PALYNOMORPH RETENTION ON CLOTHING UNDER DIFFEREING CONDITIONS

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ABSTRACT

Palynology has been used in a number of criminal cases where pollen and spores (palynomorphs) on clothing has featured as evidence. Pollen and spores are microscopic, generally morphologically unique to a plant genus and often species, resistant to decay, produced in large amounts and are components of soil. These unique features of pollen and spores make palynology a highly valuable forensic tool. Clothing is an excellent collector of pollen and spores as they become trapped in the fabric weave when clothing is brushed against flowering plants, comes into contact with dust, soil or air-borne pollen.

Most forensic palynologists have found that palynomorphs from a crime scene may remain on clothing after washing or several days wear. No empirical research has been conducted on the retention of palynomorphs on clothing under differing conditions. Research of this kind is required to provide support for the future presentation and validation of palynological evidence in court.

This project examined the relative retention of palynomorphs on clothing that had been worn during a simulated assault in a sheltered garden on the grounds of St George's College, Western Australia. Three replicate control soil samples each were collected from the actual assault scene and the whole garden to provide a baseline palynological profile for comparison to the experimental (Evidentiary) clothing samples. Forty pollen samples from the predominant species of plants in the garden and surrounds were collected, processed and databased as a reference for palynomorph identification.

Standard T-shirts and jeans were chosen as the research clothing. During the simulated assault the knees of the jeans and the backs of the T-shirts came into abrasive contact with the soil of the garden for approximately one minute. The clothing then underwent three 'conditions' to simulate 'real life' situations. Three clothing sets were immediately collected after the assault (E1), three sets were worn for a period of three days after the assault (E2) and three sets were washed after the assault (E3). Samples from each clothing set

were then collected and analysed to determine the palynological profile and calculate the relative magnitude of palynomorphs retained by each item. Passive (Background) pollen collection was also investigated. Samples were collected from three clothing sets worn for three days but not used in the simulated assault (Background Clothing).

Twenty key species were identified in the control soil samples. Despite the conditions that the clothing sets underwent, they all retained a palynological profile that was closely similar to the profile of the control soil samples. The E1 samples were most closely similar to the control soil with 19 key species. The E2 and E3 each retained an average of 16 key species in similar amounts to those of the control samples. The Background clothing samples did not have a profile similar to the research garden but the profiles collected from each set reflected the areas to which they were worn.

The number of palynomorphs per gram of garden soil ranged from thousands to tens-of-thousands of palynomorphs. The total number of palynomorphs collected by the E1 samples ranged from 100,000 to millions per clothing item. The E2 samples retained 1000's to tens-of-thousands of palynomorphs and the E3 samples retained 100's to 1000's of palynomorphs. The background clothing samples collected 1000's to tens-of-thousands of palynomorphs.

These results confirm that jeans and T-shirts worn during an assault then worn for a period of days, or washed, will still contain pollen and spores characteristic of the assault area. This highlights the importance of investigating police enquiring where and for how long clothing of interest has been worn before and after an event, or if the clothing has been washed since the event. The results of this study will provide forensic palynologists with supportive data for future casework involving clothing.

TABLE OF CONTENTS

	Page
Title Page	
Abstract	
Table of Contents	i
List of Figures	iv
List of Tables	v
List of Appendices	vii
Acknowledgements	viii
Statement of Candidate Contribution	ix
INTRODUCTION	1
1. BACKGROUND	3
1.1 Palynology	3
1.2 Pollen Morphology	3
1.3 Pollination (Production and Dispersal)	5
1.4 Degradation and Recycling	7
1.5 Forensic Palynology	8
1.6 Case Study Examples	9
1.7 Previous Research	10
2. MATERIALS and METHODS	13
2.1 Experimental Design	13
2.2 Research Area	14
2.3 Vegetation Survey	15
2.4 Sample Collection and Storage	16
2.5 Sample Preparation	19
2.6 Microscopy and Analysis	22

Page

3. VEGETATION 24
   3.1 Site Description 24
   3.2 St George's College Gardens 25
   3.3 Significant Plants in the Research Garden 28

4. RESULTS – Percent Occurrence of Palynomorphs 34
   4.1 Grouping of Major Families and Morphological Types 34
   4.2 Control Soil Palynological Profiles 36
   4.3 Background Samples 43
   4.4 Forensic Clothing Samples (Evidentiary) 50
   4.5 Other Species of Importance (Additional Key Species) 55
   4.6 Evidentiary and Control Sample Comparisons 65

5. RESULTS – Quantitative Palynology 69
   5.1 *Lycopodium* Spike 69
   5.2 Control Soil Samples 70
   5.3 Background Clothing Samples 71
   5.4 Evidentiary Clothing Samples 72
   5.5 Comparison of TPN Numbers for the Control Soil, Background Clothing and Evidentiary Samples 75

6. STATISTICAL ANALYSIS 77
   6.1 Statistical Analysis 77
   6.2 Results 78
   6.3 Combined Control Soil and Evidentiary Clothing Sample Relationships 80

7. DISCUSSION AND CONCLUSIONS 84
   7.1 Percent Occurrence of Palynomorphs 84
   7.2 Total Pollen Number (TPN) 87
   7.3 Conclusion 90
   7.4 Future Research 91

REFERENCES 93

PLATES 1-6

APPENDICES 1-5

LIST OF FIGURES

		Page
Figure 1.1	Example of pollen wall sculpture and structure	4
Figure 1.2	Example of pollen wall structure	5
Figure 2.1	Layout of experimental design	14
Figure 2.2	Aerial view of St George's College	14
Figure 2.3	Example of the clothing worn during the simulated assault	17
Figure 2.4	Photograph of the simulated assault in the Research Garden	18
Figure 3.1	Research site located in the North-West corner of St George's College's front garden	25
Figure 3.2	Schematic of research garden site showing position of plants	26
Figure 3.2	*Araucaria heterophylla*	28
Figure 3.3	*Argyranthemum frutescens*	29
Figure 3.4	*Asplenium australasicum*	29
Figure 3.5	*Cyathea cooperi*	30
Figure 3.6	*Harpephyllum caffrum*	30
Figure 3.7	*Hebe* Autumn Glory	31
Figure 3.8	*Hedera helix*	31
Figure 3.9	*Nephrolepis cordifolia*	32
Figure 3.10	*Liquidambar styraciflua*	32
Figure 3.11	*Philodendron selloum*	33
Figure 3.12	*Quercus suber*	33
Figure 4.1	Schematic of research garden site detailing the scene and local sample collection areas	37
Figure 6.1	NMDS of all replicate Control Soil, Background and Evidentiary samples	79
Figure 6.2	NMDS of all replicate Control Soil, Background and Evidentiary samples with grouping of replicates	79
Figure 6.3	NMDS of combined replicates of Control Soil, Background and Evidentiary samples	81
Figure 6.4	NMDS of all replicate Control Soil, Background and Evidentiary samples with grouping of replicates	82

LIST OF TABLES

		Page
Table 2.1	List of Background Samples and locations worn	17
Table 2.2	List of Evidentiary Samples and locations worn	18
Table 2.3	List of the Evidentiary 3 samples and locations worn	18
Table 3.1	List of the known major flora of St George's College	25
Table 3.2	Flora identified in and near the research garden site at St George's College	27
Table 4.1	Percent occurrence of all pollen groups in the control soil samples	39
Table 4.2	Total Percent occurrence of Key species in the control soil samples	40
Table 4.3	List of the Background samples and the locality worn	44
Table 4.4	Percent occurrence of pollen in the background clothing samples	44
Table 4.5	Percent occurrence of pollen in two compost samples	48
Table 4.6	Percent occurrence of pollen from Key species in the Evidentiary One samples and Control sample averages	50
Table 4.7	Percent occurrence of pollen from Key species in the Evidentiary Two samples and Control sample averages	52
Table 4.8	Percent occurrence of pollen from Key species in the Evidentiary Three samples and Control sample averages	53
Table 4.9	Percent occurrence of pollen from Key and Additional Key species in the Control Soil samples	57
Table 4.10	Percent occurrence of pollen in the Evidentiary 1 (Immediate analysis) samples and Control sample averages	58
Table 4.11	Areas to which the Evidentiary 3 samples were worn	60
Table 4.12	Percent occurrence of pollen in the Evidentiary 2 (3 day wear) samples and Control sample averages	62
Table 4.13	Percent occurrence of pollen in the Evidentiary 3 (wash) samples and Control sample averages	64

Table 4.14	Percent occurrence of pollen from the Key species in the Control and Forensic samples	65
Table 5.1	Total palynomorph number per gram of soil of the Control Soil Samples	70
Table 5.2	Total palynomorph number per item of clothing for the Background Clothing samples	71
Table 5.3	Total palynomorph number per item of item of clothing for the Evidentiary One Samples	73
Table 5.4	Total palynomorph number per item of clothing for the Evidentiary Two Samples	74
Table 5.5	Total palynomorph number per item of clothing for the Evidentiary Three Samples	74
Table 5.6	Total palynomorph number calculated for the Control Soil, Background Clothing and Evidentiary Samples	75
Table 6.1	Key for Figures 6.1 and 6.2	78
Table 6.2	Key for Figures 6.3 and 6.4	81

LIST OF APPENDICES

Appendix 1. Percent occurrence of all pollen types identified in the Control Soil and Evidentiary Clothing samples

Appendix 2. Percent occurrence of all pollen types identified in the Background Clothing samples

Appendix 3. Percent occurrence of all pollen types identified in the Background Compost samples

Appendix 4. Untransformed data for the Control Soil and Evidentiary Clothing replicate samples to be used in statistical analysis

Appendix 5. Bray – Curtis Index transformed data for the Control Soil and Evidentiary Clothing replicate samples

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STATEMENT OF CANDIDATE CONTRIBUTION

I declare that the research presented in this 48 point thesis, as part of the 96 point Master degree in Forensic Science, at the University of Western Australia, is my own work. The results of the work have not been submitted for assessment, in full or part, within any other tertiary institute, except where due acknowledgement has been made in the text.

Louise Rowell
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INTRODUCTION

Palynology is the scientific study of fossil and modern pollen, spores and other acid resistant microscopic plant bodies commonly referred to as palynomorphs. Palynology is used in many scientific disciplines including geology, geography and immunology. As a forensic tool, palynology dates back almost 50 years and has been described as a sub-discipline of botanical ecology (Horrocks *et al.*, 1998; Brown *et al.*, 2002; Brown, 2006; Mildenhall, 2006b; Mildenhall *et al.*, 2006).

Pollen and spores provide plants with a means of reproduction. Pollen contains the male sex cells of flowering and cone-bearing plants (higher vascular plants). Spores are the asexual reproductive cells of ferns, mosses and fungi (lower vascular plants) (Milne *et al.*, 2005). For the purpose of this study, the word pollen will be used to cover both pollen and spores. Pollen is microscopic in size, with most pollen grains ranging from approximately 10-70 μm (5 μm – 200 μm in greatest range) in diameter. Most plants produce and release pollen in vast quantities to facilitate fertilisation (Mildenhall, 1990; Milne, 2005). As a result, pollen can be found in dust, soil and on any surface exposed to the air. Pollen is generally morphologically unique to each plant species and the pollen wall is highly resistant to decay (Milne, 2005). It is these qualities of pollen which make palynology an ideal forensic tool.

Forensic palynology follows Locard's Principle which states that when two items come into contact there is a transfer of material (Mildenhall *et al.*, 2006). For example, pollen may be transferred from soil on to shoes or may be transferred on to clothing that has touched a flowering plant or been in contact with soil. The application of palynology in forensic case-work may refute or substantiate a claimed story, may help in placing an object or individual at the scene of a crime, may support or refute an alibi or may assist in clarifying the events of a crime (Brown *et al.*, 2002).

Palynology has been used in many criminal cases including assaults, murders and various forms of theft. Very little research as been conducted in forensic palynology and most knowledge comes from case-work (Wiltshire, 2006a).

During day to day wear, clothing collects pollen and spores which may stay trapped in the weave of the clothing fabric, visibly undetected, for some time even after the item is washed (Mildenhall, 2006b; Wiltshire, 2006a).

Although forensic palynologists know that palynomorphs from a crime scene may remain on clothing after washing or several days wear, no empirical research has been conducted on the retention of pollen on clothing in these or other conditions after an event. The only palynological research that has been conducted on clothing to date has not used 'real life' situations. Research into the quantity and longevity of palynomorph retention after an event is vitally important to support and validate the future presentation of palynological evidence involving clothing in courts.

The aim of this research is:

To investigate pollen and spore retention on clothing under different conditions.

This will be achieved by the study of:

- background pollen collected on clothing that has been worn for several days;
- the relative retention of palynomorphs on clothing a short time after a simulated sexual assault and after a period of three days; and
- the difference in palynomorph retention on clothing if the item has been washed after an assault.

Chapter 1 of this thesis contains background information on palynology, with a specific focus on its use as a forensic tool. Chapter 2 outlines the materials and methods used in this research; Chapter 3 discusses the vegetation at the research site; Chapters 4 presents the results obtained from the analysis of the soil and clothing samples; and Chapter 5 discusses the forensic significance of this research and presents final conclusions. Photographic plates 1-6 contain photomicrographs of reference and research palynomorphs and Appendices 1-3 contain the raw results from the analysis of clothing and soil samples.

1. BACKGROUND

This chapter will outline the science of palynology, its scientific applications, forensic use and implications and the current status of research in forensic palynology.

1.1 Palynology

Pollen and spores are part of the reproductive cycle of plants. Pollen contains the male sex cells of flowering and cone-bearing plants (higher vascular plants). Spores are the asexual reproductive cells of ferns, mosses and fungi (lower vascular plants) (Bryant, 1990; Stanley, 1991; Jarzen and Nichols, 1996; Milne *et al.*, 2005). For the purpose of this research the word pollen will be used to cover both pollen and spores. Pollen is generally morphologically unique to a plant genus and often species, it is resistant to decay, produced in large amounts, is found almost everywhere and is a component of soil. It is these unique features of pollen that make palynology a highly valuable forensic tool. Other scientific applications of palynology include immunology (aerobiology), geology, geography (palaeoclimatology), anthropology, archaeology and botany.

In order to understand the usefulness of palynology in a forensic setting we must understand pollen structure and the variety of methods of pollen dispersal to effect pollination.

1.2 Pollen Morphology

1.2.1 Structure and Composition

Pollen varies from species to species in size, shape, aperture type and surface sculpture. Apertures are the openings from which the male sex cell escapes during pollination. These may be circular ('pores') or slits ('colpi') or a combination of both, and may be found in varying numbers and on different parts of the pollen grain. The sculpture is the ornamentation or patterns on the pollen grain surface and may relate to the method of dispersal (Milne, 2005; Milne *et al.*, 2005). Ornamentation may also assist the pollen grain to stick to visiting insects so that it may be carried to another plant of the same species (Milne, 2005).

The wall of the pollen grain is chiefly comprised of three layers (Wodehouse, 1959; Moore, 1978; Punt *et al.*, 1994; Jarzen and Nichols, 1996; Milne *et al.*, 2005) (Figure 1.1 and 1.2). The outer layer, the exine, is predominantly composed of sporopollenin, a biopolymer highly resistant to degradation. The exine consists of two layers, the sexine and the nexine (or footlayer). The sexine is comprised of the tectum and infratectum. The endexine layer follows and may be patterned or internally laminated. The inner most wall, or intine, is composed of cellulose and is not unlike the structure of standard plant cell walls (Milne *et al*., 2005).

It is due to the hardy outer wall that pollen may survive many years following release from the plant, depending on the environment in which it was deposited (Moore, 1978; Mildenhall, 1990; Bruce and Dettmann, 1996; Horrocks *et al.*, 1998; Szibor *et al.*, 1998; Horrocks and Walsh, 2001; Brown *et al.*, 2002; Milne *et al.*, 2005; Brown, 2006; Mildenhall, 2006b; Mildenhall *et al.*, 2006; Wiltshire and Black, 2006). During fossilisation, weathering or chemical processing of pollen, the inner content (protoplasm) is decomposed or digested, leaving the resistant exine (Moore, 1978; Milne *et al.*, 2005). The exine carries the unique morphological features that are specific to a plant taxon (Moore, 1978; Milne, 2005).

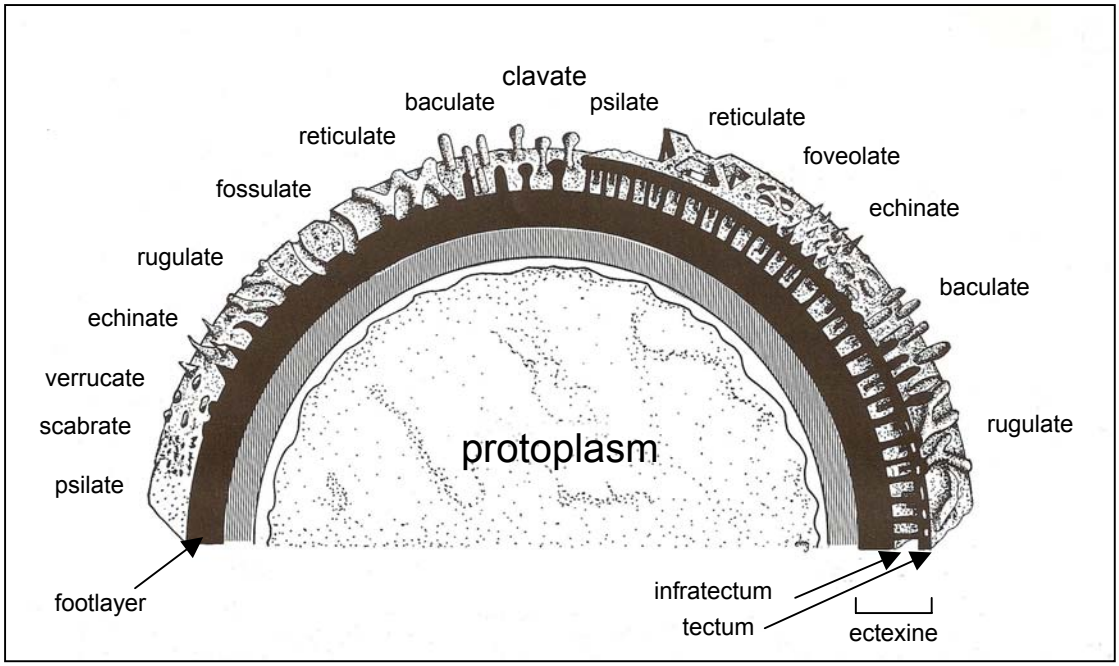

Figure 1.1 Example of pollen wall sculpture and structure. Adapted from Jarzen and Nichols (1996)

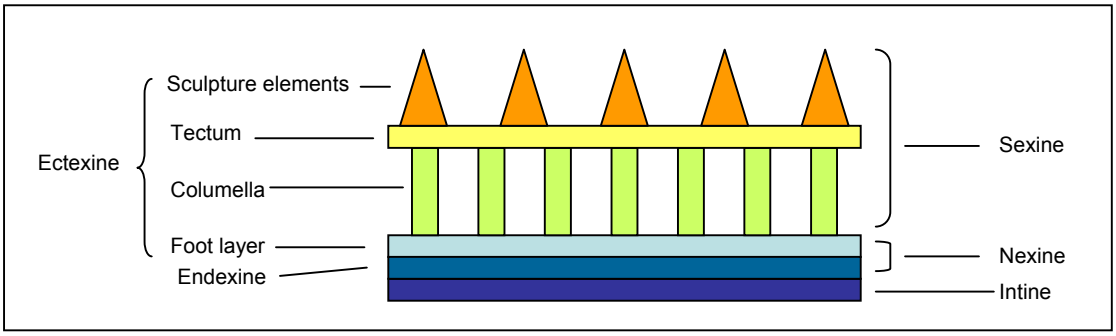

Figure 1.2 Example of pollen wall structure. Adapted from Punt *et al*. (1994)

1.3 Pollination (Production and Dispersal)

As plants are unable to move to find a mate to sexually reproduce, they disperse their pollen (pollination) to other plants by various methods including by wind and water, via insect or animal carriers, or they may self pollinate. It is fundamentally important to understand the pollination vectors to allow correct assessment and accurate interpretation of results from the examination of any given pollen sample (Bryant *et al.*, 1990; Milne *et al.*, 2005). This is because the way a plant disperses its pollen and how much pollen it produces will affect the discovered frequency of pollen from a particular species in a given sample (Moore, 1978). Although it is important to understand all methods of dispersal, the methods of most interest for forensic palynology are wind and insect or animal dispersal.

1.3.1 Wind dispersal (Anemophily)

Wind dispersal is used by gymnosperms and many angiosperms, including grasses and most forest trees, and spore producing plants (Newman, 1984; Bryant *et al.*, 1990). Anemophilous plants produce large volumes of 'light weight' pollen grains that are released and carried by the wind. This is a relatively inefficient method of pollination as it is 'hit and miss' as to whether the pollen will reach a flower of another plant of the same species or not (Bryant *et al.*, 1990; Milne, 2005; Milne *et al.*, 2005; Wiltshire, 2006a). Pollen of these plants subsequently occur in dust and soil and may be found some distance from the parent plant, even if the plant is not growing locally (Horrocks *et al.*, 1998; Horrocks and Walsh, 2001; Horrocks, 2004; Milne, 2005; Mildenhall *et al.*, 2006; Wiltshire, 2006a). However, the majority of

this type of pollen falls within a short distance (i.e. 95% within 2 km) of the parent plant (Mildenhall *et al.*, 2006). Although wind dispersed pollen may be 'overrepresented' in a pollen assemblage, it is still forensically significant as its presence in a sample will indicate the regional pollen rain.

1.3.2 Insect or animal pollination (Zoophily)

Zoophilous pollination occurs when an insect or other animal (e.g. bee, ant, bird) facilitates pollination by carrying the pollen on its body from one flower and depositing the pollen on a neighbouring flower of the same species (from the anther of one plant to the stigma of another) (Bryant *et al.*, 1990; Mildenhall *et al.*, 2006). This method of pollination is common to most flowering plants including the Australian dominants Proteaceae, Myrtaceae and Acacia. As this is a relatively efficient method, plants that utilise this type of pollination produce fewer pollen grains. The grains are commonly large in size and highly ornamented to facilitate adherence to the animal (Milne *et al.*, 2005; Mildenhall *et al.*, 2006). Due to the pollination vectors and the low pollen production of these plants, their pollen will be found predominately in samples taken from beneath or within a few metres of the parent plant (Horrocks *et al.*, 1998; Horrocks and Walsh, 2001; Horrocks, 2004; Montali *et al.*, 2006; Wiltshire, 2006a). Because the grains fall near the parent plant, pollen from zoophilous plants is considered highly significant if present in a forensic sample (Bryant *et al.*, 1990; Milne *et al.*, 2005).

1.3.3 Pollination in Water (Hydrophily)

Hydrophilous plants, commonly plants submerged in water, use water as a dispersing agent to facilitate pollination (Bryant *et al.*, 1990; Milne *et al.*, 2005; Mildenhall *et al.*, 2006). These plants produce large amounts of thin-walled pollen that is highly susceptible to degradation, and are not commonly found in forensic samples (Bryant *et al.*, 1990; Milne *et al.*, 2005).

1.3.4 Self Pollination (Autogamy)

Self pollination is a highly efficient method of pollination as pollen need only travel a short distance from the anther to the stigma of the same flower, and can be facilitated by wind or animal (Bryant *et al.*, 1990; Milne *et al.*, 2005). Autogamous plants produce very few pollen grains. This means that pollen from autogamous plants is

not commonly found in the soil or in forensic samples, but is considered highly significant if present (Bryant *et al.*, 1990; Milne *et al.*, 2005; Mildenhall *et al.*, 2006).

1.3.5 Closed Pollination (Cleistogamy)

Cleistogamous plants use a closed system to facilitate pollination (i.e. the flowers do not open) and produce very few pollen grains. As a result, pollen from cleistogamous plants is rare in forensic samples but is considered highly significant if present (Milne *et al.*, 2005; Mildenhall *et al.*, 2006).

1.4 Degradation and Recycling

Not all pollen types have the same durability. The longevity of pollen exposed to the elements is affected by both the composition and structure of the pollen grains and the environment in which they are deposited. For example, pollen from hydrophilous plants lack sporopollenin in the cell lining and as a result it is highly susceptible to degradation and oxidises rapidly (Bryant *et al.*, 1990; Mildenhall, 2006b). Comparatively, pollen from zoophilous plants has a thick exine and may be preserved for many years (Bryant *et al.*, 1990; Mildenhall, 2006b). Pollen preserves best in acidic environments (Milne *et al.*, 2005).

