Consideration of some taphonomic variables of relevance to forensic palynological investigation in the United Kingdom

Patricia E.J. Wiltshire*

Department of Geography & Environment, University of Aberdeen, Elphinstone Road, Aberdeen AB24 3UF, United Kingdom

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Abstract

Palynology is a long established and respected branch of environmental science that has been applied to criminal investigation in a meaningful way only in recent years. It has proved to be remarkably versatile in many kinds of criminal enquiry. It is not, however, an absolute science; palynological data are on a par with the suites of symptoms which allow medical practitioners to make diagnoses. Taphonomic variability is the main factor complicating interpretation of forensic palynological data. Palynological taphonomy may be defined as “all the factors that influence whether a palynomorph (pollen, spore, or other microscopic entity) will be found at a specific place at a specific time”.

If taphonomic variability is anticipated, and regularly tested, palynology will continue to keep its place in the armoury of useful forensic methods. Some assumptions made by palynologists engaged in palaeoecology and archaeology have been shown to be untenable in the forensic context. Palynological and botanical profiling of crime scenes has demonstrated anomalies which challenge received wisdoms. It has proved impossible to obtain palynological population data because every site is unique—expectations of any palynological profile can only be crude. The palynological status of any place must be tested every time. Without a body of analytical data from the actual crime scene, it is difficult to see how any palynologist can hope to present credible arguments under cross-examination. The statements made in this paper relate mainly to work carried out in the United Kingdom.

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1. Introduction

The application of palynology to criminal investigation has achieved momentum only in recent years. Those of us (and we are few), who are routinely engaged in forensic investigations have only our training, experience, and enthusiasm for continued and painstaking research to enable us to be of real practical help to the investigating authorities. None of us was trained as a forensic palynologist, and there are no established courses in forensic palynology. This is a subject not easily taught as it requires a great deal of botanical, ecological, and other environmental experience. The palynologist also needs to have the personal resources necessary to cope with the challenges presented by the police and the witness box. The value of the forensic palynologist thus depends on the individual skill and experience of the practitioner. As yet, there is no absolute benchmark for validation. Accreditation comes from repeated achievement of outcomes that prove to be of real value in criminal investigation—ones that are scrutinised by peers employed by the Defence, yet pass the test.

All competent palynologists, whatever their basic environmental training, will have knowledge of the literature that will aid interpretation of their data. However, when viewed critically, there is surprisingly little published information that is of practical value in the forensic context. Much of it is highly theoretical [1], or based on remarkably small data sets [2,3] in which case they would certainly be insufficient to stand cross-examination in the British courts. To date, the most useful papers are those which demonstrate principles through case studies [3–5]. Even here, the actual numerical and statistical data presented are sparse because the authors are attempting to present general principles. However, because of the dearth of results, they are often difficult to evaluate critically.

Palynological data are probabilistic, and their quality is constantly under scrutiny in British criminal cases. Generally,
little credibility is given to analyses where data are few but, in some cases, the results are clear and provide compelling evidence [6]. In others, even where extensive results are obtained, they can be so complex and varied that they defy interpretation. Whatever the nature of the case, standard protocol should ensure that sufficient comparator (control) samples are collected to facilitate an adequate understanding of palynological patterning at scenes of crime, and/or the palynological profiles associated with critical items. A comparator sample can be taken from any surface, matrix, or object which might have been contacted by any exhibit being investigated. Palynomorph assemblages from comparator samples are for comparison with those from the pertinent exhibits. Some investigations are highly complex and it is often necessary to obtain comparator samples from surfaces, matrices, or objects other than those associated with the crime scene itself. This is because of the phenomenon of multiple deposition of palynomorphs during normal use or wear of the exhibit. Such “innocent” comparators are necessary for elimination purposes.

Over the last few years in Britain, forensic palynology has helped to:

1. Link offenders or their belongings with places and other objects (trace evidence).
2. Predict the location of places from the pollen assemblages associated with suspects or their belongings (search and location).
3. Estimate the length of time human remains, or objects, have lain in situ.
4. Differentiate murder sites from the places victims are dumped.
5. Give information on the peri-mortem fate of victims.
6. Provide limited temporal information on post-mortem interval through gut analysis.
7. Establish food intake prior to death through gut analysis.

