Mark Horrocks,[1] *Ph.D.; Sally A. Coulson,*[2] *Ph.D.; and Kevan A. J. Walsh,*[2] *M.Sc.*

Forensic Palynology: Variation in the Pollen Content of Soil on Shoes and in Shoeprints in Soil

REFERENCE: Horrocks M, Coulson SA, Walsh KAJ. Forensic palynology: variation in the pollen content of soil on shoes and in shoeprints in soil. J Forensic Sci 1999;44(1):119–122.

ABSTRACT: Soil samples taken from and between consecutive shoeprints within a localized area were analyzed for pollen and compared with each other and with soil samples from the shoes that made the prints. The purpose was to establish the forensic value of using such samples to determine whether or not there is an association between people and crime scenes. This was done by determining the degree to which pollen assemblages in shoeprints in soil from within the same localized area differ, and the degree to which pollen assemblages in soil on shoes differ from assemblages in shoeprints in soil made by those shoes. The samples from and between the shoeprints showed a high degree of similarity, suggesting that pollen assemblages of such samples from within a localized area are homogeneous. A change in sampling depth from 1 mm to 20 mm did not significantly alter the pollen content of samples. The pollen content of the two soil samples from the shoes showed a close similarity to each other and to the soil samples from and between the shoeprints, indicating that pollen assemblages from soil on shoes do not differ significantly from assemblages in shoeprints in soil made by those shoes.

KEYWORDS: forensic science, criminalistics, pollen, palynology, soil samples, shoes, shoeprints

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), Bruce and Dettman (7), Eyring (8), Horrocks et al. (9,10), and Horrocks and Walsh (11,12).

In an earlier study (10) we considered that many crime scenes (e.g., the break-and-entry point of a building or a rape scene under a tree) may be defined as ''localized areas'' since they are generally restricted to only a few square meters. 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. Since localized areas of even similar vegetation ''type'' (e.g., open grassy areas) have significantly different pollen assemblages (10), corresponding assemblages of pollen types found in soil samples may therefore very strongly suggest that the samples are from the same source (11,12).

Shoes that are worn at a wet or muddy crime scene will collect soil from that localized area. Soil on a suspect's shoes can be analyzed for pollen and compared with control soil samples from the crime scene, especially (but not necessarily) with those taken directly from any shoeprints present. However, it may be argued that pollen assemblages of soil samples from shoeprints from within the same localized area may, in fact, differ significantly from one another due to normal sampling variation, or due to sampling from differential depths as a result of some shoeprints being deeper than others.

The aim of this study was to assess the variability to be expected in pollen assemblages of soil taken from shoes. Experiments were conducted to determine, firstly, the degree to which pollen assemblages of soil from shoeprints from within the same localized area differ; secondly, the degree to which pollen assemblages of soils from shoes differ from those of soil in shoeprints made by those shoes; and thirdly, the degree to which pollen assemblages of soil differs with depth of the sample.

Methods

An open grassy area, measuring approximately 15 by 6 m, was selected in Western Springs Reserve, Auckland, as the study area. (This was the site used in our previous study (10).) This localized area forms a hollow approximately 2 m below surrounding terrain and is surrounded by lawn and scattered shrubs and trees (Fig. 1).

A pair of clean shoes was walked once back and forth along the same line of travel (approximately 3.5 m) across a muddy part of the grassy hollow. The resulting tracks consisted of seven pairs of shoeprints, with pair members side-by-side and 2 to 5 cm apart. Each member of a pair was thus going in an opposite direction to the other (Fig. 1). From the heel of each shoeprint, a soil sample (1 mm surface scraping) was taken with a scalpel blade. A further two samples were taken from between each pair of shoeprints. The first of these consisted of a 1 mm surface scraping, the second was a gouge with the thumb and forefinger in the same place to a depth of approximately 20 mm. The deeper samples were taken to determine whether or not pollen assemblages in surface samples change significantly within this depth. Finally, a sample of soil was taken from the bottom of each of the soles of the shoes, using a fine pick to remove soil from between the treads. The quantity of soil taken for samples was 1 to 2 cc. (In our experience, this amount of soil, and often much less, will contain sufficient pollen for forensic analysis, although very sandy soils may require larger samples.)

Soil samples (approximately 1 cc of each) were prepared for pollen analysis by the standard KOH (deflocculation), acetylation (cellulose and organic matter removal) and hydrofluoric acid (silicate removal) method (13). Bleaching (further organic matter removal) was also carried out. A binocular microscope at $\times 400$

[1] School of Environmental & Marine Sciences, University of Auckland, Private Bag 92-019, Auckland, New Zealand.

[2] Institute of Environmental & Scientific Research Ltd., Mt. Albert Science Centre, Private Bag 92-021, Auckland, New Zealand.