It is important that the forensic palynologist considers preservation and degradation of the different pollen types in an assemblage. Pollen that is susceptible to oxidation and degradation may be 'missing' from a sample. This will affect the pollen profile. Well preserved pollen deposited at an earlier period may be mixed into the sample as a result of deep digging, turning of the soil or the exposure of old sediments (Bryant *et al.*, 1990). As a result, 'unexpected' pollen grains that are not from current vegetation may appear in a sample. This may make the expected vegetation of the sample appear quite different to the actual vegetation at the site from where the sample came (Bryant *et al.*, 1990). These factors may also increase the uniqueness and evidentiary potential of a sample (Bryant *et al.*, 1990).

1.5 Forensic Palynology

1.5.1 Forensic Applications and Considerations

Almost all objects or surfaces potentially contain pollen. The transfer of pollen from one substrate to another may result from direct contact with a flowering plant or by contact with substances containing pollen such as soil and mud (Mildenhall *et al.*, 2006). The presence of pollen on evidentiary items may refute or substantiate a claimed story, may help in placing an object or individual at the scene of a crime, may support or refute an alibi or may assist in clarifying the events of a crime (Horrocks *et al.*, 1998; Horrocks and Walsh, 2001; Brown *et al.*, 2002; Horrocks, 2004). For example, pollen may be transferred from soil onto shoes or may be transferred onto clothing that has touched a flowering plant or been in contact with soil. The geographical province of a sample, for example the origins of drugs and foodstuff, may also be determined (Stanley, 1991, 1992). A pollen assemblage, which is all the pollen types together in a soil or dust sample, may be quite unique to a particular site and even sites only meters apart (Horrocks *et al.*, 1998; Horrocks and Walsh, 1999, 2001; Horrocks, 2004; Mildenhall 2004).

There have been many criminal cases in which palynology has been successfully used. These include rapes, sexual assaults, murders, drug manufacture and trafficking and various forms of theft (Bryant *et al.*, 1990; Haile, 1990; Mildenhall, 1990; Stanley, 1992; Bryant *et al.*, 1996; Bryant and Mildenhall, 1998; Horrocks and Walsh, 1998; Horrocks and Walsh, 1999; Brown *et al.*, 2002; Mildenhall, 2004; Brown, 2006; Mildenhall, 2006a; Mildenhall, 2006b; Wiltshire, 2006b). Palynology has even been used to investigate the cause of a plane crash (Lewis, 1997), settle land rights issues (Mathewes, 2006) and has been used on the Shroud of Turin in an attempt to establish its authenticity (Frei, 1982; Bryant, 2000; Danin and Baruch, 2001; Milne, 2005). Forensic palynology has been used routinely in New Zealand since the 1980's and in the UK for the last 15 years. It is used as a novel application in the United States of America and Australia but is rarely used elsewhere. Bryant and Mildenhall, (1998) suggest that the lack of specialists, facilities and funding result in the limited use of forensic palynology. As a result, very little research has been conducted in this field (Wiltshire, 2006a).

Clothing is one of the best carriers of pollen. Pollen becomes trapped in the weave of clothing as it is brushed against a flowering plant, is rubbed in the soil or captures air-borne pollen. Once on clothing, pollen is visibly undetectable and can remain over time even after the item is washed (Mildenhall, 2006b; Wiltshire, 2006a). The following cases illustrate where pollen on clothing has been integral to forensic investigations.

1.6 Case Study Examples

Case 1 – Milne *et al.* (2005) and Mildenhall (2006a) report on an indecent assault and burglary case in New Zealand where *Hypericum* pollen found in large amounts on a suspect's jacket linked the suspect to the crime scene. The suspect had brushed against a flowering *Hypericum* bush when exiting the scene. The abundance of *Hypericum* pollen on the suspect's clothing indicated direct contact with a *Hypericum* plant. The offender was found guilty of sexual assault and burglary.

Case 2 - Another case reported by Mildenhall (2004) used the pollen from *Nothofagus menziesii*, a New Zealand mountain plant, to disprove an alibi. In this case the suspect claimed not to have been in the region where these plants grow. Pollen of *N. menziesii*, plants which did not grow in the areas where the suspect claimed to have been, was found in significant amounts on his clothes. The suspect eventually admitted that he had been in the area, but was not convicted of the crime.

Case 3 - A case reported by Horrocks and Walsh (1999) illustrates the use of pollen found on the pants and jacket of a suspect's clothing to differentiate a crime scene and an alibi scene that were only seven metres apart. In this instance, the pollen content in the soil samples from the two sites was significantly different enough to differentiate the two sites and place the suspect at the crime scene.

Case 4 – In 1996 the body of a woman was discovered among flowering wattle shrubs (*Acacia sophorae*) in Noosa. Her car was discovered in Gympie where she lived. It was suspected that her car was used to transport the body to Noosa as wattle flowers were discovered in the car. *Acacia sophorae* pollen was found in the victim's car and on a suspect's clothing. The suspect claimed not to have been in Noosa

recently. *Acacia sophorae* does not grow in Gympie. The palynological evidence was used to arrest the suspect. The case went to trial in 1997 and the suspect was found guilty of murder (Milne, 2005).

Case 5 - In a recent sexual assault case in Western Australia, in which the accused was acquitted, clothing was collected from an alleged suspect several days after the assault was alleged to have taken place (L. Milne *pers. comm.*). A pollen assemblage matching that of the alleged crime scene was recovered from the suspect's and victim's clothing. The judge raised questions as to the current knowledge on the retention of pollen on clothing over time. Although it is common knowledge to palynologists that some pollen remains in fabric even after washing, this is difficult for laypersons to understand. The questions raised were valid and illustrate the importance of further research to produce hard facts that courts will more readily accept. Current knowledge and understanding of pollen retention on clothing has been derived chiefly from case-work experience (Wiltshire, 2006a) and not from research.

1.7 Previous Research

Although the retention of pollen on clothing is commented on in various papers (Bryant *et al.*, 1990; Mildenhall, 1990; Bryant *et al.*, 1996; Horrocks *et al.*, 1997; Mildenhall, 1998; Horrocks *et al.*, 1999; Horrocks and Walsh, 1999; Horrocks and Walsh, 2001; Horrocks, 2004; Mildenhall, 2004; Milne *et al.*, 2005; Brown, 2006; Bryant and Jones, 2006; Mildenhall, 2006a, 2006b; Mildenhall *et al.*, 2006; Wiltshire, 2006a), no comprehensive empirical study has been conducted on how long and how much pollen is retained in clothing under different conditions. General research on forensic aspects of palynology that has been conducted to date includes that conducted by Clarke (1993 *unpub.*), Horrocks, Bedford and Morgan-Smith (1997), Horrocks, Coulson, Kevan and Walsh (1999), Riding, Rawlings and Coley (2007) and Zavada, McGraw and Miller (2007).

Horrocks *et al.* (1997) examined the relative pollen retention of various household fabrics, such as towelling, bedding, stockings, T-shirt material and calico, used to filter hash oil. They examined the palynological assemblage of the hash oil prior to and after filtration with different fabrics to see if the hash oil could still be identified

as coming from the same source (Horrocks *et al.*, 1997). They found that in most instances, despite different filtration fabrics, the pollen assemblage of the filtered hash oil could be related to the pre-filtered hash oil pollen assemblage.

Horrocks *et al.* (1999) established a link between people and crime scenes by examining shoes. In this research, soil samples were collected for palynological analysis from shoeprints and from the immediate area around and between shoeprints. The palynological assemblages from the shoeprints and surrounds were then compared to the palynological assemblage taken from the soil remaining in the shoes that formed the prints. They found that pollen assemblages from the shoes that made the prints could be related to the pollen assemblage of the soil within the prints.

Riding *et al.* (2007) also examined footwear as pollen collectors, showing that palynomorphs are collected by the footwear from each place where the footwear has been worn. Furthermore, the predominant palynological profile on the footwear 'reflects' the last location that was visited.

The studies by Clarke (1993 *unpubl.*) and by Zavada *et al.* (2007) are the closest in-depth research of palynomorph retention on clothing conducted to date, but they lack the 'human touch' required to emulate conditions in which palynological evidence on clothing could be used in a forensic context. While Clarke (1993) 'wears' the fabric examined in her research, as well as leaving fabric 'un-touched' on the ground and vegetation of the research site for a period of time, none of the items were worn 'in full' (pieces of fabric were pinned to the upper and lower body parts). The fabric examined, therefore, cannot be considered beyond the collection of background pollen, as none of the fabric was 'worn' or came into firm or abrasive contact with the soil of the research area beyond being placed on the topsoil.

Similarly, Zavada *et al.* (2007) examined the palynomorph retention potential of various fabrics ('passive pollen collectors') by creating a 'pollen collecting apparatus' whereby the various fabrics were pinned to hoops at various collection sites and allowed to swing freely in the wind for a 24 hour period. Although research such as this is of great value in showing that clothing collects pollen, no research into the retention of pollen and spores on clothing that has been worn to a particular site,

involved in a simulated assault and undergone various treatments, has ever been conducted.

2. MATERIALS AND METHODS

This chapter will describe the experimental design, research area, vegetation survey, sample collection and storage, sample preparation and analysis techniques used in this study.

Standard T-shirts and jeans were chosen as the clothing for this research as these are the common casual choice of attire. A small garden within the grounds of St George's College was chosen as the research site for the simulated assault due to its rich flora and secluded nature.

2.1 Experimental Design

Four test conditions for the clothing sets were chosen. These are illustrated in Figure 2.1 and outlined below.

- **Background Clothing:** Clothing worn for three consecutive days then analysed to *examine background pollen collection*.
- **Evidentiary 1:** Clothing worn during a simulated sexual assault then immediately analysed *to investigate the relative retention of palynomorphs from the research site (pollen and moss, fern and fungal spores) on clothing a short time after a simulated sexual assault*.
- **Evidentiary 2:** Clothing worn during a simulated sexual assault then for a further three consecutive days and then analysed *to investigate the relative retention of palynomorphs from the Research site on clothing that has been involved in the simulated assault after a period of three days* ().
- **Evidentiary 3:** Clothing worn during a simulated sexual assault and then washed before analysis *to determine the palynomorph retention on clothing if the item has been washed after an assault*.

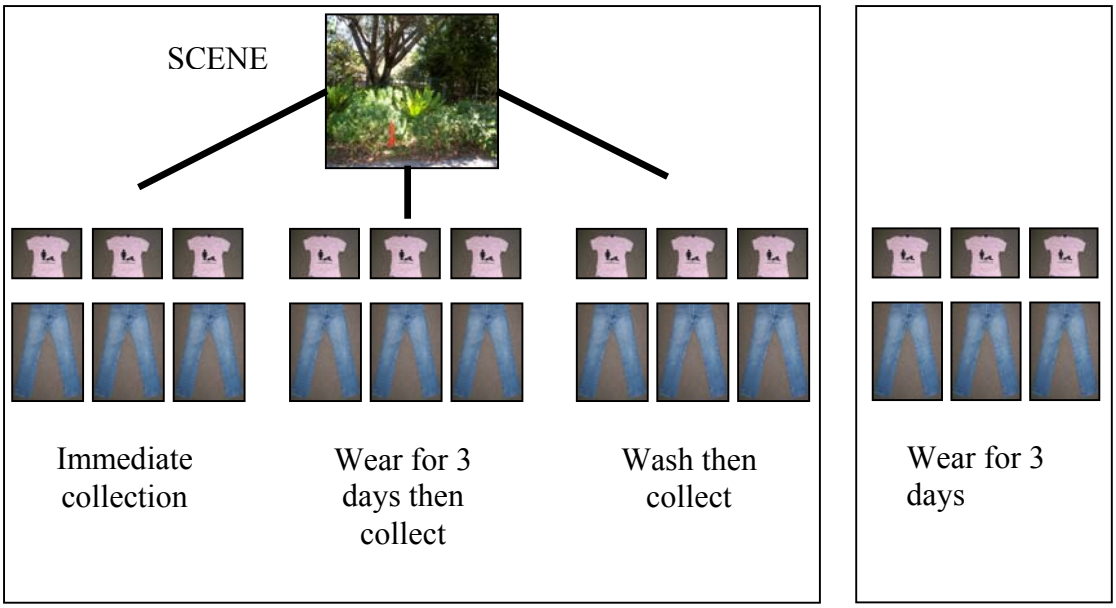

Figure 2.1 Layout of experimental design.

2.2 Research Area

The grounds of St George's College, located on Mounts Bay Rd, Crawley, Western Australia, were chosen as the research area for this study due to the rich botanical history of the residential college site.

Figure 2.2 Aerial view of St George's College garden. College boundaries are marked in red, the research garden is marked in blue. Adapted from Google Earth (http://earth.google.com/).

St George's College was founded on Australian native bushland in 1928. Many of the original native plants, (e.g. *Eucalyptus, Melaleuca* and *Banksia*) were removed to make way for a garden that was to resemble an 'informal English garden' (Wills-Johnson, 1981). The original college gardeners, George White and Ted Melbourne, set out to transform the building site with a mix of native and exotic plants designed to 'partly obscure and partly reveal', but overall complement, the grand college buildings which were completed in 1930 (Wills-Johnson, 1981).

The main feature of the grounds was to be the extensive five tiered rose garden, set at the front of the college. To achieve this, more than 100 hybrid tea bushes of various species were planted (Wills-Johnson, 1981). Although the original rose bushes are long-gone, an extensive rose garden still exists as the main feature at the front of the college buildings.

In the now heritage registered gardens (registered with the National Estate by the Australian Heritage Commission in 1980 [Wills-Johnson, 1981]) many of the original plants no-longer exist. However, given that the soil is regularly turned and dug as new plants replace old, it would be expected that pollen from plants previously planted in and around the gardens would appear in the top-soil samples. A list of the major pre-existing plants is outlined in Chapter 3.

2.3 Vegetation Survey

In cases where objects or individuals are related to particular scenes, it is vital that a comprehensive vegetation survey of the given scene is conducted and that comparator soil samples are collected (Wiltshire, 2006). These studies yield a palynological profile of the scene which may then be compared to evidentiary samples to support or exclude a suspect (Wiltshire, 2006). The vegetation at the chosen site was identified by Martin McLeish of Program Maintenance Services, Gardener for St George's College; Cate Tauss, botanist with Ecologia Environmental Consultants; and Henry and Adrian Boogard of Boogard's Nursery.

2.4 Sample Collection and Storage

2.4.1 Reference Samples

Pollen samples were collected from 27 plants in the research area. Only one failed to produce enough pollen for processing. For exotic species that could not be collected, the private collection of Dr Lynne Milne was utilised and various palynology reference books consulted. Some species were identified by Dr Lynne Milne during examination of the control sample slides. These were then photographed and used as reference images.

2.4.2 Control Soil Samples

Soil from two areas, the immediate 'scene' and the immediate garden area surrounding and including the 'scene', were collected as control samples. Samples were collected in triplicate to ensure a true representation of the palynological profile for the area. The 'pinch' method of soil collection, as described by Milne *et al*. (2005), was used to collect the samples. Each sample comprised of approximately 30 regularly spaced pinches taken from the top one centimetre of soil (each pinch equals approximately half a teaspoon in volume) over the sampling area.

Two compost soil samples were collected from a freshly composted flower bed four meters directly north-east of the research garden area. This was to investigate the possible palynomorph contribution from compost previously laid on or near the site.

2.4.3 Evidentiary Samples

There are many different types of fabrics used in the manufacture of clothing. Horrocks *et al.* (1997) noted that it would be impossible to test all types of clothing given the time and funding constraints. Considering this, 'standard' denim jeans and 'standard' short sleeved T-shirts, all of the same make and fabric, were chosen to use as the victim's and suspect's clothing.

As four experiments using the clothing, repeated in triplicate, were to be performed, 12 T-Shirts and 12 pairs of jeans were used. Due to funding and time constraints for this project only the T-shirts were used for 'victim' samples, and the jeans for

'suspect' samples. Each item was second hand but washed before use and stored separately in clean sealable bags.

Figure 2.3 Example of the clothing worn during the simulated assault

Background Samples

Three sets of clothes (one T-shirt and one pair of jeans) were worn for three consecutive days to accumulate a general airborne background pollen assemblage. The localities to which each set of clothing was worn was recorded (Table 2.1) so that the abundance of certain pollen types could be accounted for.

Table 2.1 List of Background Samples and locations worn

Clothing Set Number	**Locations Worn**
Set One	Perth City, Crawley and Fremantle.
Set Two	Myaree, Perth City, Crawley
Set Three	Innaloo

At the conclusion of three days (approx. 24 hours of 'wear-time') each item was placed into a clean, labelled sealable bag ready for sample preparation.

Evidentiary Samples (Table 2.2)

The remaining clothing items were used in the 'assault'. Each item was put on and the assault acted out by the wearer. The back and shoulder regions of the T-shirts and the shins regions of the jeans were preferentially rubbed in the soil for approximately one minute.

Figure 2.4 Photographs of the simulated assault in the Research Garden

The back and shoulder region of the T-shirt and knees and shin region of the jeans were targeted

Table 2.2 List of Evidentiary Samples and locations worn.

Sample Group	**Treatment after simulated assault**
Evidentiary 1	Clothing immediately analysed
Evidentiary 2	Clothing worn for three consecutive days before analysis
Evidentiary 3	Clothing washed before analysis

Evidentiary 1: The jeans and T-shirts (three of each) were individually placed into clean, appropriately labelled, sealable bags ready for sample preparation.

Evidentiary 2: The jeans and T-shirts were stored after the simulated assault in clean, appropriately labelled sealable bags until each item could be worn. The location each was worn during the three day period is noted in Table 2.3.

Table 2.3 List of the Evidentiary 3 samples and locations worn.

Clothing Set Number	**Locations Worn**
Set One	Perth City, Crawley
Set Two	Perth City, Crawley
Set Three	Innaloo, Crawley

At the conclusion of three days (approx. 24 hours of 'wear-time') each item was placed into a clean, labelled and sealable bag ready for sample preparation.

Evidentiary 3: The jeans and T-shirts were placed into clean, appropriately labelled and sealable bags and taken for laundering. Each item was washed separately from the other experimental clothes to avoid cross contamination or additional pollen deposits due to the increased amount of soil in the washing machine. To emulate real events, each item was washed with a full load of other washing (i.e. other items of clothing, worn as normal). Radiant washing powder was used, and each item was dried on a drying rack kept in a closed draft-free room until dry. Each item was then transferred to a clean, labelled and sealable bag ready for sample preparation.

2.5 Sample Preparation

Pollen preparation involves various chemical and physical treatments to remove minerals and extraneous organic matter. Methods of palynological processing vary in different laboratories. The methods used in this study follow those of Phipps and Playford (1984) and Milne *et al.* (2005). Palynological preparation was conducted in the Palynology and Hydrofluoric acid laboratories at the School of Earth and Environment (SEE), the University of Western Australia (UWA).

To prevent contamination, all glassware and re-usable plastic ware was thoroughly washed with Decon detergent in warm water and then rinsed with distilled water before and after use.

Safety measures included the use of fume cupboards and protective clothing. Laboratory coats and gloves were worn for general preparation and full body protective suits, gloves and rubber boots were worn for hydrofluoric acid preparation.

For the control soil samples and compost samples collected, at least half of the sample was retained for repeat processing if required. All of the clothing wash samples were used.

2.5.1 Palynological Processing

Palynological processing involves the following steps:

1 Hydrochloric acid (HCl) treatment (to remove carbonates)

2 Coarse sieving (to remove large debris)

3 Hydrofluoric acid (HF) treatment (removal of silicates, e.g. clays and quartz)

4 Acetolysis (to remove extraneous cellulose material and the inner contents of pollen)

5 Potassium hydroxide (KOH) treatment (to further remove humic materials)

6 Residue concentration (e.g. fine sieving)

7 Slide mounting

All soil samples and clothing samples underwent all of the above steps, with the exception of the 'background' clothing samples and the clothing samples that had been washed prior to analysis. These samples did not undergo HF treatment because they did not contain clay or quartz. Pollen reference samples only required steps 4 to 7.

Prior to preparation, all clothing samples were soaked in a 0.03% DeCon detergent and distilled water for 24 hours. Each garment was gently agitated and manipulated to free pollen from the fabric. The wash water was centrifuged and the residue amalgamated into one 50 ml tube for processing.

Following each preparation procedure, samples were washed in distilled water and centrifuged (three minutes at 3000 rpm) three or more times between each procedure.

To each evidentiary sample, one *Lycopodium* tablet (containing 13,911 $\pm$ 689 *Lycopodium* spores) was added. This is used during pollen analysis to calculate the number of pollen grains in each sample. Each tablet was first dissolved in a few drops of HCl.

1. Hydrochloric Acid Treatment (HCl) (carbonate removal):

Samples were placed in glass beakers in 20% HCl and were placed onto a hotplate for approximately five minutes (or until 'fizzing' ceased), with constant agitation. The sample was then transferred to sterile 50 ml tubes. The sandy residue in the bottom of

the beaker was discarded after it was thoroughly rinsed to ensure that all pollen was removed.

2. Sieving (removal of unwanted organic and extraneous material):

Soil samples were sieved with 1 mm mesh to remove large fragments of wood and leaves.

3. Hydrofluoric Acid (HF) Treatment (silicate removal):

As HF is an extremely dangerous chemical and can be lethal, this step was conducted in a dedicated HF laboratory. All required safety gear was worn and Health and Safety procedures followed.

Samples were placed in 250 ml plastic screw-top containers to which 30 mls of HF were added. The containers were placed on a mechanical stirrer and left for 48 hours. Samples were then washed / centrifuged three times with hot distilled water.

In order to remove the fluorosilicates that form during this treatment, samples were returned to glass beakers and 100 to 200 mls of 50% HCl solution added. Samples were heated for ten minutes then rinsed three times with hot distilled water.

4. Acetolysis (cellulose removal):

Samples, in 10 or 50 ml screw-top centrifuge tubes, were first dehydrated with two acetic acid washes. Acetolysis mixture (1 part concentrated sulphuric acid [H_2SO_4] to 9 parts acetic anhydride [$(CH_3CO)_2O$]) was then added to each sample. Sample tubes were placed in a boiling water bath for 5-8 minutes, with agitation every few minutes. Tubes were then filled to capacity with acetic acid (which mixes with both Acetolysis mixture and water, enabling the sample to be returned to a hydrated environment) and centrifuged. Two further washes with acetic acid and three to four with distilled water followed.

5. Potassium Hydroxide Treatment (KOH) (dissolves humic material):

To each sample tube 10% KOH was added. Tubes were placed in a boiling water bath for a three minutes. Numerous washes with distilled water followed until the sample supernatant was clear. Approximately 29 mls of 10% HCl was added to the

tubes which were placed into a boiling water bath for 5 minutes then rinsed three to four times with distilled water. This procedure is to return the samples to an acidic environment.

6. Residue concentration:

To further concentrate the pollen size fraction of the residue, it was sieved with fine mesh. Reference pollen samples were sieved with 100 μm – 200 μm mesh. Clothing samples were sieved with 200 μm mesh. Residue in the mesh sieve was microscopically examined to ensure that only unwanted residue was sieved out.

7. Slide Mounting:

The water supernatant was removed from all pollen samples, leaving approximately 2 ml of water in each. Pollen was re-suspended in the remaining supernatant. One to two drops (depending on sample quantity) of pollen suspension was mixed with a weak PVA glue solution on a clean, new 40 x 22 mm cover-slip. The pollen was fixed to the cover-slip by placing the slip onto a hotplate, and allowing the PVA and water to evaporate. Once dry, the cover slip (containing the pollen sample) was inverted onto a clean, new glass microscope slide containing several drops of Eukitt, a plastic mounting medium. The slides were labelled and allowed to dry for several days.

Four slides were prepared for each soil sample, and where possible the clothing samples. The background clothing and clothing that was washed prior to analysis had only enough sample to produce two slides each. Two slides were produced for all reference pollen samples.

2.6 Microscopy and Analysis

Microscopic examination was conducted at the SEE Microscopy Laboratory at UWA. All slides were examined using an Olympus BX60 transmitted light microscope (TLM) at x400 and x1000 with scanning at x200. Palynomorphs were photographed at x1000 (unless otherwise stated) using a Nikon DS5M digital camera attached to the TLM. The images were formatted using the Adobe®Photoshop®5.0 programme.

For each reference sample collected, 10 grains were measured to give an average size and size range and basic morphological features were noted to later aid identification in the soil and clothing samples.

Pollen assemblages for each soil and clothing sample were established by counting and identifying 200 – 300 pollen grains per sample and scanning the remaining slides not used in the count to identify additional pollen types not encountered in the count. A count of 200 grains is considered acceptable for the relative pollen percentages in a given assemblage (Bryant, 2000).

3. VEGETATION

This chapter describes the project garden, the major plants within it, their habit and pollination vectors. A schematic diagram of the research garden is provided in Figure 3.2 and a full list of plants identified in and near the research garden is recorded in Table 3.2.

