CASE REPORT

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Fine Resolution of Pollen Patterns in Limited Space: Differentiating a Crime Scene and Alibi Scene Seven Meters Apart

ORIGINAL

REFERENCE: Horrocks M, Walsh KAJ. Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart. J Forensic Sci 1999;44(2):417–420.

ABSTRACT: In an alleged rape case, the pollen content of soil samples from the suspect's clothing was compared with that of soil samples from the alleged crime scene (an alleyway) and from the alibi scene (next to a driveway) to determine whether or not the suspect had been at the alleged crime scene. Although only 7 m apart, the two scenes had significantly different soil pollen representations due to their different vegetation. Because of this close proximity, however, these differences in pollen representation were in the amounts of the same pollen types rather than in the numbers of different pollen types. The clothing samples showed a very strong correlation with each other and with the sample from the alleged crime scene in the amounts of pollen types present, very strongly supporting the contention that the suspect had been at the alleged crime scene.

KEYWORDS: forensic science, pollen, palynology, soil samples, clothing, spatial resolution

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 by Horrocks and Walsh (11,12).

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 (10). Localized 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 may therefore very strongly suggest that the samples are from the same source (11,12).

If the boundary between two localized areas is sharp, such as between a forest and paddock where tall dense vegetation directly gives way to open pasture, pollen assemblages may correspondingly change dramatically within the same short distance. However, as the edges of the two localized areas are nevertheless in close proximity, it would be expected that the changes would occur mainly in the *amounts* of the same pollen types rather than in the *number* of different pollen types.

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Received 27 April 1998; and in revised form 23 July 1998; accepted 27 July 1998.

Alleged Crime

The complainant in this case, a prostitute, alleged that the defendant had raped her in a short, V-shaped alleyway between two buildings in a commercial area in Auckland (Fig. 1). On the night of the alleged offense, the defendant parked the car in which he and the complainant were traveling, front first, in a driveway near the alleyway. The complainant alleged that the defendant failed to produce payment for sexual services in advance as agreed earlier. She said she then began to feel uncomfortable with the actions of the defendant and got out of the car through the front passenger's door, intending to leave the area. She alleged that the defendant followed her out of the car through the same door and forced her into the alleyway where he raped her.

Upon investigation by the police, an apparently freshly disturbed area of ground was found in the alleyway. This part of the alleyway was approximately 7 m from the edge of the driveway where the car was parked.

The defendant agreed that he had got out of the car through the front passenger's door but denied having been in the alleyway, stating that he had not moved more than about 1 m away from the car. He also denied having had sex with the complainant, stating that his only physical contact with her was to grab her by the wrist when she attempted to leave with money he had paid her in advance for sexual services. He said that soil on the jacket and track suit pants he was wearing at the time came from the ground next to the driveway where he had got out of the car, and not from the alleyway.

An examination of the scenes for the presence of shoeprints or seminal fluid staining did not provide any such evidence. In the absence of evidence from the suspect at either of the scenes, it was necessary to consider the evidence of the soil on the suspect's clothing. Considering the close proximity of the scenes, it was unlikely that a petrological (i.e., chemical and mineral) soil analysis would be able to differentiate the crime and alibi scenes. It was decided that pollen analysis and comparison of the soil samples would be the best means to differentiate the scenes.

A soil sample was taken from the defendant's jacket and another from the knees of his pants. A soil sample was also taken from

