Forensic palynology has been a law enforcement tool for over 50 years. Forensic palynology is the application of pollen and spores in solving legal issues, either civil or criminal. Pollen and spores can be obtained from an extremely wide range of items, including bodies. Pollen and spores provide clues as to the source of the items and the characteristics of the environments from which the material on them is sourced. Their usefulness lies in a combination of their abundance, dispersal mechanisms, resistance to mechanical and chemical destruction, microscopic size, and morphology. Their often complex morphology allows identification to an individual parent plant taxon that can be related to a specific ecological habitat or a specific scene. Pollen and spore assemblages characterise different environments and scenes and can easily be picked up and transported away from scenes of interest without providing any visual clue to a suspect as to what has occurred.

With so many publications and high-profile cases involving forensic palynology and environmental analysis now receiving publicity, the future of this branch of forensic science is assured. Furthermore, with the development of multi-disciplinary approaches to environmental analyses of crime scenes, far more detailed information is now available to law enforcement agencies, enabling them to determine with greater accuracy what may have happened during the commission of criminal activities.

1. Introduction

Forensic palynology is the study of modern and fossil spores, pollen and other acid resistant micro-plant remains in a legal context. It is not a new science; as a forensic tool, it has been sparingly used since at least the 1950s [1–3] and probably well before that, but without documentation or publicity. More recently, forensic palynology has been used regularly as evidence in criminal trials in Australia [4], New Zealand [5], and the United Kingdom [6], in particular, and probably far more sparingly elsewhere [7–9]. Not only are modern pollen used, but in a number of cases, the presence of fossil pollen in association with a criminal action has also been useful in determining what happened and where it occurred [1,7].

Over the last decade or so, a number of papers on various aspects of forensic palynology have been published. Many have given advice on the collection of forensic palynological samples [5,9–13], on how to interpret variations in pollen distribution in soil [14–17], on how various materials, including clothing, retain pollen [18–20], on assessing the value of the palynological evidence [21,22], and even on determining when the murder occurred [8]. A number of case histories have now been published [23–25] demonstrating the various ways in which forensic palynology can be used towards establishing the truth behind a criminal or civil action, only some of which are given in the references. However, it is our hope that researchers wishing to follow up on forensic palynology will be able to access additional literature by reference to those papers listed, and to take care in who they follow on internet sites now appearing in increasing frequency.

These published case histories demonstrate the use of forensic palynology in cases as broad as forgery, rape, homicide, genocide, terrorism, drug dealing, assault and robbery, arson, hit and run, counterfeiting, and illegal importation, as well as civil cases involving geopreservation, illegal fishing, and pollution [26]. In general, palynology can be used to [5,13]:

- relate a suspect to the scene of a crime or discovery scene,
- relate an item left at the crime scene or discovery scene to a suspect,
- relate an item at the discovery scene to the crime scene,
- prove or disprove alibis,
- narrow down a list of suspects,
- determine the travel history of items, including drugs [27],
- provide information as to the environment that an item has come from,
- provide information as to the geographic source of items,
- aid police in their lines of inquiry,
- help locate clandestine graves and human remains [8,28],
- help determine the peri-mortem fate of a victim [29], and
- help to determine the deposition period of human remains.
The complexity of forensic palynology and the associated necessity of needing to train in all aspects of environmental analysis at the trace level means that skilled practitioners are needed and these people are few and far between. In New Zealand and the United Kingdom forensic palynology is an accepted technique, but still it is not always being used as routinely as it should be nor in as timely a fashion. It has yet to be fully accepted elsewhere.

The protocols followed in different countries differ dramatically. In Great Britain, the palynologist always collects comparator (control) and evidential samples, visits the crime and associated scenes and undertakes vegetation surveys, and collects vegetation samples. In most, if not all, other countries the samples are more often collected by generalist crime scene examiners such as Scene of Crime Officers (SOCOs) or other law enforcement agents and sent to the palynologist. Vegetation surveys may not be undertaken and the palynologist may never visit the crime scene at all or may visit the scene just before appearing in court. The ideal situation is that occurring in Great Britain. For the palynologist to be able to fully contribute to the development of possible crime scene scenarios, he or she must be deeply involved at the start of the investigation and collect relevant samples as soon as the crime has been discovered. Many of the comments in this introduction are aimed at those law enforcement agencies who do not take advantage of the skilled palynologists available to help them determine what has occurred at crime scenes.

2. Why use spores and pollen?

Following usual convention, when the term pollen is used it also includes spores from ferns and fern allies. Pollen carries the male sex cells of higher cone- and flower-bearing plants from one plant to another of the same species. Spores are the sexual or asexual propagules and reproductive bodies of the lower plants and plant allies, including algae, ferns, fungi, mosses, liverworts, and lichens. Most spores are very small, conservative in morphology, go unnoticed and are difficult to identify and attribute to a specific taxon.