After being engaged in forensic palynological investigations in all parts of Britain for many years, the author has collected a large database of palynological profiles from very many comparator samples taken from crime scenes, and other places pertinent to specific criminal cases. This record also includes profiles obtained from a wide range of objects, and from human remains. While there are too many data to present here, some idea will be given of the extreme spatial heterogeneity of pollen and spore assemblages.

The body of results accrued over the years has made it clear that the only truly predictable aspect of the spatial patterning of pollen spectra is its unpredictability. This is due to the mass of taphonomic variables inherent in the pollen and spore accumulation in any natural or man-made environment. No crime scene can be taken for granted. It must be evaluated both by eye, and through analysis. Furthermore, very little accurate prediction can be made of the profiles that may be retrieved from various objects and from cadavers. The success of palynology in forensic investigation depends on rigorous analysis, the botanical and ecological experience of the analyst, and the cooperation of investigating officers.

In terrestrial environments, spores are released from the sporangia of mosses, liverworts, and ferns (and their allies). Pollen grains are released from the male cone sporophylls in conifers, and from the anthers of flowering plants. The spore needs to land on a suitable substrate for germination and development of the next generation. The pollen grain’s function is to reach the female and facilitate fertilisation of the ovule; this leads to seed production. The spores of mosses and ferns, and the pollen grains of conifers and flowering plants, lack motility and are carried away from the sporangium or anther by vectors [7–10].

The method of dispersal evolved by any plant species must ensure that sufficient spores or pollen will reach their functional destination; dispersal mechanisms will, therefore, influence the level of production. Successful plant spores will germinate to form new generations of mosses and ferns. Successful pollen grains will reach the female members of the species and fertilise the ovule within the ovary. But the vast majority will simply land on the ground (or some other surface) and die very quickly. The dead spore or pollen grain might decompose and disappear, or remain intact for variable lengths of time.

Palynological taphonomy may be defined as all the factors that influence whether a palynomorph (pollen, spore, or other microscopic entity) will be found at a specific place at a specific time. It is obvious that this is highly complex and involves a multiplicity of variables, including those discussed below, such as production, dispersal, and survival. For any one situation, many of these variables will remain unknown.

2. Production and dispersal

Obviously, the numbers of spores and pollen grains produced by plants, and their mode of dispersal, will affect the frequency with which they are encountered away from the parent plant. Most pollen is transported various distances away from donor plants and, in temperate ecosystems, the main vectors are insects (entomophily), and wind (anemophily).

A great deal of effort has been made to gain some insight into the amount of pollen produced by various species of plant. Some workers have estimated the absolute numbers of grains produced in each anther, by each flower, or shoot [11,12] whilst others have estimated relative pollen production of plant species and even attempted to apply correction factors for the various plant taxa [13–15].

Generally, pollen production in entomophilous taxa is relatively low, and the plants’ resources are channelled into features which encourage insect vectors. These involve production of nectar, perfume, colour, and a tendency towards zygomorphic flowers to support, or even trap, the insect during its visit. Some, such as Salix (willow), have exposed anthers so that wind can also play an additional role in dispersal, but the reliance on insects of others, such Hyacinthoides non-scripta (bluebell), and Digitalis purpurea (foxglove) means that their pollen rarely, if ever, moves far from the immediate vicinity of the plant [16]. In plants such as Aristolochia (birthwort), Arum (arum lily), the Orchidaceae (orchids), and others, their extreme adaptation to entomophily
means that their pollen is seldom out of contact with the donor plant, recipient plant, or the vector. It might only be released to the ground when the flower dies and decomposes. If the pollen of such plants were found in a forensic context, it could be of significant relevance. Certainly, the pollen of insect-pollinated plants often provides excellent forensic indicators of contact.

Because of the high risk of failure in locating the female stigmatic surface, anemophilous plants such as Pinus (pine) and Poaceae (grasses) produce very large amounts of well-dispersed pollen. It is not surprising, therefore, that they are often well represented (even over-represented compared to the absolute abundance of parent plants) in the air, especially during peak periods of their respective pollen release. Unlike insect-pollinated taxa, the morphology of such plants has evolved to ensure free dispersal of pollen away from the parent without impediment.