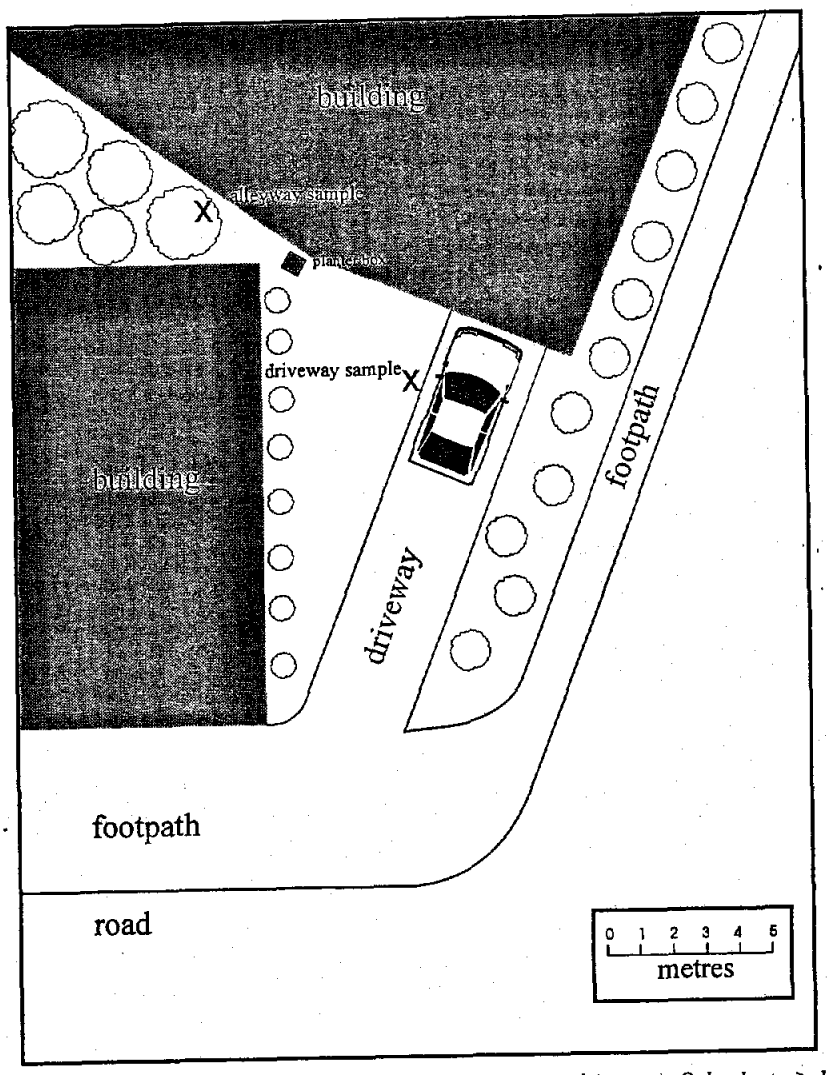

FIG. 1—*Diagram of the alleged crime scene (alleyway) and alibi scene (next to driveway). Only plants > 1 m in height are shown.*

the disturbed area of ground in the alleyway (the alleged crime scene) and from beside the driveway (the alibi scene) where the defendant said he had got out of the car (Fig. 1). These samples were analyzed for pollen to determine whether or not any of the soil on the defendant's clothing had come from the alleyway.

Methods

Areas of soil on the clothing were cut off and heated in 10% potassium hydroxide (KOH) for approximately 20 min to remove soil. All 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 $\times 400$ to $\times 1000$ magnification was used for pollen identification and counting. Some of each sample was retained for possible further analysis, e.g., pollen analysis by other parties, or for analysis of other soil components such as minerals.

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 comprise pollen-producing plants while the third comprise plants that produce spores. Spores are included in the term "pollen types." The pollen sum comprises at least 220 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 (10 out of 40) 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. There was a correlation in the types of pollen present in the alleyway and driveway samples. This is to be expected from soil samples taken from within short distances of one another. However, there were significant differences in the amounts of some of the pollen types present. The alleyway sample contained a very large amount of *Coprosma* pollen (76%) and very small amounts of the other pollen types present (<8%). The driveway sample contained a significantly smaller amount of *Coprosma* pollen (8%) than the alleyway sample and significantly larger amounts of some other pollen types, especially pine (35%). Also, the driveway sample contained a significantly larger amount of grass pollen (14%). This is to be expected because there was grass growing next to the driveway but not in the alleyway.