The usefulness of pollen as a forensic tool results from their small size, their resistance to mechanical, biological, and chemical degradation allowing them to be preserved on and within a variety of media, their abundance in the environment, and in their morphology which allows, within limits, identification to specific plant taxa.

The forensic palynologist utilises Locard’s Exchange Principle that states whenever two objects come together there is always a transfer of material. It is clear that Locard regarded the exchange of physical evidence to include footprints, and consequently the effects on the plants underfoot, as well as material exchange of mainly dust sized particles [30]. Thus, not only can one demonstrate a connection between two sources of physical evidence but one can also deduce what type of action led to the exchange. Pollen provides one source of commonly transferred material, which often is exchanged within the context of a transfer of soil, mud, and dust particles. However, pollen can also be transferred by direct contact with a part of a plant containing spores or pollen.

2.1. Size

Most wind-dispersed pollen grains fall within a short distance of the parent plant despite their very small size. Their small size, usually between about 20 and 60 μm (some are as small as 7 μm and others are as large as 200 μm) means that often they can be picked up from a scene of interest in clothing, hair, under fingernails, and on other items and transported away from the scene without any visible sign that this has happened. The forensic palynologist is often asked to determine where an item or suspect has been, what they have been in contact with, or whether a particular scene has pollen that characterises that place. They may subsequently want to know if pollen from a point of interest can also be recovered from the clothing or other items directly associated with a suspect. In many cases, there is more than one location involved. For example, a murder may take place at one scene (crime scene), the body taken elsewhere (discovery scene), and the murder weapon disposed of at a third scene, while clothing or other items can be disposed of at other places.

While pollen size is useful in broad terms, it cannot be used as a means of identification. Pollen size can vary within a single anther let alone between species, so over-reliance on size criteria might be imprudent for identification. Many studies confirm that pollen size can also vary with the processing techniques used to extract the pollen from the items under investigation or even the type of mounting media used.

Other morphological features are more useful for identification. These include shape (spherical, ovoid, triangular, etc.); sculpture patterns on the surface of the grains (granules, spines, papillae, reticulation, etc.); aperture type and number (pores, colpi, or both; inaperturate, 1- , 2-, 3-, 4-apertured, or multi-apertured); wall structure (columellae, tectate, perforate, etc.); wall thickness, and variations in thickness around the pollen grain [1,31–34]. Most pollen examinations and identifications will be undertaken using a transmitting light microscope but additional details of the surface sculpture can be obtained, if required, by examination of specific pollen grains using a scanning electron microscope (SEM). However, in most routine criminal cases, SEM is of limited value since whole assemblages of pollen must be identified in mixtures obtained from exhibits.

Forensic palynologists need more than a microscope to identify pollen. They need access to good pollen reference collections, not only of their native taxa but also of other species from all around the world. Preliminary identifications may be made by reference to illustrations (photographs usually, since drawings are too risky to use as they are an individual’s interpretation of how a pollen grain appears) in pollen atlases, journal articles, and books or book chapters. Illustrations should only be used as a guide to the appropriate part of the spore/pollen herbarium to look for reference material to compare with the unknown pollen. No one accessible reference collection is totally comprehensive. Many countries have their
own published pollen reference atlases and books that are useful for comparison, but none are as good as having access to original material. For example, comprehensive or selected published pollen references for Argentina, [35], Chile [36], China [37,38], Europe [39], New Zealand [40,41], Philippines [42], and Taiwan [43,44] exist. The above is only a partial list of available sources. In addition, there is also a gradually increasing number of pollen reference sites posted on the internet that provide good illustrations of key taxa. Although illustrations are useful, the palynologist must obtain reference material for any unknown taxon encountered in a criminal case. In this instance, it is often necessary to obtain material from herbaria or horticultural societies. A picture is never as good as the actual pollen grain, and the seriousness of forensic investigation demands rigour in analysis, so it is important to stress that no-one should give expert evidence having identified pollen from only illustrations.

2.2. Preservation

Pollen are highly resistant to chemical attack even though the innermost cell wall surrounding the protoplasm decays relatively quickly, usually within a few months. The cell itself can lose all viability even more quickly; wheat has been shown to lose viability within a few minutes while rye lives for only about 15 min. However, the outer cell wall (exine) contains a number of different compounds including sporopollenin (a large, randomly, cross-linked molecule containing a carbon–hydrogen–oxygen ratio of 4:6:1, lacking a repetitive large-scale molecular structure that would allow enzymatic and chemical agents to dissolve and reduce it to small atomic components [45]). Differences in the specific molecular structure within the exine of a pollen grain will determine which types will be more or less susceptible to various types of chemical deterioration. Although the exine is composed mostly of cellulose, various types of proteins, and lipids, pollen walls have interlocking coils and strands of the highly durable material called sporopollenin [46]. On rare occasions pollen can be preserved in a very dry state where the protoplasm is preserved as a tight dried-out ball retained within the pollen grain.

