Caulton et al, 1998). Pollen calendars for lime (*Tilia spp.*) and nettles (*Urtica spp.*) are in preparation, based on ten and fourteen years respectively. Many of the Centre’s resource books present data in the form of pollen calendars, for example (Ciampolini & Cresti, 1981; Dominguez-Vilches et al, 1984; Lejoly-Gabriel, 1978). The form of presentation figured in most pollen calendars published in books and papers are of the horizontal block type – where the x axis represents time and vertical y axis is a measurement of quantity, the

thickness of horizontal bars being proportional to numbers of pgm^3air . Occasionally pollen calendars take the form of single horizontal lines which are restricted to the length of the pollen season, giving no indication of abundance. One other form which is visually complicated, is the “butterfly” style, where abundance above the zero line is reflected similarly below the line – it looks attractive but is not so easy to read! The Centre has always favoured the horizontal bar design.

At the end of each season, a diagram showing daily pollen counts (pgs/m^3 air) correlated with rainfall (mm) and temperature ($^{\circ}\text{C}$) is produced. Figs. 3 and 4 give the first (1988) and most recent (2000) diagrams.

Comparison between Edinburgh’s data and those of Belfast (Northern Ireland) is remarkably close, less so with data derived from sites in midland and southern England due to geographical factors (distance and terrain) and weather factors (temperature and rainfall). However, overall patterns, such as diurnal periodicity/rhythmicity, show some degree of correlation. Beyond the United Kingdom, there is little or no comparison possible owing to differing climatic regimes, pollen types and seasons. Comparisons between different sites for similar taxa are nevertheless very interesting and worthwhile, e.g. in grasses (*POACEAE*) and *URTICACEAE* (nettle family).

2.4 Research Aspects Derived from the Pollen Count

Two aspects of research have over the past fourteen years emerged to form the basis of data information facilitating analysis and subsequent publication of papers and articles. The two aspects are a) pollen rain studies and b) the pollen count. The pollen count service as stated above (2.1) began in 1988 and some two years before the emergence of the national coordinating function of the BAF (3.1 below). Data accumulation required the passage of time, and in the Scottish context of variable weather conditions within and between seasons, some years of data are necessary if meaningful interpretation of results are forthcoming. It was not until 1992 therefore (Jäger & Mandrioli, 1992) that the pollen centre’s data saw the light of day in the multi-authored paper of that year. It was gratifying that Scottish data contributed directly to the geographical and temporal surveys undertaken (1992 op.cit. & Jäger &

Mandrioli, 1994). By 1994 sufficient data from ten sites in the UK enabled a study of variation of the start of the grass pollen season. As generally recognised in other fields of activity involving plants (e.g. horticulture), Scottish pollen seasons lag behind the south and midland areas of England by up to a fortnight, and sometimes up to four weeks, due to the extremely severe winter experienced in northern Britain during 2000/01.

As mentioned in 2.2.4 above in the context of EAN, data relating to the birch pollen season were added to the European mapping project and in view of the highly allergenic nature of birch pollen and grass extremely high counts experienced in central and western Europe from time to time (Hodal & Rasmussen, 2000). In the UK, birch pollen occurs each season rising to high levels especially in alternate years allowing for very favourable weather conditions – Cardiff, London & Derby recording the highest counts (Corden et al 2000). During the 2nd European Symposium on Aerobiology held in Vienna during August 2000, a special satellite symposium was devoted to birch. A preparative document was prepared by the author for distribution to delegates (Caulton, 2000). A Danish contribution referred to birch pollen as: ‘is known to be a major cause of pollen allergy in Denmark’ (Hodel & Rasmussen, 2000). Whilst birch pollen is an important allergen in the UK (Emberlin 1997), it is overshadowed by the extent of allergenicity exhibited by grass in the population generally. One early and two recent contributions in the study of variations in the grass pollen seasons have emphasised the spatial or geographical as well as the weather parameters most importantly influencing grass flowering and anthesis in the UK (Emberlin et al, 1994) and western Europe (Bagni et al, 1976; Emberlin et al, 2000). Data from the pollen centre were a contribution to the latter publication.

2.5 Research aspects derived from pollen rain studies

The nature of pollen rain and its effect was promulgated by W. Lüdi in a series of papers published between 1937 and 1947: ‘enormous quantities of pollen are liberated, float in the air for a shorter or longer period, and are eventually sifted over the surroundings as the dense and even pollen rain. The evenness of the pollen rain should not be over-rated. In a mixed vegetation plants flower at different periods, and wind and turbulence may vary. Thus, the

pollen rain from a particular patch of vegetation may be deposited in different places in different years as shown by Lüdi and others. (Lüdi 1947, Faegri & Iversen, 1966). A series of studies on pollen rain were published in 1978 within the Institut de Géographie, Université Catholique de Louvain (Lejoly-Gabriel, 1978): 'L'aeropalynologie étudie le contenu pollinique de l'air. Elle est une discipline de l'aerobiologie qui envisage toutes les particules organiques se trouvant dans l'atmosphère. Les domaines de recherches qui relèvent de l'aeropalynologie concernant les caractéristiques et l'identification des pollens transportés par le vent ainsi que leurs processus de production, d'émission, de dispersion et le dépôt'. (Aeropalynology (is) the study of the pollen content of the air. It is an aerobiological discipline which embraces all organic particles which are found in the atmosphere. The province of researches relevant to aerobiology concern the characteristics and identification of pollens transported by the wind involving the processes of production, emission, dispersal and deposition). The components of pollen rain may consist of pollen transported over long distances. However, most pollen rain components sampled in mainland areas have their origin within a 50km radius of the pollen sampler. Pollens normally regarded as entomophilous often become airborne and are frequently trapped (e.g. *Erica*, *Tilia*, *Salix* etc.) (Proctor et al, 1996). Long distance transport of pollen and spores has been investigated by a number of workers (Hirst et al, 1967; Tyldesley, 1973; Dodson, 1982; Lacey & McCartney, 1991). Sampling of long distance airborne pollens (and spores) has received attention in the antarctic region: Jan Coll Island, South Georgia (Chalmers et al, 1996) and Lord Howe Island in the Pacific, some 850 km north-east of Sydney, Australia (Dodson, 1982). The author was one of the palynology consultants involved in the Jan Coll Island survey. Pollen of the southern beech (*Nothofagus*) was trapped on the rotorod sampling tapes – this pollen travelled some 2,000 km from its source, Tierra del Fuego.

2.6 Pollen rain studies on herbivore faeces

An attempt was made to see whether or not pollen rain falling on the faeces of sheep might possibly provide a short-cut tool for assessing the local pollen flora by means of extracting the pollen from treated faecal material and observing the pollen spectra obtained. (Moe, 1983). There were problems

with the methodology – freely admitted – that being subject to a transhumance regime each autumn and spring demanded by the rigours of the Norwegian winter conditions, the sheep under study were fed for several months on grain. Moe's experiment was repeated by the author, in the Vogrie Country Park, Midlothian. Within the park, one of the field areas has for some years housed a flock of 21 Jacob sheep which belong to a rare breed specialist who leased the paddock. With the assistance of the park ranger botanist, a quadrat analysis of the field's vegetation was undertaken. Faecal samples were collected over a period of 3 years (1985-1987) and were treated to extract the pollen (Caulton & Gibson, 1988). A methodology of treatment of faeces was established during this project (Caulton, 1988). Results confirmed Moe's findings that the pollen spectra of faecal material exposed to pollen rain did not necessarily reflect the local flora's pollen source. However it was observed by us that the sheep were quite selective in their choice of herbage, including inflorescences, that it may be possible to use the sampling technique ecologically. Many plants, especially herbs, are indicator species with respect to habitat and environmental conditions. This approach has been very positive – faecal samples of sheep (*Ovis aries*), red grouse (*Lagopus lagopus*), camel (*Camelus dromedarius*) were treated and results were indicative of both preferred diet and habitat in each case (Caulton & Gibson, 1988). Two other studies on pollen rain were undertaken at the pollen centre: one involved the examination of turf and straw components of the 17th Century Collegehill House at Rosslyn, Midlothian. A localised earth tremor occurred, the suspected cause of which was the collapse of old subterranean coal mine galleries. As a result of the tremor, part of the roof tiling which overlay the original turf roof was displaced exposing the earlier roof. From the pollen spectra obtained, it was possible to reconstruct to some extent the floral landscape of two centuries earlier (Caulton & Fiskin, 1993). The second study examined the pollen rain which fell on the surface of selected moss polsters and mats, the thalloid form of dog lichen, *Peltigera canina* and the arbuscular form of reindeer moss, *Cladonia sp* in order to assess whether or not a relationship existed between the pollen spectra of the pollen rain, and the area vegetation whereby moss polsters and lichens could be used as tools for pollen analysis interpretation (cf the sheeps' faeces experiments of Moe, 1983; and Caulton & Gibson, 1988, referred to above).

The application of pollen rain studies involving the declining populations of elms (*Ulmus spp*) in south-east Scotland, resulted in a paper which traced the 20 year record of elm trees killed by Dutch elm disease and attempted a correlation with pollen data recorded over the twenty year period, 1976-1996 (Caulton et al, 1998). There is a steady decline in elms but this is not reflected to any significant degree in the air-borne pollen record. This is considered to be due to the persistent production of flowering suckers from stumps which have survived albeit for a few more years into the 21st Century.