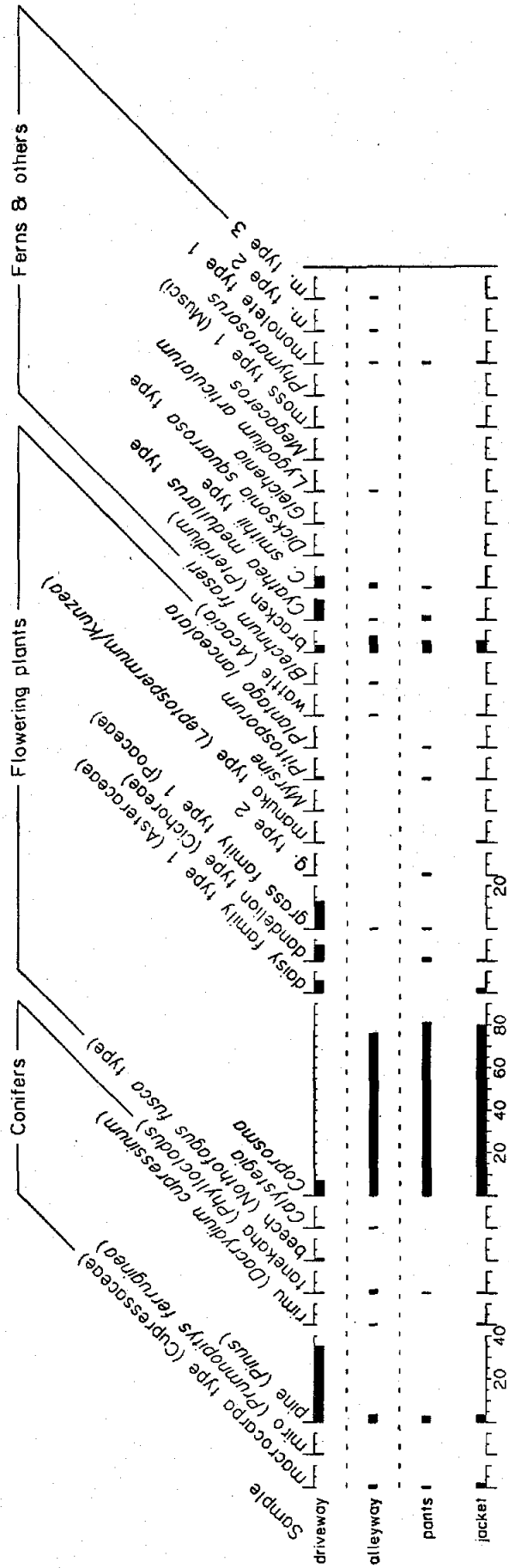

FIG. 2—*Pollen analysis of soil samples.*

The pants and jacket samples showed a very strong correlation with each other and with the alleyway sample in both the types and amounts of pollen present (i.e., both clothing samples recorded approximately 80% for *Coprosma* pollen and <8% for the other pollen types present). This evidence very strongly supports the proposition that these three samples are from the same localized area.

Discussion and Conclusions

The pollen evidence very strongly supports the allegation that the soil on the rape suspect's clothing came from the alleyway (the alleged crime scene) and not from the driveway (the alibi scene). It could be argued that the very strong correlation between the clothing and alleyway samples was coincidental and that the soil on the clothing came from another area elsewhere with similar vegetation to that of the alleyway. However, Horrocks et al. (10) showed that localized areas of similar vegetation type even within the same geographic region, have significantly different pollen assemblages.

Such fine spatial pollen resolution (only 7 m) was possible in this case due to the close proximity of the two quite different localized areas (Fig. 1). As expected from this proximity, the differences in their pollen assemblages were in the amounts of the same pollen types rather than in the numbers of different pollen types.

The alleyway vegetation was dominated by *Coprosma* bushes up to approximately 2.5 m in height. The ground surface in the alleyway was mainly bare soil due to their heavy shading effect. The tendency of plants to deposit most of their pollen directly beneath their branches would in this case have been increased due to the high density of the vegetation canopy and the wind-sheltering effect of the two buildings defining the alleyway. Also, the narrow end of the alleyway was partially blocked by a large, empty wooden planter box and hanging branches, and the wider end of the alleyway was almost completely blocked by branches. These combined effects would have thus resulted in a high deposition rate of *Coprosma* pollen in the alleyway, at the same time restricting the entry of air currents bearing pollen from outside this localized area.

Conversely, the localized area next to the driveway (the alibi scene) was open, with the vegetation consisting of grasses and other herbaceous plants, mainly less than 0.2 m in height. Also, the high density of these low growing plants meant that there were no patches of bare soil at the alibi scene, unlike in the alleyway. In fact, with the exception of the alleyway, there were no patches of bare soil in any of the other areas (i.e., those shown in Fig. 1) immediately surrounding the alibi scene. Bushes and small trees partially surrounded the scene but did not overhang, freely allowing deposition of wind-borne pollen, especially pine pollen, from other areas (including *Coprosma* pollen from the alleyway).