Pollen evidence can remain at a scene for many years after the event under investigation occurred. For example, in New Zealand, a hydroponics Cannabis operation shifted from farm to farm every few years to avoid detection and to avoid authorities noticing a continuous high electricity usage. Pollen analysis of ropes and surfaces of rafter beams showed that cannabis had either been stored or grown in these buildings even though it was 5–6 years after the suspects had left the premises. In this case, several male plants were allowed to flower at each location providing pollen which spread throughout the buildings used. No Cannabis pollen was found in appropriate sites outside the buildings. Pollen analysis did not help in dating when the buildings were last in use, nor did it help in identifying the suspects, but it confirmed lines of inquiry. Identifying the suspects and associating them with the building was done by other means. In another forensic situation in New Zealand, fern spores were found well-preserved in soil samples from a sports field 30 years after the ferns had been eliminated from the area. In a murder case in Wales, United Kingdom, pollen from Juglans (walnut) persisted in a soil for nearly 80 years after the tree had been cut down. This pollen was transferred to the offenders and was important in linking them to the crime scene. It was particularly significant because the tree is absent from that part of the country today, and was the only recorded specimen in the area 80 years before. Walnut pollen therefore provided a highly specific marker for that one place in that part of Wales today.

This persistence of pollen at scenes means that the forensic palynologist is usually dealing with a pollen rain covering many years. For example, shoes worn while walking over damp ground or bodies involved in a struggle on dry, dusty ground, do not just pick up the current season’s pollen, but can also capture pollen deposited over many years. This means that there is no direct relationship between the airborne pollen of a particular season and material picked up during the commission of a crime. However, from the time an item or body comes to rest, airborne pollen can settle on them, but these grains are usually rare relative to those picked up from direct contact, especially with the ground. From the standpoint of helping a jury understand what value the palynological evidence might be, it is unfortunate that often the data presented by a forensic palynologist is challenged by evidence presented by aeropalynologists who lack experience in forensic palynology and are often embarrassed by the lack of relevance their pollen evidence has to the issue at hand. This is not to say that forensic pollen evidence should not be challenged, but that the challenge should come from someone with appropriate experience. That experience will come as forensic palynology becomes more widely accepted and organisations equip themselves to undertake such research and servicing roles.

2.3. Abundance and dispersal

Pollen are distributed by wind, water, or some animal vectors. Plants that produce wind-dispersed pollen (anemophilous plants) often produce vast numbers of pollen grains. For example, a single male pine cone can produce anything between 100,000 and 1.5 million grains, a single cannabis flower can produce between 60,000 and 80,000 pollen grains, and a single tassel of alder can produce 4–6 million grains. Spruce trees produce so much pollen that in Southern and Central Sweden it has been estimated that 68,000 metric tonnes of pollen is produced each year [47,48]. These wind dispersed pollen grains are useful forensically in that they are generally aerodynamic, relatively simple in morphology with thin walls, preserve easily, and are found on most surfaces well after the end of the flowering season. About 95% of all pollen grains produced by anemophilous plants will fall within 2 km of the parent plant and in many cases within 100 m of the dispersal source [49,50]. However, great care must be taken in applying palaeoecological sinking speed models to all forensic scenes as experimental transect evidence shows that pine can fall from 80% at source to 4.0% within 50 m. Residuality, redeposition, local disturbance, etc., are other issues that must be considered.
All these can be much more powerful taphonomic factors than sinking speeds.

Rarer grains can be dispersed by wind hundreds to even thousands of km away and form part of the background pollen rain of a region. For example, Ambrosia pollen originating from the eastern USA has been found in deposits in western Scotland [51] and up to 10% of the pollen on Chatham Island originates from New Zealand over 700 km away [52].

Overall, a large number of factors determine just how far an individual pollen grain will travel including:

- height of release,
- the release mechanism,
- strength of wind and the presence of updrafts or downdrafts,
- weight, shape, and aerodynamics of the grain,
- atmospheric conditions (humid, wet, dry, hot, and cold), and
- obstructions between the source plant and its surroundings.

These, and other factors, will determine the “sinking speed” of each pollen grain. This sinking speed can vary between 2 and 3 cm/s for tiny pollen such as alder (Alnus) to an average of 80 cm/s for large pollen grains such as fir (Abies) and milkweed (Asclepias) [49].

Plants that disperse their pollen by water (hydrogamous1 plants) also produce large numbers of grains that are relatively small, unornamented, and do not preserve well because their exines are made of cellulose and lack the durable sporopollenin (e.g. Utricularia). A similar situation is seen in some land plants such as Juncus. These pollen types are not very useful forensically but when they are recovered and identified they can be very important in determining the nature of a wetland environment. Since these pollen types are generally oxidized during the laboratory procedures needed to recover most pollen, it is important for the forensic palynologist to visit crime scenes and collect their own samples. In that way they will know whether to search for these delicate types of pollen and how to optimise the extraction procedures to ensure recovery of all important pollen types from the samples.