In 1994 the author, whilst travelling on an Inter-City train, observed a note in the complimentary Inter-City magazing LIVEWIRE August/September issue. It described a report extracted from the EVENING NEWS (a Scotsman newspaper) entitled: 'Airborne spores put Edinburgh on cancer alert'. The report ended, 'human health fears were sparked when it induced cancer in laboratory mice'. The source of the original experimental findings are unknown. However, *in vitro* studies on mice fed with bracken spores were undertaken by Povey in 1996 (Povey et al 1996). Studies on airborne spores of bracken (*Pteridium aquilinum*) are few in the literature. The risks to human health from spores of bracken were reviewed recently (Simán et al, 1999). One UK study undertook measurement of airborne concentrations of the spores (Lacey & McCartney, 1994). It was decided to investigate the airborne concentrations of bracken in the rooftop airstream over Edinburgh. Lacey & McCartney's investigations were undertaken in a rural agricultural situation, whereas, by contrast, the rooftop airstream passing the pollen centre's trap at Merchiston, is essentially urban. A five year study was undertaken in the first instance (Caulton et al, 1995b) which was subsequently extended to ten years, 1989-1998 (Caulton et al, 1999, 2000). The concentrations found in the Edinburgh study were so low as not to prove hazardous to health. This study is ongoing and there are plans to include a survey of other UK sites and, where possible, continental sites also.

2.7 The importance of allergenic fungal spores in the indoor and outdoor environments.

Recent outdoor studies, one emanating from the pollen centre (Richardson, 1996) monitored the spore concentrations of the aeroallergen *Didymella*, over

the three year period 1992-94. Other studies (Corden & Millington, 1994; Cordon & Millington, 2001) concentrated on the occurrence of *Didymella* spores and on the long-term trends and seasonal variation of the aeroallergen *Alternaria*, which occurred during the twenty year period, 1979-1999. A multiple-site study of the aeroallergens *Cladosporium* and *Alternaria* was recently undertaken in five sites in Southern and Central Poland (Stepalska et al, 1999). In the above European studies, the Burkard Volumetric Spore Sampler was the principal means of sampling

In "Microorganisms in Home and Indoor Work Environments" (Flannigan et al, 2001), a chapter discusses allergenic fungal spores and other microbial particles. An example of one study of grass pollen comparing outdoor with indoor environments is shown as Fig. 5 (*op.cit.*)

3. APPLICATIONS OF THE RESEARCH PROGRAMME

3.1 British Aerobiology Federation

By 1990 awareness of the impact of asthma and hay fever on the medical services and loss of working hours had reached a point where the need and advantage of setting up of a body to coordinate the growing number of pollen monitoring sites was evident.

In 1990 some sixteen aerobiologists involved in monitoring allergenic pollens and spores, met together in the Harley Street, London consulting rooms of Dr W Frankland, one of Britain's leading pioneer allergologists. The outcome of the meeting was the formation of the British Aerobiology Federation (BAF). The author attended this meeting and is one of the foundation members. Since its inception almost forty pollen and spore monitoring stations have come on line reporting (at least) grass pollen counts during the summer months. BAF sampling sites occur at a variety of departments: located in hospitals, local authorities, research institutes, universities and others, like the Scottish Centre, independent. Thus respiratory diseases, community health, plant breeding and pathology, botanical research are all aspects within the wider discipline of aerobiology. The BAF has developed a scheme to ensure consistent methods of sampling, counting and data recording for pollen are maintained in the UK. A series of biannual workshops is organised for the training in aerobiological techniques. Competence and reliability in the recognition of important allergenic pollens and spores is vital to effective reporting to the media (cf 3.3 below). The author participates in these workshops, demonstrating the slide preparation technique. A manual for the guidance of samplers has been compiled and published (Caulton et al, 1995a). The organisational centre of BAF operations is the National Pollen Research Unit (NPRU) based at University College, Worcester, under the direction of Professor Jean Emberlin. The NPRU organises the annual quality control exercise for pollen counters, which involves the testing for accuracy in identification of grass pollens (in particular) along pre-determined transects on pre-prepared slides.

The author himself is subject to the same Quality Control exercise for all counters. In addition, as an EAN counter, additional pollen taxa are required to be identified and counted. Results have always been well within the accepted limits of statistical error set for the exercise, and on the last occasion (2001) a score of 100% accuracy was obtained.

In addition, the NPRU receives and collates the pollen data for birch and grass that are transmitted daily by the pollen counters in the appropriate season. The grass data from selected sites is forwarded each morning to the computer base at Vienna (q.v. 3.2 below). The NPRU negotiates on behalf of the BAF the sale of the national pollen data to the media: press, radio and television networks and also with a major pharmaceutical drug company for the sponsorship of a national telephone pollen count service available throughout the grass pollen season. The reason for confining the phone-in service to the grass season – late May (in the south of the UK) to the end of July (in the north) is that the overwhelming number of hay-fever sufferers are allergic to grass pollens. On the continent, where Birch pollen is much more abundant than in the UK, although equally allergenic, the pollen count season is advanced to late March until May to include daily counts for public consumption. The current season (2002) has included Birch pollen counts in the daily transmissions to NPRU for the first time. Publications based on data produced by the various sites in the UK appear from time to time (Emberlin et al, 1994; Corden et al, 2000) and present a progressive picture of the allergenic pollens present in the UK during the respective time scales involved. A biannual Newsletter is produced for BAF members, the first six issues of which (1991-1997), were edited by the author.

3.2 The European Aeroallergy Network

In 1990 representatives of pollen monitoring sites in most countries of Western Europe founded the European Aeroallergy Network (EAN). The driving force behind this endeavour was Professor Paolo Mandrioli of Bologna University. The Italian aerobiologists were prominent during the 1980s in developing a national network of pollen and spore sampling stations – similar networks were developed in Spain, France and Finland. As indicated above, the UK sites formed a network in 1990.

Thirteen of the UK sampling sites agreed to join the EAN as the European Aeroallergy Network (UK), sending the grass counts each day during the season to the NPRU, Worcester for onward transmission to the Vienna data base, which had been organised and managed by Professor Siegfried Jäger of the General Hospital, Währinger Gürtel, Vienna.

Publications which have utilised the Centre's data were multi-authored for purposes of surveying in particular grass and birch pollen in the UK (Emberlin et al, 1994; Jones et al 1994, abstract only; Corden et al, 2000). An agreement was reached among the EAN contributors at the outset of the formation of EAN (UK) that use of each other's data for the purpose of a publication warranted co-authorship on the part of the contributor whose data were being used. The Pollen Centre's data in each case involved grass, birch and general allergenic taxa for pollen count comparative surveys (grass and birch) and forecasting by means of predictive modelling.

In 1985, the Bologna group launched a journal entitled *Aerobiologia*. Initially, the journal published papers submitted by European workers. In 1988, the journal took on an international role as 'The International Journal of Aerobiology', but retaining its main original title *Aerobiologia*. One of the first major international collaborative efforts resulted in the publication of a series of maps showing the progressive spread of grass pollen during the 1990 season throughout Europe (Jäger & Mandrioli, 1992). Some 171 contributors from 18 countries supplied data. Data received from the Scottish Centre were acknowledged (op. cit. p39). The above collaborative exercise in mapping the grass season, as it progressed in time across Europe, was repeated two years later (Jäger and Mandrioli, 1994). Maps relating to Birch were also included in the project. On this occasion 105 contributors from 17 countries were involved. Data received from the Scottish Centre were acknowledged under the UK (op.cit p.11) *Aerobiologia* is now run by an editorial committee of 28 drawn from the principal pollen monitoring countries in Europe as well as the USA, Canada and Australia. The author regularly acts as a referee for *Aerobiologia*.

The Aims and Scope of *Aerobiologia* as issued by its new publishers (Kluwer Academic Publishers, Dordrecht, Netherlands) include the interdisciplinary fields of aerobiology: bioaerosols, transport mechanisms, biometeorology, climatology, microbiology, aeromycology aeropalynology with links to respiratory allergology, plant pathology, biological weathering, indoor air-quality industrial aerobiology and cultural heritage.

3.3 Media application

Perhaps the most important application of the research programme is the provision of allergenic pollen and spore data to the media. Radio, press and television are all involved. Whilst a limited amount of information is disseminated by individual centres/sites, the data passed under contract to the national media reaches the population rapidly with daily listening, reading and viewing. The Scottish Centre has for the past ten years supplied under separate contract, pollen counts (total and grass) with a forecast each weekend for the weekend newspapers: SUNDAY POST and SCOTLAND ON SUNDAY.

From time to time, the author has been invited to the BBC studio to participate in a 'phone-in' or a telephone interview concerning the pollen count (Caulton, 1985-1990). A television interview took place in "Reporting Scotland" in June 1989. Press and magazine articles involving the work of the Pollen Centre have occurred at regular intervals since 1988 (e.g. Nelson, 1991). Some articles have been either solicited or offered to more general scientific publications (Caulton, 1994; 1996; 2001).

3.4 Contributions to Research Publications

The need to develop a reasonably reliable pollen forecasting service has been realised. Research into data analysis, which, by means of using data of selective taxa (e.g. oak and ash), the onset of birch pollen release may be predicted. The application of pollen curves from different regions in the UK (including Scotland) is being employed to produce predictive computer models (Jones et al, 1994).

3.5 Medical Application

The increasing incidence of both pollinosis and asthma in the population at large has involved, among other, pollen and spore data concerned being included in publications emanating from respiratory diseases, community health and medical practices (D'Amato et al, 1991; Spiewak, 1995; Corden & Millington, 2001). One of the earlier British studies investigating the relationship between pollen and spores and allergy was published by Hyde (1972). The Scottish Centre has been involved in two studies involving medical application of data (Mackay et al, 1992, Agius, 1999, unpublished). The ever increasing attention to and research into the application of aerobiology to medicine is exemplified by the publications involving the relationship between aerobiology and allergology (Morrow-Brown, 1994) and the airborne fungal populations in British homes and the health implications (Hunter & Lea, 1994). Between ten and twenty per cent of the world's population is considered to be city dwellers (Hunter & Lea, 1994). The changing health patterns reflect this shift from the rural environment, no more so than in the increase in allergies recorded including pollinosis, seasonal rhinitis and asthma. As a consequence, the future application of aerobiological studies will have an increased emphasis on indoor aerobiology. As city dwellers spend a major part of their lives in the indoor environment – working, at leisure, eating and sleeping – the need to investigate the aerobiological environment for causes of allergies is both paramount and urgent. Public transport, libraries, schools, hospitals, restaurants and community/leisure centres as well as homes, can harbour pollen, fungal spores, house/dust mites, danders and other biological allergenic agents (Rantio-Lehtimäki, 1991; Riponen, 1994; Verhoeff, 1994; Nikkels et al, 1996; Garrett et al, 1997; Flannigan et al, 2001 (& Fig. 5)).