Even though wind-pollinated plants can be over-represented in the air and on the ground, they may still be important markers in forensic investigation. Certainly, if there is a large amount of pine pollen in a sample, it is highly likely that there is at least one mature pine tree close to the pollen site. When prolific pollen producers are represented at low level, their presence is sometimes considered to be a “long distance” component, and relatively unimportant. Dispersal characteristics of individual taxa within specific stands of vegetation have received attention by many palaeoecologists [17,18] and, over the last 40 years, a very large body of data has been assembled from various parts of the world. However, in some of these studies, data sets from disparate investigations have been amalgamated, while in others the pollen was collected over very large areas with relatively few samples in each specific stand of vegetation. Even where an attempt was made to address pollen dispersal over time in a relatively small area, the sample interval was crude (only 3 sample sites over 1.5 km) [19]. To be of use in the forensic context, sampling frequency needs to be high. In a series of studies with students, Wiltshire (unpublished) showed that, at two sites in different parts of the United Kingdom where surface samples were taken at 5.0 m intervals along transects, pine pollen dropped from values in excess of 80.0% of total pollen and spores (TPS) to about 4.0% within 50 m of the woodland edge. Pine could hardly be regarded as a “long distance component” here since there were dense stands of mature trees within a very short distance of the pollen site. However, the pollen of some plants is certainly known to travel very considerable distances from source. For example, Ambrosia pollen was found in deposits on the Isle of Skye in the Hebrides [20]. The only possible source was the USA. It has also been found in organic deposits at the top of the foreshore of the River Mersey in Liverpool, United Kingdom [21]. In the forensic context, these occurrences are so rare that they have little relevance for most criminal investigations. Whether the vector is wind or insects, in every case most of the pollen produced falls near the parent; and local obstacles and obstructions have enormous impact on the distance travelled by the pollen grain.

2.1. Relevance of pollen production and dispersal in the forensic context

It is useful for the forensic palynologist to have some knowledge of pollen production, especially where an investigation involves searching for important places such as clandestine burial sites. Obviously, if an exhibit which had had contact with a specific place had very high levels of a certain pollen taxon, it might be assumed that the plant was relatively abundant in the place in question. This may or may not be the case but, in the absence of any other information, it would be the only intelligence available to the scientist trying to find the location.

But, the practising forensic palynologist must be aware of anomalies, and much of the information regarding pollen production (considered to be of paramount importance by palaeoecologists attempting to reconstruct ancient environments) is largely irrelevant to forensic investigation; accurate information on the pollen production of any plant species is unimportant. Furthermore, other than a broad understanding of trends and tendencies, published data on pollen dispersal are also largely irrelevant. It is the representation of taxa in the whole pollen assemblage at the crime scene (or other pertinent place) that is critical. Knowledge of this can only be gained by vegetation survey, and adequate collection of comparator samples from the relevant locations. A comprehensive analysis of targeted comparator samples at the crime scene will give the forensic palynologist an accurate picture of the within-site heterogeneity in pollen patterning. Therefore, a planned and adequate sampling strategy (as early as possible in an investigation) is vital. The forensic palynologist cannot rely on theoretical models for interpretation. Data must be obtained which demonstrate, as comprehensively as possible, the palynological profile at the crime scene.

The following recent criminal cases exemplify some of the anomalous situations that could confound interpretation. In a murder case in Wales [22], the victim was found buried in a shallow grave in a dense plantation of Picea sitchensis (Sitka spruce) at an upland location. It was chastening to find that the comparator samples contained less than 1.0% spruce pollen, even though hundreds of hectares of the landscape were planted with this tree. Indeed, pine pollen was far more abundant in the comparator samples, in soils both from the surface and within the victim’s grave. Forestry records showed that the woodland had been planted less than 40 years previously so that most of the trees had not reached sexual maturity. The large amounts of pine pollen in the surface soil were derived from a single, small (though mature) pine tree growing about 100 m from the deposition site of the victim. The pine pollen in the grave profile could have been derived, at least in part, from the single pine but also from trees growing in the catchment before the spruce was planted. If the grave had been sought from assemblages yielded by the footwear and spades of the suspects, the pollen percentages would have provided a very false picture of the extant vegetation at the site.

In a case in Birmingham, United Kingdom [23], a young girl had been found dying in an alleyway opposite a schoolyard and garden. Macroscopic leaf fragments from her hair and shoes,
and the same evidence from the shoes of the accused, showed that they had both stood, and been on the ground, close to a young (but mature) *Fagus sylvatica* (beech) tree. There were abundant beech fruits on the ground so it was obvious that the tree had produced pollen (there were no other beech trees in the vicinity). It was remarkable to find that none of the comparator samples taken from that end of the garden contained even a single beech pollen grain although other plants growing there were well represented in the profile.