3.1 Site Description

The research area chosen is in the north-west corner of the front garden of St George's College, Crawley, Western Australia. The garden is 6 m x 6.5 m (39 m^2) and is located behind a residential house with office buildings at its front (see Fig. 2.2 in Ch. 2). A fenced parking lot runs along the western side and a walkway runs along the eastern side. The garden is relatively over-grown with an ivy ground-cover, *Nephrolepis cordifolia* (fishbone ferns), *Hebe* bushes and features several large *Asplenium australasicum* (Bird's nest fern). A large *Harpephyllum caffrum* (Kaffir Plum) tree grows on the other side of the western fence and its canopy overhangs the garden, concealing it from street view. Garden beds immediately north of the site contain *Agapanthus praecox, Camellia japonica, Chlorophytum comosum*, *Cyathea cooperi* (Tree fern), *Galtonia candicans*, *Philodendron selloum*, *Punica granatum, Pittosporum undulatum* and *Rondeletia amoena* plants. Other garden beds containing rose bushes and numerous exotics are located within 10 to 20 m south, south-east and east of the site. Large trees, including *Liquidambar styraciflua*, *Jacaranda mimosifolia*, *Quercus suber* (Cork Oak) and *Araucaria heterophylla* (Norfolk Island pine) grow in the immediate area surrounding the garden (within 20 m). Numerous other large trees grow within the vicinity of St George's College front garden.

The research site was chosen because of the rich botanical make-up of the garden and its surrounds and the secluded nature of the area. The garden is 'typical' of a location where an assault may take place. Horrocks and Walsh (1999) and Mildenhall (2006) describe rape and assault cases that occurred in secluded garden locations flanked by buildings.

Figure 3.1 Research site located in the North-West corner of St George's College's front garden.

3.2 St George's College Gardens

St George's College was founded on native Australian bushland. The garden has been planted and re-planted many times over the years and as a result pollen from plants previously growing in gardens is likely to occur in the soil. A list of the major plants that were originally planted in the garden was recorded by Wills-Johnson (1981) and is listed below (Table 3.1). Some of these plants are still present in the gardens today.

Table 3.1 List of the known major flora of St George's College from Wills-Johnson (1981)

Family	Botanical Name
Araucariaceae	*Araucaria heterophylla*
Bignoniaceae	*Jacaranda mimosifolia*
Cupressaceae	*Cupressus macrocarpus*
Fabaceae	*Cassia fistula*
Fagaceae	*Quercus coccinea*
Fagaceae	*Quercus suber*
Moraceae	*Ficus benjamina*
Moraceae	*Ficus hilli*
Myrtaceae	*Eucalyptus marginata*
Pinaceae	*Pinus pinaster*
Pittosporaceae	*Pittosporum*
Rosaceae	*Rosa* sp.
Salicaceae	*Populus* sp.
Sterculiaceae	*Brachychiton acerifolium*

Table 3.2 lists the plants in and near the research garden site identified by Martin McLeish of Program Maintenance Services, Gardener for St George's College; Cate Tauss, botanist with Ecologia Environmental Consultants; and Henry and Adrian Boogard of Boogard's Nursery.

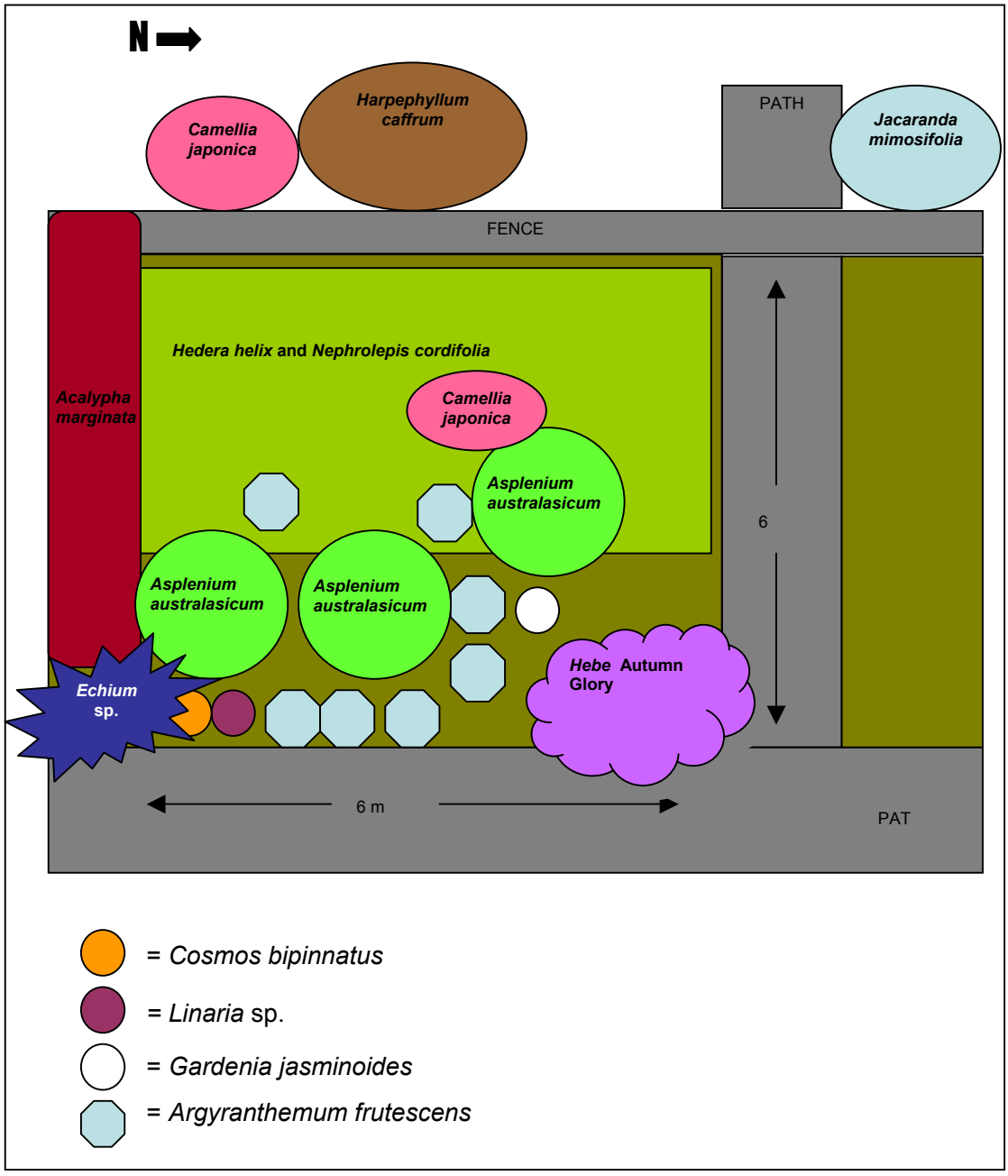

Figure 3.2 Schematic of research garden site showing position of plants. Measurements are approximate.

Table 3.2 Flora identified in and near the research garden site at St George's College

Botanical Name	Family	Common Name
Acalypha marginata	Euphorbiaceae	Acalypha 'copper leaf'
Acer pseudoplatanus	Sapindaceae	Sycamore
Agapanthus praecox	Alliaceae	Agapanthus
Agonis flexuosa subsp. *flexuosa*	Myrtaceae	Native Peppermint
Amaranthus sp.	Amaranthaceae	Amaranthus
Araucaria heterophylla	Araucariaceae	Norfolk Island Pine
Argyranthemum frutescens	Asteraceae	Marguerite Daisy
Asplenium australasicum	Aspleniaceae	Birds Nest Fern
Bauhinia sp.	Caesalpiniaceae	Bauhinia
Brachychiton acerifolius	Sterculiaceae	Illawarra Flame Tree
Brachyscome iberdifolia	Asteraceae	Swan River Daisy
Brunfelsia sp.	Solanaceae	Yesterday, Today and Tomorrow or Kiss-Me- Quick
Callistemon sp.	Myrtaceae	Bottlebrush
Camellia japonica / sasanqua	Theaceae	Camellia
Cassia fistula	Fabaceae	Yellow Tree
Chlorophytum comosum	Agavaceae	Spider Plant
Cosmos bipinnatus	Asteraceae	Cosmos
Cupressus sp.	Cupressaceae	Cypress
Cyathea cooperi	Cyathaceae	Tree Fern
Echium sp.	Boraginaceae	
Eucalyptus globulus	Myrtaceae	Tasmanian blue gum
Galtonia candicans	Liliaceae	
Gardenia jasminoides	Rubiaceae	Gardenia 'Florida'
Harpephyllum caffrum	Anacardiaceae	Kaffir Plum
Hebe Autumn Glory	Scrophulariaceae	Hebe – Veronica 'Autumn Glory'
Hedera helix	Araliaceae	Ivy
Hibiscus rosa-sinensis	Malvaceae	Hibiscus (Wilders White)
Impatiens sp.	Balsaminaceae	Busy Lizzie
Jacaranda mimosifolia	Bignoniaceae	Jacaranda
Jasminum sp.	Oleaceae	Jasmine
Leptospermum laevigatum	Myrtaceae	Victorian Teatree
Leucojum aestivum	Amaryllidaceae	Snowflake
Ligustrum sp.	Oleaceae	Privet
Linaria sp.	Plantaginaceae	
Liquidambar styraciflua	Altingiaceae	Liquidambar
Lophostemon confertus	Myrtaceae	Oueensland Box Tree
Magnolia grandiflora	Magnoliaceae	Magnolia evergreen
Melaleuca linariifolia	Myrtaceae	Snow in Summer
Melaleuca quinquinerva	Myrtaceae	Paperbark
Nephrolepis cordifolia	Davaliaceae	Fishbone Fern
Nerium oleander	Apocynaceae	Oleander
Pennisetum clandestinum	Poaceae	Kikuyu Grass
Philodendron selloum	Araceae	Philodendron
Pittosporum undulatum	Pittosporaceae	Pittosporum
Punica granatum	Lythraceae	Pomegranate
Quercus robur	Fagaceae	Common Oak
Quercus suber	Fagaceae	Cork Oak
Rhaphiolepis delacourii (pink)	Rosaceae	Indian hawthorn
Rhaphiolepis indica (white)	Rosaceae	Indian hawthorn
Rondeletia amoena	Rubiaceae	Rondeletia
Rosa sp.	Rosaceae	Rose
Salvia farinacea	Lamiaceae	Salvia
Syagrus romanzoffiana	Arecaceae	Cocos Island Palm
Tamarix aphylla	Tamaricaceae	Athel Pine
Triadica sebifera	Euphorbiaceae	
Trifolium sp.	Fabaceae	Clover
Vinca roseus	Apocynaceae	Vinca
Viola sp.	Violaceae	Violet

3.3 Significant Plants in the Research Garden

Although the garden has a wide variety of plants there are some species that are prominent within the garden itself and in the areas surrounding the garden. The habit, pollination type and general information on these plants is discussed below, this information is important to help understand the amount of pollen produced and its presence or absence in the samples. For example, if there was a high amount of daisy pollen (an insect pollinated plant) in the control soil and forensic clothing samples, it could be explained by the fact that there are several daisy plants growing and flowering in the research garden, or that the clothing was brushed against a flowering daisy plant during the assault. Likewise, a plant may be in abundance at the research garden, but little to none of its pollen may be seen in the control soil samples and forensic clothing samples because it produces very little pollen.

3.3.1 *Araucaria heterophylla* 'Norfolk Island Pine' (Araucariaceae):

Araucaria heterophylla is an evergreen, cone-producing tree native to the Norfolk Island. It can grow to a height of 60-70 m with a spread of 6 m and grows best in well-drained soils (Bodkin, 1986; Wilson, 1994). *Araucaria heterophylla* is pollinated by wind (Owens *et al.*, 1998).

Figure 3.3 *Araucaria heterophylla*

An *Araucaria heterophylla* tree grows within 20 m east of the research garden. As it is wind pollinated, some *A. heterophylla* pollen would be expected in the control soil and forensic clothing samples even though it is not within the research garden. Some pollen would also be expected on the background clothing samples that were worn around the St George's College grounds.

3.3.2 *Argyranthemum frutescens* 'Marguerite Daisy' (Asteraceae):

Argyranthemum frutescens is an erect or ascending shrub native to the Canary Islands (Spencer, 2002). It grows to a height of 90 cm and produces white flowers. Over 70 cultivars with varying petal colours exist (Spencer, 2002). *Argyranthemum frutescens* is from the Asteraceae family and is pollinated by insects (Florabase, 2008).

Several *A. frutescens* bushes grow along the eastern border of the research garden and were flowering at the time of the simulated assault. Although these plants are insect pollinated, some pollen is expected in the control soil and forensic clothing samples due to the number of flowering plants in the garden.

Figure 3.4 *Argyranthemum frutescens.*

3.3.3 *Asplenium australasicum* 'Birds-nest Fern' (Aspleniaceae):

Asplenium australasicum is a large tropical fern native to Queensland, New South Wales and Lord Howe Island, Australia, and the Pacific Islands. In its native habitat *A. australasicum* often grows as an epiphyte on trees, or lithophyte on rocks, and prefers filtered light and humic soils. The 'leathery' fronds grow up to 1 m in length and 20 cm in width, forming a large 'nest' in which to catch debris for nutrients (Greig, 1987; Spencer, 1995). *Asplenium australasicum* produces spores on the underside of the fronds that are carried by the wind when released (Goudey, 1988). *Asplenium australasicum* is often confused with *A. nidus* which also grows in Queensland and tropical Asia and Africa. *Asplenium nidus* requires glass-house conditions to grow in temperate regions.

There are several large *A. australasicum* growing in the understorey of the garden, all of which were producing spores at the time of the simulated assault. High amounts of *A. australasicum* spores would be expected in the control soil and forensic clothing samples.

Figure 3.5 *Asplenium australasicum.*

3.3.4 *Cyathea cooperi* 'Tree Fern' (Cyathaceae):

Cyathea cooperi is an evergreen tree fern native to New South Wales and Queensland, Australia. It grows in moist rich soils, prefers shaded areas and can grow up to 10-14m in height with a 4-6 m spread. Maturity is reached at approximately four years when spore production begins (Bodkin, 1986; Goudey, 1988; Spencer, 1995; Edwards, 2005). Spores are produced on the underside of the fronds and are released and carried by wind.

Figure 3.6 *Cyathea cooperi.*

Two mature *C. cooperi* plants grow within a 2 m distance north-west of the research site. *Cyathea cooperi* spores would be expected in the control soil and forensic clothing samples.

3.3.5 *Harpephyllum caffrum* 'Kaffir Plum' (Anacardiaceae)

Harpephyllum caffrum is an evergreen tree native to South Africa that can grow up to 15 m in height and flowers in the summer period. In its natural habitat, *H. caffrum* attracts many insects and birds, indicating pollination by insects or other animals (Exell *et al.*, 1966; Plamer and Pitman, 1972; Palgrave, 1977).

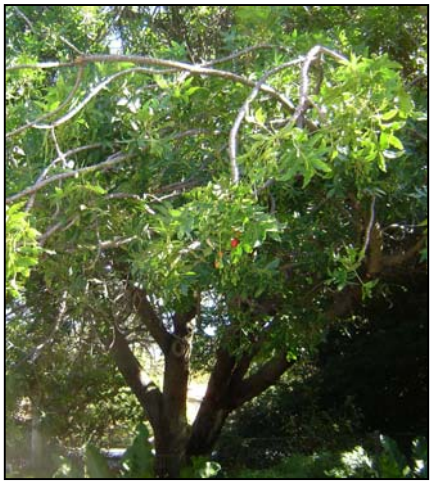

Figure 3.7 *Harpephyllum caffrum.*

Harpephyllum caffrum grows at the western border of the garden with its branches forming the upper storey of the garden. Although *H. caffrum* is insect pollinated some pollen is expected in the control soil and forensic clothing samples, as the plant grows directly over the garden. As this tree is mature, pollen released over previous years will have accumulated in the soil of the garden.

3.3.6 *Hebe* Autumn Glory (Scrophulariaceae):

Hebe is a low-growing evergreen shrub native to Australia and temperate South America, with cultivated species native to New Zealand and the United Kingdom. The cultivar Autumn Glory is thought to have originated in Ireland. It grows to a height of 45-60 cm and flowers in the summer periods. Flowers appear as purple 'spikes' and are hermaphroditic (Garnock-Jones, 1993; Spencer, 2002; Metcalf, 2006).

There are numerous *Hebe* plants in the research garden which were flowering at the time of the simulated assault. As these plants produce very little pollen, little to no pollen is expected in the control soil or forensic clothing samples.

Figure 3.8 *Hebe* Autumn Glory.

3.3.7 *Hedera helix* 'Ivy' (Araliaceae)

Hedera helix is an evergreen woody climber native to central Europe, North Africa and West Asia (Bodkin, 1986; Fitter and Fitter, 1993; Spencer, 2002). It has become naturalised in New South Wales and Victoria, Australia. It can grow as a ground cover, with older plants becoming 'shrub-like' (Spencer, 2002). *Hedera helix* produces small green or yellow flowers in summer, but only when 'the plant has grown above its support' (Bodkin, 1986). There are various cultivars of *H. helix* (Spencer, 2002).

Hedera helix grows as a ground cover in the research garden; however never appeared to flower during the duration of this study.

Figure 3.9 *Hedera helix*

3.3.8 *Nephrolepis cordifolia* 'Fishbone Fern' (Davaliaceae):

Nephrolepis cordifolia is a perennial fern native to north-eastern Australia, New Zealand and South-East Asia. Growing best in damp soils in a protected environment, *N. cordifolia* can grow up to 1 m in height and width, and when mature produces spores on the underside of the fronds. The spores are released and carried by the wind (Bodkin, 1986; Goudey, 1988; Spencer, 1995; Mabberley, 1997).

Nephrolepis cordifolia is a prominent ground cover of the garden, occupying approximately 50% of the total garden space. *Nephrolepis cordifolia* produces a large number of spores that are predominantly distributed by wind. Its spores would be expected in both the control soil and forensic clothing samples.

Figure 3.10 *Nephrolepis cordifolia.*

3.3.9 *Liquidambar styraciflua* 'Liquidambar' (Altingiaceae):

Liquidambar styraciflua is a large wind pollinated deciduous tree native to America which can grow to a height of 40 – 50 m with a spread of 5 m. It prefers rich, moist soils such as swampy woods but is adaptable to other environments. The flowers of *L. styraciflua* are unisexual, without petals and produce a spiny fruit (Preston, 1949; Correll and Hohnston, 1970; Wisniewski and Bogle, 1982; Bodkin, 1986).

Liquidambar styraciflua occurs within 20 m south-east of the research garden. As *L. styraciflua* is wind pollinated, some of its pollen would be expected in control and forensic clothing samples, despite the tree being some distance from the actual garden site. *Liquidambar styraciflua* pollen would also be expected on the background clothing worn around the St George's College grounds.

Figure 3.11 *Liquidambar styraciflua*

3.3.10 *Philodendron selloum* (*bipinnatifidum*)'Philodendron' (Araceae):

Philodendron selloum is an ever-green shrub native to tropical America that thrives in moist, rich, but well-drained soil and prefers shaded areas (Bodkin, 1986). This tree-like shrub can grown to a height and spread of 2 m and produces small white and brown flowers which thermoregulate (warm up to approximately 35-42^{o} C) to attract beetles as pollen carriers (Bodkin, 1986; Seymour and Schultze-Motel, 1997).

Several *P. selloum* grow approximately 14 m north of the research garden so a limited amount of *P. selloum* pollen would be expected in the research garden soil.

Figure 3.12 *Philodendron selloum.*

3.3.11 *Quercus suber* 'Cork Oak' (Fagaceae):

Quercus suber is a large ever-green tree native to the Mediterranean which grows in non-carbonated, sandy soils. It can grow up to 20 m in height with a spread of up to 4 m. *Quercus suber* flowers in summer in the northern hemisphere and is pollinated by wind. Both male and female flowers are produced on the same individual tree (Polunin, 1976; Mitchell, 1978; Humphries *et al.*, 1981; Bodkin, 1986; Gomez-Casero *et al.*, 2004; Pausas *et al.*, 2006). *Quercus suber* has thick, corky bark and was commonly planted for commercial use (Polunin, 1976; Mitchell, 1978; Humphries *et al.*, 1981).

A large *Q. suber* tree, planted when the gardens of St George's College were originally established, grows approximately 10 m from the research garden. Considerable amounts of *Q. suber* pollen are expected in the control soil, forensic clothing and background clothing samples.

Figure 3.13 *Quercus suber.*

4. RESULTS - Occurrence of Palynomorphs

Pollen assemblages from the Control Soil samples, Evidentiary and Background Clothing samples are presented and discussed in this chapter. Where possible, the pollen from each sample was identified to the lowest possible taxonomic level by comparison to the reference pollen samples collected from the vegetation survey of St George's College gardens, slides from the Balme reference collection and LAM reference collections available in the School of Earth and Environment (SEE) at the University of Western Australia (UWA), databases on international websites and additional identification by Dr Lynne Milne. Pollen that could not be identified to species level has been grouped by family or by morphological type (e.g. Poaceae spp, Tricolporate sp.1). Florabase, the Database of Western Australian Flora, managed by the Western Australian Herbarium, was used to gather information on the Australian plants.

Pollen types identified in all samples are listed in Appendix 1-3. Some pollen grains could not be identified due to degradation and loss of external characteristics required for identification. Degraded pollen grains and grains that could not be identified due to the grain folding or breaking have been recorded as Gen *et* sp. indet. Plates 1-6 are selected photomicrographs of pollen grains from reference collections and soil and clothing samples. In Tables 4.1, 4.2, 4.4, 4.5, 4.6 - 4.10, 4.12 and 4.13 key species have been identified (highlighted) and most other species have been grouped into major families and morphological types.

4.1 Grouping of Major Families and Morphological Types

Pollen from some plant families is ubiquitous in Western Australian soils and are often difficult to identify to a species level. For the purpose of this research they have been grouped.

4.1.1 Asteraceae: There are many species of plant belonging to this family, commonly called the Daisy Family. Pollen from the Asteraceae family is tricolporate (three pores and three colpi) and is characterised by its spiny surface sculpture (echinate). Several *Argyranthemum frutescens* (Marguerite Daisy) plants and a single *Cosmos bipinnatus* (Cosmos) plant were growing in the research garden. Pollen

collected from these plants is very similar in size, shape and sculpture. There were five distinct types of Asteraceae pollen present in the control soil samples and forensic clothing samples. These were named Asteraceae sp. 1-5. Pollen from both *A. frutescens* (Marguerite Daisy) and *C. bipinnatus* (Cosmos) that occur in the garden is very much like Asteraceae sp. 3. Asteraceae pollen that could not be identified to one of these groups was placed in Asteraceae spp.

4.1.2 Myrtaceae: Myrtaceae is a large family with 47 genera and 1174 species in Western Australia (current in Florabase). Major genera include *Eucalyptus* and *Corymbia* (Eucalyptus), *Callistemon* and *Calothamnu*s (bottlebrushes) and *Melaleuca* (paperbarks). Myrtaceae pollen is triangular and has three pores and three colpi that merge or anastomose at the poles (syncolporate or parasyncolporate). Myrtaceae pollen is ubiquitous in Western Australian pollen assemblages and is often difficult to separately identify to the species level based on pollen characteristics. For the total pollen count (Appendix 1-3), myrtaceous pollen was divided into three groups for the clothing and control soil samples; Myrtaceae sp. 1 (thick-walled Eucalypt-type) Myrtaceae sp.2 (moderately thick-walled Eucalypt / Melaleuca-type) and Myrtaceae sp.3 (Melaleuca-type aperture on grain apices). For percent occurrence and statistical comparison of all samples in this chapter (Control soil, Forensic clothing and Background samples) (Tables 4.1, 4.4, 4.5, 4.9, 4.10, 4.12 and 4.13) the myrtaceous groups were combined into the group Myrtaceae spp. No key species from the research garden were of the Myrtaceae family but plants in the surrounds of St George's College include both *Eucalyptus* and *Melaleuca*. A variety of myrtaceous species grow on the northern border of the St George's College Gardens (Fig 2.2) as this borders with Kings Park and Botanical Gardens.

4.1.3 Poaceae (grasses): There are approximately 640 species listed by Florabase that belong to the Poaceae family in Western Australia. Poaceae pollen is spherical in shape with a single pore (monoporate). Beyond size considerations, it is often difficult to distinguish between Poaceae species by their pollen morphology. Like Myrtaceae pollen, Poaceae pollen is ubiquitous in Western Australian soils. No key species in the garden belonged to the Poaceae family. All Poaceae pollen has been grouped into Poaceae spp.

4.1.4 Monocolpate and Tricolporate groups: There were numerous pollen grains that could not be identified beyond their morphological characteristics that fall into this group. These consisted of monocolpate grains (single colpus) and tricolporate grains (three colpi and three pores). There were three distinct types of monocolpate pollen that were divided into three groups (Monocolpate sp. 1-3). Unidentified monocolpate pollen that did not fit into these groups was placed in Monocolpate spp. There were 11 distinct groups of tricolporate pollen (Tricolporate sp. 1-11). Unidentified tricolporate pollen that did not fit into these groups was placed in Tricolporate spp.

