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Forensic Palynology: Variation in the Pollen Content of Soil Surface Samples

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ABSTRACT: Surface soil samples from several localized areas were analyzed for pollen and compared using an ordination analysis. The aim was to objectively establish the forensic value of using soil samples to link people or objects to crime scenes. This was done by determining the degree to which pollen assemblages of surface soil samples from within the same localized area differ, and the degree to which the pollen assemblage of a surface soil sample from within a localized area differs from distant localized areas of similar vegetation type. Samples from within the same localized area (the control site) showed a high degree of similarity, suggesting that pollen assemblages of surface soil samples from within a localized area are homogeneous. Results also showed that localized areas of similar vegetation type, even within the same geographic region, have significantly different pollen assemblages.

KEYWORDS: forensic science, pollen analysis, palynology, soil samples

Forensic palynology is the science of deriving evidence for court purposes from pollen and spores. Various methods and examples have been described by Mildenhall (1–3), Bryant et al. (4), Stanley (5,6), Horrocks et al. (7) and Horrocks and Walsh (8). Its main forensic application is in providing associative evidence, i.e., assisting to prove or disprove a link between people and objects with places or with other people. For example, soil on a suspect's shoes or clothing can be analyzed for pollen and compared with control soil samples from the crime scene.

Soil surfaces contain pollen and spores which may be collected and analyzed. Wind-pollinated plants generally produce abundant pollen which may be dispersed long distances (up to 100's of km), whereas insect-pollinated plants produce much smaller amounts of pollen, most of which is deposited on the ground within a few meters of the parent plant. Spores from non-pollen-producing plants (e.g., ferns) also vary in dispersal distance. The difference between species' pollen production and dispersal results in pollen representations which may change considerably over just a few meters (9).

In New Zealand, surface soil samples may contain up to about 50 different pollen types. Some of these (e.g., pine, grass and plantain pollen) are wind dispersed and therefore commonly found in samples regardless of whether or not the parent plants are, or have been, locally present. Insect-dispersed pollen types, however, being poorly dispersed, tend to be found only in soil samples taken from within a few meters of parent plants.

Many crime scenes (e.g., the break and entry point of a building or a rape scene under a tree) are typically restricted to only a few square meters, i.e., they are "localized areas." These areas will have a particular combination of plant species comprising the local and surrounding vegetation that produces a particular pollen combination or "assemblage" in their soil. Corresponding assemblages of pollen types found in soil samples, especially those containing rare (insect-pollinated) types, may therefore very strongly suggest that the samples are from the same source (8). A suspect may thus be associated with a crime scene by pollen evidence. However, Bryant et al. (4) stated that although pollen assemblages from different areas are "unique in their own way," the use of complex mathematical calculations and time-consuming analyses of many pollen control samples is required to show this. It may be argued that pollen assemblages of samples from within the same localized area may in fact differ significantly from one another due to normal sampling variation, or that the pollen assemblages of these localized areas will be similar to those of other localized areas of the same vegetation "type." For example, it could be argued that the pollen assemblage of a particular crime scene (an open grassy area, say) would be indistinguishable from that of other open grassy areas.

The aim of this study was to determine firstly, the degree to which pollen assemblages of surface soil samples from within the same localized area differ, and secondly, the degree to which the pollen assemblage of a surface soil sample from within a localized area differs from those of other localized areas of similar vegetation type.

Methods

An open grassy area, measuring approximately 15 by 6 meters, was selected in Western Springs Reserve, Auckland, as the control site. It forms a hollow approximately 2 meters below surrounding terrain and is surrounded by lawn and scattered shrubs and trees. Twenty-one control soil samples ("Control" 1–21) were taken at a depth of 1 mm (a surface scraping with a scalpel blade) and approximately 1 meter apart on a grid pattern across the site. A further 14 evenly spaced samples were taken from a 7 meter (approximately) diagonal line across the center of the site. Seven of these ("Surface" 1–7) were taken to a depth of 1 mm and the remaining seven ("Below Surface" 1–7) were a gouge taken using the thumb and forefinger directly below samples Surface 1–7 to a depth of approximately 2 cm. The deeper samples were taken to determine whether or not pollen assemblages in surface samples

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change significantly within this depth. In addition, a further sample ("6 Months") was taken from within the center of the control site six months later to determine whether or not seasonal changes had significantly affected the pollen content of surface samples from the site.