A similar situation prevailed inside a stand of mature *Acer pseudoplatanus* (known in UK as sycamore) in Telford, Shropshire [24] where there was an attempted rape of a young girl. None of the comparator samples in, or immediately adjacent to, the crime scene contained any *Acer* pollen grains at all. In spite of the very large number of sycamore trees, only a very low percentage of sycamore pollen was found on the grassy area outside the stand of trees. It was even more surprising that the comparator samples within the wooded area were dominated by pine pollen (>60% total pollen). This had been derived from a young pine tree growing slightly up a slope on the edge of the sycamore woodland. In view of the dense canopy, the pine pollen had probably blown in through the sycamore trunk space. The observed assemblage could never have been predicted, especially if the palynologist had not been given the opportunity to visit the crime scene. An offender was never identified and the case was not pursued further by police.

Another example of a false impression gained of the local landscape by pollen assemblages came from comparator samples collected from a badger sett near Stafford [25]. The sett had been constructed very close to a boundary hedge in a species-rich pasture. The landscape was largely open with many small pastures and crop fields bounded by ancient hedges. Immediately next to the sett, the hedge had had remained untrimmed for some time and consisted of *Crataegus* (hawthorn), *Lonicera* (honesuckle), *Sambucus* (elder), *Acer campestre* (field maple), *A. pseudo-platanus* (sycamore), *Hedera helix* (ivy), *Rubus fruticosus* (bramble), and *Rosa canina* (dog rose). There were extensive stands of *Pteridium aquilinum* (bracken) growing with and through the bramble. Most significantly, there were two large *Quercus robur* (oak) trees in the hedge, each about 60 m away from the sett, and 120 m from each other. The comparator samples yielded a profile that might have been mistaken for a mixed, deciduous woodland. Although herbs were recorded, the profiles were dominated by oak and the woody plants in the hedge. Flowering of pasture plants was much diminished through heavy grazing by stock animals, and the untrimmed hedge provided a woodland signal. Again, without a field visit, the palynologist could have been forgiven for providing completely spurious information.

In cases where investigators are desperate to gain information on the location of a clandestine grave, and they have items belonging to a suspect, the pollen assemblages retrieved from those exhibits provide the only intelligence for finding the human remains. In this kind of case, any information might be valuable but, at all times, the palynologist must keep in mind the unpredictability in production and dispersal when interpreting data. There have certainly been cases where the palynological profile obtained from offender’s belongings have allowed an eerily accurate picture of the place where a victim has been dumped [26], but there have been others where it has proved impossible to envisage a location precisely even though the nature of the landscape could be described in broad terms [27].

### 2.2. The value of predictive modelling in forensic context

The mixture of spores and pollen grains in the air is known as the “airspora” and it is envisaged as falling as “pollen rain”. The airspora tends to be dominated by plant taxa which exploit turbulent air for dispersal; many trees and shrubs, but also considerable numbers of herbaceous plants, are wind-pollinated and are frequent components of the airspora. The study of airborne palynomorphs is fraught with difficulty, and there can be very great variation in results. This phenomenon has been a matter of study, modelling, and debate between palaeoecologists for many years [28–34], and it is becoming increasingly clear in more recent research projects (such as the Pollen Monitoring Programme and POLLANDCAL [35–38]) where modern pollen data are being used to generate predictive models. Just as with the early work on pollen productivity and dispersal, current research is concerned with obtaining information to enhance interpretation of pollen diagrams covering long periods of vegetation history. In an attempt to interpret past vegetation more accurately, it aims to relate patterns of pollen deposition on the ground to modern vegetation.

Researchers in the Pollen Monitoring Programme have set themselves a huge task, even for the “broad brush approach” in modelling at the landscape level. They use various kinds of pollen trap to estimate pollen influx (number of grains cm-2 year-2) for comparing estimates of the airspora at the various sampling sites. Sample size is strictly standardised so that meaningful comparisons can be made between estimates. This quantitative method is considered to provide a more objective basis for interpreting vegetation in the environs of the trap.