4.1.5 Genus and Species Indeterminate (Gen. *et* sp. indet.): Pollen grains that could not be identified due to degradation or mechanical distortion of the morphological features (e.g. fragmentation of the grain) were grouped in Gen. *et* sp. indet. during the initial pollen count. Tables of the initial counts are in Appendices 1-3. For the tables in the results chapters (chapters 4 and 5), pollen that occurred rarely and was not considered significant in the analysis of results have also been grouped into Gen. *et* sp. indet.

4.2 Control Soil Palynological Profiles

Triplicate control soil samples were collected from two areas of the research garden. One area was the immediate 'scene' (Scene Area) and is approximately 2 m x 1 m and the second area was the entire garden site, approximately 6 m x 6.5 m (Local Area). The two areas are indicated in Figure 4.1.

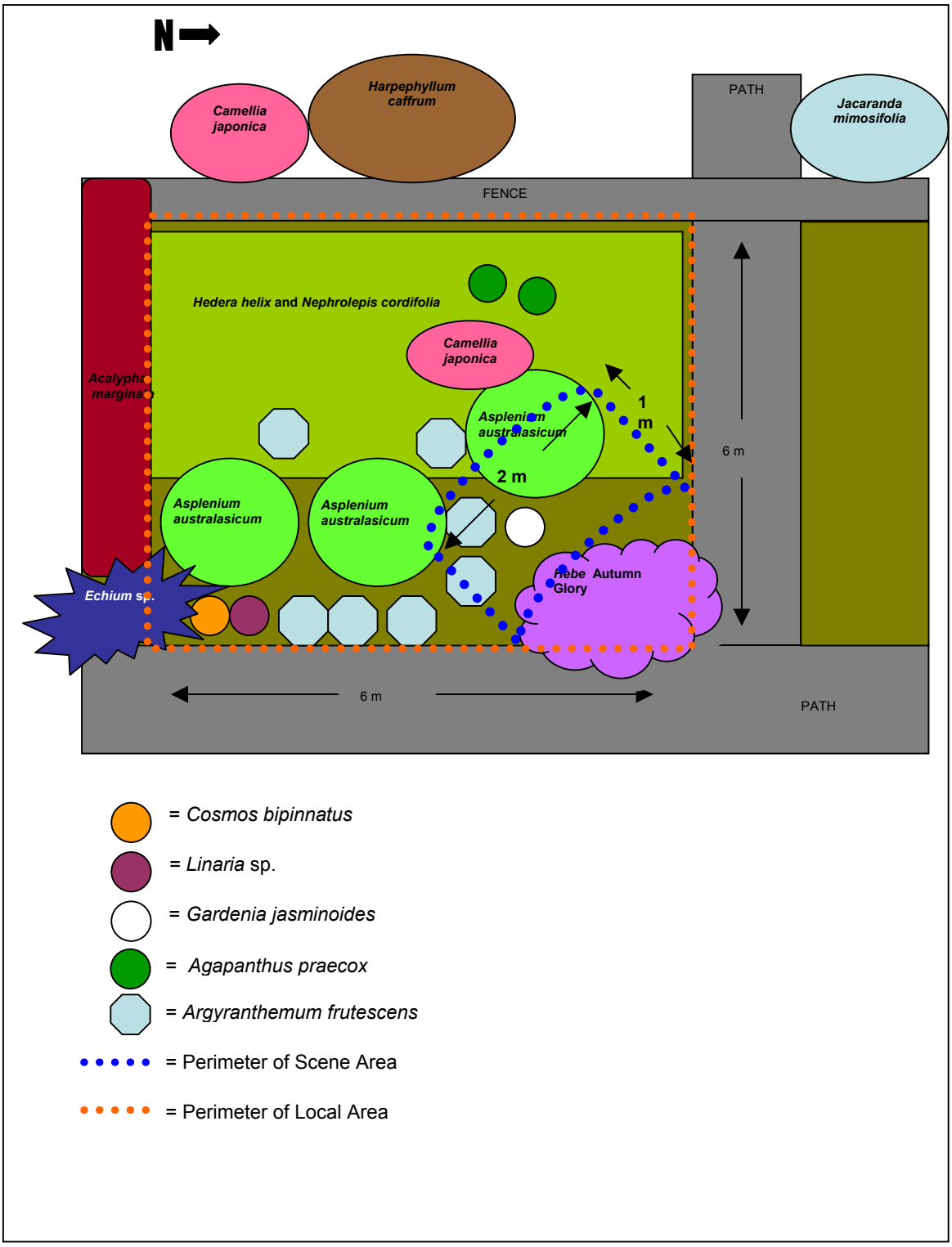

Figure 4.1 Schematic of research garden site detailing the scene and local sample collection areas. Measurements are approximate.

The palynological profiles of the two soil samples are expected to be similar, but with some minor differences because some plants are more abundant in the Local Area compared to the Scene Area or are absent altogether (Fig. 4.1). For example, a higher

pollen percentage of *Argyranthemum frutescens* (Marguerite Daisy) pollen would be expected in the Scene soil compared to the Local soil, as the soil taken for the Scene control sample has come from directly beneath several *A. frutescens* plants. Likewise, the Local control sample is expected to have a higher percentage of *Nephrolepis cordifolia* (Fishbone Fern) spores as there is an abundance of this species growing towards the back area of the garden, and not so much in the Scene Area.

4.2.1 Significance of Species

In the tables of the results presented in this chapter (Tables 4.1, 4.2, 4.4, 4.5, 4.6 - 4.10, 4.12 and 4.13), major pollen types not identified as 'key species' have been grouped.

The species highlighted in red have been identified as the **Key plant species** currently growing in or near the research garden. In total, there are 11 Key species. These are: *Araucaria heterophylla* (Norfolk Island Pine), *Asplenium australasicum* (Birdsnest Fern), Asteraceae sp. 3 (Marguerite Daisy and Cosmos), *Cyathea cooperi* (Tree Fern), *Gardenia jasminoides* (Gardenia), *Harpephyllum caffrum* (Kaffir Plum), *Hebe* Autumn Glory, *Liquidambar styraciflua* (Liquidambar), *Nephrolepis cordifolia* (Fishbone Fern), *Philodendron selloum* (Philodendron) and *Quercus suber* (Cork Oak).

Many of the plant species growing in the garden are insect pollinated. Finding pollen from these species on the forensic clothing, even in low percentages, is highly significant as the item of clothing must come into direct contact either with the flowering plant itself or the soil nearby. The trees *Q. suber*, *A. heterophylla,* and *L. styraciflua* grow near the garden and a significant level of their pollen is expected in the control soil samples because they are high pollen producers and their pollen is dispersed by wind.

Pollen from the families Myrtaceae, Poaceae, Chenopodiaceae, Casuarinaceae, Liliaceae and from *Pinus radiata* are not considered key due to their general occurrence in most Western Australian soils. However, their overall percentage occurrence is considered in the general comparison of pollen assemblage

characteristics because high or low percentages of common species are often an important comparative factor.

Table 4.1 Percent occurrence of all pollen groups in the control soil samples
* indicates that species is present in the sample but was not encountered during the 200 grain count. Blue highlighted species indicate the identified 'Key' plant species that are growing in or near the garden.

	Control Soil Samples							
	Soil Scene				Soil Local			
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.
Palynomorphs								
Araucaria heterophylla	0.5	1		0.5	0.5	*		0.17
Asplenium australasicum	0.5	0.5	1	0.67	2	0.5	0.5	1
Asteraceae sp. 3	0.5	*	*	0.17		*	1.5	0.5
Asteraceae spp	5.5	3.5	5.5	4.83	15	15	12.5	14.17
Casuarinaceae spp	*	2		0.67	1	0.5		0.5
Chenopodiaceae spp	1.5	1.5	1.5	1.5	2.5	2	0.5	1.67
Cyathea cooperi	*	*		*	0.5		0.5	0.33
Gardenia jasminoides						0.5		0.17
Harpephyllum caffrum	3.5	0.5	4	2.67	1.5	1.5	3.5	2.17
Hebe Autumn Glory			1	0.33	0.5			0.17
Liliaceae spp	1	2	1	1.33	*	1.5	1.5	1
Liquidambar styraciflua					0.5	*		0.17
Myrtaceae spp	29	32	24.5	28.5	20.5	21.5	19.5	20.5
Nephrolepis cordifolia	1	1	0.5	0.8	9	11.5	10	10.17
Philodendron selloum	2	1	0.5	1.17	1	0.5	0.5	0.67
Pinus radiata	1	1	1.5	1.17	2.5	0.5	1	1.33
Poaceae spp	33	36.5	40	36.5	25.5	31	30	28.83
Proteaceae spp	0.5	0.5		0.33				
Quercus suber	2	2	1.5	1.83	3	2	0.5	1.83
Tricolporate spp	5.5	1	5.5	4	1.5	5	4.5	3.67
Gen. *et* sp. indet.	13	14	12	13	13	6.5	13.5	11
Total Key Species	10	6	8.5	8.17	18.5	16.5	17	17.33

4.2.2 Pollen Assemblage Characteristics of the Scene and Local Control Soils

Key Species

The most abundant pollen of the key species growing within the research garden is that of *Harpephyllum caffrum* which ranges from 0.5 – 4 %. This is expected, as the foliage of this tree provides the canopy of the research garden. Other key species within the garden include *Nephrolepis cordifolia* (0.5 -9%), *Hebe* Autumn Glory (0.5 – 1%), *Gardenia jasminoides* (0.5%), Asteraceae sp. 3 (0.17 – 1.5%), and *Asplenium*

australasicum (0.5 – 2%). The total number of Key species as opposed to all other pollen types in the control soil samples (labelled Others) is shown in table 4.2.

Species designated as Key species that are not growing in the research garden but are within close proximity include *Quercus suber* (0.5 – 3%), *Philodendron selloum* (0.5 – 2%), *Liquidambar styraciflua* (0.5%), *Cyathea cooperi* (0.5%) and *Araucaria heterophylla* (0.5 – 1%).

Table 4.2 – Total Percent occurrence of Key species in the control soil samples
* indicates that species is present in the sample but was not encountered during the 200 grain count. Blue highlighted species indicate the identified 'key' plant species that are growing in or near the garden.

	Control Soil Samples							
	Soil Scene				Soil Local			
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.
Palynomorphs								
Araucaria heterophylla	0.5	1		0.5	0.5	*		0.17
Asplenium australasicum	0.5	0.5	1	0.67	2	0.5	0.5	1
Asteraceae sp. 3	0.5	*	*	0.17		*	1.5	0.5
Cyathea cooperi	*	*		*	0.5		0.5	0.33
Gardenia jasminoides						0.5		0.17
Harpephyllum caffrum	3.5	0.5	4	2.67	1.5	1.5	3.5	2.17
***Hebe* Autumn Glory**			1	0.33	0.5			0.17
Liquidambar styraciflua					0.5	*		0.17
Nephrolepis cordifolia	1	1	0.5	0.8	9	11.5	10	10.17
Philodendron selloum	2	1	0.5	1.17	1	0.5	0.5	0.67
Quercus suber	2	2	1.5	1.83	3	2	0.5	1.83
Others	90	94	91.5	92	81.5	83.5	83	82.67
Total Key Species	10	6	8.5	8.17	18.5	16.5	17	17.33

Non-Key Species

Poaceae spp (25.5 – 40%) and Myrtaceae spp (19.5-29%) are the dominant groups in all control samples. The group Gen. *et* sp.indet. comprises 6.5-14% of the total pollen percentage and Asteraceae spp at 3.5 – 15% and Tricolporate spp at 1-5.5% are the next most prominent groups. The high level of Asteraceae pollen is unusual as 1-2% of Asteraceae pollen is considered normal as background levels in most West Australian soils. However, the research garden and the St George's College grounds contain various plants belonging to the Asteraceae family. The low percentage of pollen from wind pollinated species such as Casuarinaceae spp at 0.5 – 2%, Chenopodiaceae spp at 0.5 – 2% and *Pinus radiata* at 0.5 – 2.5 %, is also expected as their pollen is also ubiquitous in small amounts in Western Australian soils. Plants of

these species were observed growing in the greater Crawley area. The research garden is sheltered by dense vegetation on three sides (Fig 4.1) which likely reduces the amount of wind-borne pollen that settles in it. Rare Proteaceae spp at 0.5% include *Stirlingia* sp., an uncommon wind pollinated Proteaceae species, *Banksia / Dryandra* species, *Grevillea* Robyn Gordon and several unidentified species. Although none of these plants were currently growing in the research garden, *Grevillea* Robyn Gordon is a common garden plant and the remainder of these occur in Kings Park Botanical gardens and would have been part of the native vegetation prior to the establishment of the St George's College gardens. Liliaceae spp pollen is also present in the control soil in low amounts (1-1.5%).

4.2.2.1 Comparison of triplicate soil samples

Scene Soil Replicates

On average, nine of the 11 Key species are present in the Scene Control soil sample. There is general consistency in the pollen percentage occurrence of each species between each replicate. Of the Key species, only minor variations between replicates is noted. The variations occur in species present in low numbers. This includes *A. heterophylla* (0.5% in replicate one [R1] and 1% in replicate two [R2]), *C. cooperi* (noted in R1 and R2), Hebe Autumn glory (1% in replicate three [R3]) and *N. cordifolia*. The non-key pollen groups, which include Asteraceae spp (3.5-5.5%), Casuarinaceae spp (2% in R2 and noted R1), Chenopodiaceae spp (01.5%), Liliaceae spp (1- 2%), Myrtaceae spp (29-32), *Pinus radiata* (1-1.5%), Poaceae spp (33-40%), Proteaceae spp (0.5% in R1 and R2) and Tricolporate spp (1-5.5%) do not vary considerably between each replicate, with only two groups, Casuarinaceae spp and Proteaceae spp not having been counted in all of the replicates.

Local Soil Replicates

On average, all 11 Key species are present in the Local Soil and there is general consistency in pollen percentages between each replicate. Of the Key species identified, at least five are present in each replicate. The species which are not present in each replicate are once again the species occurring in low amounts. This includes *A. heterophylla* (0.5 % in R1 and noted in R2), Asteraceae sp. 3 (1.5% in replicate three and noted in replicate two), *C. cooperi* (0.5% in R1 and R3), *G. jasminoides* (0.5% in R2), *Hebe* Autumn glory (0.5% in R1) and *L. styraciflua* (0.5% in R1 and

noted in R2). Pollen percentages within the non-key species are closely similar in all replicates, with only Casuarinaceae spp (1% in R1 and 0.5% in R2) not noted in R3 of the Local soil samples.

In general, pollen from insect and animal pollinated species will be under-represented in a soil sample whereas pollen from wind pollinated plants will be over-represented. The pollen of *L. styraciflua* and *A. heterophylla*, and spores of *C. cooperi*, are distributed by wind but their parent plants are some distance from the research garden. This, combined with the protected nature of the garden, explains the low abundance of pollen from these Key species in the Control pollen assemblages.

Gardenia is insect pollinated and *Hebe* is self pollinated (Florabase), so little pollen is produced and little to none will be found in the soil. The *Gardenia* plant was very young and not flowering at the time of the simulated assault. These factors, combined with the 'dilution' effect, and that only a fraction of the soil mix is actually observed, explains why species such as *Hebe* and *Gardenia* do not appear in high percentages or are absent from the soil samples. The dilution effect occurs when pollen from insect or self pollinated species are present in low amounts and are swamped by a large number of palynomorphs from wind pollinated species. When the pollen from one of the insect or self pollinated species does occur, the forensic value is high due to the low expected pollen occurrence.

In an actual forensic case where palynological evidence is to be heard in court, much larger counts (1000 + grains counted per sample) would be conducted to ensure that no, or few, species are missed due to its low abundance in a given sample (Milne, *pers. comm*.).

The counts of the replicates for the Scene and Local samples were totalled to make a pollen count of 600 grains per sample, and then converted to percentages. The more grains counted the more accurate the representation of the relative occurrence of palynomorphs in the sample (Bryant, 2000). Major differences in the average pollen percentages between the Scene and Local sample include a greater number of *N. cordifolia* spores and Asteraceae spp pollen and a lower amount of *H. caffrum* in the Local sample compared to that in the Scene sample. This is a result of vegetation

change over the sample area. As Figure 4.1 illustrates, there are numerous *N. cordifolia* ferns growing to the west side of the research garden and many *A. frutescens* (Asteraceae) plants growing in the middle and on the east side of the research garden. The soil collected for the Scene sample was taken from the north-east side of the garden where fewer to no *N. cordifolia* and *A. frutescens* grow. The lower amounts of *H. caffrum* likely occurs because there are simply more plants in the Local area compared to the Scene area, and therefore more palynomorphs of various species exist, diluting the *H. caffrum* pollen count.

4.2.3 Conclusion

The Key species growing in or near the research garden have been identified and their relative percentages in the control soils established. The combination of these species and their percent occurrence provide a unique pollen assemblage for the research garden. If these species are present in similarly relative amounts in the forensic samples, a link between them and the garden may be established.

With the exception of some pollen types that are present in very low amounts in the soil, the pollen percentages of the Key species within each replicate are close. The minor difference in the species pollen percentage occurrences between the averaged Local Area and Scene Area pollen assemblage is attributed to the different species present and their concentration within each area (Fig. 4.1).

4.3 Background Samples

Two types of background samples were collected:

- Clothing (three replicate sets of T-shirts and jeans) was worn for three consecutive days before the pollen samples were collected from them and analysed (Background Clothing).
- Two compost soil samples were collected from a freshly composted flower bed adjacent to the research site (Background Compost).

The purpose of these collections was to see the amount and type of 'background' pollen that may be collected by clothing if it were worn for several days prior to an assault, and if compost would significantly contribute to the palynological profile of a garden sample.

4.3.1 Background Clothing

The clean Background Clothing (three T-shirts and three jeans in total) was worn for three consecutive days to accumulate a general airborne background pollen assemblage. The locality to which each set of clothing was worn is listed in Table 4.3. The percent occurrence of pollen for each sample is recorded in Table 4.4.

Table 4.3 List of the Background samples and the locality worn

Clothing Set Number	Locations Worn
Set One	Perth City, Crawley and Fremantle.
Set Two	Myaree, Perth City, Crawley
Set Three	Innaloo, Crawley

Table 4.4 Percent occurrence of pollen in the background clothing samples
Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Un-highlighted species are all other species present on the clothing samples.

	Background One Samples							
	Background T-Shirt				Background jeans			
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.
Palynomorphs								
Acacia spp	0.67	1.33		0.67		1	*	0.2
Araucaria heterophylla	60.67	1.67	11.7	24.68	66	14.33	8.33	29.6
Asteraceae spp	0.33	1.33	1	0.89	0.33	6.67	2.33	3.11
Banksia/Dryandra spp	*			*				
Betula sp.	*			*				
Casuarinaceae spp	1.33	1	3	1.78	0.33	0.67	0	0.33
Chenopodiaceae spp					0.33	1.67	6.67	2.89
Cryptogam spore spp							0.67	0.22
Cyathea cooperi	*	0.33		0.1				
Grevillea Robyn Gordon					*			*
Harpephyllum caffrum		0.33	1	0.44				
cf. Harpephyllum caffrum			1	0.33			*	*
Liquidambar styraciflua	3.33	45	34.67	27.67	3.33	33	23.33	19.89
Monocolpate sp. 2							0.33	0.11
Monocolpate spp	3.67			1.22	1.33			0.44
Myrtaceae spp	3.33	17.33	19.67	13.44	6.67	17.67	12.67	12.34
Nephrolepis cordifolia	0.33	0.33	0.33	0.33				
Pinus radiata	14	9.33	2.3	8.54	14.67	8.33	8.67	10.56
Poaceae sp. 1	*			*			2.33	0.78
Poaceae spp	0.67	5	3.33	3	0.33	7.67	7	5
Stirlingia spp					*			*
Tricolporate spp	4.67	11	4.33	6.67	4	5.67	10.33	6.67
Gen *et* sp. indet.	7	6	17.67	10.22	2.67	3.33	17.33	7.78
Total Key Species	64.33	47.67	47.67	53.22	69.33	47.33	31.67	49.44

Pollen is found almost everywhere, and as a large number of plant species are pollinated by wind, it is expected that there will be some 'background' pollen deposited on clothing as it is worn during general daily activities. The areas in which each set was worn (Table 4.3) are reflected in the pollen assemblages recovered from each item. Set one was worn to three locations - Perth City, Crawley area (including St George's College grounds and the University of Western Australia [UWA]) and Fremantle. Set two was worn around the suburb Myaree, Perth City and the Crawley area (including St George's College grounds and UWA). Set three was worn around the suburb Innaloo and Crawley area (UWA only).

In general, the palynological profiles of the T-shirts and jeans sets worn at the same time are closely similar to each other but different from the profiles from the other clothing sets (i.e. T-shirt 1 and jeans 1 were worn together). For example, the set one (S1) T-shirt and jeans both have very high percentages of *Arauacaria heterophylla* (60.17% and 66% respectively) pollen. This is because these items were worn to Fremantle where a large number of A. *heterophylla* occur in parklands in the city area.

The pollen assemblages from replicate sets two (S2) and three (S3) are different from S1 but are quite similar to each other in the percent occurrence of the species identified. The S2 and S3 sets both have a high occurrence of *Liquidambar styraciflua* (33-45% for S2 and 23.33 – 34.67% for S3) and Myrtaceae spp (17.3 – 17.67% for S2 and 12.67 – 17.67% for S3) and a lower occurrence of *A. heterophylla* (1.67 – 14.33% for S2 and 8.33 – 11.7% for S3) and *Pinus radiata* (8.33 – 9.33% for R2 and 2.3 – 8.67% for R3). These results, especially those of R1, demonstrate how palynology can be used to determine where an item of clothing has been worn if dominant wind pollinated species are present at the location.

Between the T-shirts and jeans in each replicate set there are some minor differences. There are *Nephrolepsis cordifolia* spores (all at 0.33%) on each of the T-shirt replicates, but none on the jeans. Likewise, there is Chenopodiaceae spp pollen (0.33 – 6.67%) present on all of the jeans replicates but none on the T-shirts. This is not unexpected as during wearing, the jeans are exposed to different surfaces than the T-shirts. Each of the jeans had long trouser legs that may have brushed against grasses

and weeds. The seat of the jeans may also be exposed to different pollen and spores as many people will sit on grassed areas. What must also be considered is the presence of pollen on each item before its experimental use. Although each item was washed prior to use in the simulated assault, any pollen collected on the clothing before washing and pollen collected during washing (i.e. from the other items washed with the clothes and from the water in which the clothes were washed) may have remained trapped in the weave of the fabric.

In total, only five or less of the 11 Key species described in section 4.2 are present in the Background Clothing sets (Table 4.4). These include *A. heterophylla* (1.67 – 66%), *Cyathea cooperi* (0.33%), *Harpephyllum caffrum* (0.33 – 1%), *Liquidambar styraciflua* (3.33 – 45%) and *N. cordifolia* (all 0.33%). Only the T-shirt of R2 contains all five of the key species, four of which are common wind-pollinated species (*A. heterophylla, L. styraciflua, C. cooperi* and *N. cordifolia*). The jeans of all three replicates contain only *A. heterophylla* and *L. styraciflua.* The percent occurrence of each key species present is **considerably different** to the percent occurrences of the key species in the control soil samples (Tables 4.1 and 4.2) and four of the five species (*A. heterophylla, L. styraciflua, C. cooperi* and *N. cordifolia*) are common wind pollinated plants.

More of the Key species are present on the T-shirt replicates (with R1 and R3 retaining four of the key species and R2 retaining five), than the jeans. All three of the jeans replicates contained only two of the key species, *A. heterophylla* and *L. styraciflua*.

Both S2 and S3 were exposed to the same wind pollinated species as found in or near the research garden, however the pollen percentage of these species on the background clothing sets is quite different to those in the control sample pollen assemblages. From these results it is obvious that the background clothing sets have not come into direct contact with the soil in the research garden site but have been in a similar area.

4.3.2 Compost samples

To investigate the possible palynomorph contribution from compost previously laid on or near the site, two compost soil samples were collected from an adjacent, freshly composted flower bed. The relative percent occurrences of pollen in the two compost samples are listed in Table 4.5.

Plants that were growing in the garden that was sampled for compost included *Acalypha marginata, Camellia japonica / sasenqua, Cyathea cooperi, Hydrangea* sp., *Linaria* sp., *Pittosporum undulatum, Salvia farinacea* species and a *Violaceae* sp. ground cover. The compost was a commercial style mix of manure, bark and other organic matter. The compost had been added to the soil approximately a week prior to the sample collection and the soil of the garden had been mixed through with the compost. Some pollen and spores from the plants growing in the garden and nearby would be expected to be mixed into the compost in low amounts.

Compost is comprised of decomposed organic matter and can be home-made or commercially bought. Its function is to hold moisture and provide support and nutrients for growing plants. Compost often consists of animal waste, food products and plant material. The palynological inputs of these ingredients are of interest as the palynological profile of soil in a garden may be influenced by the addition of compost.