Plants that use an animal vector to disperse their pollen (zoogamous2 plants) produce relatively few pollen grains which are usually large, sticky, highly ornamented, thick-walled, and readily preservable. They are useful forensically in that they are usually found very close to dispersal source areas and are unlikely to be a contaminant in any forensic sample. Animal vectors include bees, ants, bats, beetles, birds, butterflies, flies, small marsupials, monkeys, moths, mosquitoes, rodents, wasps, etc.

There are two final categories of pollen—autogamous and cleistogamous types. Autogamous plants have an open self-pollinating system where the pollen producing anthers and the fertile receptive stigma develop at the same time and the plant uses various means to get the pollen the short distance from the anther to the stigma, including using wind or animals. Not many pollen grains are needed for this effective type of fertilization procedure so not many are produced. Therefore, these pollen types are rarely found in forensic samples, but if found indicate the close proximity of the parent plant (e.g. tomatoes).

Cleistogamous plants have an even more efficient reproductive strategy than autogamous plants. They have a closed self-pollination system in which the plants retain the pollen produced within an unopened flower. The number of grains produced is very limited and they have no evolutionary need for ornamentation. These grains can be important because many cereal grasses have this type of dispersal mechanism. Nevertheless, even these plants can lose some pollen grains from the flower, such as by attaching themselves to people brushing passed them. In a murder case in England, wheat pollen was shown to have been transported to a body deposition site more than 100 m from the source and through a hedge.

2.4. Sample collection

Detailed information on how to collect and store comparator (control) and evidential samples is given in a number of publications [9–15]. The collection of comparator (control) samples depends greatly on the nature of the case under investigation and often the control collection techniques used at one scene would not be appropriate at another scene. The most useful way to collect such samples depends on the needs of the scene in question and the experience of the collector. If possible, scene samples will be taken by an experienced forensic palynologist.

All law enforcement agents should be aware of the usefulness of forensic botany and in particular forensic palynology. Ideally, the forensic palynologist should be called to the crime scene(s) as soon as possible before any potential contamination or inadvertent destruction of the scene takes place. This means that the palynologist should be called to visit the crime scene very early so appropriate control samples can be collected from the scene, even if no useful evidential material is subsequently obtained. In some countries, it is not practical for the forensic palynologist to be called in and this type of collection is done by the police Scene of Crime Officer (SOCO) or scene-attending forensic scientists. However, the collection of forensic palynology and associated vegetation samples, site surveys, and subsequent sample storage, should preferably be done by an experienced palynologist to reduce the likelihood of any sampling problems, to stop continued biological activity (microbial destruction) in the samples, and to avoid continual transfer of evidential and control materials. Every crime scene is different and the palynologist needs to consult the investigating team as to an appropriate sampling strategy. Financial budgets are a great issue, and much unnecessary expense can be incurred by inappropriate sampling. Furthermore, when untrained staff attempts to obtain samples, they often miss critical microsites or, indeed, the most important ones. Comprehensive site surveys and photos should always be undertaken to enhance the interpretation of the pollen

1 Strictly the term should be hydrophilous; hydrogamous would mean pertaining to the sexual reproduction of water.

2 Strictly the term should be zoophilous; zoogamous means pertaining to the sexual reproduction of animals.
samples, explain how samples were collected, and to rationalise the processing techniques to be used in extracting pollen from the samples.

Unfortunately, too often control samples have been collected by untrained personnel and thus even before processing, are clearly different from the evidential material. While on occasions this is because the evidential material did not come from the crime scene, on too many occasions subsequent investigations have determined that the Scene of Crime Officers thought that “any material” from the ground would satisfy as a control, or that a sample collected anywhere near the scene would do, or that even the depth they dug into the ground for a sample was irrelevant. In Great Britain, this situation is much improved but it still requires action in some other countries. Care should be taken that the samples are collected with clean implements, placed into sterile containers that can be tightly sealed, and that sampling implements are thoroughly cleaned after each sample is taken or preferably use new ones each time. If samples are collected by hand then sterile gloves should be worn and changed after each sample is gathered, if only to avoid any possible challenge in court as this is unnecessary if sampling a single place.