Essentially, there are two main kinds of trap (and modified versions of them)—the Burkard spore trap (modified from the Hirst spore trap), and the Tauber trap (and its derivatives). The Burkard trap is a volumetric instrument; measured volumes of air are drawn in for known periods of time and the airborne palynomorphs adhere to a sticky surface. The relative abundance of palynomorph taxa in the airspora can then be compared from place to place for specified periods. The Tauber trap is a non-volumetric sedimentary sampler and relies on gravity to evaluate the palynomorph composition of the air. Again, fallout from the airspora can be compared from place to place. Details of these mechanisms, and their relative limitations and merits, can be obtained from various sources [39,40]. In essence, trapping efficiency depends on wind velocity, relative size and mass of particles, and size of trap aperture; there can be considerable bias in the results depending on the kind of trap employed. Furthermore, to allow comparability with other similar data
sets, traps must be placed in situations where there can be little or no impediment to free flow of air, or free fall of palynomorphs. It is vitally important to remember that, for reasons outlined below, these kinds of study have little relevance to forensic palynology.

2.2.1. Absolute counting techniques

Forensic data need to be expressed as percentages of the total pollen and spores counted in a sample. In most cases, it is impossible to obtain samples of known area, volume, or concentration from police exhibits, and the forensic palynologist simply has to retrieve as much of the pollen load as possible from the item. It would certainly be difficult to standardise a pollen sample from footwear, a fur coat, a vehicle foot well, or a pair of trousers. Therefore, since they demand samples of quantifiable size, so-called absolute counting techniques such as volumetric [41–43], weighing [44], and exotic marker [45–47] methods, and estimation of pollen influx, have had little or no value in forensic investigation.

2.2.2. Partial assemblages

Another reason for the limited value of the Monitoring Programme to forensic palynology is that the database is restricted to influx values for the major trees and ecologically significant shrubs and herbs; the aim of the Programme is to evaluate pollen deposition across transition zones between forests and open landscapes. In forensic palynology, every taxon at a crime scene has potential significance; wherever possible, the presence of each one (either as a growing plant or as a palynomorph in a comparator sample) must be noted. Furthermore, obtaining influx data from comparator samples at crime scenes, and other locations pertinent to the criminal investigation, would be both impossible and irrelevant. Rather than trying to estimate what might have fallen from the airspora in the period during and after the crime has been committed, it is important to know as comprehensively as possible the nature of the assemblage transferred to an offender’s belongings. This assemblage might have accumulated on a surface over many years.

3. Other factors affecting palynological heterogeneity in forensic samples

Many years of experience have shown that pollen and spore assemblages at ground level are exceedingly heterogeneous and unpredictable [48], especially in urban and domestic environments. This is a function of the contribution made from the pollen rain at any one time of year, but mostly of the many years of accumulation on the ground surface. The airspora will change from day to day and year to year. In most cases, that on soil, vegetation, and litter will yield a cumulative sample. This means that pollen loads in soil and on vegetation will exhibit less temporal variation than that observed in the pollen trap.

Experience has also shown that any area of ground will have a unique palynological signature because of the multiplicity of taphonomic factors affecting the persistence of the assemblage: (a) accumulation history, (b) potential for promoting or inhibiting decomposition of palynomorphs, (c) effects of precipitation, rain splash, and wind (d) action of animals, (e) effects of people and barriers, and (f) many other unknown taphonomic factors.

Obstacles will certainly affect palynological assemblages in samples taken from traps or the ground [49]. Stands of vegetation, walls, fences, or banks will distort airflow and bias the results obtained from comparator samples at crime scenes. Vegetation can also act as effective filters of pollen laden air, while palynomorphs can become impacted onto walls, tree trunks [50], and other obstacles. It has been shown that samples from a wall can reflect the local vegetation better than those taken from adjacent ground [51]. The effect of barriers on the palynological profile at any one site is unpredictable.

4. Persistence of palynomorphs in soil, sediments, and on vegetation

The persistence of pollen grains and spores on surfaces, or within soils and sediments, depends on ambient conditions and the robustness of the spore/pollen outer wall. This appears to depend on the relative amounts of sporopollenin in the outermost part of the pollen wall (the sexine). A number of studies have shown that some pollen taxa are more vulnerable to decomposition than others [52,53]. Some decompose so quickly that there is little likelihood of them being found even in comparator samples. These include the Juncaceae (rushes) and Orchidaceae (orchids). Others such as the spores of Lycopodium (clubmoss), Polypodium (polypody fern), Alnus (alder), and Tilia (lime) appear to be much more robust and resistant to decay; where conditions promote decomposition, they are often over-represented and biased profiles can be obtained. In recent years, frequent analysis of modern soils has demonstrated that observed effects do not always agree with published results. In forensic palynological study, an open mind must be kept at all times; it is imprudent to adhere strictly to the findings in published experimental work when interpreting forensic material. They must only be regarded as a general guide.