Two highly allergenic airborne pollen allergens are those of mugwort (*Artemisia vulgaris*) and ragweed (*Ambrosia artemisiifolia*). Pollen from mugwort is trapped occasionally at Merchiston, but in very low amounts, so that a pollen calendar for this taxon has not been feasible. Ragweed, a native of North America, has been introduced into Europe and is well known in Mediterranean countries. It is gradually spreading north and is currently a subject of great concern due to its severe allergenic pollen (Déchamp, 1995).

If the predicted climatic warming takes place, then the spread of Ragweed northwards in Europe might well see it established in the British Isles. A recent report from the Baltic States refers to small pollen counts recorded from some twenty local sources of Ragweed over a three year period (Saar et al, 2000). Concern has also been expressed as to an allergy risk posed by Ragweed in Sweden in the event of climatic warming (Dahl et al, 1999). A 14-year study in Vienna recently reported correlation between sensitization rates with the amount of inhaled Ragweed pollen (Jäger, 2000).

3.6 Veterinary Application

The Scottish Centre has been involved in two veterinary investigations – one concerning horses, the other relating to dogs. The Royal (Dick) School of Veterinary Studies, Edinburgh, asked the author to investigate suspected pollinosis in two horses in Perthshire (Dixon et al, 1992). The cause of the condition, apparently common in the United States but erstwhile not reported in the UK, was feeding adjacent to a profusely flowering willow tree. Normally pollinated by bees, willow catkins are erect and exposed and its pollen is readily dislodged during anthesis and transported by air currents, some of which bring the grains that impact on our rooftop sampling slides. The second investigation involved young German Shepherd dogs undergoing training for blind-guide duties (Fraser et al, 2001). The method used in both investigations involved analysis of faeces containing ingested grains (Caulton, 1988). In the former investigation, willow pollen was found to be the cause of the condition, whilst in the latter investigation, pollen of both pine and grass were suspected as causing the symptoms of pollinosis observed.

3.7 Cultural Application

The role of bioaerosols, moulds, lichens and bacteria in the deterioration of buildings, archival materials and works of art are well documented. The effect of expired moist air with the relatively high carbon dioxide concentration during visits to subterranean limestone caves containing prehistoric cave paintings such as those at Lascaux and Altamira, in France and Spain respectively, have necessitated either complete closure or very restricted visits by tourists. A potential impact of pollen in subterranean conditions would be

as a surface contaminant, pollen attached to visitors' clothing being dislodged by movement and carried by air currents to adjacent walls. Recent Italian studies on biodeterioration caused by bacteria and moulds have clearly demonstrated the deleterious effect of air-borne microorganisms (Maggi et al, 2000; Montacutelli et al, 2000; Monte & Ferrari, 2000).

3.8 Meteorological Application

The role of climate on the large scale and weather in the more localised situations have a major impact on aerobiology, especially relating to the transport locally or over long distances of pollen and spores (Tyldesley, 1973). Studies undertaken on the presence of pollen grains in the upper reaches of the atmosphere over the Atlantic produced interesting results (Erdtman, 1937; Lacey & McCartney, 1991). The application of airborne pollen distribution and concentration over time to meteorological parameters of rainfall, humidity, temperature, wind direction and speed, enable correlations to be made and an explanation of observed results (Pedgley, 1980). In coastal areas, sampling sites of airborne pollen and spores are much affected by wind speed and direction, probably more so than those whose sites are inland.

3.9 Forensic, Criminal and Legal Application

Palynological evidence used as corroborative evidence to secure prosecutions in criminal cases is known, but, for obvious reasons, such forensic evidence on police files is not widely publicised (Faegri & Iversen, 1966). Suspected art forgeries, especially paintings by Spanish, French and Italian artists, who characteristically worked in the open air, and whose valuable works are eagerly sought after by collectors at auctions, may be subject to palynological tests in order to provide evidence and resolve doubts as to the genuineness or otherwise of the painting. A minute spot of pigment is removed and analysed for the pollens present. Their presence can confirm or otherwise whether or not the airborne pollen spectrum observed belonged to or originated in the area where the painting was actually undertaken (Emberlin, pers.comm.)

The pollen centre has been involved in a legal case, where information as to the pollen count and weather conditions was required as evidence in relation to a traffic accident leading to possible prosecution (Caulton and Logan, 1996).

The application of pollen analysis in determining date and location of bodies drowned in peat bogs or buried in ice following an avalanche has proved to be of critical value. The role of pollen analysis in resolving the problems of approximate date (centuries) of bodies deposited in peat bogs and subsequently brought to light, was revealed in a series of discoveries made in Denmark in the 1950s – culminating in the discoveries of Tollund and Grauballe Men. For over 200 years the remains of some 35 bog people have been recorded from Danish bogs alone (Glob, 1971). In 1991 a male body became exposed in the ice on an alpine ridge on the Italian-Austrian border. The body of the “ice-man” was subjected to intensive and extensive forensic examination (Spindler, 1994). In both, the Bog People and the “ice-man” respectively, pollen analysis played an important role, where indicator species among the total pollen spectra were able to be used to reconstruct the contemporary landscape, and alongside other dating techniques (e.g. C14), fix approximate dates of death. Both pollen analysis dating and radio carbon dating in the Danish examples were found to correlate exactly (0-400 years AD/0-1600 years BC) corresponding with the early Iron Age to the Roman Iron Age. In the case of the Tyrolean “ice-man”, (subsequently named Ötzi), some 2,222 pollen grains were recovered from the vicinity of his body. ‘High figures for pine (*Pinus*), alder (*Alnus*) and meadow grass (*Poa*); a medium count for mugwort (*Artemisia*), whilst only individual counts for birch (*Betula*), hazel (*Corylus*), spruce (*Picea*), elm (*Ulmus*) and beech (*Fagus*) along with ivy (*Hedera*), fern (*Pteridium*), stinging nettle (*Urtica*) and plantain (*Plantago*), which indicated that their main dispersal was over. It follows therefore that the ice in which the ice-man rested was formed between late summer and early autumn (September/October). (Spindler, 1994). His last supper before being killed has been described from his stomach contents: various seeds along with conifer pollen. (New Scientist, 2002).

A final example of the application of pollen analysis was only recently revealed, which proved the time of year (season) in which a disputed mass execution of prisoners had occurred during the closing years of World War II

European Theatre when Russian armies were overrunning Germany. The mass execution was shown by the pollens present on the clothing of the exhumed remains to have been carried out by the invading army, not the retreating one, i.e. the Red Army (Radford, 1999).

The mutilated torso of a coloured boy, aged between 5 and 6 years, was found in the River Thames in September 2001. His death is believed to be the result of a ritual killing. The identification and origin of the boy named "Adam" has proved difficult. 'The only clue police have is from pollen particles found in his stomach, which were of a type not found in the northern hemisphere, suggesting that he arrived in Britain from Africa a few days before his death'. (The Scotsman, 10.07.02).

Thus pollen analysis has become a valuable tool to the forensic scientist not only as corroborative evidence but in its own right. Whilst the Pollen Centre's data application has been limited to community health and identification of diet and habitat it has not, as yet, been involved in the exciting and fascinating aspects of forensic investigations; nevertheless, what application there has been and is ongoing, is regarded as very important and of value to the community at large.

There are ongoing long-term studies on the medication and treatment rendered to pilgrims passing over Soutra in the upland moorland of the Scottish borders country, where a mediaeval hospice was an important outpost of healing and short term respite. Investigations involving a prominent role played by pollen analysis has shed valuable light on mediaeval herbal remedies and nursing practice of the time (Moffat, 1989). These studies have linked mediaeval medicine with archaeological excavations of the site.

3.10 Forecasting

The application of aerobiology, especially with regard to allergenic pollens and spores, to forecasting the onset of the pollen seasons, has obvious implications for community and individual health (Ong et al, 1997; Stark et al, 1997; Peeters, 1998; Schäppi et al, 1998) and also for the national or local economy (González Minero & Candau Fernandez-Mensaue, 1996). The

combination of meteorological information (i.e. rainfall and temperature) allows for an estimation of the potential daily pollinosis symptoms during the grass pollen season (Schäppi et al, 1998). Forecasting is at present an inexact science, but is receiving increasing attention in Europe, the United States and Australia, where years of cumulative pollen/spore and meteorological data allows for computerised models to be constructed (Jones et al, 1994). In areas where less stable climates exist, for example the UK, forecasting requires a long period over which data can be analysed and statistically correlated to produce even estimated forecasts.

The utilisation of parameter data from aerobiological, phenological and phytogeographical studies (Zerboni and Manfredi, 1998) is another good example of the value of interdisciplinary work related to allergology.