Samples to a depth of 1 mm were also taken from two sites near to the control site. The first site ("Open Close") was also an open grassy area, approximately 75 meters away, and the second site ("Under Trees Close") was under a closed area of shrubs and small trees approximately 30 meters away. A further sample was taken from each of three open grassy areas 0.5–1.0 km in different directions away from the control site. The first of these ("Open A") was approximately 0.5 km distant and still within the Reserve. The remaining two ("Open B" and "Open C") were roadside verges approximately 1 km away from the Reserve.

A further 10 soil samples were included in the analysis, eight of which were from open grassy sites ("Open D" to "Open K") and two from under trees ("Under Trees A" and "Under Trees B"). These were samples sent to our laboratory for forensic pollen analysis, five of which (Open E, Open G, Open H, Under Trees A and Under Trees B) were still within the central Auckland area (i.e., up to 15 km from the control site). The remaining five were between 35 and 210 km from the control site. Sampling depth for these 10 samples was less than 2 cm.

Samples were prepared for pollen analysis by the standard acetylation and hydrofluoric acid method (10). Bleaching was also carried out.

In the pollen diagram, the pollen types were assigned to the following three groups: (1) conifers, (2) flowering plants, and (3) ferns and others. The first two groups are comprised of pollen-producing plants while the third is comprised of plants that produce spores. Spores are included in the term "pollen types." The pollen sum is comprised of at least 250 pollen grains and spores for all samples except the 10 case samples, in which the pollen sum was at least 100 pollen grains and spores. To reduce the size and complexity of the pollen diagram, pollen types unmentioned in the text that did not record more than 2% of the pollen sum (37 out of 73) are not shown. The software packages TILIA and TILIAGRAPH [E. Grimm, Illinois State Museum, Springfield, Illinois] were used to construct the pollen diagram.

An ordination (DECORANA) was carried out on the pollen samples. Ordination implies continuous variation in composition, placing each sample in relation to one or more axes in such a way that a statement of its position relative to the axes conveys the maximum information about its composition (11). Each point on the ordination figure represents a pollen sample and the distance between points represents their degree of similarity or difference. A DECORANA (detrended correspondence analysis) ordination mathematically ensures that similar differences between samples are expressed by similar distances on the figure (12). Pollen types that did not comprise greater than 1% of the pollen sum were excluded from the analysis (13). The software package TILIA was used for this analysis.

Results

Pollen analysis results for surface soil samples are shown in Fig. 1. None of the control site samples (i.e., Control 1–21, Surface 1–7, Below Surface 1–7 and 6 Months) differ significantly from one other. All are dominated by grass pollen (28–55% of the pollen sum) and bracken spores (11–45%), including the sample taken six months later (6 Months). All other pollen types make up less than 10% of the pollen sum, except in three grid samples where willow pollen is recorded at 13–18%.

Samples from all other sites contain pollen assemblages significantly different from those of the control site and of each other. The open grassy area near the control site (Open Close) records a very high percentage of reed and sedge pollen (35% of the pollen sum) and the area under trees near the control site (Under Trees Close) records higher percentages for pine and *Cyathea medullaris* type (both 15%). Under Trees Close is also distinguished from the control site by the presence in small amounts (<3%) of *Griselinia* and *Pittosporum* pollen, two rare pollen types (i.e., poorly dispersed) of which the parent plants were on site.

Samples from remaining open grassy sites record some very high levels of rare pollen types, e.g., Open D is dominated by ash pollen (40% of the pollen sum) and Open J by Kenilworth ivy pollen (32%). The remaining sites from under trees are dominated by *Coprosma* pollen (Under Trees A) and spore type 1 (Under Trees B), both pollen types being recorded at 76% of the pollen sum.

An initial DECORANA ordination of all 51 samples indicated two outliers (Under Trees A and Under Trees B). This had the effect of compressing remaining samples into an unsatisfactorily small space. Following Gauch's (14) recommendation, these outliers were removed and a subsequent analysis carried out. The results are given in Fig. 2.