Where microbial activity is inhibited (for example by low water potential (pF), low redox potential (Eh), and extremes of pH), palynomorphs can persist for many thousands of years. Pollen and spores are preserved in sedimentary rocks, ancient sediments, lake and riverine sediments, peat and fen deposits, and palaeosols. Indeed, the resistance of pollen and spores to decay has allowed the reconstruction of prehistoric landscapes, and the palaeoecological literature is very extensive. In the last 20 years or so, a very large body of publication has also accrued from analysis of archaeological buried soils and features such as pits, ditches, and water holes. It has been possible to reconstruct not only a picture of ancient landscapes, but also past farming practices and diet [54–59], and even ancient shipwrecks [60]. Palynology has also provided information about prehistoric cadavers [61], and even indicated the use of medicines by Iron Age medical practitioners [62]. But, preservation of palynomorphs in the forensic context can be problematical.
In every case, good preservation is only possible when the physico-chemical environment within the matrix is inhibitory to micro-organisms, particularly to bacteria such as the Actinomycetes; these have the enzymes necessary for hydrolysis of highly recalcitrant molecules [63,64]. Although no sporopollenin has yet been identified, its presence in some of the Protista is certainly implied [65]. It would seem that conditions which are conducive to pollen decay are the ones in which Actinomycetes and other bacteria thrive.

In well-aerated, microbially-active soils, a whole season’s load of palynomorphs can disappear within 4 months (or possibly less time). For example, in Sussex, southern England, the soil of a fertilised, ploughed field under wheat yielded a rich assemblage of pollen in July; the pollen spectra reflected the immediately local vegetation very closely. In February the following year, further sampling, at the same locations within the field, showed that all palynomorphs had decomposed in the intervening period; not even corroded grains were observed in the preparations [66]. On the other hand, a soil profile, under Carpinus betulus (hornbeam) coppice in Hertfordshire, yielded such a rich, well-preserved palynological assemblage (from the surface to a depth of 50 cm) that detailed vegetation history of the site could be constructed [67]. Comparison of the soil profile’s pollen sequence with historical records indicated that the soil had preserved at least a 100 years of vegetation history at this crime scene. In contrast to the soil in the Sussex wheat field, that in the Hertfordshire hornbeam coppice was an acidic, nutrient-poor, tannin-rich compacted clay where microbial decomposition was inhibited. It was of considerable interest, therefore, that a subsequent experiment at the site, which involved digging a grave and enriching the soil with a pig carcass, created “islands” of intense bioactivity and pollen decomposition [68–70].

Whether palynomorphs will be present in soils can sometimes be deduced by the vegetation growing on them. If the plants are mostly nutrient-demanding and favour light, well-drained soils, the chance of good preservation is much less than if the plants indicate an acidic, water-logged, badly aerated conditions.

4.1. Residuality

It has been stressed that Burkard and Tauber traps are of little value in most criminal investigations where knowledge of the residuality of current and previous seasons’ palynomorphs on surfaces (including those of soil and vegetation) is more important. Soils, moss polsters, leaf litter, and surface foliage of growing plants, including grassy turf, are all useful for studying pollen assemblages at any one place [71,72]; they have the advantage of representing longer periods of pollen rain than traps. However, each kind of sample material will have inherent biases for both the range and abundance of palynomorph taxa [72].

Depending on the vulnerability or recalcitrance of the individual pollen taxa at the pollen site, more than one, and sometimes very many, years’ worth of pollen fallout can be retrieved from moss polsters. Leaf litter can give many months’ record, while that yielded by surface vegetation (such as hedge foliage or grassy turf) will depend on frequency of removal by mowing, cutting, or even grazing [73,74]. In this case, the palynological profile will represent the fallout between the periods of removal. This can be of considerable advantage when temporal aspects are relevant to an investigation. The abundance of pollen is usually much lower in such samples, but when taken between mowing, they can provide specific information for the period in question. If the cutting frequency is known, the pollen profile for that period can be measured [75]. However, the results obtained from young vegetative growth can be seasonally variable and, in much of Britain, particular care must be taken when formulating sampling strategies at crime scenes, especially in spring/early summer, and where wind-pollinated trees are present in the catchment. It has been found that pollen assemblages obtained from grass and other plant clippings can exhibit dramatic differences within 1–2 weeks, and that these effects can vary over relatively small distances [75]. This means that sampling delays may cause problems for comparing the palynological profiles from exhibits with those from crime scenes.