3.11 Pollen Calendar Leaflet

One recent project directly involving analyses of twelve pollen taxa monitored at the Merchiston site, has resulted in the production of a leaflet showing the pollen calendars of each of twelve highly, moderately and weak pollen allergens (Plate 5). On the reverse side of the leaflet, in seven languages, is a short explanatory paragraph featured against the appropriate national flag (Plate 6). The purpose of the leaflet, which is based on ten years of data (1988-1997), is to provide guidance for individual activity during the pollen season of a taxon relative to the Hay Fever/Pollinosis suffered. The leaflet is aimed at both residents and visitors to Scotland, particularly in the Central Belt where the majority of the Scottish population lives. Circulation is currently organised among Community Health Centres, NHS Primary Care units, Tourist Boards, General Practitioners and Pharmacies.

The author was solely responsible for the concept of such a leaflet being applied to the Scottish scene, and also for the organisation, production and subsequent distribution. Recipients have already expressed interest in the use of the leaflet and its value to sufferers of hay fever (seasonal rhinitis).

3.12 Melissopalynological Application

CALENDAR OF ALLERGENIC POLLENS IN SCOTLAND 1988 - 1997

- Highly allergenic
- Allergenic
- Least allergenic

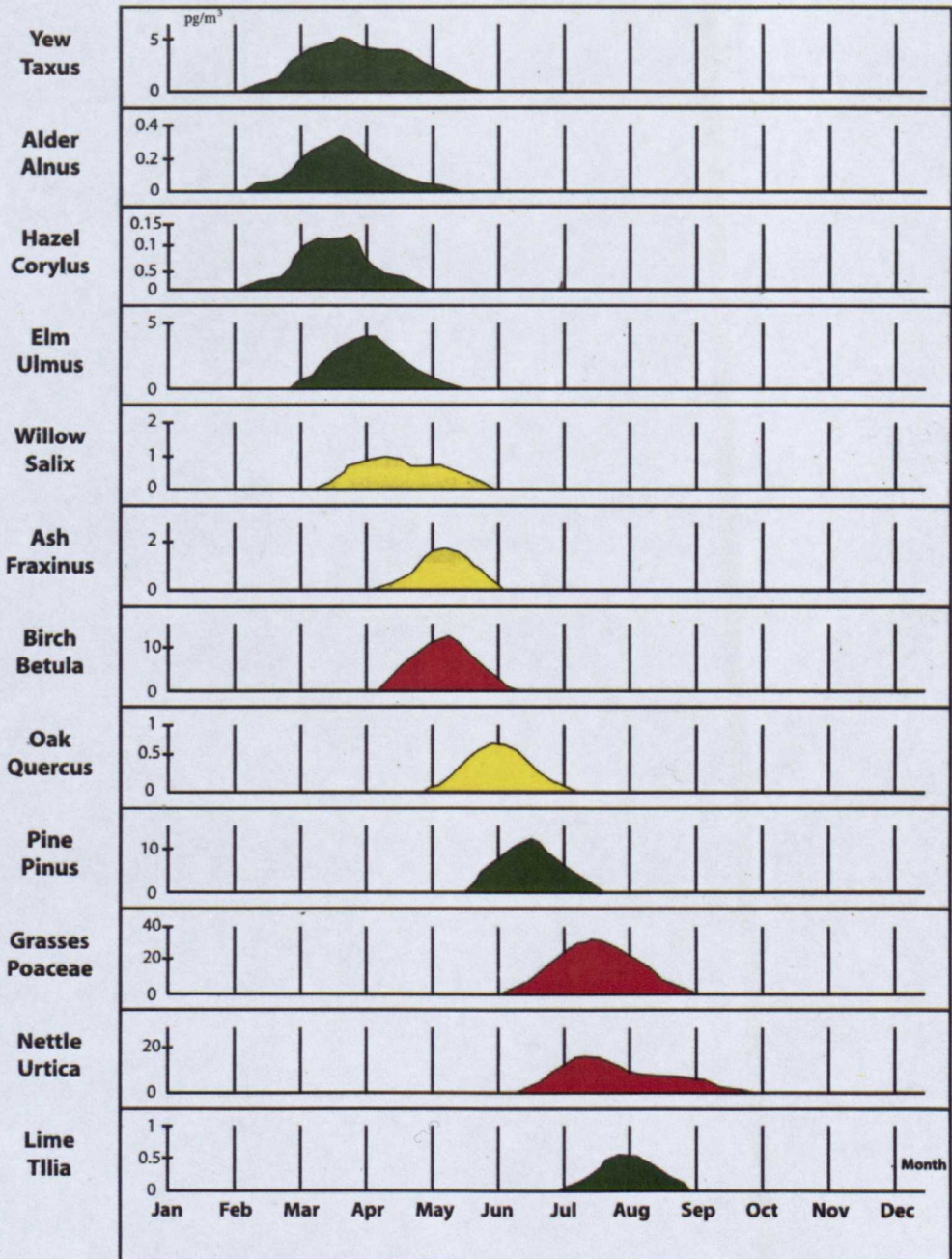


Plate 5



"Hay Fever" (Seasonal Rhinitis) affects about ten per cent of human populations. Itchy and streaming eyes, sneezing and a general feeling of not being well, are characteristic. Allergenic pollens are the commonest cause of Hay Fever. Pollen may also "trigger" attacks of asthma. The purpose of this leaflet is to help visitors to Scotland avoid exposure to those pollens to which they are allergic, by timing their visits before or after the "peaks" of high pollen counts. The pollen calendar shown refers to allergenic pollens present in the air over Edinburgh during the decade 1988-1997.



Circa il 10% della popolazione è afflitta da "raffreddore da fieno". I sintomi caratteristici sono prurito agli occhi e lacrimazione, stimolo e starnutire, et un generale stato di malessere. La principale causa del raffreddore da fieno è l'allergia al polline. Il polline puo anche agire da fattore scatenante per attacchi di asma. Questo opuscolo si propone di aiutare turisti che intendono visitare la scozia., ad evitare i pollini a cui sono allergici.. Con l'aiuto del calendario dei pollini qui riportato, potranno programmare i viaggi prima o dopo i periodi in cui la quantità di polline presente nell'aria raggiunge i valori massimi. Il calendario qui riportato si riferisce ai pollini presenti nell'area di Edimburgo durante il periodo 1988-1997.



Le "Rhume des Foins (rhinite) affecte environ 10% de la population mondiale. Il se caractérise par un état de fatigue générale, des éternuements ainsi que des yeux irrités. Les allergies aux pollens sont les principales causes des rhumes des foins. Le pollen pourrait donc être un "facteur déclenchant" de l'asthme. Cette fiche d'information a pour but d'aider les visiteurs de l'Ecosse, à repérer les espèces de pollen auxquelles ils sont sensibles, et de prévoir leur visites avant et après la période où la quantité de pollen dans l'air est maximale. Le calendrier pollinique représente différentes espèces de pollen présent dans l'air d'Edinburgh durant la decennie 1988-1997.



Etwa zehn Prozent aller Menschen leiden unter "Heuschnupfen", d.h. jahreszeitlich bedingtem Schnupfen, der sich vor allem durch juckende und tränende Augen und allgemeines Unwohlsein bemerkbar macht. Die häufigste Ursache für Heuschnupfen sind Pollen, die sogar Asthmaanfälle hervorrufen können. Dieses Merkblatt soll den von Heuschnupfen betroffenen Besuchern des Schottlands helfen, den Zeitpunkt ihrer Reise so zu planen, daß sie Zeiten, in denen mit einem erhöhten Pollenflug zu rechnen ist, meiden können. Der abgebildete Pollenkalender bezieht sich auf Messungen, die 1988-1997 in Edinburgh durchgeführt wurden..



La "fiebre del heno" (rinitis estacional) afecta casi a un 10% de la población humana. Los síntomas característicos son picor y lagrimeo de ojos, estornudos y malester general. La causa más común de fiebre del heno se debe a granos de polen alergénicos. El polen puede también provocar ataques de asma. El propósito de este folleto es ayudar a los visitantes de Escocia a evitar la exposición a aquellos pólenes a los que son alérgicos, mediante la programación de sus visitas antes o después de los picos de altas concentraciones polínicas alérgicos presentes en el aire de Edimburgo durante un periodo de 10 años, 1988-1997.



A "Febre dos Fenos" (rinite) afecta Cerca de dez por cento da população mundial. Os sintomas caracterizam-se por um estado de fadiga geral, espirrar com alguma frequência nos olhos. As Alergias ao pólen constituem as causas principais da "Febre dos Fenos". O pólen pode também ser responsável por crises de asma.. O objectivo principal deste panfleto é divulgar alguma informação respeitante à abundância de pólen alérgico durante o ano, de forma que quem visita a Escócia possa organizar a sua visita e evitar periodos em que a quantidade de pólen no ar é bastante elevada. O calendário de pólen aqui representado inclui diferentes espécies de pólen presente no ar, em Edimburgo, entre 1988 e 1997.



10人に1人がかかるといわれる花粉症（季節性鼻炎）。その症状は、目のかゆみ、涙、くしゃみ、不快感で、アレルギーを誘発する花粉が一般的な原因です。また、花粉はぜんそく発作をもたらす可能性もあります。この案内書が、スコットランド南東地方を訪れる方々にとって、花粉量が最大となる前後の時期にいらっしゃり、アレルギーを起こす花粉に悩まされず過ごされるよう、お役に立てば幸いです。なお、花粉カレンダーは、過去10年間(1988-1997)のエジンバラにおける花粉の空中飛散データを参考にしています。

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Material compiled by: Caulton, E., Carmichael, R., Minebois, E. & Vaschetti, C.