The palynologist needs to know all the details of the collection strategy if Scene of Crime Officers take the evidential and control samples rather than call in the palynologist. Great care must be taken at all times to avoid any contamination during or after samples are collected. Likewise all samples should be well-labelled and sealed in sterile, zip-lock plastic bags, or screw-top plastic (in preference to glass) containers. Samples should not be stored in cloth or paper bags, which may already contain ambient pollen. However, one must put vegetation samples into new paper envelopes and dry them in drying ovens, if necessary, as microfungi proliferate in storage if there is any dampness. The standard protocols of detailed documentation of the movement (sample history) of all samples must be undertaken to avoid any suggestion of possible contamination and to establish continuity of possession at all times. The packaging for each exhibit (or preferably the exhibit itself) should be initialled by the examiner and identified with a unique laboratory number. A record of the continuity of possession should be kept for every exhibit. This record should be signed, countersigned, dated and perhaps the time recorded every time it is handed to a new person. It is imperative that this documentation should include the date and time a sample bag has been opened and under what conditions. This is especially important for evidential material such as clothing that may be investigated thoroughly under conditions where pollen in the air could get onto the clothing before any pollen samples are taken. In Great Britain, everything happens in strictly controlled and inspected laboratories and continuity of possession is very strictly monitored.

Because no obvious staining occurs on clothes or items under investigation, it does not mean that pollen from the crime scene have not been picked up. Often, excellent sources of evidence have been missed because no indication of the existence of trace pollen evidence could be seen by the naked eye. Thus, it is possible for the clothes of new arrivals into a country to carry pollen from the place they originated from. Determining this origin could be very important in view of the present climate of international terrorism and with the appearance of so many people on international borders without passports, claiming refugee status. With fire occasionally being used to destroy evidence of a murder and an increase in terrorism even fragments of clothing from a burnt murder victim and suicide bombers might contain pollen evidence as to the person’s origin.

Why there is a need to collect comparator (control) samples needs to be understood. Control samples collected at random often do not answer the types of questions likely to be asked in court such as, for example, the size of the source area that the evidential samples may represent. Control samples should be collected to encompass the potential area represented by evidential samples and to eliminate other areas that could not be represented by the evidential samples. Control samples may be skewed by an over abundance of one pollen type because the sample was collected too close to a plant or the sample may include the anther from a plant. Often dandelion pollen occurs abundantly in one sample but is relatively rare in others from a compact scene. This possibility and problem can be reduced by using pinch samples from a number of locations around a scene [53]. The purpose of pinch samples (small samples of surface soil collected at different locations within a small area and then combined into one sample) is to get a composite image of the background pollen rain over a relatively homogenous area from which any one evidential sample may have originated. Alternatively, surface samples (top 1.0 mm) over about 900 cm$^2$ ($30\times30$ cm) surface area can be taken. These are taken in a targeted fashion and this means that the palynologist requires information from the investigators regarding the alleged circumstances of the crime. Each sample is then homogenised and an aliquot taken. This should represent the average pollen profile for that area and a composite picture developed that is much more comprehensive than taking pinch samples.

It is not possible to suggest a set number of control samples required at any scene because the needs will vary from case to case. The only way to determine how many need to be taken at a crime scene is to try and predict what types of questions are likely to be asked by the prosecution and defence and which sampling locations might support or refute the presence of the defendant or other items related to the case at the crime scene. It is important to take sufficient control samples at a given location to work out the variation in the pollen rain over an area and to be able to show the evidential material does, or does not, fall within the range of variation covered by the control samples. In summary, it is always better to collect too many than not enough control samples. One does not always need to examine every control sample, but it is comforting to know all the specific control samples were collected, if needed.

In Great Britain, all crime scenes relevant to individual cases are visited by the forensic palynologist, but this is not the situation in most other countries. If a palynologist or botanist cannot visit the crime scenes, then control samples should also
include samples of the local vegetation, especially cones, flowers, seeds, fruit, and leaves, if possible still attached to a branch, so the palynologist can determine what plants dominate the scene and estimate their significance. When sampling a crime scene ample photographs should be taken not only of the actual crime scene but also of each control sample location and of the plants in the local vegetation assemblages. This is important because from our experience plant identifications conducted by police or local gardeners are rarely accurate, and failing to find the pollen of an incorrectly named plant, the flowers of which an offender was known to have been in direct contact with, can cause credibility problems for the forensic palynologist in court. Plant samples collected at a crime scene should not be packaged in plastic bags because potential moisture can encourage microbial decomposition. In a recent homicide case, three magnolia flower petals (the trinity) placed on the body of a murdered woman were sent in a plastic bag to a palynologist for formal identification; however, they had become a brown mush when finally received. Even so, some magnolia pollen was still identified from the mush. Since most crime scenes are thoroughly photographed, often photos include some of the plants, particularly at outdoor scenes. Nevertheless, unless emphasis in given beforehand to ensure ample photos of “all” local plants and vegetation, and a 360° panoramic view of the geography of the scene or scenes, the resulting photo record is often partial rather than thorough. When photographs are available, they should always be made available to the palynologists and all scenes requiring the forensic palynologist should be adequately photographed.