Table 4.5 Percent occurrence of pollen in two compost samples

Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Un-highlighted species are all additional species present in the compost samples.

Background Compost Samples			
Samples	**Rep 1**		**Rep 2**
Palynomorphs			
Asplenium australasicum			1
Casuarinaceae spp	*		2
Chenopodaceae spp	1		*
Asteraceae spp	2.5		2
Nephrolepis cordifolia	0.5		
Poaceae spp	27.5		40
Cryptogam spore spp	*		*
Monocolpate spp	1		
Myrtaceae spp	25		21.5
Pinus radiata	1.5		0.5
Cyathea cooperi	1.5		
Tricolporate spp	14		10.5
Gen *et* sp. indet.	25.5		22.5
Total Key Species	2		1

The compost samples are dominated by pollen of the Myrtaceae (21.5 – 25%) and Poaceae (27.5 – 40%) families, undetermined species (Gen. *et* sp. indet.) (22.5-25.5 %) and various fungal spores (Appendix 3). Of the Key species identified in section 4.2, only three species are present in low numbers. These include *Asplenium australasicum* (1% in replicate two only), *N. cordifolia* (0.5% in replicate one only) and *C. cooperi* (1.5% in replicate one only). As these species grow in and around the composted garden it is likely that they have come from the garden itself rather than from the compost.

It could not be said from these results that the addition of compost would greatly influence or change the Key species for the control soil samples collected from the research garden. The organic and moist nature of compost is favourable to fungal growth and many small fungal spores of various types were present in the compost samples analysed. The presence of a variety of fungal spores in a sample can be as significant as any palynomorph in defining the unique characteristics ('fingerprint') of the soil. For the purpose of this research, fungal spores have not been identified beyond what is recorded in the original count-tables (Appendix 1 to 3).

The pollen assemblages from the two compost samples are fairly close in individual species percentages with some minor differences in low occurring species. For example, Monocolpate spp pollen is present in Compost replicate one (C1) at 1% but is absent from Compost replicate two (C2). *Nephrolepis cordifolia* spores are present at 0.5 % in C1 but not in C2. The likely reason these species have been detected in low amounts in one sample and not the other sample is because only a fraction of the mixed soil is actually analysed and because only 300 grains were counted per sample.

Additional species present include Casuarinaceae spp (2% in C2) Chenopodiaceae spp (1% in C1), Asteraceae spp (2 – 2.5%), Monocolpate spp (1% in C1), *Pinus radiata* (0.5 – 1.5%), Tricolporate spp (10.5 – 14%) and Gen. *et* sp. indet. (22.5 – 25.5%). With the exception of Tricolporate spp and species Gen. *et* sp. indet., all additional species are present in low amounts. It is important to note that Asteraceae spp and Tricolporate spp recorded here are general counts of pollen belonging to these broad groups, and not the same as the Asteraceae species identified as Key in the control samples.

The high amount of Myrtaceae spp and Poaceae spp pollen indicates that the main contribution that this particular type of compost would have on a soil pollen assemblage would be 'background' pollen originating mainly from wind pollinated species. Very few of the key species are present in this sample despite some of the plants (e.g. *N. cordifolia*) being directly above the site where the compost was collected. This is likely because the compost is fresh and, although mixed in with the topsoil, the pollen assemblage from the plants in the composted site has been diluted by the compost. Considering this, the large amounts of Tricolporate spp pollen and Gen. *et* sp. indet. pollen is likely to have come from the compost itself and not from the plants in the composted garden. This is important to consider when analysing the pollen percentages of the soil and samples from the research site, as pollen from these 'groups' may have also originated from compost rather than from the plants from the garden itself. Although these species may be unidentified, they are still extremely important as clothing that has come into contact with soil containing compost will still carry a palynological profile that includes pollen from compost that has been added to the soil or may even be an important marker.

4.4 Forensic Clothing Samples (Evidentiary)

Three test conditions for the forensic clothing samples were chosen. Each clean clothing set was first used for a simulated rape in the research garden. Samples were then collected from one set immediately (Evidentiary 1), from another set after being worn for three consecutive days (Evidentiary 2) and the third set after being washed (Evidentiary 3).

4.4.1 Evidentiary 1 (Immediate analysis)

The items of clothing for the Evidentiary 1 (E1) condition were involved in the simulated sexual assault then immediately analysed. The percent occurrence of pollen from the Key species present is listed in Table 4.6. The group labelled 'Others' contains all other species present. All pollen types identified in all samples are listed in Appendix 1-3.

Table 4.6 Percent occurrence of pollen from Key species in the Evidentiary One samples and Control sample averages. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden.

	Evidentiary One Samples								Control Soil Samples	
	Immediate T-Shirt				Immediate jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
Araucaria heterophylla		1.5	1	0.83	0.5		1	0.5	0.5	0.17
Asplenium australasicum	5.5	9	7	7.17	6.5	10.5	12.5	9.83	0.67	1
Asteraceae sp. 3	2	1	1.5	1.5	1	1	1	1	0.17	0.5
Cyathea cooperi		0.5		0.17			1	0.33	*	0.33
Gardenia jasminoides		0.5		0.17		0.5		0.17		0.17
Harpephyllum caffrum	1.5	5	4.5	3.67	3	2	7	4	2.67	2.17
Hebe Autumn Glory					0.5			0.17	0.33	0.17
Liquidambar styraciflua	1	1	1.5	1.17		0.5	1	0.5		0.17
Nephrolepis cordifolia	2	2.5	0.5	1.67	0.5	0.5	1	0.67	0.8	10.17
Philodendron selloum	0.5	1	0.5	0.67	1.5	1		0.83	1.17	0.67
Quercus suber	1	1	2.5	1.5	1.5	0.5	1	1	1.83	1.83
Others	86.5	77	81	81.5	85	83.5	74.5	81	92	82.67
Total Key Species	22	27	21	23.3	20.5	20.5	33	24.7	8.17	17.33

On average, 10 of the Key species are present in the E1 T-shirt replicates. Pollen and spores of *A. heterophylla,* Asteraceae sp. 3, *C. cooperi, G. jasminoides, Hebe, L. styraciflua, N. cordifolia, P. selloum* and *Q. suber* fall within a range of 0.5-2.5%.

Asplenium australasicum and *H. caffrum* are the most prominent Key species with a range of 5.5-7% and 1.5-5% respectively.

The jean replicates contain all 11 Key species. *Araucaria heterophylla* (0.5-1%), Asteraceae sp. 3 (1%), *C. cooperi* (1%), *G. jasminoides* (0.5%), *Hebe* (0.5%), *L. styraciflua* (0.5-1%), *N. cordifolia* (0.5-1%), *P. selloum* (1-1.5%), *Q. suber* (0.5-1.5%) fall within a range of 0.5-1.5. Like the T-shirt replicates, *A. australasicum* and *H. caffrum* are the most prominent Key species with a range of 6.5-12.5% and 2-7% respectively.

Figure 4.1 shows that the simulated attack took place directly near an *A. australasicum* fern. This explains the increase in percent occurrence of *A. australasicum* spores in the E1 samples compared to the Control Soil samples, as the clothing came into contact with soil directly under the fern and with the actual fern. Conversely, there are few *N. cordifolia* plants growing in this immediate area, explaining the smaller percent occurrence of this species compared to the control soil samples.

With the exception of *Hebe*, the percentages of the 600 grain count for both T-shirt and jeans is closely similar. The difference between the T-shirt and jeans 600 grain count (i.e. the 'Average' columns) for the total Key species is less than 1.5%. This is an example of how a greater pollen count provides more consistent comparative results.

4.4.3 Evidentiary 2 (3 Day Wear)

The items of clothing for the Evidentiary 2 (E2) condition were involved in the simulated sexual assault and then worn for three consecutive days before the sample was collected for analysis. The percent occurrence of pollen from the Key species is listed in Table 4.7**.**

Table 4.7 Percent occurrence of pollen from Key species in the Evidentiary Two samples and Control sample averages. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden.

	Evidentiary Two Samples								Control Soil Samples	
	3 Day Wear T-Shirt				3 Day Wear jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
Araucaria heterophylla	1.5	*		0.5	0.5			0.17	0.5	0.17
Asplenium australasicum	2.5	1.5	3.5	2.5	4.5	1.5	29.5	11.83	0.67	1
Asteraceae sp. 3	0.5	1		0.5	1.5	0.5	1	1	0.17	0.5
Cyathea cooperi	1.5	0.5	1	1	*	0.5	1.5	0.67	*	0.33
Gardenia jasminoides										0.17
Harpephyllum caffrum	1	2	2.5	1.83	1.5	*		0.5	2.67	2.17
Hebe Autumn Glory									0.33	0.17
Liquidambar styraciflua	3.5	2.5	5	3.67	3	3.5	2.5	3		0.17
Nephrolepis cordifolia	0	1	1	0.67	3	2	1.5	2.17	0.8	10.17
Philodendron selloum	2.5	2	1.5	2	1	0.5		0.5	1.17	0.67
Quercus suber	3.5	20.5	7.5	10.5	2	1.5	5.5	3	1.83	1.83
Others	83.5	69	78	76.83	83	90	58.5	77.17	92	82.67
Total Key Species	21	32.5	24.5	26	22	15	43	26.67	8.17	17.33

On average, nine of the Key species are present in the E2 T-shirt replicate samples. Pollen of *A. heterophylla, A. australasicum,* Asteraceae sp. 3, *H. caffrum, N. cordifolia* and *Philodendron selloum,* and spores of *C. cooperi*, fall within a range of 0.5-3.5 %. *Quercus suber* is the most prominent Key species with a range of 3.5-20.5%.

The jean replicates contain the same nine Key species. *Araucaria heterophylla,* Asteraceae sp. 3, *C. cooperi, H. caffrum, L. styraciflua, N. cordifolia* and *P. selloum* fall within the same range as the T-shirt replicates (0.5-3.5 %). *Asplenium australasicum* and *Q. suber* are the most prominent Key species with a range of 1.5 – 29.5% and 3.5-20.5% respectively. As for the E1 samples, the higher percent occurrence of *A. australasicum* and Asteraceae sp. 3 in the E2 samples as compared to the control samples can be explained by the location where the simulated assault occurred within the garden (Figure 4.1).

The E2 clothing was worn around the Crawley area, particularly around the St George's College gardens as noted in Table 4.11 above. This is reflected in the

higher percentage of *C. cooperi, L. styraciflua* and *Q. suber* in the E2 samples compared to the control samples because these plants currently grow in the St George's College Gardens and in the surrounding Crawley areas. *Liquidambar styraciflua* and *Q. suber* are wind-pollinated trees and *C. cooperi* is a spore producing fern. The pollen and spores of these species will be abundant in the air.

Percentages of the 600 grain count ('Average' column, Table 4.7) for both T-shirts and jeans are similar. Minor differences include a higher percentage of *A. australasicum, L. styraciflua* and *N. cordifolia* in the jeans replicates and a higher percentage of *Q. suber* in the T-shirt replicates. The difference between the T-shirt and jeans 600 grain count for the total Key species is less than 0.7%.

4.4.4 Evidentiary 3 (Wash)

The Evidentiary 3 (E3) clothing was involved in the simulated sexual assault then washed before a sample was collected for analysis. This was to determine if a pollen assemblage collected after washing retains the palynological characteristics of the assault site. The percent occurrence of pollen from the Key species in the E1 samples is listed in Table 4.8**.**

Table 4.8 Percent occurrence of pollen from Key species in the Evidentiary Three samples and Control sample averages**.** Blue highlighted species indicate the identified Key plant species that are growing in or near the garden.

	Evidentiary Three Samples								Control Soil Samples	
	Wash T-Shirt				Wash jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
Araucaria heterophylla	0.5	1.5	4	2	1.5	2.5	0.5	1.5	0.5	0.17
Aspleniaceae	6	1	2.5	3.17	6	4	2.5	4.17	0.67	1
Asteraceae sp. 3	0.5	0.5		0.33	1			0.33	0.17	0.5
Cyathea cooperi		1.5	0.5	0.67	*		0.5	0.17	*	0.33
Gardenia jasminoides					0.5			0.17		0.17
Harpephyllum caffrum	1.5	2.5	2.5	2.17	2	2	2.5	2.17	2.67	2.17
***Hebe* Autumn Glory**									0.33	0.17
Liquidambar styraciflua	4	8.5	5.5	6	8	7.5	2	5.83		0.17
Nephrolepis cordifolia	0.5	1	0.5	0.67	2	2	0.5	1.5	0.8	10.17
Philodendron selloum		0.5		0.17			0.5	0.17	1.17	0.67
Quercus suber	2	1.5	1	1.67	*	1		0.33	1.83	1.83
Others	85	81.5	83.5	83.33	79	81	91	83.67	92	82.67
Total Key Species	16	18.5	19	17.83	23.5	22.5	10	18.67	8.17	17.33

On average, nine of the Key species are present in the E3 T-shirt replicates. *Araucaria heterophylla, A. australasicum,* Asteraceae sp. 3, *C. cooperi, H. caffrum, N. cordifolia, P. selloum* and *Q. suber* all fall within a range of 0.5-4%. The most prominent Key species with a range of 1-6% and 4-8.5% are *A. australasicum* and *L. styraciflua* respectively.

The jeans replicates contain the same nine key species as in the T-shirt replicates with the addition of *G. jasminodies* in replicate one (R1). *Araucaria heterophylla, A. australasicum ,* Asteraceae sp. 3, *C. cooperi, H. caffrum, N. cordifolia, P. selloum* and *Q. suber* all fall within a range of 0.5-2.5%. As for the T-shirt replicates *A. australasicum* and *L. styraciflua* are the most prominent Key species with a range of 2.5-6% and 2-8% respectively.

The higher percentage of *A. australasicum* spores in both the T-shirt and jeans replicates of the E3 samples compared to the control samples is explained by the location where the simulated assault occurred within the garden (Fig. 4.1). It is also possible that some of this pollen was contributed by the other items that the E3 samples were washed with.

With the exception of *G. jasminoides*, the percentages of the 600 grain count for both T-shirt and jeans is similar. There is a minor increase in *N. cordifolia* in the jeans replicates and an increase of *Q. suber* in the T-shirt replicates. The difference between the T-shirt and jeans 600 grain count of the total Key species is less than 1.5%.

Discussion

No less than nine of the 11 Key species remains in each of the Evidentiary samples despite the differing conditions that each Evidentiary item underwent. The E1 clothing pollen assemblages are most closely similar to the control sample assemblages as compared to the other Evidentiary samples with 10 of the Key species present at a similar percent occurrence to the Control samples. This is because each item was taken immediately for analysis and had little movement to lose soil adhering to the garments and little exposure to other surfaces or locations. The percent

occurrence of each Key species in the E1 samples is also most closely similar to the Control samples compared to the other Evidentiary samples. There is also minimal difference in pollen assemblages between the T-shirt and jeans replicates of the E1 samples. This shows the importance of collecting clothing evidence as soon as possible after an 'event' as the palynological evidence will be at its 'best' because minimal soil (pollen and spores) will be lost from or added to (from other sources) the garments.

Although the E2 clothing was worn for three consecutive days after the simulated assault, the pollen assemblages from the E2 samples are still closely similar to the Control Soil samples. The nine Key species present still have a similar percent occurrence to the Control samples despite some loss of soil during the three-day wear period. This is a remarkable and very important quality of palynological evidence, as the palynological evidence is not lost despite the garments being worn and then collected several (three) days after an 'event'.

The E3 clothing samples, although not as closely similar to the Control Soil samples as the E1 and E2 samples, are still similar in the percent occurrence of the nine Key species remaining as to those in the Control Soil samples, despite being washed after the simulated assault. Pollen and spores would have been lost (and added) during the washing process, however the percent occurrence of the Key species on the clothing demonstrates that the palynological evidence is still present. These results demonstrate that, although it is important to collect evidentiary samples as soon as possible (to reduce pollen and spore loss due to mechanical movement or addition from other sources), palynological evidence may still be present despite garments having been washed.

4.5 Other Species of Importance (Additional Key Species)

After analysing the forensic samples and comparing them with the Control soil samples it became evident that additional palynomorphs that could not be attributed to the plants currently growing in the garden were common to both the Control and Evidentiary samples.

Although these species were present in the control samples, they do not appear to have originated from any of the plants currently growing in the research garden or within the grounds of St George's College. This group includes the palynomorphs Asteraceae sp. 1 and 4, Cryptogam spore sp. 1, *Jasminum* sp, Tricolporate sp. 3, 7, 8 and 9 and *Typha* sp. cf. *muralis*. All of the plants growing in the research garden were sampled for pollen where possible, however some, including *Acalypha marginata, Echium* sp. and *Hedera helix,* did not produce flowers during the research period or no pollen on flowers collected was recovered.

It is believed that most of these Additional Key species belong to plants that were growing in the garden prior to this research, belonged to plants that did not produce any pollen at the time of sampling or may have been transferred to the garden from added compost. The St George's College gardens are frequently tended and re-planted, with smaller plants removed or replaced as their flowering season ends. Pollen from these plants will remain in the soil and contribute to the unique palynological profile of the research garden.

The following section discusses the occurrence of the Additional Key species and other species of interest in the replicate samples for each evidentiary set. The percent occurrence of Key species and Additional Key species of the three replicates from the Control samples have been averaged and listed in Table 4.9 for comparison.

Table 4.9 Percent occurrence of pollen from Key and Additional Key species in the Control Soil samples. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Beige highlighted species indicate the additional Key species.

	Control Soil Samples							
	Soil Scene				Soil Local			
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.
Palynomorphs								
Araucaria heterophylla	0.5	1		0.5	0.5	*		0.17
Asplenium australasicum	0.5	0.5	1	0.67	2	0.5	0.5	1
Asteraceae sp. 1	0.5	0.5	1.5	0.83		1		0.33
Asteraceae sp. 3	0.5	*	*	0.17		*	1.5	0.5
Asteraceae sp. 4		*		*	*			*
Asteraceae spp	5	3	4	4	15	14	12.5	13.83
Casuarinaceae spp	*	2		0.67	1	0.5		0.5
Chenopodiaceae sp.1	1	1.5	1.5	1.33	1	0.5		0.5
Chenopodiaceae spp	0.5	*	*	0.17	1.5	1.5	0.5	1.17
Cryptogam spore sp. 1	*	*	*	*	0.5		0.5	0.33
Cyathea cooperi	*	*		*	0.5		0.5	0.33
Gardenia jasminoides						0.5		0.17
Harpephyllum caffrum	3.5	0.5	4	2.67	1.5	1.5	3.5	2.17
***Hebe* Autumn Glory**			1	0.33	0.5			0.17
***Jasminum* sp.**	0.5	0.5		0.33	1			0.33
Liliaceae spp	1	2	1	1.33	*	1.5	1.5	1
Liquidambar styraciflua					0.5	*		0.17
Myrtaceae spp	29	32	24.5	28.5	20.5	21.5	19.5	20.5
Nephrolepis cordifolia	1	1	0.5	0.8	9	11.5	10	10.17
Philodendron selloum	2	1	0.5	1.17	1	0.5	0.5	0.67
Pinus radiata	1	1	1.5	1.17	2.5	0.5	1	1.33
Poaceae spp	33	36.5	40	36.5	25.5	31	30	28.83
Proteaceae spp	0.5	0.5		0.33				
Quercus suber	2	2	1.5	1.83	3	2	0.5	1.83
Tricolporate sp. 3	1	*	0.5	0.5		*	*	*
Tricolporate sp. 7	1		2	1	1.5	1	0.5	1
Tricolporate sp. 8		*		*		0.5		0.17
Tricolporate sp. 9						0.5	1	0.5
Tricolporate spp	3.5	1	3	2.5	*	3	3	2
Gen. *et* sp. indet.	12.5	13.5	12	12.67	11.5	6.5	13	10.33
Total Key Species	10	6	8.5	8.17	18.5	16.5	17	17.33

4.5.1 Evidentiary 1

The percent occurrence of pollen from the Key species, the Additional Key species and species of interest are shown in Table 4.10.

Table 4.10 Percent occurrence of pollen in the Evidentiary 1 (immediate analysis) samples and Control sample averages**.** Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Beige highlighted species indicate the Additional Key species. Purple highlighted species indicate pollen types of interest that are not Key species. Un-highlighted species are all other species present on the clothing samples.

	Evidentiary One Samples								Control Soil Samples	
	Immediate T-Shirt				Immediate jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
Araucaria heterophylla		1.5	1	0.83	0.5		1	0.5	0.5	0.17
Asplenium australasicum	5.5	9	7	7.17	6.5	10.5	12.5	9.83	0.67	1
Asteraceae sp. 1	1	1		0.67		0.5	0.5	0.33	0.83	0.33
Asteraceae sp. 3	2	1	1.5	1.5	1	1	1	1	0.17	0.5
Asteraceae sp. 4		0.5	*	0.17	0.5	0.5	0.5	0.5	*	*
Asteraceae spp	2	4.5	3.5	3.33	5.5	5.5	2.5	4.5	4	13.83
***Banksia/Dryandra* spp**					1	*	0.5	0.5	*	*
***Betula* sp.**		0.5		0.17		*		*	*	0.33
Casuarinaceae spp	2.5	1	1	1.5	1.5	1	1	1.17	0.67	0.5
Chenopodiaceae sp. 1	2	1.5	0.5	1.33		*	1	0.33	1.33	0.5
Chenopodiaceae spp	0.5	3	0.5	1.33		0.5		0.17	0.17	1.17
Cryptogam spore sp. 1	2	*		0.66	0.5		0.5	0.33	*	0.33
Cyathea cooperi		0.5		0.17			1	0.33	*	0.33
Gardenia jasminoides		0.5		0.17		0.5		0.17		0.17
Harpephyllum caffrum	1.5	5	4.5	3.67	3	2	7	4	2.67	2.17
***Hebe* Autumn glory**					0.5			0.17	0.33	0.17
***Jasminum* sp.**									0.33	0.33
Liliaceae spp					0.5			0.17	1.33	1
Liquidambar styraciflua	1	1	1.5	1.17		0.5	1	0.5		0.17
Myrtaceae spp	23	20	21.5	21.5	23.5	20.5	22	22	28.5	20.5
Nephrolepis cordifolia	2	2.5	0.5	1.67	0.5	0.5	1	0.67	0.8	10.17
Philodendron selloum	0.5	1	0.5	0.67	1.5	1		0.83	1.17	0.67
Pinus radiata	2	1.5	2.5	2	0.5	*	1	0.5	1.17	1.33
Poaceae spp	32.5	31	33.5	32.33	32.5	36	28.5	32.33	36.5	28.83
Proteaceae spp	*			*					0.33	
Quercus suber	1	1	2.5	1.5	1.5	0.5	1	1	1.83	1.83
Romula rosea	0.5			0.17		*		*		
***Rosa* sp.**					0.5			0.17		
Tricolporate sp. 1		*	0.5	0.17						
Tricolporate sp. 2						0.5		0.17		
Tricolporate sp. 3	0.5	0.5	*	0.33		0.5	0.5	0.33	0.5	*
Tricolporate sp. 4						0.5		0.17	0.5	0.33
Tricolporate sp. 5		1		0.33	0.5	0.5	0.5	0.5	0.17	*
Tricolporate sp. 6					0.5			0.17	0.17	
Tricolporate sp. 7	2	*	0.5	0.83	1	1	0.5	0.83	1	1
Tricolporate sp. 8	1	*	0.5	0.5		1	2.5	1.17	*	0.17
Tricolporate sp. 9		0.5	0.5	0.33	3	0.5	1.5	1.67		0.5
Tricolporate sp. 10	0.5	1	2.5	1.33	1	1.5		0.83		0.33
Tricolporate spp	2	2	1.5	1.83	0.5	2.5		1	2.5	2
Gen. *et* sp. indet.	12.5	7.5	12	10.83	12	10.5	11	11.17	11.83	9.33
Total Key Species	**13.5**	**23**	**19**	**18.5**	**15**	**16.5**	**25.5**	**19**	**8.17**	**17.33**

Additional Key Species

Eight of the nine Additional Key species are present in the E1 samples. This includes Asteraceae sp.1 and sp. 4 (0.5-1% and 0.5% respectively), Cryptogram spore sp. (0.5-2 %) and Tricolporate sp.3 (0.5%), sp.7 (0.5-2%), sp.8 (1-2.5%) and sp.9 (0.5-3%).

Additional Non-Key Species

Pollen types common to the Control soil samples and the E1 replicates that are not nominated Key or Additional Key species include *Banksia/Dryandra* sp. (0.5-1%), *Betula* sp. (0.5%), *Romula rosea* (0.5%), *Rosa* sp. (0.5%) and Tricolporate sp.1 [0.5%], sp.2 [0.5], sp.4 [0.5%], sp.5 [0.5-1%], sp.6 [0.5%] and sp.10 [0.5-2.5%]. However, it is important to note that some of these species, such as *Banksia/Dryandra* sp., *Betula* sp. and Tricolporate sp. 4-6 are present in low amounts in the Scene, Local or both Control Soil samples. In the E1 samples, not every replicate contained the Non-Key species. For example, the *Banksia/Dryandra* species is only present in low amounts in the jeans replicates. These species may have come from the garden itself (i.e. from plants previously planted) but were not identified in the 600 grain control Scene and Local counts or from external sources (i.e. collected on the clothing from the wind, or have been on the clothing prior to the simulated assault).