Plate 6

Honey production is an ancient and important aspect of human nutrition. Bee-keeping is a widespread activity both commercially and privately in the UK, where blending of honey types is often a characteristic of the former. In Scotland, bee-keeping and honey production may be described as a “cottage industry”. Imported honeys may not always be what the labels on the jars indicate. Pollen analysis of honey has received the attention of the author and bee-keeping colleagues in the Centre’s early days. It was indeed through a talk given by the author to the Edinburgh Bee-keepers’ Association in 1987, on pollen, that the two first scientific volunteers, who were enthusiastic bee-keepers, assisted in the setting up of the Pollen Centre. Pollen analysis of honey is an extremely time-consuming task. On two occasions, the author was commissioned to investigate and identify the honey types supplied. The first occasion was part of a random survey of supermarket stocks of commercially produced honeys being undertaken by the Consumers’ Association “WHICH?” reports. Sixteen unknown (i.e to the author) honeys were supplied for pollen analysis and typing. The task took several weeks, amounting to some 300 hours of work!

The results of the sixteen sample analyses were expressed as a “honey type” for each sample. The honey type was determined after counting and evaluating the percentage present of each pollen type. The principal honey types were ‘mixed floral’ (meadow flowers), tree blossom (e.g. lime), highland (heather) and rape (oil seed rape). The object of the exercise by WHICH? was to check by random sampling the accuracy of the labels on the jars taken directly from supermarket shelves.

The second assignment was undertaken for the local office of Fair Trading Overseas. Two honey samples labelled “Orange-blossom” and “Pine” had their origin in Mexico and Turkey respectively. The Mexican honey labelled “Orange-blossom” turned out to contain predominantly *Euphorbia* pollen, and the Turkish sample labelled “Pine” was predominantly *Eucalyptus* – both samples being mis-labelled (Sawyer, 1981, 1988).

Pollen present in honey could be implicated in triggering a type of hay-fever condition (Dr A Sutherland pers.comm.). The condition exhibited by two

patients resembled hay-fever – the only aspect held in common when questioned, was they each had recently eaten quantities of honey.

3.13 Archaeological Application

Pollen analysis has a long history as a tool of the archaeologist particularly with regard to post glacial climatic history and vegetation history as revealed by the technique applied to peat deposits. This area of application and its significance in human evolution and cultural development is now widely recognised (Wood, 1968).

As pollen analysis has developed over the past half century, so the emphasis in application has changed. As a student undergraduate (1947-50), palynology was a minor aspect of study; the emphasis being more concerned with the cytology of pollen development rather than a study in its own right. Plant ecology as developed by Tansley (Tansley, 1953) made only passing reference to pollen analysis, then only emerging as a significant tool, but subsequently underwent important change with the recognition and identification of communities (plant sociology) and the concomitant concept of *indicator species* of habitat. Thus pollen analysis of peat and lake deposits became a useful tool in the reconstruction of past vegetation history (Godwin, 1975; Pennington, 1969). This aspect was also of interest and relevant to Biogeography (Tivy, 1971; Watts, 1971; Cox and Moore, 1993). From this point onwards as inter-glacial and post-glacial vegetation studies became more widely undertaken, the community nature, including indicator species, became more emphasised. The concept of *fossil assemblages* developed and the whole vegetation analysis with pollen analysis at the core took on both a prehistoric and an ecological stance (West, 1968; Birks and Birks, 1980). The condition and nature of plant fragments alongside pollen *in situ* has also played a part in discoveries relating to fossil remains of man (Wood, 1968; Day, 1969).

The Centre was involved in a short study of a sample of what was, at the time described as “gunge” – an amorphous substance dredged up from the side of a crannog in Loch Tay. The material had been extracted from the corner of a broken, submerged dish. The sample labelled “P”, was given to the author to analyse. The report revealed much alder pollen, but also of particular interest

was the presence of numerous intact fern sporangia with developing spores and a large number of released mature spores of bracken (*Pteridium*). Such artefacts and their contents are used to attempt to reconstruct the nature of Crannogs, their function and the day to day activities of the occupants. It was obvious from the analysis results of sample "P", that bracken fronds had been gathered as floor covering and bedding, to be gathered up when the fresh fronds were becoming dry and less serviceable and tossed overboard, to be replaced by fresh material. The (then) brittle nature of the sporangial pedicels (stalks) caused them to break releasing the microscopic sporangia and their immature contents as well as mature spores not yet shed. Thus sporangia and spores became part of the air-borne dust and doubtless, when settling contaminated the open cooking vessels such as the one dredged up. The substance "P" was nicknamed "Bronze-age Butter" (Dixon, 1982). Because of the condition of the sporangia which were intact, it was possible to assign the month of use of the fronds to August-September of that particular year, some 6,000 year BP.

4. CRITICAL ANALYSIS AND SYNTHESIS OF RESEARCH PUBLICATIONS

4.1 The Research Programme

The author's nine publications arranged, chronologically within major topic areas, are listed below (4.2). The nine submissions represent the author's personal research programme undertaken since 1988, the first season of sampling (Fig. 3). The topic areas of his research have included : the pollen count; pollen rain studies; pollen analysis of faeces and a biographical study. All the field, laboratory and information retrieval work was, in every instance, undertaken by the author.

4.2 Publications

The Pollen Count

1. Mackay, T.W., Wathen, C.G., Sudlow, M.F., Elton, R.A. & **Caulton, E.** (1992). Factors affecting asthma mortality in Scotland. *Scottish Medical Journal* 37, 005-007.
2. Emberlin, J., Jones, S., Bailey, J., Caulton, E., Corden, J., Dubbels, S., Evans, J., McDonagh, N., Millington, W., Mullins, J., Russell, R. & Spencer, T. (1994). Variation in the Start of the Grass Pollen Season at Selected Sites in the United Kingdom, 1987-1992, *Grana* 33 (2),. 94-99.
3. **Caulton, E.**, Keddie, S., Carmichael, R., & Sales, J. (2000). A ten year study of the incidence of spores of bracken (*Pteridium aquilinum* (L.) Kuhn) in an urban rooftop airstream in south-east Scotland. *Aerobiologia* 16, 29-33.
4. Corden, J., Millington, W., Bailey, J., Brookes, M., **Caulton, E.**, Emberlin, J., Mullins, J., Simpson, C. & Wood, A. (2000). UK Regional Variations in *Betula* Pollen (1993-1997). *Aerobiologia* 16, 227-232.

Pollen Rain Studies

5. **Caulton, E.**, Arnott, J. & Primiani, F. (1996). Monitoring of pollen rain in moss polsters and mats in view of their suitability as control for determining the air-borne component of pollen spectra in herbivore faeces. *Aerobiologia* 12, 75-84.

6. **Caulton, E.**, Aitken, W. & Rashid, N. (1998). Aerobiological aspects of Elm (*Ulmus* spp.) in South-east Scotland in relation to elm decline from Dutch Elm disease (1976-1996). *Aerobiologia* 14, 147-153.

Pollen analysis of faeces

7. **Caulton, E.** & Gibson, W. (1998). Étude du Contenu pollinique des matières fécales d'herbivores sélectionnés. *Inst. Fr. Pondichery, trav. Sec. Sci. tech.* LXXV, 301-38. Actes. X^e Symposium APLF Bordeaux, 28.9.87-02.10.87.
8. Fraser, M.A., **Caulton, E.** & McNeil, P.D. (2001). Examination of faecal samples as a method of identifying pollen exposure in the dog. *The Veterinary Record* 149, 424-426.

Biographical Study

9. Chanda, S. and **Caulton, E.** (1999). David Douglas Cunningham (1843-1914): a biographical profile. *Aerobiologia* 15, 255-258.

4.3 Critical Analysis of Publications

4.3.1 The Pollen Count

- 4.3.1.1 Mackay, T.W., Wathen, C.G., Sudlow, M.F., Elton, R.A. & **Caulton, E.** (1992). Factors affecting asthma mortality in Scotland. *Scottish Medical Journal* 37, 005-007.

The above publication reflected the vital importance of the need for intensive and ongoing studies into this aspect of respiratory disease. Scotland has the unenviable position of having one of the highest mortality rates due to asthma in developed countries. The need to identify the causal factors leading to death is paramount due to the urgency of the current situation. The pollen data was expressed as total pollen as opposed to pollen grains per cubic metre of air. This was not the author's preferred presentation but the pattern of the histogram would have been similar in either form of presentation. Separate grass pollen counts for the period of study were also sent and incorporated due to their particular allergenicity.

The author's pollen data drawn on in this paper was an integral and important component of the ongoing study. It was concluded in this study that pollen may not play as important a factorial role in Scotland as previously thought. It may well be that allergenic fungal spores, especially those of *Alternaria* and

Cladosporium may prove to be a more important factor in asthma mortality than pollen. Little work however has been published involving specifically Scottish studies relating to airborne allergenic fungal spores to date. The author is currently working on a five year (partly retrospective) study into the incidence of airborne spores of *Alternaria* in the rooftop airstream over Edinburgh with a view to rectifying to some extent the lack of information extant.

4.3.1.2 Emberlin, J., Jones, S., Bailey, J., **Caulton, E.**, Corden, J., Dubbels, S., Evans, J., McDonagh, N., Millington, W., Mullins, J., Russell, R. & Spencer, T. (1994). Variation in the Start of the Grass Pollen Season at Selected Sites in the United Kingdom, 1987-1992. *Grana* 33 (2) 94-99.

4.3.1.3 Corden, J., Millington, W., Bailey, J., Brookes, M., **Caulton, E.**, Emberlin, J., Mullins, J., Simpson, C. & Wood, A. (2000). UK Regional Variations in *Betula* pollen (1993-1997). *Aerobiologia* 16, 227-232.

UK studies of patterns in grass and birch pollens

The two multiauthored papers (Emberlin et al, 1994 and Corden et al, 2001) together with the study published in Abstract only (Jones et al, 1994) were pioneer publications in UK aerobiological studies. In each case the author's pollen data for the two types of pollen were included to give a Scottish dimension to the UK picture. The two first publications were the result of analysis of data extending over five years, whereas the Abstract component was based on seven years of data. The study undertaken by Jones et al (op.cit.) was designed to construct a predictive model.