So, what types of evidential material should one collect for pollen? The answer is “almost anything.” Nearly everything contains pollen or has pollen attached to it. There are some exceptions such as some highly processed illegal drugs, but even these, if dispersed and cut at different sites, can produce a pollen signature from these sites [27]. Some unusual materials that one would not suspect to contain pollen, but actually do in many cases, include enamel painted wood, counterfeit banknotes, condoms, painted works of art, cow pats (from the road and on a car used in a hit and run), birds cooked in bread, grease on guns, lungs, real and fake medicinal tablets, stomachs, wood shavings in food, antique furniture, stuffed animals, forgeries of antique furniture, and dusty foot impressions.

Multiple evidential samples often need to be taken. The mapping of pollen types found on clothing can often lead to additional clues and evidence. For example, someone walking through a field of corn or maize brushing against flower heads might have far more pollen around and just below the shoulders than around the legs. Someone hiding under trees may have more pollen attached to headwear and shoulders than the rest of the body; a person leaning against some surface would carry away pollen from that surface on only one part of his/her shirt or jacket; kneeling on the ground forces pollen into the mid areas of trousers; and walking through low-lying herbs and bushes will cause pollen to be concentrated on the legs, possibly on one side of the leg only, cuffs, and shoes. Multiple pollen samples from cars in forensic cases could provide evidence of more than one crime scene or location. For example, in one case in New Zealand pollen evidence proved that a car was at another crime scene that Police did not know about, as well as showing where the car was usually parked, and even its orientation in the parking area. In a recent murder case in Great Britain, foot pedals provided a similar palynological profile to the spoiler on a car, but there were sufficient differences for the palynologist to give an accurate description of where the car had been driven, and where the offender had walked in relation to the car. Footwear is another good source for multiple sampling to indicate more than one scene.

One problem with the collection of evidential samples is trying to determine which forensic recovery techniques are likely to be the most important (i.e. soil analysis, fibres, trace elements, DNA, etc.) to undertake on the sample and their relative priorities. Palynology is often not the only technique that is likely to be used and when other techniques are involved, then palynology will almost always be last in the sequence. This is because the pollen extraction techniques are usually destructive. Soil studies of a sample may include clay and heavy mineral analysis while other types of studies might examine hairs and other fibres, various chemical signatures, insect remains, and even soil DNA. It is our belief that palynology and mineralogy are the most important aspects of soil analyses especially with the use of QEMSCAN, a scanning electron microscope-based automated mineral analysis system [54]. The advantage of QEMSCAN is that it produces rapid and statistically reliable repeatable data from most soil samples. What can also be important are diatom and ostracod analyses. If palynology is to be used, then all other tests should be carried out in an environment that permits the final use of palynology without possible contamination or compromising of the samples. This can usually be solved by the provision of aliquots and working in a co-ordinated way with all investigators.

Thus, the size of the collected samples needs to be considered carefully. If palynology is to be the only test, then very small samples need to be taken, often only 1 g or so is sufficient and that is quite a lot. There is often no control over the size of the evidential samples, which may vary from a few hundredths of a gram to slightly larger amounts. Therefore, control samples could be taken to reflect the evidential samples and the environmental range of their possible source or sources. The size of samples will vary greatly depending on the nature of the samples concerned and the likely abundance of pollen. We need to know as much as possible about the palynological profile of a pertinent site so that we know what to expect. We do not try to match volume of sample. That would be pointless.

We also want to stress that searches for forensic pollen need not be restricted to crime activity undertaken outdoors. Several unpublished incidences have occurred in New Zealand where transfer of pollen from indoor flowers has provided evidence as to whether the room was entered by a suspect. Likewise, we recognize that the lack of pollen on a suspect does not prove he/she did not enter a room because the suspect may not have picked up any pollen; nor does the presence of specific pollen of interest on a suspect necessarily prove beyond doubt that he/she was in a specific room, unless those pollen types are particularly unusual, or not normally expected in the local environment.
Brightly coloured fresh flowers often found in vases in houses are generally insect-pollinated. As such, those flowers do not produce much pollen, but in a confined space with ample airflow, some pollen might be dispersed well enough to provide evidence. Some brightly coloured flowers used for decoration are wind-pollinated types and thus do produce pollen that will often disperse into a room.

Storage of palynological samples prior to submission to the palynologist should be undertaken with great care. Some alcohol or phenol could be added to samples after collection to eliminate any microbial activity, but this could compromise the samples especially if soil DNA analysis is required. Preferably everything should be frozen or dried. Dry samples should be stored in a secure, evidence storage room, but damp samples must be dried, kept in a refrigerator or frozen to eliminate and prevent bacterial, fungal and other biological activity that could adversely affect the pollen. Any damp clothes are automatically dried in special, sterile drying ovens. Damp soils are frozen, and damp vegetation is put into paper bags and dried at room temperature. Freezing does not destroy pollen and research has shown that repeated freezing and thawing will also not adversely affect them either [55,56]. In this way, samples can be kept almost indefinitely and certainly within the time frame of any case coming to court.