Discussion

The presence of the Additional Key species in the E1 samples as well as in the control samples further substantiates their similarity and leaves little doubt that the two assemblages are from the same locality. Additional Non-Key species of interest are present in low percentages and are not considered forensically important in the comparison of the E1 samples to the control samples. However, Non-Key species should not be ignored in evidentiary samples, as the presence of Non-Key species may indicate where an item (and thus the wearer) has been prior to an ‘event’. Pollen and spores will be picked up by clothing and shoes at each location that is visited (including from the place of manufacture). It is reasonable to expect that items for forensic investigation will contain pollen and spores from locations other than the crime scene, as these items may have been worn to different locations before and after the crime. In an investigation, Non-Key species may assist in identifying the other locations that the clothing has been worn. .

4.5.2 Evidentiary 2

The items of clothing for this condition were worn for three consecutive days before the samples were collected and analysed. The localities to which each set of clothing was worn was recorded (Table 4.11), so that the abundance of certain pollen types could be accounted for.

Table 4.11 Areas where the Evidentiary 3 samples were worn.

Clothing Set Number	Locations Worn
Set One	Perth City, Crawley (St George's College)
Set Two	Perth City, Crawley (St George's College)
Set Three	Innaloo, Crawley

The percent occurrence of pollen from the Key species, the Additional Key species and other species of interest are listed in Table 4.12**.**

Additional Key Species

Seven of the ten Additional Key species are present in the E2 pollen assemblages, although not in every replicate. The Additional Key species include Asteraceae sp.1 (T-shirt R3 and jeans R2: 0.5%), Chenopodiaceae sp. 1 (T-shirt R1, R2 and R3: 0.5-1% jeans R1: 1.5%), Cryptogram spore type 1 (T-shirt R1: 0.5% and noted in T-shirt R3), *Jasminum* sp. (T-shirt R1 and jeans R1: 1% and noted in jeans R2), Tricolporate sp. 7 (0.5-4.5%) sp. 8 (jeans R1: 0.5%) and sp. 9 (T-shirt R3: 0.5%, jeans R1: 1% and noted in T-shirt R2). These species were also identified as species of interest in the E1 samples and are present in low percentages in the control samples. They would be significant in an actual case.

Additional Non-Key Species

Several pollen types are common to some replicates of the E2 samples but were not identified as Key or Additional Key species. These include *Acacia* spp (0.5% in T-shirt R3 and noted in T-shirt R2 and jeans R2), *Banksia/Dryandra* sp. (noted in jeans R1 and R3), *Betula* sp. (T-shirt R2: 2.5%, R3: 0.5% and 3.5% in R2 and noted in R3), Monocolpate sp. 1 (T-shirt and jeans R2: 0.5% and noted in T-shirt R3), Monocolpate sp. 2 (jeans R2: 0.5% and noted in T-shirt R2), Monocolpate sp. 3 (T-

shirt R1 and R2: 0.5 %), *Pelargonium* spp (T-shirt R2 and R3: 0.5%) and Tricolporate sp. 10 (0.5-3%). Some of these species, such as *Banksia/Dryandra* sp., *Betula* sp. and Tricolporate sp. 10 are present in the Control Soil samples in low amounts and in an investigation would be important indicators. The Non-Key species in E2 are not all the same as the Non-Key species of the E1 samples. The species that are the same have a significantly different pollen percentage between the E1 and E2 samples. For example, there is a higher percentage in general of Tricolporate sp. 10 in the E2 samples.

Discussion

As for the E1 samples, the presence of the Additional Key species in the E2 samples, as well as in the control samples verifies their similarity. This strongly suggests that the two assemblages are from the same locality, despite the collection of 'background' pollen that occurred during wearing after the 'event'. Pollen types present in the E2 samples that do not appear in the control soil, such as the Monocolpate species, may be additions from the general environment (background pollen) during the 3-day wear period. As discussed in section 4.3.2, the clothing will pick up pollen from the other places that they have been worn, which may provide helpful information in an investigation.

Table 4.12 Percent occurrence of pollen in the Evidentiary 2 (3 day wear) samples and Control sample averages. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Beige highlighted species indicate the Additional Key species. Purple highlighted species indicate pollen types of interest that are not Key species. Un-highlighted species are all other species present on the clothing samples.

	Evidentiary Two Samples								Control Soil Samples	
	3 Day Wear T-Shirt				3 Day Wear jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
***Acacia* spp**		*	0.5	0.17		*		*		
Araucaria heterophylla	1.5	*		0.5	0.5			0.17	0.5	0.17
Asplenium australasicum	2.5	1.5	3.5	2.5	4.5	1.5	29.5	11.83	0.67	1
Asteraceae sp. 1			0.5	0.17		0.5		0.17	0.83	0.33
Asteraceae sp. 3	0.5	1		0.5	1.5	0.5	1	1	0.17	0.5
Asteraceae sp. 4									*	*
Asteraceae spp	3.5	1	1	1.83	1.5	5.5	1.5	2.83	4	13.83
***Banksia/Dryandra* spp**					*		*	*	*	*
***Betula* sp.**		2.5	0.5	1		3.5	*	1.17	*	0.17
Casuarinaceae spp	1	1	2.5	1.5	2.5	2.5	3.5	2.83	0.67	0.5
Chenopodiaceae sp.1	0.5	1	1	0.83	1.5			0.5	1.33	0.5
Chenopodiaceae spp	0.5			0.17		0.5		0.17	0.17	1.17
Cryptogam spore sp. 1	0.5		*	0.17					*	0.33
Cyathea cooperi	1.5	0.5	1	1	*	0.5	1.5	0.67	*	0.33
Gardenia jasminoides										0.17
Harpephyllum caffrum	1	2	2.5	1.83	1.5	*		0.5	2.67	2.17
***Hebe* Autumn Glory**									0.33	0.17
***Jasminum* sp.**	1			0.33	1	*		0.33	0.33	0.33
Liliaceae spp						0.5		0.17	1.33	1
Liquidambar styraciflua	3.5	2.5	5	3.67	3	3.5	2.5	3		0.17
Monocolpate sp. 1		0.5	*	0.17		0.5		0.17		
Monocolpate sp. 2		*		*		0.5		0.17		
Monocolpate sp. 3	0.5	0.5		0.33						
Myrtaceae spp	19	22	15	18.67	21.5	34.5	15.5	23.83	28.5	20.5
Nephrolepis cordifolia	*	1	1	0.67	3	2	1.5	2.17	0.8	10.17
***Pelargonium* spp**		0.5	0.5	0.33						
Philodendron selloum	2.5	2	1.5	2	1	0.5		0.5	1.17	0.67
Pinus radiata	2	4	3.5	3.17	2	1.5	3	2.17	1.17	1.33
Poaceae spp	40	24	37.5	33.83	32.5	19	20	23.83	36.5	28.83
Proteaceae spp									0.33	
Quercus suber	3.5	20.5	7.5	10.5	2	1.5	5.5	3	1.83	1.83
Tricolporate sp. 3									0.5	*
Tricolporate sp. 7	2.5	0.5	0.5	1.17	1	4.5	1.5	2.33	1	1
Tricolporate sp. 8					0.5			0.17	*	0.17
Tricolporate sp. 9		* (cf)	0.5	0.17	1			0.33		0.5
Tricolporate sp. 10	2	1.5	1.5	1.67	3	1	0.5	1.5		0.33
Tricolporate spp	1	2	2.5	1.83	4.5	5	2.5	4.17	2.5	2
Gen. *et* sp. indet.	9.5	7.5	10.5	9.17	10.5	10.5	10.5	10.5	12.67	9.83
Total Key Species	**16.5**	**31**	**22**	**23.33**	**17**	**10**	**41.5**	**22.83**	**8.17**	**17.33**

4.5.3 Evidentiary 3

The percent occurrence of pollen types that were observed in the E3 samples are listed in Table 4.13**.**

Additional Key Species

Six of the ten Additional Key species are present in the E3 samples, although not in every replicate. The Key species include Asteraceae sp.4 (T-shirt R1: 0.5% and jeans R1: 1%), Chenopodiaceae sp. 1 (T-shirt R3: 1% and jeans R1 and R2: 0.5%), Cryptogram spore sp. 1 (T-shirt R3: 1% and jeans R1 and R2: 0.5%), *Jasminum* sp. (jeans R1: 0.5% and noted in jeans R2), Tricolporate sp. 7 (T-shirt R1 and R3: 0.5%, jeans R3: 2.5% and J3:1%) and sp. 9 (T-shirt R3: 0.5%).

Additional Non-Key Species

The presence of pollen types other than the Key and Additional Key species is quite minimal with no species appearing beyond 1% on average, or above 4% in a single replicate. Several of these species, such as *Betula* sp.(0.5-2%), *Banksia/Dryandra* spp (T-shirt R1: 0.5%) and Tricolporate sp. 5 (0.5%) were present in the Scene and Local Control Soil sample which suggests that they may have originated from the garden itself, or that they are commonly found in most metropolitan garden soils.

Discussion

The washing of the E3 samples did not affect the relative percent occurrence of the Key and Additional Key species. On average, 10 of the 11 Key Species were present in a relative percent occurrence similar to the Control Soil samples. The presence of five Additional Key species in the E3 samples at a percent occurrence similar to the Control samples, also supports that the two assemblages are from the same locality.

A low number of Additional Non-Key species, all at relatively low percent occurrence, are present in the E3 samples. There are three obvious possibilities to account for the presence of these species. They may have been on the clothing prior to the simulated assault or are from the research garden itself. It is also possible that some of the other species identified that are not common to the Control soil samples may have come from the washing water or from the items the clothes were washed with. The results show that there is minimal contribution of these Non-Key species.

Table 4.13 Percent occurrence of pollen in the Evidentiary 3 (wash) samples and Control sample averages. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Beige highlighted species indicate the Additional Key species. Purple highlighted species indicate pollen types of interest that are not Key species. Un-highlighted species are all other species present on the clothing samples.

	Wash T-Shirt				Wash jeans				Scene	Local
Samples	Rep 1	Rep 2	Rep 3	Av.	Rep 1	Rep 2	Rep 3	Av.	Av.	Av.
Palynomorphs										
***Acacia* spp**			1	0.33		*		*		
Araucaria heterophylla	0.5	1.5	4	2	1.5	2.5	0.5	1.5	0.5	0.17
Aspleniaceae	6	1	2.5	3.17	6	4	2.5	4.17	0.67	1
Asteraceae sp. 1									0.83	0.33
Asteraceae sp. 3	0.5	0.5		0.33	1			0.33	0.17	0.5
Asteraceae sp. 4	0.5			0.17	1			0.33	*	*
Asteraceae sp. 6		0.5		0.17			1	0.33		
Asteraceae spp	5	0.5	1.5	2.33	4	2.5	4.5	3.67	4	13.83
***Banksia/Dryandra* spp**	0.5			0.17					*	*
***Betula* sp.**	0.5	0.5	0.5	0.5	2.5		0.5	1	*	0.17
Casuarinaceae spp	5	1.5	5	3.8	5	4.5	4.5	4.67	0.67	0.5
Chenopodiaceae sp.1			1	0.33	0.5	0.5		0.33	1.33	0.5
Chenopodiaceae spp	0.5	0.5	0.5	0.5	*	1		0.33	0.17	1.17
Cryptogam spore sp.1			0.5	0.17	0.5	0.5		0.33	*	0.33
Cyathea cooperi		1.5	0.5	0.67	*		0.5	0.17	*	0.33
Gardenia jasminoides					0.5			0.17		0.17
Harpephyllum caffrum	1.5	2.5	2.5	2.17	2	2	2.5	2.17	2.67	2.17
***Hebe* Autumn Glory**									0.33	0.17
***Jasminum* sp.**					0.5	*		0.17	0.33	0.33
Liliaceae spp									1.33	1
Liquidambar styraciflua	4	8.5	5.5	6	8	7.5	2	5.83		0.17
Myrtaceae spp	17.5	42.5	23.5	27.83	31.5	28	45	34.83	28.5	20.5
Monocolpate sp. 1			0.5	0.17		0.5		0.17		
Monocolpate sp. 2					0.5			0.17		
Nephrolepis cordifolia	0.5	1	0.5	0.67	2	2	0.5	1.5	0.8	10.17
***Pelargonium* spp**	0.5			0.17				0		
Philodendron selloum		0.5		0.17			0.5	0.17	1.17	0.67
Pinus radiata	5.5	2.5	3.5	3.83	2.5	1	3	2.17	1.17	1.33
Poaceae spp	35.5	17.5	29.5	27.5	19	26.5	23	22.83	36.5	28.83
Proteaceae spp									0.33	
Quercus suber	2	1.5	1	1.67	*	1		0.33	1.83	1.83
Tricolporate sp. 3									0.5	*
Tricolporate sp. 5	0.5	0.5		0.33					0.17	*
Tricolporate sp. 7	0.5		0.5	0.33		2.5	1	1.17	1	1
Tricolporate sp. 8									*	0.17
Tricolporate sp. 9			0.5	0.17						0.5
Tricolporate sp. 10	2		1.5	1.17	0.5	1.5	1	1		0.33
Tricolporate sp. 11		4		1.33						
Tricolporate spp	1.5	3.5	1	2	0.5	3.5	1	1.67	2.5	2
Triporate sp. 1			1	0.33		1		0.33		
Gen. *et* sp. indet.	9.5	7.5	12	9.67	10.5	7.5	6.5	8.17	12.5	9.83
Total Key Species	15	18.5	16.5	16.67	21	19	9	16.33	8.17	17.33

4.6 Evidentiary and Control Sample Comparisons

The three replicates for each of the Control and Evidentiary samples were combined to form a 600 grain count. The higher grain count provides a more representative relative percent occurrence of the palynological profiles for each sample. The averages of the Control and Evidentiary samples (600 count) are listed in Table 4.14.

Table 4.14 Percent occurrence of pollen from the Key species in the Control and Evidentiary samples. Blue highlighted species indicate the identified Key plant species that are growing in or near the garden. Beige highlighted species indicate the Additional Key species. Un-highlighted species are all other species present on the clothing samples.

	Averages of Control and Evidentiary samples							
	Control Soil		Evidentiary One		Evidentiary Two		Evidentiary Three	
Samples	Scene	Local	T-Shirt	jeans	T-Shirt	jeans	T-Shirt	jeans
Palynomorphs								
Araucaria heterophylla	0.5	0.17	0.83	0.5	0.5	0.17	2	1.5
Asplenium australasicum	0.67	1	7.17	9.83	2.5	11.83	3.17	4.17
Asteraceae sp. 1	0.83	0.33	0.67	0.33	0.17	0.17		
Asteraceae sp. 3	0.17	0.5	1.5	1	0.5	1	0.33	0.33
Asteraceae sp. 4	*	*	0.17	0.5			0.17	0.33
Asteraceae spp	4	13.83	3.33	4.5	1.83	2.83	2.33	3.67
Casuarinaceae spp	0.67	0.5	1.5	1.17	1.5	2.83	3.8	4.67
Chenopodiaceae sp.1	1.33	0.5	1.33	0.33	0.83	0.5	0.33	0.33
Chenopodiaceae spp	0.17	1.17	1.33	0.17	0.17	0.17	0.5	0.33
Cryptogam spore sp. 1	*	0.33	0.66	0.33	0.17		0.17	0.33
Cyathea cooperi	*	0.33	0.17	0.33	1	0.67	0.67	0.17
Gardenia jasminoides		0.17	0.17	0.17				0.17
Harpephyllum caffrum	2.67	2.17	3.67	4	1.83	0.5	2.17	2.17
***Hebe* Autumn Glory**	0.33	0.17		0.17				
***Jasminum* sp.**	0.33	0.33			0.33	0.33		0.17
Liliaceae spp	1.33	1		0.17		0.17		
Liquidambar styraciflua		0.17	1.17	0.5	3.67	3	6	5.83
Myrtaceae spp	28.5	20.5	21.5	22	18.67	23.83	27.83	34.83
Nephrolepis cordifolia	0.8	10.17	1.67	0.67	0.67	2.17	0.67	1.5
Philodendron selloum	1.17	0.67	0.67	0.83	2	0.5	0.17	0.17
Pinus radiata	1.17	1.33	2	0.5	3.17	2.17	3.83	2.17
Poaceae spp	36.5	28.83	32.33	32.33	33.83	23.83	27.5	22.83
Proteaceae spp	0.33		*					
Quercus suber	1.83	1.83	1.5	1	10.5	3	1.67	0.33
Tricolporate sp. 3	0.5	*	0.33	0.33				
Tricolporate sp. 7	1	1	0.83	0.83	1.17	2.33	0.33	1.17
Tricolporate sp. 8	*	0.17	0.5	1.17		0.17		
Tricolporate sp. 9		0.5	0.33	1.67	0.17	0.33	0.17	
Tricolporate spp	2.5	2	3.83	3	1.83	4	4.83	2.5
Gen. *et* sp. indet.	12.67	10.33	10.83	11.67	13	13.5	11.5	10.33
Total Key Species	**12.13**	**20.51**	**12.34**	**14.49**	**26.01**	**26.67**	**18.02**	**18.67**

Conclusion

In total, 20 Key and Additional Key species have been identified and their relative percentages in the Control Soil samples established (Table 4.1). The combination of these species and their percent occurrence provide a unique pollen assemblage for the research garden. The pollen percentages of the Key and Additional Key species within each replicate of the control samples are closely similar. Minor differences in the pollen percentages between the averaged Local Area and Scene Area pollen assemblages is due to the concentration of different species within each area (Fig. 4.1).

The assemblages collected from the Background Clothing samples reflected the areas to which each clothing set was worn (Table 4.3) and, in general, the palynological profiles of the T-shirts and jeans worn as a set are closely similar to each other but differ from the other clothing set profiles. There were minor differences between the T-shirts and jeans of R2 and R3 as these were worn in similar areas. R1 is considerably different from the other sets as it was worn to the Fremantle area on a particularly windy day. As a result, this replicate is dominated by the pollen of the wind pollinated species *Araucaria heterophylla.*

The minor differences between the T-shirt and jeans in each set is expected as the different garments are exposed to different wear-conditions. For example, the seat and cuffs of the jeans may be exposed to different plants/surfaces than the T-shirt, such as grass, and will retain slightly different palynomorphs as a result. The weave of the clothing fabric should also be considered. The weave of the denim jeans is much tighter than the weave of the T-shirt fabric. The presence of pollen and spores on each item before its experimental use must also be considered, as palynomorphs may be trapped in the weave of the clothing from previous wearing, washing and even manufacture. These results illustrate how palynology can be used to determine where an item of clothing has been worn.

Only five or less of the 11 Key species and only one of the Additional Key species are present in the Background Clothing sets. Furthermore, only the T-shirt of R2 contains all five of the key species present. The percent occurrence of each key species present is **considerably different** to the percent occurrences of the key species in the control

soil samples (Tables 4.1 and 4.2). This strongly indicates that the two assemblages are **not** from the same precise locality, but that they have been in an area with similar vegetation.

The background compost samples are dominated by pollen from the wind pollinated Myrtaceae and Poaceae families, undetermined species and various fungal spores (Table 4.4). This indicates that the main contribution from this type of compost to a soil pollen assemblage would be 'background' pollen, mostly from wind pollinated species. Only three of the identified Key species are present, each in very low numbers. The pollen from these species is likely to have come from the garden rather than from the compost since these species grow in and around the composted garden. Inversely, the large amounts of Tricolporate spp pollen and Gen. *et* sp. indet. pollen is likely to have come from the compost rather than from the surrounding plants. This type of palynological contribution should be considered when unidentified 'groups' such as these appear in a soil pollen assemblage. Clothing that has come into contact with soil containing compost will contain a palynological profile that includes pollen from compost that may act as an important marker.

On average, 19 of the 20 Key and Additional Key species are present in the E1 samples in similar amounts, leaving little doubt that the E1 samples are from the same locality as the control samples.

In the E2 samples, an average of 16 of the 20 Key and Additional Key species are present, which also indicates that the E2 samples are from the same or a similar locality as the control samples. The additional Non-Key pollen types present in the assemblages, likely to have been collected during the three day wear period, **do not** change the profile of the Key species in the E2 samples but may play an important role in identifying other possible locations that the clothing may have been worn. This shows the importance of knowing the locations that clothing has been worn after an event (e.g. assault) as the palynological profile of the clothing will subtlety change to reflect where each item has been. Pollen and spores from the areas where the clothing was worn prior to, or after, an 'event', may also be significant and helpful in a forensic investigation.

As for the E2 samples, the 16 Key and Additional Key species present in the E3 samples indicate that they are from the same, or a similar, locality as the Control samples. Additional pollen, either from the water in which the clothes were washed, or from the other items which were washed with the clothes, **do not** diminish the link between the clothes and the research garden despite the reduction in overall pollen retention. These results demonstrate the importance of knowing if a forensic sample has been washed after the 'event', because additional palynomorphs or low numbers of key species can be explained.

The results presented in this chapter show that even after clothing has been worn for a short period of time or washed after an event, clothing that has been abrasively rubbed in the soil of a particular site will still retain a palynological profile closely similar to that of the site. These results also illustrate that it is important for the police to ask where clothing may have been worn prior to, or after, an event. The palynological profile of the clothing will subtlety change as pollen and spores are collected from each place that the clothing is worn.

5. RESULTS - Quantitative Palynology

This chapter will discuss the concentration of palynomorphs calculated to occur in the samples collected during this study. The Total Palynomorph Number (TPN) in the control soil samples per gram, and the number of palynomorphs retained by each clothing item (Background and Evidentiary clothing), were calculated by the addition of a *Lycopodium* 'spike' which acted as an internal standard. This technique is commonly used in Quaternary palynology (geography) to calculate pollen and charcoal density of sediments for the analysis of changes in palaeoclimate and palaeovegetation. The calculation of TPN has not been use in forensic palynology to date. It is used in this study to determine the amount of pollen retained by clothing under varying conditions, and to illustrate the importance of pollen occurrence compared to pollen amount.

5.1 *Lycopodium* Spike

Prior to sample processing, a single *Lycopodium* tablet (Lot number 124961) was added to each sample (with the exception of the Background Compost samples) as an internal standard.

The manufacturers instructions from the Canadian Association of Palynologists (http://www.scirpus.ca/cap/supply.htm) state that five *Lycopodium* tablets (Lot number 124961) contain 62,712 spores with a standard deviation of ± 2,081. Using the formula provided by Maher (1997), one *Lycopodium* tablet contains 12,542 spores with a standard deviation of ± 931.(Maher, 1997).

Approximate total palynomorph number in a sample can be calculated by using the formula:

$$\frac{X}{Y} = \frac{N}{Z}$$

Therefore:

$$X = \frac{N \times Y}{Z}$$

$$X = \frac{300 \times 12{,}542}{110}$$

$$X = 34{,}205$$

Where: X = total palynomorph number (unknown)

Y = *Lycopodium* spores in one tablet

N = Number of palynomorphs counted in a given sample

Z = Number of *Lycopodium* spores counted in a given sample.

For example, as illustrated above, if a given sample had a count of 110 *Lycopodium* spores for the 300 palynomorphs counted, the total palynomorph number would be approximately 34,205 ± 2,539. The calculation is repeated for the standard deviation of the *Lycopodium* spore numbers **per tablet** to give the standard deviation for the palynomorphs counted.

The above formula was used to calculate the approximate TPN for the Control Soil, Background Clothing and Evidentiary samples collected. The results are presented in Tables 5.1 – 5.5.

5.2 Control Soil Samples

Prior to adding the *Lycopodium* spike each soil sample was weighed so that the total palynomorph number **per gram** of soil could be calculated. The TPN per gram of soil are presented in table 5.1.

Table 5.1 Total palynomorph number per gram of soil of the Control Soil Samples.

	Control Soil Samples					
	Scene			Local		
Samples	1	2	3	1	2	3
Palynomorphs / gram	83,613 ± 6,207	100,336 ± 7,448	100,336 ± 7,448	83,613 ± 6,207	50,168 ± 3,724	50,168 ± 3,724

The results demonstrate the vast amount of pollen present in the control soil samples per gram with a range of 50,168 – 100,336 TPN**.** The difference between the palynomorph concentration per gram in the Scene soil and the Local soil is likely due to plant density and type and their distribution over the two areas. For example, as shown in Figure 4.1 (Ch. 4), the Scene Area contains various flowering and spore producing plants including *Asplenium australasicum, Gardenia jasminoides, Argyranthemum frutescens, Hebe* Autumn Glory, *Nephrolepis cordifolia* and *Hedera*

helix. Although in total there are more plants in the Local Area, these are spread over a greater area and thus the average total palynomorph amount per gram of soil may be less in the Local Area than in the smaller Scene Area.