The two papers by Emberlin et al and Corden et al (op.cit.) considered the UK as a whole – an holistic approach, whereas that of Jones et al (op.cit.) considered regional groupings of the data of pollen monitoring sites in the UK e.g. Belfast, Invergowrie and Edinburgh which constitute a North-British group, being some considerable distance from the nearest group south of the Scottish Border.

Unfortunately Jones et al's 1994 study has not been published to date, so her work to utilise current and past pollen output to construct predictive models, which are of much value, has not materialised. Predictive modelling for the

UK is still in its early developmental stage. There is an obvious significant advantage in attempting to predict the onset of allergenic pollens and spores, and the likely abundance in each case. Predictive modelling for the onset of the grape harvest has been well established in France, particularly by P. Cour's team at Montpellier. Birch, the onset of the pollen season of which, has been forecast to be early in 2002 (Emberlin, pers.comm).

Whilst the application of pollen curves to develop predictive models for each regional grouping of pollen monitoring sites in the UK would be extremely valuable (*vis à vis* allergenic pollens and spores), whether or not the accuracy of predictions with regard to the unsettled climatic regime in Britain, especially exposed northern parts, could be reliable, is a problem. The importance of the author's contribution to these three studies is that his comparisons over a number of years have been able to point to a distinctive pollen count – usually lower and later in starting – but nevertheless having a pattern similar to that produced elsewhere in the United Kingdom, especially at the three northern sites – Edinburgh, Invergowrie and Belfast.

- 4.3.1.4 **Caulton, E., Keddie, S., Carmichael, R. & Sales, J. (2000).** A ten year study of the incidence of spores of bracken (*Pteridium aquilinum* (L.) Kuhn) in an urban rooftop airstream in south-east Scotland, *Aerobiologia* 16, 29-33.

The above paper was an extension of an original five-year study of the incidence of airborne spores of bracken in the rooftop airstream at the author's sampling site (Caulton et al 1995b). The studies were initiated by the author in response to publicity concerning the effects of inhalation of bracken spores *en masse* during the spring season (August-September in Britain). Bracken has long been known to be highly toxic when its fronds are consumed by grazing herbivores. Lesions and severe abnormalities can result and the discovery following *in vivo* experiments on rats revealed carcinomas appearing in the respiratory tract when inhalation occurs in a spore-laden atmosphere.

The only publication arising from studies on the output of spores of bracken resulted from work in the field at Rothamsted Experimental Field Station in Hertfordshire (Lacey & McCartney 1994). The high numbers of spores

released in the vicinity of the plant combined with the discovery of the carcinogenicity, suggested to the author to establish by means of a long-term retrospective study, the concentration of bracken spores in the urban airstream to which people were exposed and, whether or not such concentration might pose a health hazard.

In both papers, the 5-year and the extended 10-year studies were undertaken retrospectively from slides prepared from 7-day exposed tapes throughout August and September. The spore counts were low with the exception of 1989, where the total number of spores counted for the spring season was over 500. That year proved to be the longest season by far of all the other nine years. The overall picture however was of a low spore count as monitored by the Burkard sampler, and it was concluded that such a low concentration did not pose a risk to health.

The reasons for low counts could have reflected the paucity of fertile stands of bracken up wind of the Burkard. Most bracken stands, and there are plenty of them in the periurban areas of the author's site, reproduce vegetatively by underground rhizomes. The nearest known spring stand to the author's sampler is some 1.6 km distance with another more distant. As with pollen, spore discharge is affected by weather conditions: humidity, hours of sunshine, temperature, wind speed and rainfall. It was established in the earlier 5-year study, that a critical period of two weeks in May influences the development of the sporangial initials and subsequently, the maturation of sporangial contents. Warm and dry weather conditions during this fortnight are crucial to spore development within the sporangium. The release of mature spores is likewise weather dependent – warm sunny conditions with a gentle breeze favours maximum spore release. Such conditions for both spore development and release existed unusually in 1989.

The author's contribution to bracken spore studies in relation to possible effects on health has been viewed as significant by the International Bracken Group. Further urban and rural comparative studies both in the UK and abroad would doubtless be valuable, but unfortunately sites monitoring the air spora outwith the pollen seasons are few in number.

4.3.2 Pollen Rain Studies

- 4.3.2.1 Caulton, E., Arnott, J. & Primiani, F. (1996). Monitoring of pollen rain in moss polsters and mats in view of their suitability as control for determining the air-borne component of pollen spectra in herbivore faeces, *Aerobiologia* 12, 75-84.

Pollen and spores when discharged, usually forcibly, describe a parabolic curve. A number of factors are involved in influencing the height attained and the distance between the points of discharge and landing. Most of these factors are microclimatic, but some relate to (spore) density and morphology of the grains themselves. The collective assemblage of pollen grains in the air descending to ground surface is termed 'pollen rain'. Whilst the major component of pollen rain consists of locally produced pollen, the overwhelming majority of pollen grains originates within a 50 km radius of the point of discharge to the point of landing. A negligible component of the pollen rain assemblage which originates beyond this radius may be regarded as 'long range transmission' (Tyldesley, 1973).

All pollen eventually descends to the ground surface, alighting on bare rock, trees, ground-cover vegetation or water. Much ground-cover vegetation comprises grasses, sedges, mosses, liverworts and lichens. Mosses are frequently compacted into cushions or polsters, others, together with some liverworts and lichens form mats. Plate-like thalli of some liverworts and lichens form recipient surfaces for pollen rain.

The author was interested in collecting, by washing technique, samples of pollen rain trapped in polsters and on mats of bryophytes and lichens, to determine the pollen spectra present and whether or not it might be possible to relate these to the pollen spectra found in herbivore faeces (rabbit).

This publication was unique among the relatively few moss polster pollen rain studies in the literature, in its objective; in its comparison of selected species of mosses, and lichens for their physical effectiveness as traps, comparison of species from open, exposed sites as opposed to closed, sheltered sites; lastly in its comparison of two opposing geographical areas namely east and west Scotland.

Whilst the technique of sampling pollen rain deposits in polsters and mats reflected well the immediate source of pollen, and is of value in this respect, as a tool for correlating the pollen sampled in the polster and mat microhabitats with the pollen spectra in herbivore faeces, it is of very limited value. Whether sampling *Sphagnum* polsters from heathland bog pools or *Thuidium* in closed woodland habitat, the necessity for regular and frequent sampling (e.g. weekly) during the study period is imperative otherwise, 'wash down' (*Sphagnum*) or 'accumulation' (*Thuidium*) distorted the orderly sequence of pollen types descending in the pollen rain. For pollen rain monitoring, bearing in mind the need for careful determination of frequency of sampling, polsters and mats from open exposed habitats, (e.g. moorland), are to be preferred to closed, sheltered (e.g. woodland) ones.

4.3.2.2 **Caulton, E., Aitken, W. & Rashid, N. (1998),** Aerobiological aspects of elm (*Ulmus* spp.) in South-East Scotland in relation to elm decline from Dutch Elm disease (1976-1996). *Aerobiologia* 14, 147-153.

The incidence of Dutch Elm disease has resulted in the death of some twenty million trees in the UK, the vast majority of which occurred in England and Wales. The "elm decline" is a well marked feature of pollen diagrams from peat and lake sediment deposits dating back to Neolithic times. Deposits of unknown date can be dated by the level of the 'elm decline' revealed, so prominent is this phenomenon. The modern decline has been compared with the Neolithic one and the possibility of similar causation, namely by an insect-borne fungal disease organism. However it is now generally considered that the Neolithic decline was due to the extensive clearance of elms for cultivation (LANDNAM) and the use of foliage for animal fodder. The 20th century outbreak however is clearly due to fungal infection introduced by three species of elm bark beetle.

A palynological investigation undertaken by Peter Moore based on pollen analysis of soil cores extracted from erstwhile elm tree sites in southern England, demonstrated the decline as measured by decreasing pollen counts revealed with time as sampling proceeded from the bottom of the cores upwards (Perry & Moore, 1987).

At a meeting of the Linnean Society Palynology Specialist Group in 1997, the author discussed the soil palynological investigation (op.cit.) with Peter Moore, who then suggested that a similar study be undertaken in Scotland. The author selected for the investigation a few sampling sites in and around Edinburgh where elms had been growing. The principal site utilised was on Corstorphine Hill which had been cleared of the dead remains of the elm stand there. The author's investigation showed that the soil pollen analysis was not as clear cut as that undertaken in the south of England. The author subsequently turned his attention to an analysis of actual records held by the capital's Parks Department, of the numbers of English elms extant over the twenty-year period, 1976-1996. The feature that distinguished the Scottish elm scene from that of southern Britain is that in Scotland, the commoner and indigenous species, the Wych elm (*Ulmus glabra*) has shown a rate of decline mainly due to it being more resistant to the disease than the English elm (*U.procera*). Added to this is the extensive planting of the Wheatley elm (*U.angustifolia* var. *saraniensis*) as an introduced amenity tree in urban areas. This species is more compact in its growth habit and weakly susceptible to the fungal disease. There is also a climatic factor which has a bearing on Dutch Elm disease in Scotland when compared with England, namely generally lower temperatures which are unfavourable to northward migration of the elm bark beetles (*Scolytus* spp) which carries and transmits the fungus spores (*Ceratocystis* sp.) – the causative organism of the disease in elms. The higher rainfall and few days of warm sunny weather experienced north of the Border also discourages the vector.

The author's investigation considered the long-term prospect of elm survival in Scotland. By extrapolation from the data over twenty years recording the rate of decline of elms, the author predicted that few if any standing elms will be seen by the end of the first decade of the twenty first century in Scotland. Such elm pollen which may continue to be represented in pollen trapped will have been produced by regenerating suckers arising from surviving elm rootstocks. These will most likely die in the course of time.