Plant leaves, flowers, and other plant material need to be kept in a botanical field press between dry sheets of clean newspapers, which should be frequently changed. These field presses, with the pressed plants inside, should be stored in a drying room with low humidity to permit the plant materials to dry. Since it is unlikely that Police would have access to field presses they could use the inside of a new newspaper, which is sterile, and put heavy weights on top. Once dried, the plant parts can be used for identification, and those materials can also be sub-sampled in the future, for DNA, plant fibres, cuticle, phytoliths, or other such materials.

Once the pollen evidence has been collected, processed, analysed, and interpreted, it should then be presented in court in a format that can be understood by juries so that those individuals can assess the value of the evidence. One way of preparing pollen data in a suitable format to present to a jury has been assessed [21,22] using the (Bayesian approach) Likelihood Ratio. The Likelihood Ratio looks at the quality of the correspondence between evidential and control samples and includes an assessment of how common the pollen assemblage from the evidential material is. Given the character of palynological evidence a perfect correspondence is rarely attained, so one has to look at the quality of the pollen correspondence (i.e. how many pollen types correspond?; are the percentages approximately the same?; and if not why not?) and how common the correspondence may be (e.g. what geographic area would that pollen assemblage represent?). This ratio of probabilities is usually expressed in court using suitable phrases to express the significance of the evidence. In terms of the contention that the evidence supports the association of the suspect with the crime the evidence may:

- weakly support,
- support,
- strongly support,
- very strongly support, or
- conclusively prove,

the contention that the suspect, or items closely associated with the suspect, were associated with the crime scene. Equally the evidence may not support the contention that the suspect was connected with the crime scene or else the evidence may be inconclusive.

The use of the Likelihood Ratio should be undertaken with a high degree of caution because it assumes a degree of randomness in the collection and dispersal of pollen grains which does not usually happen in nature. Those control samples, where there is an overwhelming dominance of one specific pollen type, for example, dandelion (Taraxacum) or plantain (Plantago), may be eliminated as being not comparable with any other. However, the dominance may have a logical environmental explanation which is not covered by a Likelihood Ratio assessment.

2.5. Other useful organic materials

Pollen are not the only potential acid-resistant palynomorphs recovered from evidential and control samples. On occasions, by eliminating various stages in the normal laboratory extraction technique, it is possible to recover a wide range of other types of materials including: fungi (e.g. Microthyriaceae, Tuberaeaceae), liverworts (Ricciaceae), hornworts (Anthocerotales), mosses such as Sphagnum, leaf hairs, plant cuticles, phytoliths (siliceous crystals produced by many plants, especially grasses), various types of specialised plant cells (tracheids, fibres, vessels), resins, diatoms, algae (including marine and freshwater acritarchs, dinoflagellates, solitary cysts of algae [Debarya, Mougeotia, Closterium (desmid), Spirogyra, Zygnemataceae, etc.], and colonial coenobia of green algae like Botryococcus and Pediasstrum), charcoal and fly ash, chitin, other types of animal remains (including insects, foraminifera, and shell [mollusc and egg] fragments), particles of rubber and foam, finger-nails, and other types of material as well. The “ghosts” of Foraminifera are also often seen and identified. Attempts to recover all of these other types of materials should be considered as on occasions they could also prove important in determining the geographical environment and source. Limited processing in the recovery of pollen can also assist in the determination of a broad time frame by study of the nature of the cell contents remaining in the individual pollen or spore, but cell contents can remain in pollen grains after being stored under ideal conditions for over 20 years.

3. A multidisciplinary approach

We have stressed the need for considering forensic palynology in many of the crime scenes that are investigated. It is equally important that, wherever possible, forensic palynology should not be used in isolation and that a multidisciplinary approach always be undertaken with analyses of the full range of botanical, zoological, and inorganic evidence available. Essentially this means that a full environ-
mental analysis of the various crime scenes, and items and people associated with those scenes should be undertaken, and such studies should not be limited only to aspects of botany or geoscience [57,58]. Typically, environmental profiling might involve:

- palynology, including spores, pollen, algal, and fungal remains, and many non-palynological remains that are often associated in samples such as leaf trichomes, copepods, insects, egg cases, cuticles, etc.,
- all other aspects of botany including plant identification and anatomy, diatoms, seeds, leaves, conducting elements, full vegetation analysis, phytoliths, leaves, cuticles, cones and cone scales, galls,
- analysis of charcoal, wood fragments, fly ash, and/or micro-charcoal,
- analysis of insect and other aquatic and terrestrial zoological remains, including bacterial DNA, animals,
- geological analysis including mineralogy, petrology, analysis of brick, clays, concrete, microfossils, micro-stratigraphy,
- geochemical analysis, including stable isotope analysis,
- soil analysis, including soil chemistry and the identification of clays, polish and rounding of soil particles, soil mapping, organic content,
- geophysical analysis including resistivity, ground penetrating radar,
- age analysis including radiocarbon dating, OSL, isotopic analysis, plant recovery times, insect developmental times,
- archaeology,
- osteology and forensic anthropology with respect to the identification and reconstruction of human remains, and
- comprehensive site analyses.