Even if a flowering plant has been contacted and one taxon is dominant in the palynological profile from an exhibit [76], other taxa will be picked up; and although there are exceptions, the offender rarely picks up only the current season’s pollen deposition. The persistence of palynomorphs deposited from the pollen rain will depend on their susceptibility to decomposition. However, if they land in environment where ambient conditions inhibit decay, they can remain in good state of preservation for many years, and be available for transfer to any object with which they come into contact.

When several or many seasons’ pollen deposition are represented in a sample, differential decomposition might be very obvious, and this can be useful for identifying pollen sources on exhibits [77,78]. However, where the ambient environment inhibits microbial growth, it is often difficult to differentiate between old and new pollen [79]. It must also be kept in mind that residual palynomorphs can be recirculated by eddying of turbulent air as well as by rain splash and other forms of physical disturbance. By their very nature, these effects are localised and unpredictable, and contribute to the heterogeneity of palynomorph assemblages encountered in surface samples.

It is important to stress that failure to find a palynomorph taxon in any sample does not necessarily mean that it is not present; but if a taxon is found (and there was no likelihood of contamination during laboratory preparation), it is obvious that it was a component of the sample. The failure to find a taxon might simply be a function of the numbers of pollen and spores counted. A count of 300 grains is generally considered acceptable and, after this number, the law of diminishing returns operates [80,81]. Nevertheless, higher counts will enhance the possibility of locating the rarer components of any assemblage. It is often necessary to achieve counts of several thousand palynomorphs per sample, especially if the *in situ* vegetation is swampy more pertinent, underlying signals.
5. Exhibits—some taphonomic considerations

It is possible that any object standing in the free air will pick up airborne or soil/vegetation-borne palynomorphs. The object might be a vehicle, article of clothing, footwear, rubbish bins, or bicycle—indeed any article that might be important to a criminal investigation. Any item can pick up pollen from a polleniferous surface; even things made of plastic, such as petrol cans and toys, retain palynomorphs for considerable (though unknown) periods [82]. Digging implements such as spades, shovels, and garden forks often yield information of clandestine graves, or demonstrate links between offenders and their victim’s dump site [22,26] even years after the event. Well-preserved pollen has been retrieved from spades more than 5 years after being used for criminal activity [78].

Unless an enquiry is intelligence-led by reliable witness testimony, or compelling independent evidence from, for example, foot or tyre marks, the actual surfaces contacted by an offender’s belongings at a crime scene can only be surmised. It is often the case that there is no knowledge of the offender’s approach path so that obtaining comparator samples has to be achieved by using “common sense” tactics. In such cases, those surfaces most likely to have been contacted by the offender are sampled. If a victim has been buried, then it is known without doubt that the offender must have contacted the grave and its immediate environs [16,22]. This, therefore, would be an important target site for sampling. In view of the high degree of heterogeneity in palynological assemblages at any place, identification of an offender’s approach path to a crime scene can be of pivotal importance for obtaining the most relevant comparator samples [82]. At best, such samples can only represent a very small part of any crime scene, so every feasible approach path should be systematically sampled for comparison with exhibits. It is often necessary to obtain large numbers of such samples, especially when there is little intelligence about the movements of the offender. But targeting of the most likely areas can reduce the cost and time involved in the investigation. These are always very important considerations by holders of police budgets.

The different kinds of object and exhibit that have been analysed over the years are so many and varied that further consideration must be given elsewhere. Only a small number of objects that are frequently important in criminal investigation will be discussed here.

5.1. Vehicles

Car owners in Britain are certainly aware of the heavy pollen coatings that appear on their vehicles when parked near or under flowering plants, particularly in late spring. But, in criminal investigations, the importance of such accumulation will depend on the length of time the pollen is retained. In reality, cars are often cleaned assiduously (particularly if an offender is “forensically aware”), and there is little doubt that it is easy to remove palynomorphs from car body work. Other parts are more retentive [83] and vehicles have proved to be important sources of palynological trace evidence; delicate sampling has yielded profiles that have contributed towards many convictions.