The author's investigation demonstrated the effect of geographical and climatic factors in addition to biological factors relating to the varying degrees

of resistance seen and distribution of the bark beetle north of the Border. The author was at the time invited to participate in a Manchester University survey of elm bark beetle distribution in the UK. Samples of pheromones were designed to attract any of the three species of *Scolytis* in the vicinity of the garden fence site. The beetle samples were returned to the team at Manchester, but the author was not involved in any subsequent publication.

The author's investigation has evoked some interest outwith the UK and may well have value in other areas of plant pathology.

4.3.3 Pollen Analysis of Faeces

- 4.3.3.1 **Caulton, E. & Gibson, W.** (1988). Étude du contenu pollinique des matières fécales d'herbivores sélectionnés. *Inst. Fr. Pondichéry, trav. sec. sci. tech. LXXV, 301-308* Actes X^e Symposium APFL Bordeaux 28.9.87-02.10.87.

A paper published in *Grana* in 1983 aroused the author's interest as it concerned a contemporary study on the pollen spectra found in the faeces of sheep in Norway (Moe, 1983). The study aimed to establish whether or not the pollen spectra in sheeps' faeces reflected the local pollen rain. The result proved negative. Living in a Country Park in Midlothian, the author decided to investigate, by repeating Moe's study, using a flock of 21 Jacob sheep kept in one of the park's paddocks, with the same objective. Quadrat analysis of the vegetation cover grazed by the sheep was undertaken and a comprehensive list of species compiled. A list of species of the general flora outwith the paddock – trees and herbs – was also noted over the next three years, random samples of faeces were collected at fortnightly intervals during each of the three pollen seasons. Unlike Norwegian livestock farmers, transhumance is not practised on a regular basis in Scotland – the Jacob sheep were paddocked the entire year. The author's results confirmed the Norwegian study. However, the author observed a notable degree of selectivity in grazing when the pollen species represented in the faecal samples were compared to the paddock sward. Further studies were initiated by the author to investigate the pollen spectra in the faeces of other herbivores in habitats with distinctive vegetation (e.g. meadow, moorland, desert). A further discovery was made from these studies, namely that in each distinctive habitat pollens from habitat indicator species were present, which enabled the author to establish not only

the *preferred* plant diet but also *where* grazing occurred. The source of the faecal pollen present in any sample could reflect not only specific inflorescences consumed directly, but also include pollen 'contaminants' of the animals' coat whilst moving through vegetation which had been recipient of pollen rain. This latter pollen reaches the gut by virtue of coat grooming and subsequent swallowing.

The author found, after applying the technique to other species of herbivore, faeces derived from a range of habitats in Britain and overseas, that pollen analysis of faeces is a useful tool in determining the preferred diet and feeding habitat of grazing herbivores.

Pollen analysis of faecal material is not a new technique, as shown in studies on the pollen content of fossilised cave bear droppings, dating back to the late Tertiary and early Quaternary period.

The author has recently begun studies into residual pollens present in carnivore faeces derived from herbivore prey. Attention is currently focussed on bats (*Chiroptera*), particularly insectivorous species.

Pollen analysis of faeces, whether herbivore or carnivore, is reasonably reliable as a technique in view of the relative indestructibility of the pollen outer wall (exine). Such damage to the pollen grain that may be sustained, is due to physical rather than chemical factors (e.g. mastication).

The author was recently informed (Dettman, pers. comm.) of the use of his technique and findings, in an Australian Conservation public enquiry issue concerning roosting and feeding areas of flying possums. The findings, using pollen analysis of the marsupials' faeces, settled the issue in favour of the conservationists. The Queensland National parks department indicated their interest with regard to feeding and locality of Koala – and the likelihood of saving much bush rangers' time and public money in obtaining the required information (pers. comm.)

4.3.3.2 Fraser, M.A., Caulton, E. & McNeil, P.D. (2001). Examination of faecal samples as a method of identifying pollen exposure in the dog. *The Veterinary Record* 149, 424-426.

The author was contacted by Mrs Fraser, then a post-graduate student at the Glasgow Veterinary College, and asked if he could help with a problem of pollinosis detected in the current group of young dogs undergoing preliminary training for suitability as guide dogs for the blind. The author suggested using the faecal analysis technique and received random samples at monthly intervals over a period of six months during the pollen season when dogs were exercised locally. The pollen spectra derived from the samples indicated considerable intake into the gut (by self grooming) of grass and pine pollen. Other pollens were minimal and scattered randomly among the samples.

As the author's contribution constituted a part of Mrs Fraser's PhD thesis, her examiners had expressed their considerable interest in the 'novel' technique applied to that particular situation (pers. comm.)

This study undertaken by the author probably arose from an earlier request of a similar nature, but involving horses, in the current care of the Royal (Dick) Veterinary College, Edinburgh – equine pathology department. (A brief report was published in the annual report of the equine association at the time – Dixon et al, 1992). The outcome of this equine investigation was to identify willow pollen as the allergen causing the condition of pollinosis. Both horses were paddocked adjacent to a flowering willow at a Perthshire stables.

4.3.4 Biographical Study

Chanda, S. & Caulton, E. (1999). David Douglas Cunningham (1843-1913): a biographical profile. *Aerobiologia* 15, 255-258.

This paper, in which the author was appointed co-author, is quite different to the other eight papers analysed in this part of the thesis, in that it is biographical in content.

The study was first drawn to the author's attention by his receipt from the Editor of *Aerobiologia* of a draft manuscript submission by a Dr S Chanda, an

Indian aerobiologist. With the manuscript came the request for the author to investigate the background of the subject. Dr David Douglas Cunningham's experience and work with the Indian Army Medical Corps was researched by Dr Chanda. Information concerning Dr Cunningham's interests after his retirement to Torquay, Devonshire, was found in the library of the Royal Botanic Garden, Edinburgh. It is possible that the author's involvement in this study was linked to the fact that Dr Cunningham received his medical training at the Edinburgh Medical School and the fact that the author's laboratory base is likewise located in the capital. However that may be, the author's researches bore fruit, as to the subject's retirement activities, as well as, thanks to the author's association with the Linnean Society of London, being able to procure a good copy of the only original photograph of Dr Cunningham extant. In addition, archival material from the Torquay local press, resulted in useful information as to the subject's interest in and contributions to natural history and horticulture during his retirement years. All the author's research material was forwarded to Bologna. Shortly after, a final manuscript for proof reading was received by the author. This manuscript embodied both Dr Chanda's and the author's research results. The author discovered that his name had been unexpectedly added as co-author with Dr Chanda's!

Scientific progress and achievement is built upon previous generations of workers, some of whom make distinguished 'ground breaking' discoveries, which enable the discipline to advance more rapidly. New understanding and new bases for advancement are the outcome. The majority of workers however make significant contributions step by step to the advancement of their discipline.

David Douglas Cunningham deserves to be remembered in *Aerobiologia*, whose biographical series, which appear from time to time, as his contributions to the study of diseases of various kinds encountered by him during his term of service in India were distinguished. Aerobiological studies and experiments came within his ambit.

The author considers such biographical studies to be important, not only in enabling understanding of the developmental process of ideas and techniques

and the achievements which arise therefrom, but also to remind us all of the debt we owe to the efforts and dedication of our predecessors.

4.4 Synthesis of Publications

Of the nine publications presented in the thesis, all are concerned with Aerobiology. Eight of them are directly involved with airborne pollen – whether as part of the pollen count, pollen rain studies or as pollen present in the faeces of animals. Thus the main emphasis in each case has been a palynological contribution to the multi-disciplinary science of Aerobiology. The nature of such investigations involves team work and consequently draws on the efforts and expertise of colleagues. Where publications are based on national or international surveys involving data handling from a number of sampling sites, multi-authored papers tend to result. Three such papers occur under “Pollen Count” above, (Section 4.3), two of which were of geographical significance within the UK, whilst the third involved collaboration between a medical team and the author. It is rare for a publication in the field of aerobiology, other than a review, to be single-authored. This reflects the complexity of analysis required to ensure all relevant parameters have been considered in order to arrive at valid conclusions.

The remaining seven publications were not hypothesis-based, but involved resolving answers to basic questions by data collection and analysis e.g. *what* are the variations in the start of the grass pollen season and *what* regional variations exist of Birch pollen in the UK? Likewise, there were no preconceived ideas concerning the concentration of bracken spores in the roof top airstream and whether or not such concentration as was found might constitute a health hazard. Data collection over time with statistical analysis produced a negative answer for the Edinburgh site.

The two faecal analysis studies were designed to answer two questions: *what?* and *where?* in relation to diet and habitat in the earlier paper (Caulton & Gibson, 1988) and *What?* in relation to pollinosis in dogs (Fraser et al, 2001). Both sets of questions were resolved by means of pollen analysis of faeces. The results in both sets of studies were reached after relatively straightforward statistical tests.

The biographical study (Chanda & Caulton, 1999) was purely an information retrieval exercise in the main, involving evaluation of the significance of the subject's scientific contribution to Aerobiology.

Palynology has wide applications as can be seen from the diverse range of topics represented in the author's own work presented in this thesis; the application of pollen rain studies as a technique to facilitate monitoring airborne pollen; investigations into seasonal and geographical variations in selected taxa; the application of the concept of "indicator species" to interpret from pollen found in animal faeces the nature of preferred diet and feeding habitat; and, lastly, the assessment of the significance of the aerobiological studies undertaken by a distinguished Indian Army doctor in the 19th century.

Whilst the author's work encapsulates the diversity it by no means covers the potential range.

Whilst the "scientific method" tends to follow the time hallowed procedure: hypothesis, testing by experiment (or observation), evaluation by statistical analysis and whether or not a theory can be established, this procedure is not followed, or appropriate, in every scientific investigation. Nevertheless 'observation', collection and analysis of data and conclusions are essential components of all scientific studies.