The pollen evidence in this case very strongly supports the contention (11,12) that the soil on the rape suspect's clothing came from the crime scene and not from the alibi scene. Although only 7 m apart, the two scenes were able to be differentiated on the basis of their pollen assemblages.

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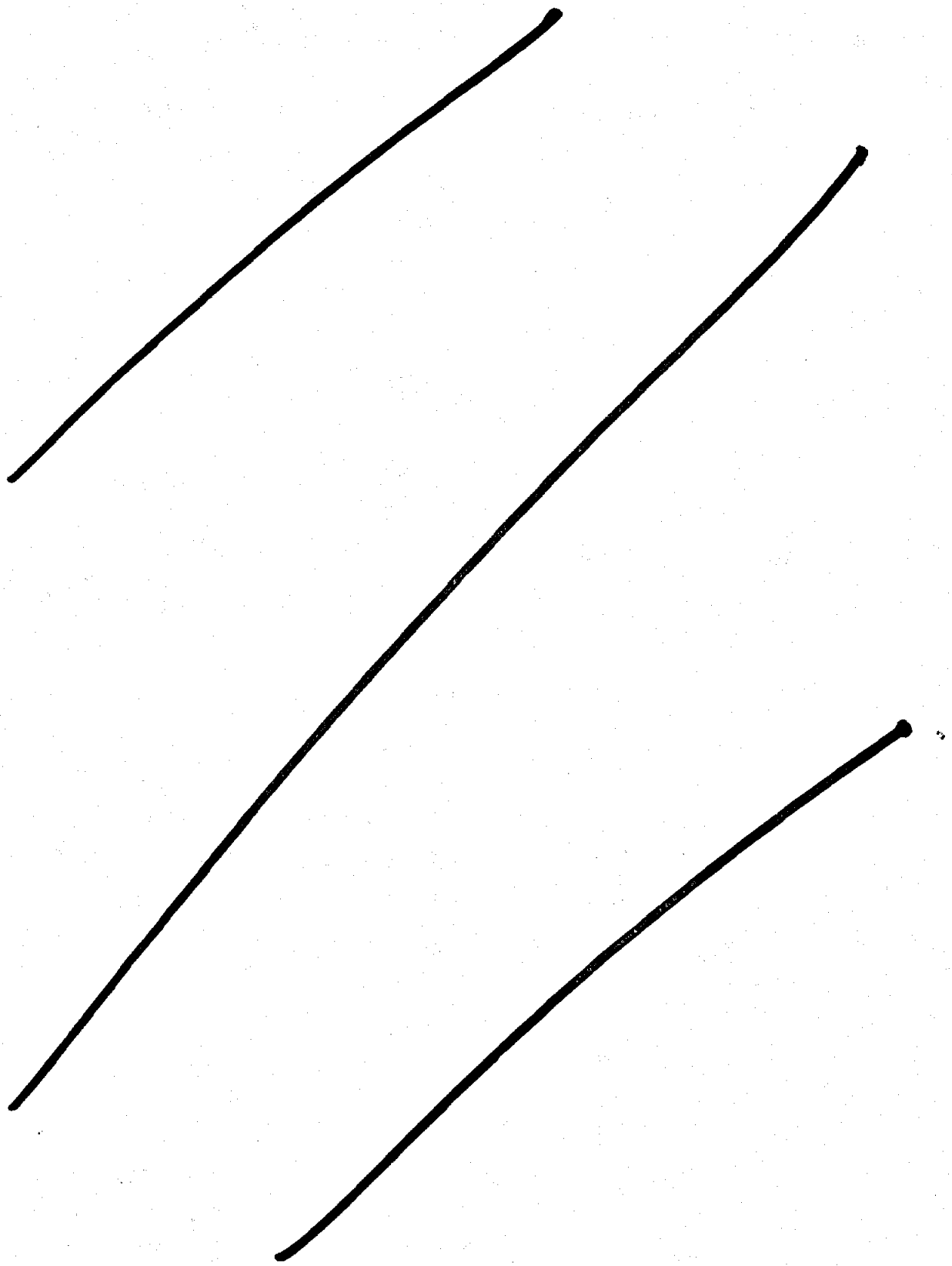