5.3 Background Clothing Samples

To examine the potential for 'background' pollen deposition on clothing, clothing was worn for three consecutive days and then analysed. The total palynomorph number for **each item** (i.e. estimated total pollen number on the clothing, not per gram) is recorded in Table 5.2. As these clothing items were not (consciously) rubbed in soil there was very little soil recovered. However, the results show that a substantial amount of pollen was deposited on, and retained by, the clothing during day to day wearing. The TPN for the Background Clothing samples ranged from 2,752 - 34,205 TPN.

Table 5.2 Total palynomorph number per item of clothing for the Background Clothing samples.

	Background Clothing Samples					
	T- Shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	34,205 ± 2,539	2,752 ± 204	3,703 ± 275	22,943 ± 1,703	5,935 ± 441	10,252 ± 761

The TPN range for the Background Clothing samples was 2,752 - 34,205. Of the replicate sets the TPN of R1 is considerably higher than R2 and R3 because of the location to which the clothing was worn and the weather conditions on the day (Table 5.2). The Background TPN falls between TPN of the E2 and E3 samples. If the R1 results of the Background samples are not counted in its TPN, the amount would be considerably less than the E2 samples and closer to the E3 samples. Although the overall Background Clothing sample TPN appeared large (Table 5.2), the percent occurrence of the Key (including Additional Key) species (Table 4.1) was not similar to those of the Control Soil samples.

The TPN for the t-shirt and jeans of a single replicate is within close range but with a slightly higher concentration on the jeans in replicate two (R2) (5,935 TPN) and replicate three (R3) (10,252 TPN). As the legs and cuffs of the jeans are in close

proximity to the ground, more pollen and spores may have been transferred from soil, grass and flowering plants as the cuffs brushed along the ground during walking.

It is interesting that there is a higher total palynomorph number retained by the t-shirt (34,205 TPN) of replicate one (R1) than the jeans (22,943 TPN). As discussed in Chapter 4, the majority of the pollen retained was from the anemophilous (pollinated by wind) *Araucaria heterophylla* (Norfolk Island Pine) trees when the clothing was worn to the Fremantle area on a windy day. The pollen from this species is large (~ 60 μm) and circular in shape with a granulate surface sculpture. It is possible that the higher retention of total palynomorphs by the t-shirt may be a result of the t-shirt weave. Denim has a tight weave compared to t-shirt fabric. The looser weave of the t-shirt may allow a larger amount of pollen to become trapped as it is forced into the weave by wind or mechanical movement.

5.4 Evidentiary Clothing Samples

As the clothing used in the simulated assault (E1, E2 and E3) was firmly and abrasively in contact with the soil, some of the soil containing palynomorphs will be retained within the fabric weave as each sample was exposed to different conditions (i.e. E1: immediately analysed, E2: worn for three days then analysed and E3: washed then analysed), some of the soil (and palynomorphs) will be lost, and some additional palynomorphs from the environment will be added. Calculation of the TPN for each Evidentiary sample will illustrate the difference in palynomorph retention considering the different clothing 'conditions'. The E1 samples had the largest amount of soil recovered after the soaking process followed by the E2 samples. Very little soil and debris was retained by the E3 samples because each clothing item was washed in a washing machine before being soaked for pollen removal.

5.4.1 Evidentiary One Clothing Samples

The items of clothing for the Evidentiary 1 (E1) condition were involved in the simulated sexual assault then immediately analysed. The TPN for the E1 samples are shown in Table 5.3.

Table 5.3 Total palynomorph number per item of item of clothing for the Evidentiary One Samples

	Evidentiary One Samples					
	t-shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	2,508,400 ± 186,200	2,508,400 ± 186,200	267,100 ± 46,550	1,254,200 ± 93,100	836,133 ± 62,067	418,067 ± 31,033

All clothing items retained a substantial amount of palynomorphs, with the lowest amount at greater than 400,000 TPN (jeans R3: 418,067 TPN), but not all replicates retained the same amount. The R3 items (t-shirt: 627,100 TPN, jeans: 418,067 TPN) retained the least amount of palynomorphs compared to R1 (t-shirt: 2,508,400 TPN, jeans: 1254200 TPN) and R2 (t-shirt: 2,508,400 TPN, jeans: 836,133 TPN). It is possible that since this was the last clothing replicate to be rubbed in the soil (following R1 and R2), the items may not have been exposed to as much loose soil as the first two replicates (as the soil would have been brushed aside or retained by the first two replicates), or that the wearer was not as vigorous in the mock assault.

The results in Table 5.3 show that the t-shirts retained a higher amount of palynomorphs at 627,100 – 2,508,400 TPN, compared to the jeans which retained 418067 – 1,254,200 TPN. As discussed in section 5.3 above, it is possible that the t-shirt fabric has greater retention potential (i.e. bigger weave) than the jeans or simply that the t-shirts had a greater surface area exposed to the soil during the simulated assaults (the backs of the t-shirts were exposed to the soil compared to the knees and shin areas of the jeans).

It is expected that the E1 samples will have retained the highest TPN compared to the other Evidentiary samples because the E1 clothing was exposed to far less movement and subsequent loss of soil.

5.4.2 Evidentiary Two Clothing Samples

The items of clothing for the Evidentiary 2 (E2) condition were involved in the simulated sexual assault and worn for three consecutive days before the sample was collected for analysis. The TPN for the E2 samples is displayed in Table 5.4.

Table 5.4 Total palynomorph number per item of clothing for the Evidentiary Two Samples

	Evidentiary Two Samples					
	t-shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	15,977 ± 1,186	24,592 ± 1,826	8,590 ± 638	14,499 ± 1,076	30,590 ± 2,271	17,064 ± 1,267

The average retention of pollen on each E2 item (Table 5.4) is far less than the E1 samples with a range of 8,590 – 30,590 TPN. Each replicate TPN was reasonably close, with replicate R2 containing the highest amount (t-shirt: 24,592 TPN and jeans: 30,590 TPN). As the E2 clothing was worn for three days, much of the soil containing pollen and spores that had been transferred to the clothing during the assault would have been lost due to movement.

5.4.3 Evidentiary Three Clothing Samples

The Evidentiary 3 (E3) clothing was involved in the simulated assault then washed before analysis. The TPN for the E3 samples is displayed in Table 5.5.

Table 5.5 Total palynomorph number per item of clothing for the Evidentiary Three Samples

	Evidentiary Three Samples					
	t-shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	746 ± 55	934 ± 69	1,165 ± 86	3,029 ± 225	1,231 ± 91	1,598 ± 119

The TPN on each item, with a range of 746 – 3,029 TPN, is considerably less than that from the other Evidentiary samples (418,067 – 2,508,400 TPN for the E1 samples and 8,590 – 30,590 TPN for the E2 samples) or Background Clothing samples (2,752 – 34,205 TPN). The jeans retained more pollen than the t-shirts, even though the E1 sample results suggest that the t-shirts retain more pollen initially after the simulated assault. Perhaps, as suggested for the Background samples, the tighter weave of the denim assists in retaining the pollen in the fabric despite rough conditions such as washing. These results show that, regardless of clothing being washed, a considerable amount of pollen may be retained.

5.5 Comparison of TPN Numbers for the Control Soil, Background Clothing and Evidentiary Samples

Table 5.6 shows the calculated TPN for the Control, Background Clothing and Forensic Clothing samples (E1, E2 and E3).

Table 5.6 Total palynomorph number calculated for the Control Soil, Background Clothing and Evidentiary samples

	Control Soil Samples					
	Scene			Local		
Samples	1	2	3	1	2	3
Palynomorphs / gram	83,613 ± 6,207	100,336 ± 7,448	100,336 ± 7,448	83,613 ± 6,207	50,168 ± 3,724	50,168 ± 3,724
	Background Clothing Samples					
	T- Shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	34,205 ± 2,539	2,752 ± 204	3,703 ± 275	22,943 ± 1,703	5,935 ± 441	10,252 ± 761
	Evidentiary One Samples					
	T- Shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	2,508,400 ± 186,200	2,508,400 ± 186,200	267,100 ± 46,550	1,254,200 ± 93,100	836,133 ± 62,067	418,067 ± 31,033
	Evidentiary Two Samples					
	T- Shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	15,977 ± 1,186	24,592 ± 1,826	8,590 ± 638	14,499 ± 1,076	30,590 ± 2,271	17,064 ± 1,267
	Evidentiary Three Samples					
	T- Shirt			jeans		
Samples	1	2	3	1	2	3
Total Palynomorphs	746 ± 55	934 ± 69	1,165 ± 86	3,029 ± 225	1,231 ± 91	1,598 ± 119

Comparison of the Evidentiary samples TPN ranges shows that the E1 samples had the highest TPN (267,100 – 2,508,400 TPN per clothing item) followed by the E2 samples (8,590 – 30,590 per item) and finally the E3 (746 – 3,029 per item) samples.

The *possible* contribution of background pollen to the E2 samples worn for three days can be seen in Table 5.6. The R1 set of the Background Clothing samples are excluded due to the exceptionally high TPN as a result of weather conditions and locality. These conditions were not repeated during the 3-day wear period of the E2 clothing. The R2 and R3 of the Background Clothing sets are considered to be more representative of 'normal' conditions. A comparison of the TPN retained by the Background Clothing (R2 and R3 only) against the E2 clothing samples shows that

the E2 clothing, on average, contains considerably more palynomorphs than the Background Clothing samples. The key species of the E2 samples are also more closely similar to the other Evidentiary and Control samples than they are to those in the Background samples.

Conclusion

As expected, the Control Soil samples had a TPN ranging into the hundreds-of-thousands **per gram** of soil. Of the Evidentiary items, the E1 samples retained the highest TPN because the clothing was taken for analysis immediately after the simulated assault. The E2 samples had the second highest TPN of the Evidentiary samples because the three day wear period following the simulated assault would have caused some loss of soil (thus palynomorphs) from the clothing. As expected, the E3 samples had the lowest TPN of all the Evidentiary samples because the sample was collected after the clothing was washed.

The TPN results discussed in this chapter should be considered in conjunction with the results in Chapter 4, as it is only with palynomorph identification (and thus relative percentage of a particular pollen type) that TPN will have significant meaning. It is aimed to demonstrate, for example, an item with a small TPN may still be considered closely similar to an item with a large TPN, as it is the presence / absence of species and thus percent occurrence (concentration) of the palynomorphs retained that is of importance. The TPN calculated here does not represent exact amounts but rather shows relative magnitude of pollen and spores retained by clothing under different conditions.

Despite the differences in magnitude of TPN from each experimental condition, all of the Evidentiary samples retained a pollen assemblage characteristic of the Research garden. The results of this chapter support the common tenet that it is not the **amount** of pollen present on an item that is important, but rather the presence or absence and relative occurrence of specific (**key) species** that characterise a pollen assemblage.

6. STATISTICAL ANALYSIS

Statistical analysis is not traditionally used in forensic palynology because, as described by Murray and Tedrow (1992), 'no two physical objects can ever, in a theoretical sense, be the same'. For example, in this study, the palynological profile of the Scene soil is different in both palynomorph type and amount to the Local soil even though the soil of each sample originated from the same garden. Another consideration is that replicates of samples are not often taken given that they have been collected from casework.

Statistics does not take into consideration the 'value' of the presence of 'rare' pollen types that occur in low percentages (for example, animal pollinated species) within a sample and so the presence of important (key) species (albeit in low amounts) may be overlooked and seem unimportant in statistical calculations. However, because this study encompasses three replicates of every sample (with the exception of the Background Compost samples, of which there are two replicates) and because key species have been identified, some statistical analysis may be applied to this data.

6.1 Statistical Analysis

The program used to conduct all statistical analyses was the statistical package R. The Bray – Curtis Index (BCI) was chosen to transform the raw data. The transformation of the data reflected the particular aspects considered to be important (the key species) and showed the dissimilarities between each data set (each sample). The results of this were then displayed via non-metric multi-dimensional scaling (NMDS). NMDS graphically illustrates the relationship between each sample (Clarke, 1993). The more similar two samples are, the closer they will appear in the two dimensional plot. This technique was used with palynological data by Lin (2008) who showed that replicate samples collected from a particular location are significantly similar in NMDS.

For the statistical analysis only the Key and Additional Key species were used. All other species were grouped into major families or Gen. *et* sp. indet. The untransformed data and the BCI transformed data is displayed in Appendix 4 and 5 respectively.

6.2 Results

The first two NMDS graphs below show the relationships between each replicate of the Control Soil and Evidentiary Clothing samples (Fig 6.1 and 6.2). The third NMDS graph shows the relationship between each grouped sample (Fig 6.3). Each sample has been given an alphanumeric symbol. The key to these symbols is shown in Table 6.1. The stress values for the ordination ('goodness of fit') of the NMDS graphs is 0.185.

6.2.1 Control Soil and Evidentiary Clothing Sample Replicate Relationships

The NMDS graph in Figure 6.1, showing the relationships between each replicate for the Control and Evidentiary samples, has been reproduced in Figure 6.2 with the replicates grouped by colour.

Table 6.1: Key for Figures 6.1 and 6.2.

Symbol	Sample	Replicate	Symbol	Sample	Replicate
Control Soil Samples			**Forensic Clothing Samples Evidentiary 1** (Immediate analysis)		
SS1	Scene	1	TSI1	t-shirts	1
SS2		2	TSI2		2
SS3		3	TSI3		3
SL1	Local	1	JI1	Jeans	1
SL2		2	JI2		2
SL3		3	JI3		3
Background Clothing Samples			**Forensic Clothing Samples Evidentiary 2** (3 day wear)		
BGTS1	t-shirts	1	TS3DW1	t-shirts	1
BGTS2		2	TS3DW2		2
BGTS3		3	TS3DW3		3
BGJ1	Jeans	1	J3DW1	Jeans	1
BGJ2		2	J3DW2		2
BGJ3		3	J3DW3		3
Background Compost Samples			**Forensic Clothing Samples Evidentiary 3** (Wash)		
C1	Compost Soil	1	TSW1	t-shirts	1
C2		2	TSW2		2
			TSW3		3
			JW1	Jeans	1
			JW2		2
			JW3		3

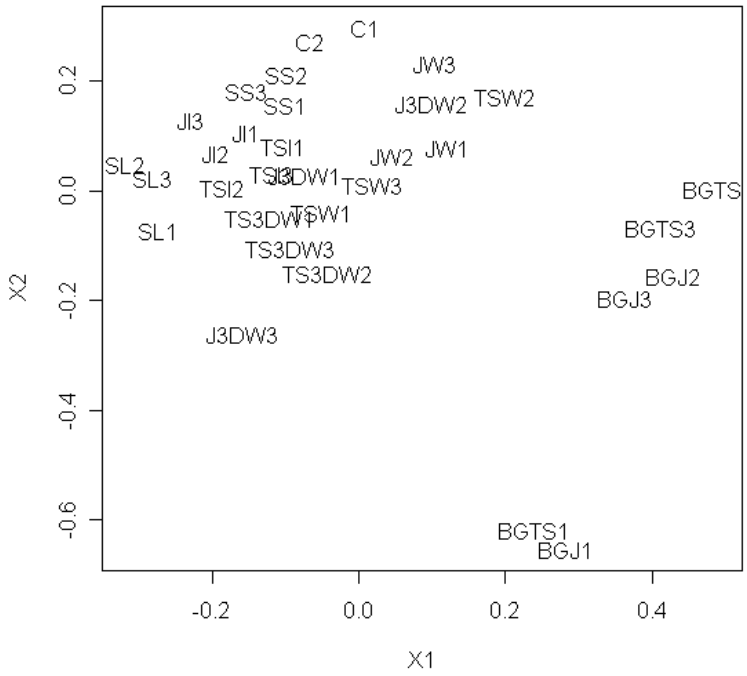

Figure 6.1: NMDS of all replicate Control Soil, Background and Evidentiary samples.

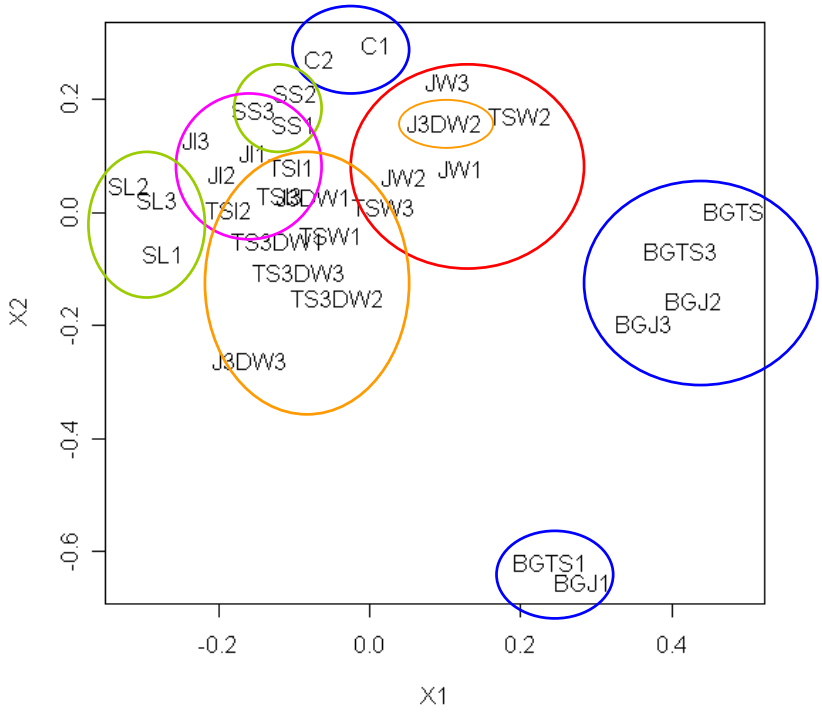

Figure 6.2: NMDS of all replicate Control Soil, Background and Evidentiary samples with grouping of replicates where Blue = Background Samples, Green = Control Soil Samples, Pink = Evidentiary 1 samples, Orange = Evidentiary 2 samples and Red = Evidentiary 3 samples.

6.2.2 Discussion

Of the replicate samples (Fig 6.1 and 6.2), the Control soil samples collected from the Scene area are the most spatially closest, and thus most closely similar, replicate set. This is followed by the soil collected from the Local area and the Evidentiary 1 (E1) samples. Replicate set one of the Background Clothing and the Compost samples are also spatially close. The Evidentiary 2 (E2) and Evidentiary 3 (E3) samples are less spatially close than the control and E1 samples. As the E2 samples were worn for three days after the simulated assault, it would be expected that they would lose some of the material collected from the garden, including the key species. The remaining garden assemblage would also be mixed with pollen collected during the three day wear thus 'diluting' the original assemblage from the garden. The E3 clothing was washed after the simulated assault, which would result in an even greater loss of key species and perhaps an addition of pollen from the other clothing washed with them.

The pollen assemblage from the Background T-shirts and jeans are closely similar to the assemblages from their corresponding replicates, however not necessarily to each other replicate set. Interestingly, Replicate set one (R1) assemblages are spatially separate from Replicate sets two (R2) and three (R3). The jeans assemblages of R2 and R3 are also spatially closer to each other than to their corresponding T-shirt assemblages. The separation of R1 from R2 and R3 is a result of the locality to which each clothing set was worn, as recorded in Table 4.3.

6.3. Combined Control Soil and Evidentiary Clothing Sample Relationships

The replicates of each sample have been grouped to produce the NMDS graphs 6.3 and 6.4 to show the relationship between the Control and Evidentiary samples. Each sample represents a 600 grain count. The NMDS graph in Figure 6.3 has been replicated in Figure 6.4 with the samples grouped by colour.

Table 6.2: Key for Figure 6.3 and 6.4.

Sample	Replicate	Sample	Replicate
Control Soil Samples		**Forensic Clothing Samples Evidentiary 1** (Immediate analysis)	
SS	Scene	TSI3	t-shirt
SL	Local	JI3	jeans
Background Clothing Samples		**Forensic Clothing Samples Evidentiary 2** (3 day wear)	
BGTS	t-shirt	TS3DW3	t-shirt
BGJ	jeans	J3DW3	jeans
Background Compost Samples		**Forensic Clothing Samples Evidentiary 3** (Wash)	
C	Compost soil	TSW1	t-shirt
		JW3	jeans

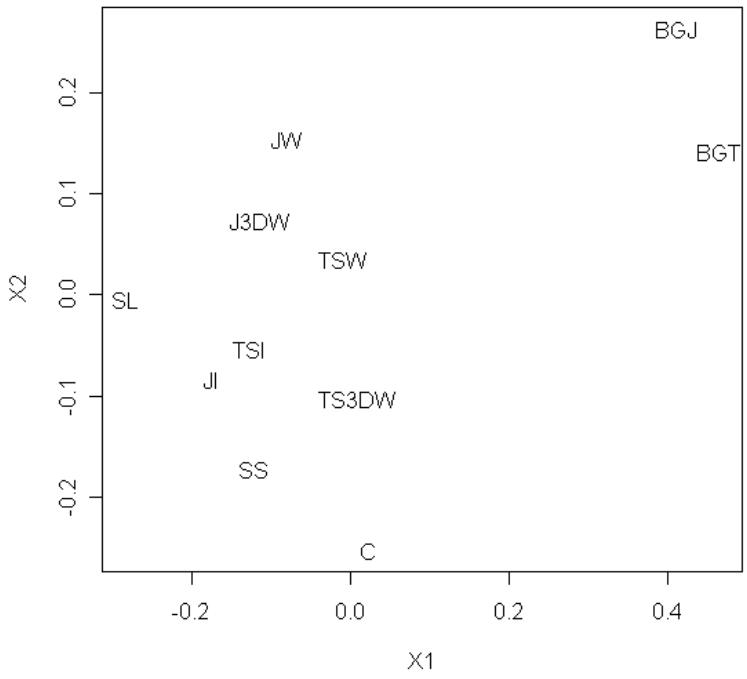

Figure 6.3: NMDS of combined replicates of Control Soil, Background and Evidentiary samples.

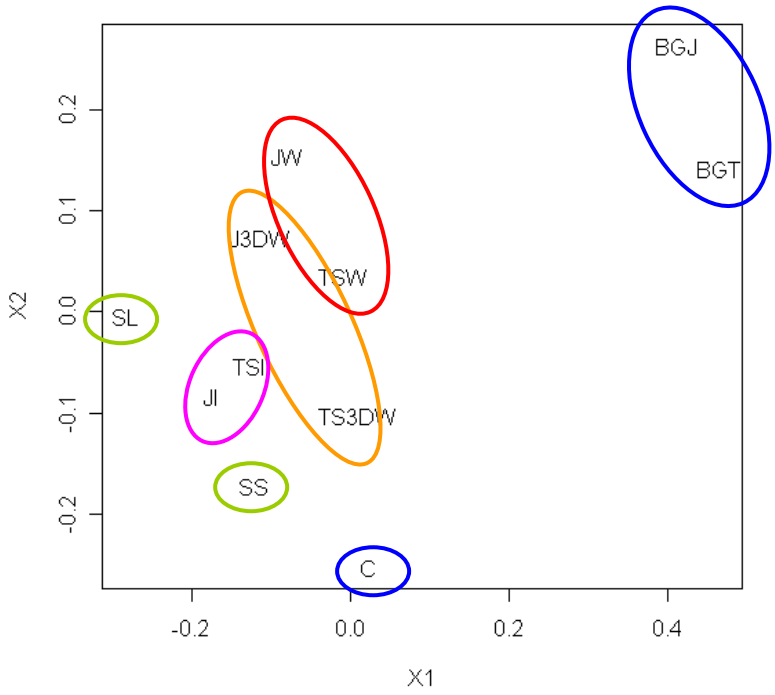

Figure 6.4: NMDS of all replicate Control Soil, Background and Evidentiary samples with grouping of replicates where Blue = Background Samples, Green = Control Soil Samples, Pink = Evidentiary 1 samples, Orange = Evidentiary 2 samples and Red = Evidentiary 3 samples.

6.3.1 Discussion

The results show that, when only the Key and Additional Key species are considered, the E1 samples are spatially closer to, and thus most closely similar to, the Control Soil samples. This is expected as these were the items that were analysed immediately after the simulated assault. The E2 samples (clothing worn for three days after the simulated assault) are the next group most spatially close to the control soil samples followed by the E3 (clothing washed after simulated assault). This indicates that the E2 samples are more closely similar to the Control samples than the E3 samples.

When the Background samples (compost and clothing) are considered, the Background compost samples are spatially closer to the Control soil and Evidentiary samples than the Background clothing samples. This is expected as the compost samples came from a garden bed four meters away from the Research garden (where the Control samples were taken) and contained some of the Key species identified in the control soil samples. The Background Clothing samples lie the furthest distance

from the Control samples, indicating that these are the least similar to the control soil samples compared to the Evidentiary and Compost samples.