Received 19 Feb. 1998; and in revised form 23 April 1998; accepted 8 June 1998.

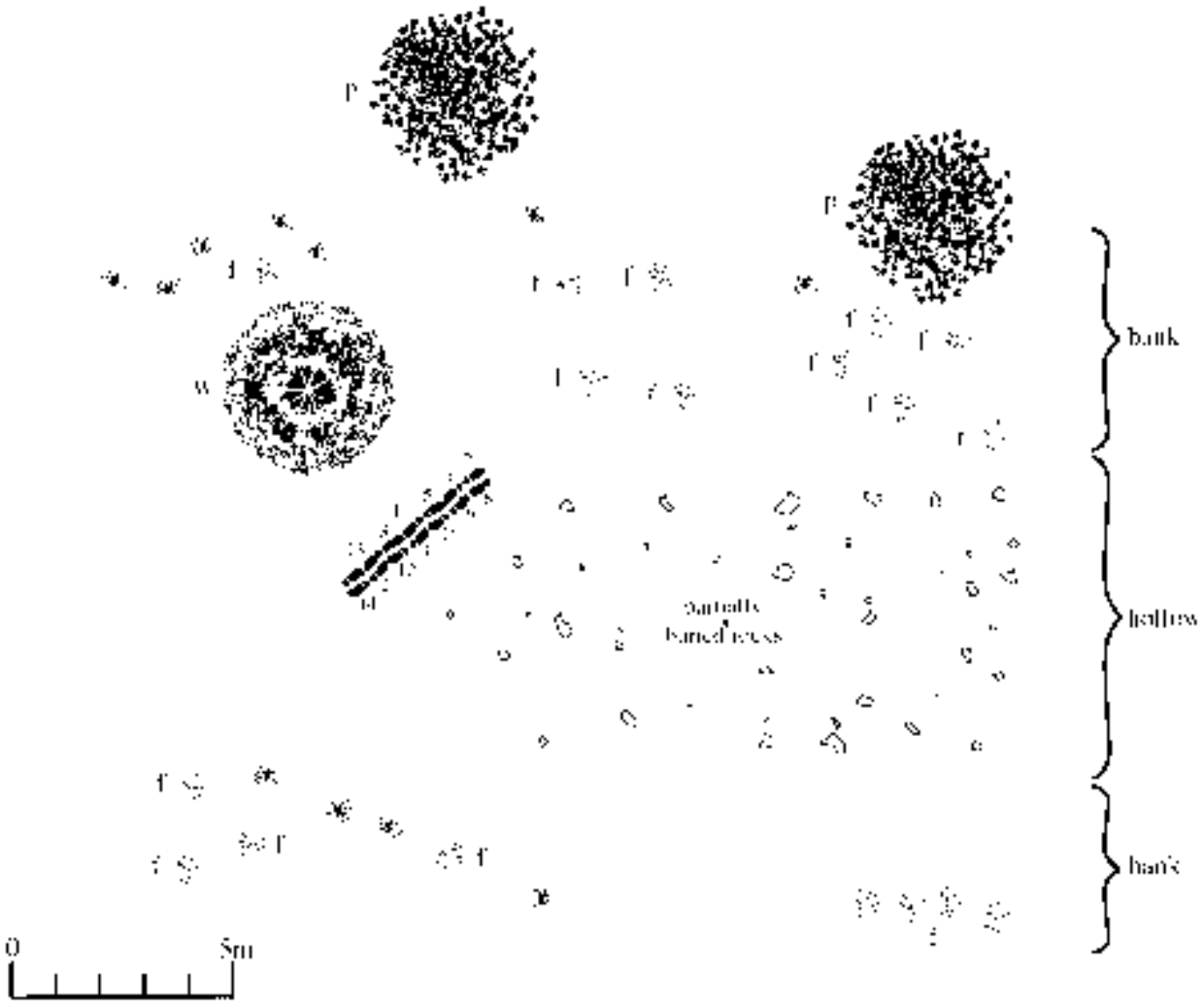

FIG. 1—*Diagram of the study area, a grassy hollow, showing shoeprints and vegetation. (Shoeprint numbers indicate ''Heelmark'' samples shown in Fig. 2. Ground cover herbs and grasses are not shown. Only relatively large parent plants of pollen types mentioned in the text are identified: f = flax bush, p = pohutukawa tree, w = willow tree.)*

to ×1000 magnification was used for pollen identification and counting. (When preparing evidential soil samples, some of each sample should ideally be retained for possible further analysis, e.g., pollen analysis by other parties, or for analysis of other soil components such as minerals and anthropogenic materials.)

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. To reduce the size and complexity of the pollen diagram, pollen types unmentioned in the text that did not record more than 0.4% of the pollen sum (16 out of 47) are not shown. The software packages TILIA and TILIAGRAPH (E. Grimm, Illinois State Museum, Springfield, IL) were used to construct the pollen diagram.