In the lower center, the 36 control site samples form a distinct, dense cluster. Six of the remaining samples (Under Trees Close, Open A, Open C, Open E, Open I and Open K) closely surround the control site group. Remaining samples are scattered widely. Distance on the ordination axes does not appear to correlate with actual distance of sites from the control site or from each other.

Discussion and Conclusions

The co-dominance of grass pollen and bracken spores is a distinguishing feature of control site samples (Fig. 1). The high percentages of grass pollen are not surprising as grasses are generally well represented in New Zealand pollen spectra, and lawn is the principle vegetation type of the Reserve and of much of the Auckland region. Bracken spores are also well represented in pollen spectra and their generally high values in control site samples, despite bracken plants not having been locally significant for about 25 years [A. Esler, personal communication], is an example of the high durability of most pollen types. The outer wall of pollen grains and spores is made of sporopollenin which, unlike most biological material, is very resistant to decay and abrasion (11).

Although samples from some of the other sites also record high percentages of pollen and bracken spores, the absence of certain rare pollen types (e.g., willow and *Epilobium*) and different percentages for other common pollen types (e.g., *Cyathea medullaris* type), differentiate them from the control site samples and from each other. The close similarity of the pollen content of control site samples clearly indicates the high degree of homogeneity of pollen assemblages of surface soil samples from within a localized area. Even a change in depth from 1 mm to 2 cm did not significantly alter the pollen content of control site samples. On soil surfaces, mixing of sediments commonly occurs due to rain and wind action and bioturbation (15). Furthermore, the sample taken six months later shows that the pollen assemblage of this area had not significantly changed after this length of time.

Statistical analysis in the form of a DECORANA ordination (Fig. 2) clearly illustrates that different areas of similar vegetation type, even within the same geographical region, have significantly