Analysis of the publications presented in this thesis highlights many of the problems both physical and biological inherent in aerobiological studies: height above ground of the trapping site; the effect of wind speed and direction; the influence of temperature and, to some extent, hours of sunshine; the volume and duration of rainfall and associated relative humidity; the size and form of pollen grains; short- and long-range transport of pollen. All these factors influence the formation, release and dispersal of pollen and understanding their various impacts and attempting valid explanations of cause and effect, can only be realised over relatively long periods of time.

The nine papers on which this thesis is based are considered by the author to have fulfilled the constituent requirements of scientific investigations.

5. CONCLUSION

The concluding section of this thesis concerns the author's personal contribution to aerobiological studies in south-east Scotland, the UK as a whole and also to the wider community in Europe, both within and outwith the POLLEN CENTRE.

Section 2 above describes the establishment and organisation of the POLLEN CENTRE. Over the fourteen years of its existence and ongoing development, the author has been indebted to the able assistance and advice of a number of volunteer scientists, each of whom is recognised in the ACKNOWLEDGEMENTS. Nowadays little scientific work is accomplished in isolation – teamwork being accepted not only as necessary but as the best approach for maximising effort and results.

It should be emphasised that the direction and final decision-making, the latter after appropriate discussion with colleagues in the team, has been entirely that of the author. It has always been the author's policy to give co-authorship to any participating colleague or student without whose contribution a publication would not have materialised.

The pollen centre has become a valuable, and in Scotland, a unique resource. As the years pass not only will the resource naturally increase but will thereby become more valuable. All the publications extant involving pollen counts, pollen calendars and pollen spectra of faecal material in which the author has been first author cited, have originated from within the pollen centre itself. The author's membership of the British Aerobiology Federation's Executive Committee has always been regarded as one of representing the centre's team and incidentally ensuring a Scottish voice in the national aerobiological scene. Articles by the author about the work of the centre in Scottish and national journals (e.g. Caulton, 1994, Caulton, 2001) and in the media, with various accounts about the work of the centre in general and the pollen count in particular, have given the author an authoritative status at the national level in Scotland. An example of the latter has been a request by a scientific author to

include aspects of the author's article (Caulton, 2001) on a web site currently being compiled.

Reference has already been made to the British Aerobiology Federation's close links via 13 of the UK pollen monitoring sites with the European Aeroallergy Network and its data base in Vienna (Sections 3.1 and 3.2 above).

The year prior to the Pollen Centre's inception (1987) the International Aerobiology Association (IAA) had held the third international conference at Basle, Switzerland. The IAA conferences are quadrennial, and subsequent ones (as well as that held in Basle) have been attended by the author: Stockholm, Sweden (1990), Bangalore, India (1994), Perugia, Italy (1998). At each of the aforementioned conferences the author was invited to chair/co-chair one of the sessions. Papers were presented at each of the three conferences in 1990, 1994 and 1998 (two papers).

In 1996 the European nationals organised the first European Symposium on Aerobiology held at Santiago de Compostela, Spain, at which the author presented a poster exhibit (on Dutch Elm disease, subsequently published (Caulton et al, 1998). The second European Symposium on Aerobiology was held in Vienna in 2000. Two papers were presented at the symposium. The author was also invited to be a member of the International Organising Committee. The author has been appointed PhD examiner on three occasions: 1996 (University of Bangalore) and 1998 and 2000 (for the Department of Botany, Visva-Bharati (College), Santiniketan, West Bengal). From 1991 to the present the author has been requested to act as referee for 28 papers submitted to *Aerobiologia* (17), *Biologist* (1), *Plant Cell Biology and Development* (8) and personal requests (2). The Association des Palynologues de Langue Francaise (APLF) hold biennial meetings. The author attended four of these: Montpellier (1985), Bordeaux (1987), Orléans (1989) and Besançon (1991). At the last three meetings papers were given (in French). Following the presentation of the first bracken paper (Caulton et al, 1995) given at the International Bracken Group's Conference: BRACKEN 94 (paper given by co-author Adrian Dyer), considerable interest was taken in the potential health hazard posed by the carcinogenic bracken spores. This led to extending the survey to cover ten years – this study (Caulton et al, 1999, 2000)

interested the Scottish Environment Protection Agency (SEPA) who invited the author to give a resumé of the paper to a meeting of farmers and landowners in the bracken infested area of the Borders. A national survey of the incidence of airborne bracken spores in urban and rural areas is highly desirable, and to date only Derby (Midlands Asthma Allergy Research Association (MAARA) has been able to supply data. It is hoped London (St Mary's hospital) and perhaps Cardiff may have records or slide resources which can be read.

The author has been an active member of the Linnean Society's Palynology Specialist group since its inception in the early 1990s. Its meetings were originally held twice a year – Spring and Autumn, but three years ago reduced the occurrence of meetings to once a year in the Autumn. The author usually attends the autumnal occasion presenting a resumé of the current topic of research.

During the year 2000, the author achieved three distinctions; election to the Fellowship of the Institute of Biology, in recognition of the contribution to science in an active research programme, outwith teaching and lecturing in ecology, extending over forty years. The second distinction was an invitation to become governor of the National Bracken Control Commission. Thirdly, an invitation to visit Szeged University in Southern Hungary to receive a medal, one of two awarded annually to scientists, who have rendered service to the University – in this case the Department of Botany.

The author has been involved in four consultancies within the field of palynology – The British Antarctic Survey on Jan Coll Island, South Georgia (Chalmers et al, 1996); The Department of Community Medicine, University of Edinburgh, 1996 (Report including the Centre's data awaiting publication; Professor F. Agius pers.comm.) The Royal (Dick) College of Veterinary Studies, Edinburgh University (Dixon et al, 1992); the University of Glasgow Veterinary College (Fraser et al, 2001).

The author was involved in a fifth consultancy in May 1987 in Kuwait. The remit of his visit is outwith the concern of this thesis but a unique opportunity was provided to collect droppings from camels which at that time were

crossing the sand sheet desert as part of the annual Bedouin migration. Two sites were sampled and analysed (Caulton & Gibson, 1988). It may seem anomalous to analyse camel faeces in the light of other herbivorous species' faeces sampled. Some 7-8 spp. Of *Plantago* (plantain) were part of the flora which had turned the desert green after the previous month's rain. However, plantain pollen dominated over all other pollen taxa from the desert sample sites, and pollen of the grass *Phalaris arundinacea* (reed grass) from the Jahra oasis site dominated the pollen spectrum.

In 1998, the author was approached by the Maltese authorities to advise on the establishment of a pollen monitoring service at Valetta. Details of equipment, infrastructure and costings were supplied. The service began monitoring in 1999.

The author was also requested in 1999 to investigate a nature reserve at Bemersyde near Gattonside in the Borders. The site, a reserve, was flooded some 200 years ago, and there was an interest in the changing land use during that period. It was considered that pollen analysis of cores extracted from soil around the perimeter of the small lake might yield some evidence of vegetation succession. However, disappointingly, the first cores proved inconclusive as no definite pollen sequence, which might reflect a changing flora accompanying change in land use, was discernible. Attempts may be repeated from drier sites outwith, but adjacent to the reserve, to analyse core samples (cores were extracted by a 30 cm Hiller Core Borer).

The above review of the author's activities in the fields of Palynology and Aerobiology, it is hoped, speak for themselves as rendering a positive, distinct and valuable contribution to Aerobiological studies in south-east Scotland, Scotland as a whole and further afield. The published papers and articles constitute a body of knowledge and, it is hoped, understanding of the importance of aerobiological studies to the community at large.

As for the future – the above successful achievements in organisation and publication, it is hoped, will continue to stimulate further development of the Pollen Centre's work.

During the past and current years, one or two projects have been completed: Protocols used in the training of visiting students on placement; Is there palynological evidence for climatic warming in south-east Scotland?; Analysis of pollens present in the roof top airstreams over Edinburgh during the winter months of the three years, 1995/96, 1997/98 and 1999/00.

The first of the above projects was submitted for publication in the *Journal of Biological Education*, and is currently undergoing revision prior to re-submission this autumn. The second project is based on the start dates (at 5% level) and duration of the pollen season of Birch (*Betula*) throughout the 13 year period 1988-2000.

Two posters exhibited at the BAF 10th anniversary celebrations held at Rothamstead in March 2000 examined the start dates and duration of seasons over the past 30 years for Birch. One set of data from London records indicated a distinct and progressively early start and length of season which together may provide positive evidence for climatic warming, whereas those from Turku and Oulu in Finland indicated a distinct cooling – later starts and shorter durations of the Pollen seasons for Birch. Whilst Scotland is not latitudinally mid-way between the south of England and Finland, there is a marked difference in climate between the two which it was felt justified even over a shorter (c.half) period to see whether or not climatic warming has influenced the start of anthesis and duration of the pollen season in Birch.

The third project concerns residual allergenic pollens present and circulating in the air stream during the winter months November, December and January of each of the three seasons sampled. Winter studies are few and far between in northern Europe and the U.S.A. – Scottish winters provide a less severe (normally) rigorous climatic regime than Scandinavia and continental U.S.A. The recent winter of 2000/01 in Scotland was quite exceptional in its severity.

Over the fourteen years during which the author has personally been involved in the active organisation, training, pollen counting and producing publications, it is not unreasonable to assume an achievement in competence, expertise in the field, and national and international recognition. The author has personally read over 5,000 slides since monitoring began.

It is on this basis that the author submits his publications and account of the commitment involved for the award of the degree of Doctor of Philosophy.

ERIC CAULTON

August, 2002

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PUBLISHED PAPER^S

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APPENDIX – Research Publications

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