Results

Pollen analysis results for soil samples are shown in Fig. 2. Palynologically, none of the 30 samples differ significantly from one other. All samples are dominated by grass pollen (37 to 59% of the pollen sum) and bracken spores (16 to 44%). All other pollen types make up less than 10% of the pollen sum.

Discussion and Conclusions

The co-dominance of grass pollen and bracken spores in all samples (Fig. 2) is to be expected because both are common pollen types, traveling in large amounts by wind up to many kilometers from parent plants. While some grass pollen and bracken spores would have come from plants that were growing or had previously grown within the sampled area, an undetermined amount would have come from outside sources. Although dominant in all samples in more or less similar amounts, these two pollen types therefore do not necessarily imply that the samples are from the same localized area. However, the presence of certain uncommon (i.e., poorly dispersed) pollen types (i.e., cleavers, *Epilobium,* flax, pohutukawa type and willow which are mainly deposited within only a few meters of parent plants) in most samples, and in similar amounts, indicates that the samples are from the same source. Plants producing these uncommon pollen types were growing in the immediate vicinity of the study area (Fig. 1).

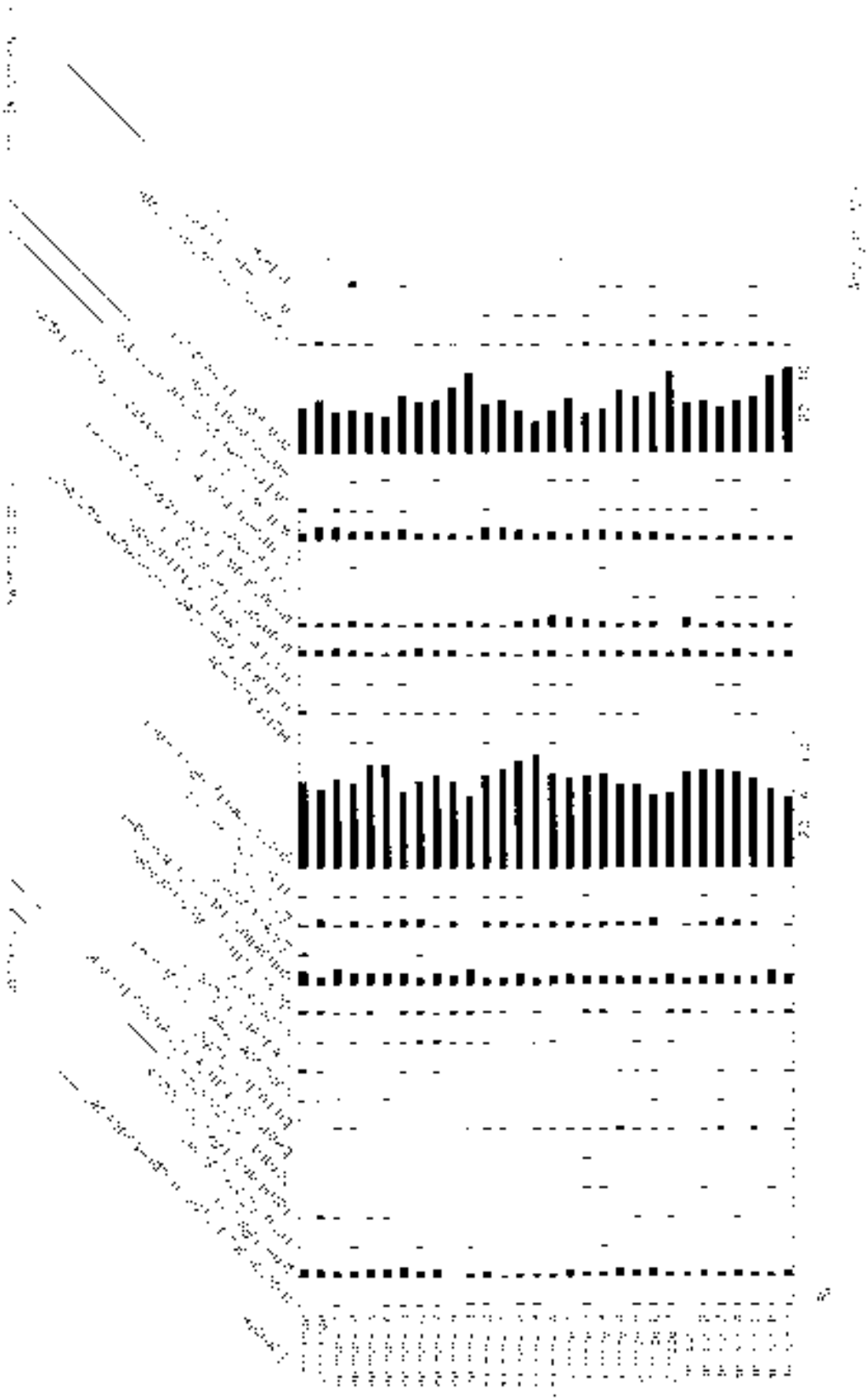

FIG. 2—*Pollen analysis of soil samples. (Bel sur = below surface.)*

The close similarity of the pollen content of shoeprint samples clearly indicates the high degree of homogeneity of pollen assemblages of such samples from within a localized area (10). Even a change in sampling depth from 1 mm to 20 mm did not significantly alter the pollen content of samples taken from between the shoeprints. On soil surfaces, mixing of sediments commonly occurs due to rain and wind action, and bioturbation (14).

The close similarity of the pollen content of the two shoe samples to each other and to the shoeprint samples suggests that pollen assemblages from shoes do not differ significantly from assemblages from shoeprints made by those shoes. Variations between the samples in some of the counts for pollen types of low relative abundance illustrate the differences that occur as a result of sampling variance. For example, the sample ''Heelmark 4'' is the only one that does not contain daisy pollen, and bulrush pollen was found in 75% of the control samples but was not present in either of the shoe samples. These findings illustrate that ''perfect matches'' between samples are neither found nor expected. However, the probability of finding uncommon pollen types may be increased by increasing the pollen count per sample, and by continuing to scan the microscope slides of samples after the initial count.