A famous case where palynological evidence from a car was critical was that of murder of Holly Wells and Jessica Chapman in the village of Soham [82], Cambridgeshire. Here, material taken from the chassis, spare wheel, and foot wells demonstrated a good match with the crime scene profile. In a sub-judice Metropolitan case, it has been possible to obtain a convincing match between mud from the murder scene and mud smears on the side walls of a car’s rear tyres [84]. The results were very surprising considering the age of the vehicle and the number of miles it had been driven. Even more remarkable results were obtained in a recent Hertfordshire murder case. Here the palynological profile from the offender’s car foot wells matched that of the victim’s woodland grave 6 months after the offence, after having been driven from England to Albania, and after having been cleaned several times [83].

Wheel arches have rarely been a useful source of forensic palynological evidence although a notable exception was in the case of search for Charlotte Pinkney [85] in Devonshire, in the west of England. Because of the long depositional history represented in many wheel arches, they are not usually the favoured parts of a car to be sampled. Recent developments in retrieval techniques are yielding more promising results from this part of motorised vehicles (personal observation). In the experience of the author, air filters have been of little use in the United Kingdom because of the small scale and high heterogeneity of landscapes.

5.2. Fabrics, clothing, and footwear

Work in numerous criminal cases has shown that pollen and spores can remain within the weave of dry fabric for many years. Fears of spontaneous oxidation appear to be unfounded when items are kept dry. If pollen is allowed to become damp, it is likely that microbial decomposition is responsible for its disappearance rather than progressive oxidation by the atmosphere. As yet there are no data on fabrics that have been kept damp, other than general observations of items becoming mouldy. This is because police are always requested to dry items as quickly as possible to prevent microfungal growth. Fungal spores cause problems by obscuring pollen grains on the prepared pollen slide, and also making it difficult to assess the fungal component in the original matrix.

The abundance of palynomorphs retrieved from clothing varies according to the retentive qualities of the fabric, and the assiduousness of cleaning after the event. A single attempt at cleaning generally fails to remove palynomorphs entirely. Rich assemblages, suitable for comparison with those obtained from crime scenes, have been retrieved from clothing even when they have been dry cleaned [86] or washed [87] immediately after the crime. Experience has shown that dressed cotton is the least retentive material while wool and a wide range of synthetic fabrics are much better. Nylon and synthetic fleece fabrics are particularly good at both at picking up pollen, and retaining it for long periods [75]. Some fabrics are such good traps of pollen and
spores that it is actually quite difficult to remove them to facilitate analysis. Certainly, out of very many cases, only one has demonstrated secondary transfer from one fabric to another and, in this case, the original garment was heavily laden with palynomorphs, and there was firm and prolonged contact between the garment and the fabric car seat cover which received the pollen [75].

The popularity of training shoes with highly structured soles has facilitated the use of forensic palynology in criminal investigations. It is very difficult indeed to remove palynomorphs completely from such footwear, even when it has been put through the washing machine (as is common practice in Britain). Length of retention is very variable, even between training shoe types and styles. However, in one case, a pair of men’s Gucci fine leather shoes yielded the profile of a burial site after being worn by the offender continuously while on remand for 4 months, and having been cleaned several times [79]. In another case against a multiple rapist [75], it was possible to differentiate two distinct crime scenes from a single pair of training shoes. In yet another criminal investigation, evidence of a specific British mixed, deciduous woodland was retrieved from the offender’s shoes many months after he had given them to his sister in Albania. She had worn them for working in the fields, but the mixed palynological profile that was obtained from them clearly reflected both the Albanian and British soils [83].

It must be remembered that the assemblage obtained from the sole of a shoe (or any other object) will never match any crime scene perfectly. By its very nature, footwear may pick up palynomorphs from more than one place. However, experience has shown that unless the offender walks on soil, vegetation, or on organic debris, shoes tend to pick up small (often insignificant) amounts of pollen [16] during general wear.

Footwear is such an interesting and valuable resource in forensic palynology that it warrants further consideration elsewhere.

6. Final comments

At the beginning of this paper, the areas where palynology has been of help in criminal investigation were listed. No detailed information on how to carry out these investigations has been given but some of the problems that the forensic palynologist must keep in mind at all times have been outlined. Forensic palynology has become a well-established part of criminal investigation in the United Kingdom and the discipline has been boosted by the support and enthusiasm of the British Police. Every case brings novelty—problems not previously encountered, and questions not previously posed. The future looks bright for the discipline in the United Kingdom and it is hoped that information presented here will help palynologists in other parts of the world to be brave enough to apply their skills to criminal investigation.

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