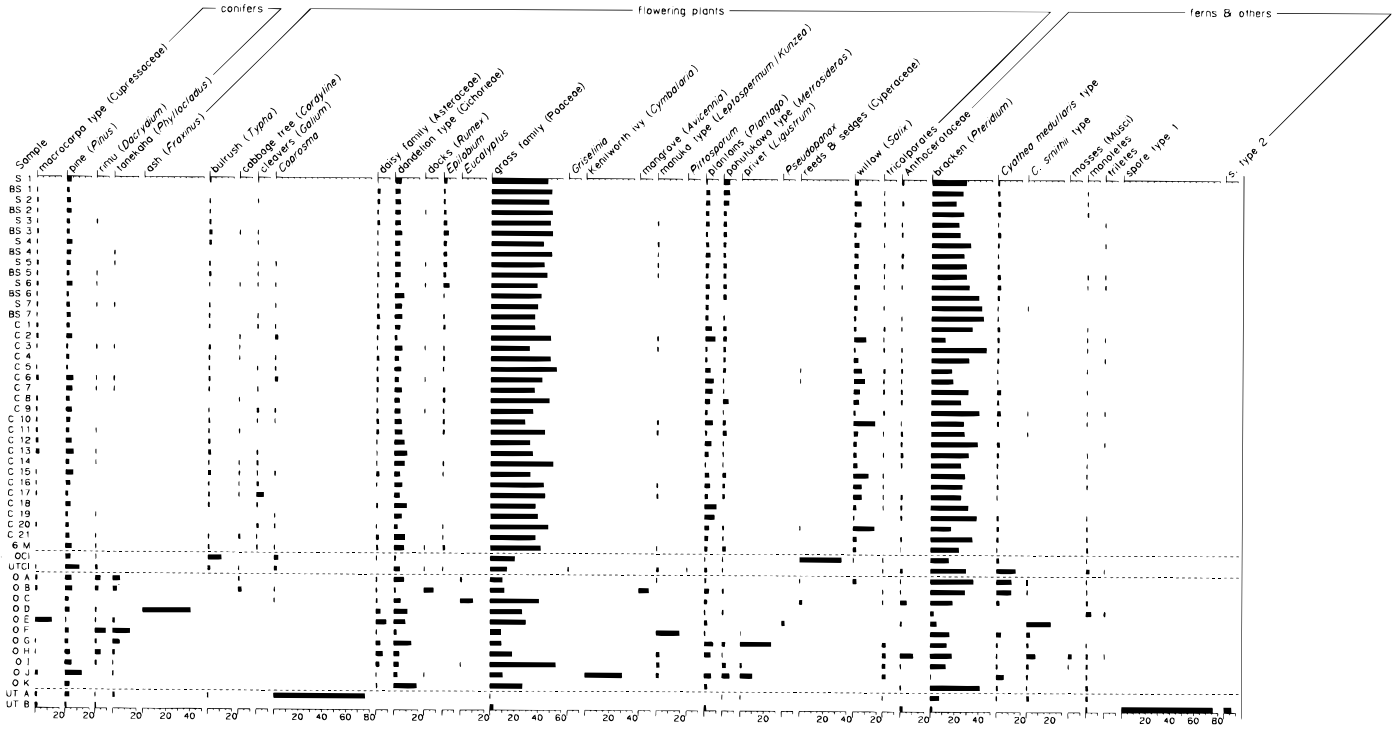

FIG. 1—*Pollen analysis of soil samples. (S = Surface, BS = Below Surface, C = Control, 6 M = 6 Months, OCl = Open Close, UTCl = Under Trees Close, O = Open, UT = Under Trees.)*

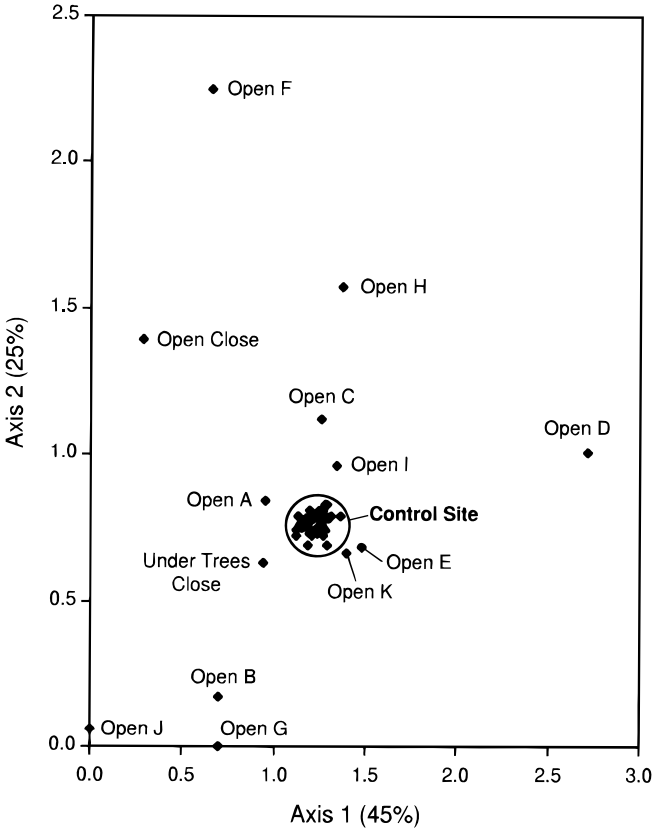

FIG. 2—*DECORANA ordination of pollen in soil samples.*

different pollen assemblages. These differences appear to be unrelated to vegetation type or distance. For example, the site under trees (Under Trees Close) near the open grassy control site is much closer to the control site cluster on the ordination than the near open grassy site (Open Close). Horrocks and Ogden (9) also found generally good differentiation between sites 20–100 meters apart, both in open vegetation types and under trees. Similarly, one of the four open grassy sites outside the Auckland area (Open I) is much closer to the control site cluster on the ordination than two of the open grassy sites within the Auckland region (Open G and Open H).

Results from this study suggest that pollen assemblages of surface soil samples from within the same localized area are homogeneous. Results also show that the pollen assemblage of a surface soil sample from within a localized area differs significantly from those of other localized areas of similar vegetation type. This illustrates that pollen analysis of soil samples is a valuable forensic tool in associating suspects and objects with crime scenes.

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