For this study, a clean pair of shoes was used and the soil was collected immediately after the shoeprints were made. For some crimes, an offender may have collected soil from other localized areas prior to or after collecting soil from the crime scene. In such instances, it would be expected that the mixing of soil would result in pollen assemblages in the soil from the shoes being significantly different to pollen assemblages in soil from the crime scene. In these cases the presence of the same uncommon (i.e., poorly dispersed) pollen types in samples from a suspect's shoes and from the crime scene might support the proposition that some of the soil on the shoes could have come from the crime scene. The strength of this kind of evidence would increase with the number of uncommon pollen types shared between samples, and in combination with other evidence, such as a correspondence of the shoeprint pattern or soil analysis, there may be sufficient combined scientific evidence to strongly support the proposition that there is an association between the suspect and the crime scene. Results from this study illustrate that pollen analysis of soil samples from shoes can provide a valuable forensic tool to determine whether or not there is an association between suspects and crime scenes.

References

1. Mildenhall DC. Forensic palynology. Geological Society of New Zealand Newsletter 1982;58:25.
2. Mildenhall DC. Deer velvet and palynology: an example of the use of forensic palynology in New Zealand. Tuatara 1988;30:1–11.
3. Mildenhall DC. Forensic palynology in New Zealand. Rev Palaeobotany Palynology 1990;64:227–34.
4. Bryant VM Jr, Jones JG, Mildenhall DC. Forensic palynology in the United States of America. Palynology 1990;14:193–208.
5. Stanley EA. Application of palynology to establish the provenance and travel history of illicit drugs. Microscope 1992;40:149–52.
6. Stanley EA. Forensic palynology. Federal Bureau of Investigation International Symposium on Trace Evidence. Washington, DC: US Government Printing Office, 1993.
7. Bruce RG, Dettman ME. Palynological analysis of Australian surface soils and their potential in forensic science. Forensic Sci Int 1996;81:77–94.
8. Eyring MB. Soil pollen analysis from a forensic point of view. Microscope 1997;44:81–97.
9. Horrocks M, Bedford KR, Morgan-Smith RK. The filtering effects of various household fabrics on the pollen content of hash oil (cannabis extract). J Forensic Sci 1997;42:256–9.
10. Horrocks, M, Coulson, SA, Walsh, KAJ. Forensic palynology: variation in the pollen content of soil surface samples. J Forensic Sci 1998;43:320–3.
11. Horrocks M, Walsh KAJ. A Bayesian approach to interpreting forensic pollen evidence. Rev Palaeobotany Palynology, Special Edition: New Frontiers in Palynology. In press.
12. Horrocks M, Walsh KAJ. Forensic palynology: assessing the weight of the evidence. Proceedings of the 9th International Palynological Congress, Houston, TX, 23–28 June 1996. In press.
13. Faegri K, Iversen J. Textbook of pollen analysis. 4th rev. ed. Chichester: John Wiley & Sons, 1989.
14. Bradshaw RWH. Spatially-precise studies of forest dynamics. In: Huntley B, Webb T III, editors. Vegetation history. Dordrecht: Kluwer Academic Publishers, 1988;725–51.

Additional information and reprint requests:
Mark Horrocks, Ph.D.
School of Environmental & Marine Sciences
University of Auckland
Private Bag 92-019
Auckland
New Zealand