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Original

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 ×400

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

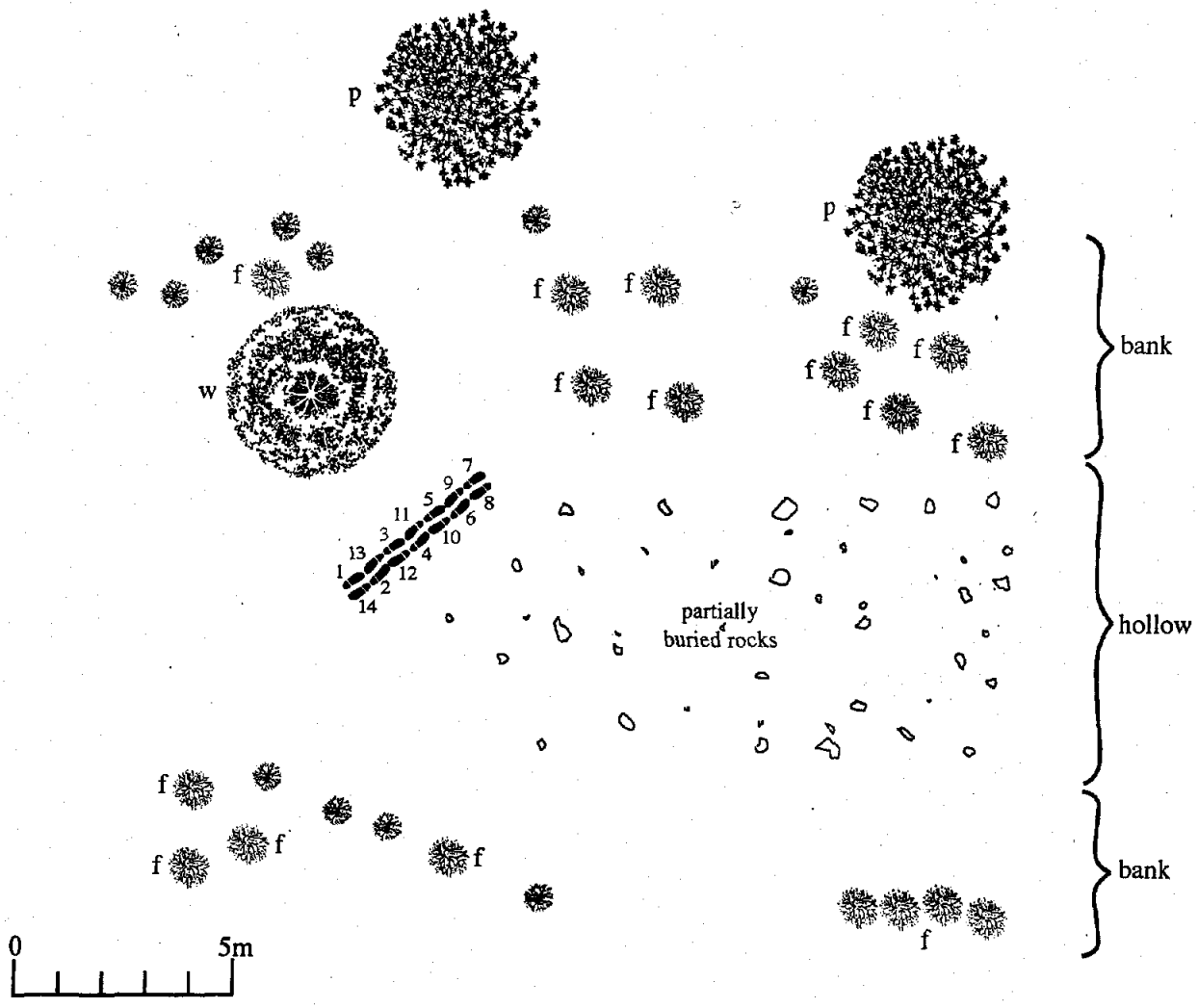

FIG. 1—*Diagram of the study area, a grassy hollow, showing shoeprints and vegetation. (Shoeprint numbers indicate "Heelmark" samples shown in Fig. 2. Ground cover herbs and grasses are not shown. Only relatively large parent plants of pollen types mentioned in the text are identified: f = flax bush,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