Not all of these analyses can, or even need to be undertaken at each crime scene, however, when used in combination they can provide potent tools in determining the nature and details of the events that may have happened at crime scenes. Clearly, the best way to accomplish such a comprehensive research and investigative team is for federal, provincial, state, or local agencies in each country to create special Forensic Ecology Units within their individual forensic organisations.

While many of the papers in this special issue on forensic pollen studies mention other types of forensic techniques, what is urgently needed, and what needs to be done to convince others of the importance of these types of combined studies, is a series of multi-disciplinary research projects based on actual and blind cases. Rawlins et al. [59] provide one such example where soil-derived materials found on items, including clothing, footwear and cars, provided very useful evidential information. They developed a blind test case, using a combination of mineralogy, environmental particles, palynology, and structural characteristics of organic matter at the molecular level to accumulate information on the source of soil samples from three different sites. In two of the cases, the information gathered was most useful while at the other site some discrepancies were evident.

4. The future

Forensic palynology will always be needed, even if the developments in DNA that are currently underway [60] eventually allow for individual plants to be identified as being involved in a criminal or civil action. Similarly, current studies on the DNA signature of bacteria in soils [61] may become a less expensive option in showing an absolute relationship between soil associated with a suspect or victim and the crime scene than the more labour-intensive forensic palynology type analysis. At present, however, DNA signatures from soil bacteria need additional testing and refining of the specific signatures before they will become routine or will be accepted in courts of law. Likewise, failure to consider collecting pollen evidence at a crime scene may result in the loss of an important source of evidence, since in many cases pollen can be picked up from crime scenes without any accompanying soil (with bacterial DNA or mineralogical signals). Another advantage of pollen grains trapped in soils is that they do not change their morphology or signatures with time even though in certain types of nutrient-rich environments they might be destroyed by oxidation or damaged by microbes. On the other hand, the bacterial signature of a soil can change very quickly with nutrient fluctuations, biochemical changes, or cycles of wetting and drying. Another obvious point is that there is no exposed soil at many crime scenes and the comparator samples consist entirely of surface vegetation.

We believe that forensic palynology has a bright future. The future scientists in this emerging discipline will come from students around the world who are being trained in the discipline of pollen studies. The problem, however, is in linking their experience as pollen analysts with the needs of doing precise types of forensic analysis. Unfortunately, at present some palynology students wanting to enter the field of forensics have not been trained by scientists experienced in case work. In addition, often the palynologists training students lack essential training in forensic crime scene studies or essential botany and ecology.

If forensic pollen studies are to become commonplace, then scientists promoting its use must demonstrate to law enforcement agencies that the agency is “getting value” for the time and money spent on these types of intensive analyses. Likewise, for agencies to ensure that they will get good value for money spent on pollen studies, these agencies must be ready to use the full potential of the technique by allowing the forensic palynologist to work closely with other members of the investigative team and ensuring a close liaison with pathologists and other scene of crime personnel. Only in Great Britain is the full potential of this technique currently being realised with the development of a new Environmental and Forensic Ecology Unit within the British Forensic Science Service. This new unit should be seen as an example of a multi-disciplinary environmental unit that could be emulated in other national forensic services.

Another problem for fledgling forensic palynologists is that they need access to large palynology databases and available pollen and spore reference collections. This often means that it is difficult for them to join and work closely within well-established forensic science organisations. The forensic palynologist needs to be a competent botanist and have direct
and ready access to palynological facilities with reference collections covering both potential native and exotic taxa that might appear at some crime scenes under investigation. Regrettably, the slow development of internet pollen and spore databases is not yet adequate for most types of forensic work. However, regardless of future internet sources and the current, limited availability of various pollen and spore atlases, nothing currently available can compensate for having direct access to actual pollen and spore reference materials. The guilt or innocence of a suspect, and/or the potential association of some object with a crime scene will always depend on the identification skills of the scientist examining the pollen evidence. Certainty of identification can only be gained when pollen or spores found in association with a crime scene can be compared directly with specimens in a reference collection.

For many regions of the world where forensic palynology is not yet accepted or rarely used, this type of evidence may not be recognized because it has yet to be court tested. Often one way that the courts will accept a new discipline is if it is shown to play a significant role in a high profile case [9,10]. Another important way to gain acceptance is through public knowledge of new forensic techniques. With the increasing number of publications now appearing in technical and public sources [17,20,62] that validate forensic palynology and demonstrate its use, its future is now assured.

The following papers add to the many ways in which forensic palynology has assisted law enforcement agents determine what happened at crime scenes.

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