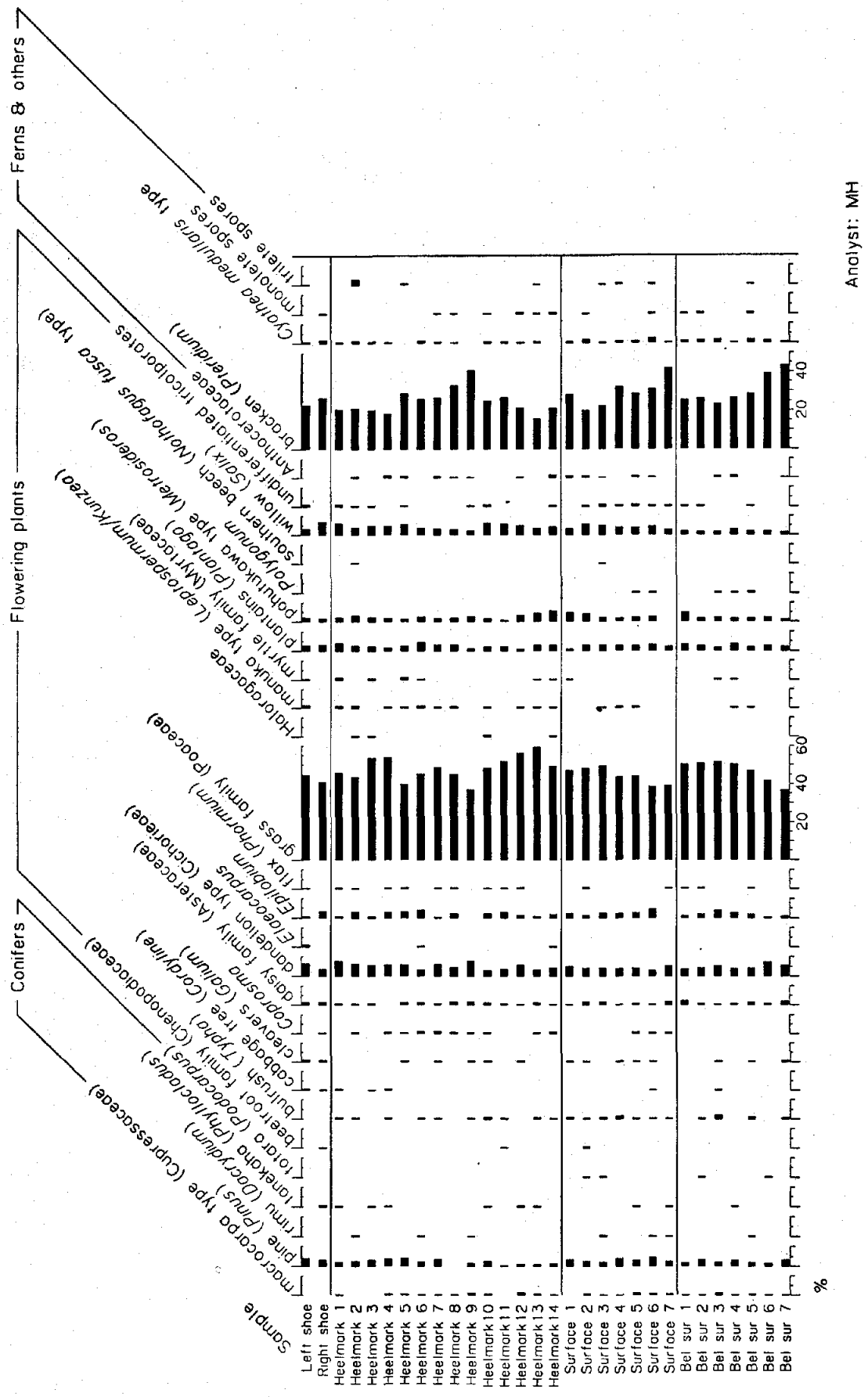

FIG. 2—*Pollen analysis of soil samples. (Bel sur = below surface.)*

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.

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