6.3.2 Conclusion

Analysis of the NMDS graphs (Fig 6.3 and 6.4) shows that the E1 samples, which were analysed immediately after the simulated assault, are most closely similar to the control samples, followed by the E2 samples, which were worn for a period of three days after the assault, and E3 samples, which were washed before samples were collected, respectively. This shows how important it is to collect palynological evidence as soon as possible. However, the results also indicate that clothing that has been worn for a period of three days or clothing that has been washed after an event will still have a palynological profile similar to the control samples to suggest that they are from the same location. The outlier positioning of the Background samples indicates their dissimilarity to the Evidentiary and Control samples and therefore it would not be possible to say that the Background samples are from the same location as the Control and Evidentiary samples.

The statistical analysis used here is simplistic in its form, allowing easy understanding and interpretation for laypersons. However, it would not be possible to apply statistical analysis to all forms of forensic palynology as often it is not feasible to collect replicate samples, especially from evidence during case work. In particular, the importance of some species over others (such as animal pollinated species) is overlooked in this type of statistical analysis, something which is very often the most significant factor of forensic palynology.

7. DISCUSSION AND CONCLUSIONS

Forensic palynology is a relatively young science with most of the current knowledge having been derived from experience (Wiltshire, 2006a). Only limited research has been conducted and very little involves the study of pollen retention on clothing. Empirical research in forensic palynology is required for the support and validation of expert evidence in court. This research investigated the pollen and spore retention on clothing under different conditions after a simulated assault in a small secluded garden.

The results and conclusions of this research presented in chapters 4 and 5 are discussed in this final chapter. Conclusions are drawn and discussed with an emphasis on how this research can be used to support palynological evidence in future criminal investigations.

7.1 Percent Occurrence of Palynomorphs

The type and relative percent occurrence of pollen and spores from the Control soil, Background Clothing and Evidentiary samples was determined and compared to demonstrate the relative retention of pollen and spores on clothing under differing conditions.

7.1.1 Control Soil Samples

The examination of the two Control soil samples (Scene and Local) from the research garden, each collected in triplicate, led to the identification of 20 key species common to both. Eleven of the key species came from plants that were currently growing in the research garden; these were called 'Key species'. The remaining nine species were common to both Control Soil samples but were not currently growing in the garden; these were called 'Additional Key species'. These may have come from plants that had once grown in the research garden but had since been removed. Together, these species are referred to as 'key species'. The presence of the key species combined with their relative percent occurrence, provided a unique pollen assemblage for the research garden that could be used as a baseline for comparison with the Background and Evidentiary samples.

7.1.2 Background Samples

Background Clothing Samples

The pollen assemblages collected from the three Background Clothing sets that were worn for three consecutive days to various locations were reflective of the areas where each clothing set was worn. On average, five or less of the 11 Key species and only one of the Additional Key species identified from the Control Soil samples were present in the T-shirt replicates. Only two of the Key species were present in the jeans replicates, both of which were from common wind-pollinated plants.

The palynological profiles of the T-shirts and jeans of each set that were worn at the same time were closely similar to each other but were generally different from the profiles of the other clothing sets. On average, the assemblages from replicate sets two and three were moderately similar to each other and contained more of the key species than set one. This is because clothing sets two and three were worn around the general area of the research garden site.

The percent occurrence of the key species in the Background Clothing samples was **considerably different** to the percent occurrence of the same key species in the Control Soil samples. It was clear from the pollen assemblages that none of the background samples were from the same specific locality as the research garden but that sets two and three may have been in an area with similar vegetation. The pollen assemblage from set one was dominated by pollen from the wind pollinated species *Araucaria heterophylla.* This set was worn around the Fremantle area near many *A. heterophylla* trees on a particularly windy day. These results illustrate how palynology is useful in determining the locations to which clothing has been worn and illustrates the importance of investigating police enquiring where clothing may have been worn before an event.

Background Compost Samples

Pollen from wind pollinated species (Myrtaceae and Poaceae families), undetermined species and various fungal spores dominated the Background Compost samples. Only three of the 20 identified key species were present, each in low amounts with a percent occurrence **considerably different** to those in the Control Soil samples.

These results indicated that the main contribution from compost of this type to a soil pollen assemblage would be 'background' pollen and fungal spores, with the majority having come from wind pollinated species. This is forensically significant, as items and clothing that have come into contact with composted soil will have a palynological profile that will include some pollen and spores from compost.

7.1.3 Evidentiary Samples

Each Evidentiary clothing set (three sets per condition) was worn during a simulated assault during which the clothes came into abrasive and prolonged (minimum one minute duration) contact with the soil of the research garden. After the simulated assault, the first Evidentiary clothing sets (E1) were taken for immediate analysis, the second clothing sets (E2) were worn for a further three days before analysis and the third clothing sets (E3) were washed prior to analysis. The differing conditions applied to the clothing sets emulated possible real life situations (e.g. it would not be uncommon for clothes to be washed after an assault). The results from this research will assist in the explanation of expert palynological evidence in court where a palynological profile has been recovered from clothing thought to have been involved in an 'event' such as that simulated here.

Evidentiary One Samples

For the E1 clothing sets, an average of 19 of the 20 key species were present in percentages similar to those in the Control samples, **strongly indicating** that the E1 samples were from the same locality as the control samples.

The 10 Additional Non-Key species that were identified in the E1 samples were present in low amounts.

Evidentiary Two Samples

The 16 key species present in the E2 clothing samples were at percentages **closely similar** to those in the Control Soil samples. This illustrates that the E2 samples still retained significant amounts of pollen from the research garden despite having been worn for three consecutive days after the simulated assault.

Eight Non-Key pollen types, generally common to two or more of the E2 replicate sets, were present in the E2 profile in low amounts. It is likely that these pollen types were either from the research garden and/or had been collected during the three day wear period. The presence of Non-Key species does not indicate that the clothing has not been in contact with the research garden, because the key species common to both the E2 clothing and the Control soil samples are present in similar percentages even after the three day wear period. Rather, the additional Non-Key species are important forensic indicators and are representative of the other locations to which the clothing may have been worn after the simulated assault.

Evidentiary Three Samples

Although the E3 clothing was washed after the simulated assault, on average 16 of the key species identified from the research garden were found in the E3 profiles with percentages similar to those in the Control Soil samples. These results signify that the E3 samples were from the **same or a similar** locality as the Control Soil samples. *These findings have great forensic significance as this demonstrates that clothing that has been in firm contact with soil at a particular site and then washed will still contain pollen from that site.*

On average, small amounts of 11 Non-Key species were identified in the E3 samples, seven of which were not common to either of the Control samples, indicating that it was unlikely that they had originated from the research garden. It is possible that the Non-Key species may have been contributed by the water in which the clothes had been washed or from the other items which were washed with the clothes.

7.2 Total Pollen Number (TPN)

The Total Pollen Number (TPN) was calculated using an internal standard (*Lycopodium* spores) **per gram** of soil for the Control Soil samples. The TPN of the clothing samples was calculated for the total number of palynomorphs recovered **per item** of clothing. The Control and Evidentiary samples TPN are not comparable to each other but show the differing magnitudes of pollen concentration.

7.2.1 Control Soil Samples

The Total Pollen Number (TPN) for the Control samples had a range in the thousands to tens-of-thousands of palynomorphs **per gram** of soil. This result demonstrates the potentially high amount of palynomorphs in the soil of a 'standard' garden. The forensic significance of this is reflected in Locard's exchange principal: When two items come into contact, a transfer of material will occur. The more palynomorphs there are in soil, the greater the chance that they will be collected by clothing that comes into contact with the soil.

7.2.2 Background Clothing Samples

The average TPN recovered from the Background Clothing samples had a range in the thousands to tens-of-thousands **per clothing item** examined. The considerable difference in TPN between the replicate sets is reflective of the location to which each set was worn. The R1 set was worn around the Fremantle area on a very windy day, which resulted in the clothing collecting very large quantities of pollen (specifically of the wind pollinated species *Araucaria heterophylla*) compared to the R2 and R3 sets which were mostly worn around the general Crawley area during calmer weather. Forensically, knowledge of where an item has been both before and after an 'event' is vital, as the amount and type of pollen collected on the item will be reflective of this.

On average, the T-shirts in the Background Clothing sets retained a higher amount of (mostly wind-pollinated species) pollen compared to the jeans. The larger weave of the T-shirt fabric may be more suitable to act as a pollen trap for wind dispersed pollen and spores, compared to the tighter weave of the denim jeans.

7.2.3 Evidentiary Clothing Samples

In summary, the E1 samples retained the highest TPN recovered **per item** of clothing, followed by the E2 and E3 samples.

Evidentiary One Samples

The TPN for the E1 samples was exceptionally high with a range between the hundreds-of-thousands to millions of palynomorphs per clothing item. This is because little soil would have been lost from the clothing as it was analysed immediately after the simulated assault. This demonstrates the importance of

collecting evidence such as this as soon as possible. The sooner an item is collected as palynological evidence, the less chance that palynological evidence will be lost.

On average, the T-shirts retained a higher amount of palynomorphs than the jeans. This may be due to the large (loose) weave of the T-shirt fabric and because the T-shirts had a greater surface area exposed to the soil during the simulated assault. This demonstrates how important it is for police/investigators to ask which item or part of an item has had prolonged contact with soil, as this is the item or area of clothing which will retain the largest amounts of palynological evidence.

Evidentiary Two Samples

The TPN of the E2 samples ranged in the thousands and tens-of-thousands. During the three day wear period some palynomorphs that were collected during the simulated assault would have been lost and some 'background' pollen would have been collected by the clothing. When the TPN of the E2 samples is compared to the Background Clothing samples (excluding R3 because it was swamped by *A. heterophylla*) the results indicate that the contribution of background pollen and spores would be much less than what was retained by the clothing after the assault and three day wear. This demonstrates that clothing that has been worn for a number of days may still contain pollen and spores collected during an event. The analysis of 'background' pollen collected by the clothing may also assist in establishing where clothing has been worn after an event.

Evidentiary Three Samples

The TPN collected from the E3 clothing, although the lowest of all the Evidentiary samples, still ranged in the hundreds to thousands; an amount high enough to establish a reliable palynological profile showing that palynomorphs from the garden were still on the clothing. These results illustrate the importance of knowing if a forensic sample has been washed after an 'event', as a low TPN may be a result or indicator of this.

7.3 Conclusion

The results of this research confirm what forensic palynologists have long believed. However, very little research to support the tenet of pollen retention on clothing under different conditions has been documented. Research into this area that has been conducted to date is either limited by the items used (such as shoes) or uses different fabrics as passive (background) collectors, rather than as a worn object in a 'real life' situation.

By the use of an internal standard it was demonstrated that one gram of soil from a 'standard' garden may contain tens-of-thousands of palynomorphs. *It would be impossible for an item, such as clothing, to come into abrasive contact with that soil and not retain some of the palynomorphs from that soil.*

The number of ('background') palynomorphs collected by clean clothing that has been worn for several days may be in the range of thousands to tens-of-thousands of grains. The palynological profile is generally reflective of the areas to which the clothing has been worn. Clothing that has been in previous contact with soil and then worn for a period of days will retain a profile which includes palynomorphs from the soil and wind pollinated species collected during the wear period.

In summary, the results from the Evidentiary samples presented in this study demonstrate that clothing collected immediately after an event where it has come into abrasive contact with soil will retain the largest amount of pollen (in the thousands to tens of thousands) with a profile (most) closely similar to that soil compared to clothing that has been worn for several days or washed after abrasive contact with soil. Even after washing or a three day wear period the clothing will retain a large number of palynomorphs with a profile similar to that of the soil. Clothing that has been worn for several days without contact with soil will collect background pollen from wind pollinated species that is representative of the areas to which the clothing has been worn.

Despite the differences in magnitude of TPN from the Evidentiary samples, each sample retained a pollen assemblage characteristic of the research garden. This is exceptionally important as it is research that demonstrates that it is not the amount of pollen present but rather the relative occurrence of species that matters.

For palynology to be more widely accepted as a forensic tool, more empirical research of this nature is required. These results highlight the need for investigating police to find out where a suspect has been before and after an event, if the clothing has been worn for several days and if the clothing has been washed before or after an event. This will assist in the palynologists interpretation of palynological profiles recovered from evidentiary items.

The results of the research conducted in this project will provide support for the future presentation and validation of palynological evidence in court, specifically in assault cases with conditions similar to those simulated in this study.

7.5 Future Research

The research conducted in this study is of a preliminary nature. Only three replicates of three conditions could be tested due to time and monetary constraints. There are innumerable variables that could be tested on the relative retention of palynomorphs on clothing under differing conditions. The specific type (including size, shape and other morphological features) of pollen and spores that are preferentially retained by different types of clothing could also be examined in depth. Different fabrics and objects could be examined to see if the nature of the fabric or item will affect potential palynomorph retention. Wet and dry conditions of the soil in which the clothing comes into contact with could also be examined to see if retention is affected by weather conditions. Further studies into the total palynomorph number retained by clothing (or other) items would be useful in ascertaining the magnitude of palynomorphs retained after various conditions.

New methods and further research into the collection of ‘background’ pollen is required so that forensic palynologists may have a better understanding of the effects

background pollen may have on a profile. For example, the palynological profile recovered from tap-water used to wash clothing in could be examined in depth.

Different gardens could also be sampled to give a better idea of the predominant species found in gardens. These could be documented to form a palynological database for garden types to be used in other forensic investigations

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PLATE 1

Plate 1 - Pollen as identified in the Control Soil samples and Evidentiary Samples

Pollen from Cyatheaceae (a-c), Davaliaceae (d-f), Cryptogram Spore (g), Rubiaceae (h-k), Anacardiaceae (l–o) and Araceae (p-s). Magnification x 1000. All samples were field collections from the Research Garden Site. **K** = Key Species, **AK** = Additional Key Species **G** = from grouped family / type, **ANK** = Additional Non-Key Species.

a-c	*Cyathea cooperi* **K**
d-f	*Nephrolepis cordifolia* **K**
g	cryptogam spore sp. 1 **AK**
h- k	*Gardenia jasminoides* **K**
l-o	*Harpephyllum caffrum* **K**
p-s	*Philodendron* sp. **K**

PLATE 2

Plate 2 - Pollen as identified in the Control Soil samples and Evidentiary Samples

Pollen from Araucariaceae (a-b), Pinaceae (c-g), Aspleniaceae (h-j) and Proteaceae (k). Magnification x 1000. All samples were field collections from the Research Garden Site. **K** = Key Species, **AK** = Additional Key Species **G** = from grouped family / type, **ANK** = Additional Non-Key Species.

a-b	*Araucaria heterophylla* **K**
c-d	*Pinus radiata* **G**
e-g	*Asplenium australasicum* **K**
h-j	*Liquidambar styraciflua*
k	*Grevillea* (Robyn Gordon) **G** (Gen. *et* sp. indet.)

PLATE 3

Plate 3 - Pollen as identified in the Control Soil samples and Evidentiary Samples

Pollen from Asteraceae (a-i), Scrophulariaceae (j-l), Proteaceae (m), Betulaceae (n), Casuarinaceae (o-p), Chenopodiaceae (o-r), Liliaceae (k) and Myrtaceae (u-z). Magnification x 1000. All samples were field collections from the Research Garden Site. **K** = Key Species, **AK** = Additional Key Species **G** = from grouped family / type, **ANK** = Additional Non-Key Species.

a-b	Asteraceae sp. 3 **K**
c-d	Asteraceae sp. 2 **ANK**
e-g	Asteraceae sp. 1 **AK**
g	Asteraceae sp. 4 **AK**
h	Asteraceae sp. 6 **ANK**
i	Asteraceae spp **G**
j-l	*Hebe* Autumn Glory **K**
m	*Banksia/Dryandra* spp **ANK**
n	*Betula* sp. **ANK**
o-p	Casuarinaceae spp **G**
q-r	Chenopodiaceae spp **G**
s	Chenopodiaceae sp. 1 **AK**
t	Liliaceae spp **G**
u-z	Myrtaceae spp **G**

PLATE 4

Plate 4 - Pollen as identified in the Control Soil samples and Evidentiary Samples

Pollen from Theaceae (a), Tricolporate spp (b-q), Poaceae (r), Typhaceae (g) and Monocolpate spp (t-v). Magnification x 1000 unless stated otherwise. All samples were field collections from the Research Garden Site. **K** = Key Species, **AK** = Additional Key Species **G** = from grouped family / type, **ANK** = Additional Non-Key Species.

a	*Camellia japonica / sasanqua*
b-d	Tricolporate sp. 2 **ANK**
e	Tricolporate sp. 1 **ANK**
f-g	Tricolporate sp. 3 **AK**
h	Tricolporate sp. 4 **ANK**
i	Tricolporate sp. 5 **ANK**
j-l	Tricolporate sp. 7 **AK**
m	Tricolporate sp. 8 **AK**
n	Tricolporate sp. 9 **AK**
o-p	Tricolporate sp. 10 **ANK**
q	Tricolporate spp **G**
r	Poaceae spp **G**
s	*Typha* sp. cf. *muralis* **G** (Gen. *et* sp. indet.)
t	Monocolpate sp. 1 (Note: Magnification x 400) **ANK**
u-v	Monocolpate sp. 2 **ANK**

PLATE 5

Plate 5 - Pollen as identified in the Control Soil samples and Evidentiary Samples

Pollen from Tricolporate spp (a-c), Rosaceae (d-f), Proteaceae (g-h), Monocolpate spp (i) and Mimosoideae (j). Magnification x 1000. All samples were field collections from the Research Garden Site. **K** = Key Species, **AK** = Additional Key Species **G** = from grouped family / type, **ANK** = Additional Non-Key Species.

a	Tricolporate sp. 6 **ANK**
b-c	Tricolporate sp. 12 **ANK**
d-f	*Rosa* sp. **ANK**
g	*Stirlingia* sp. **G** (Proteaceae spp)
h	Proteaceae spp **G**
i	Monocolpate sp. 1 **ANK**
j	*Acacia* spp **ANK**

PLATE 6

Plate 5 - Fungal spores found in the Research Garden common with the Evidentiary Samples.

Fungal spores (a-e). Magnification x 1000. All samples were field collections from the Research Garden Site.

a	Fungal spore Type 1
b	Fungal spore Type 2
c	Fungal spore Type 3
d	Fungal spore Type 4
e	Fungal spore Type 5

Plate 1

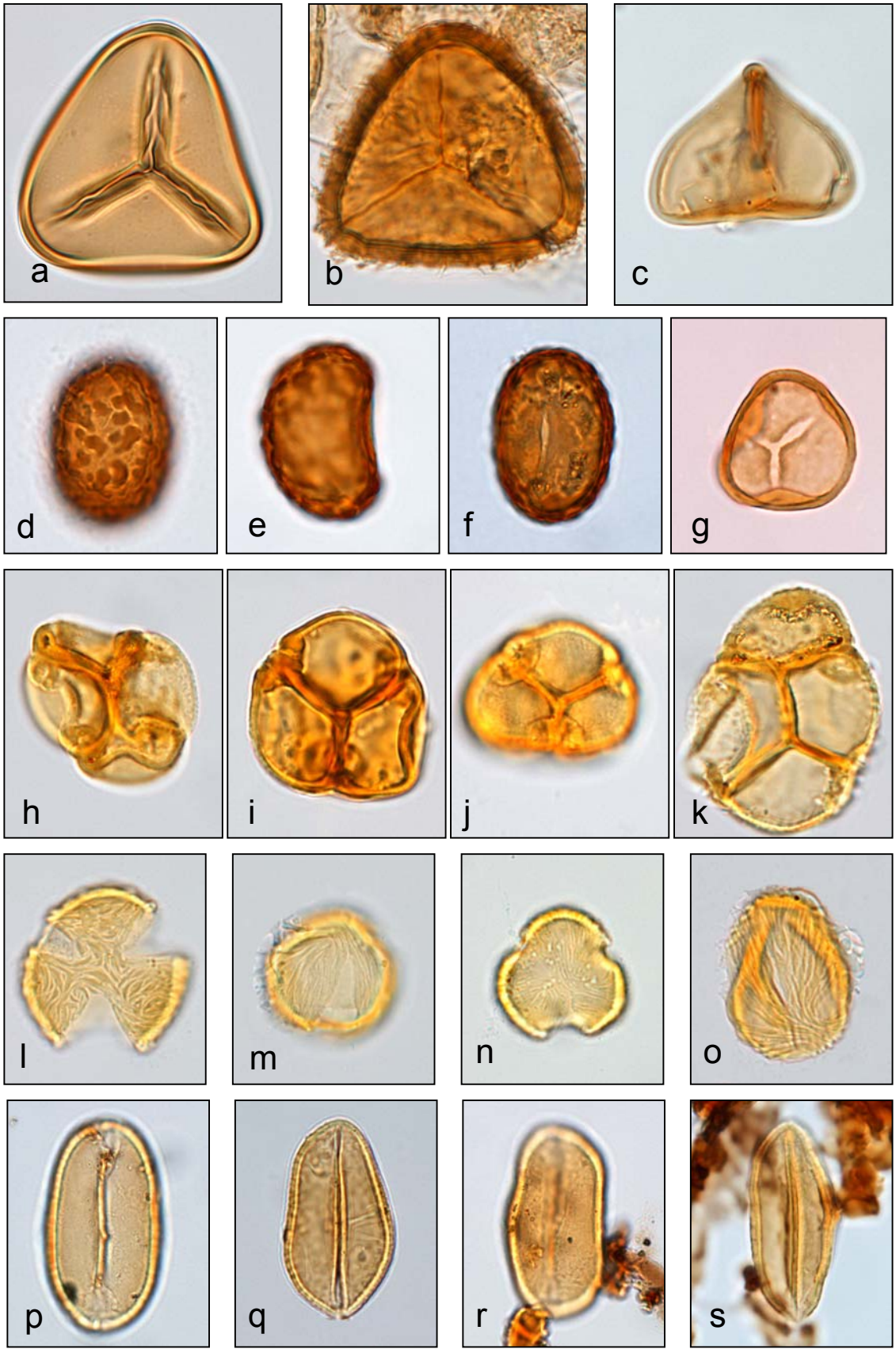
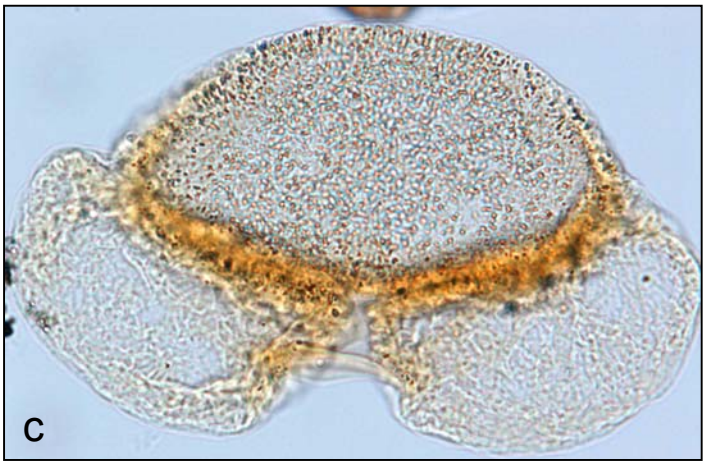
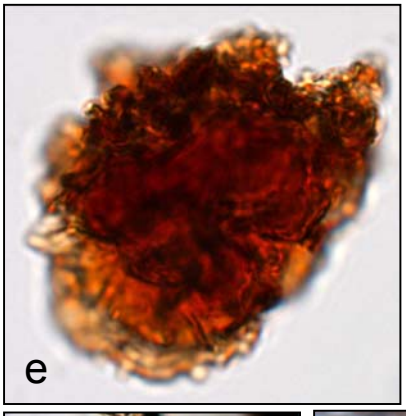
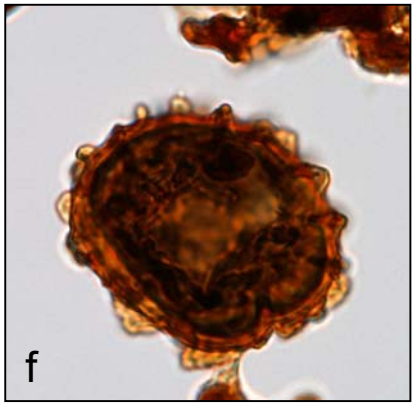
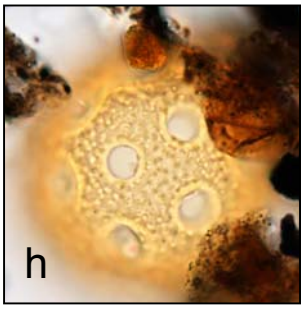


Plate 2









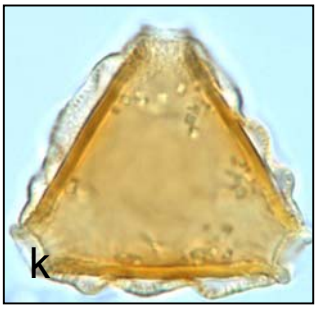


Plate 3

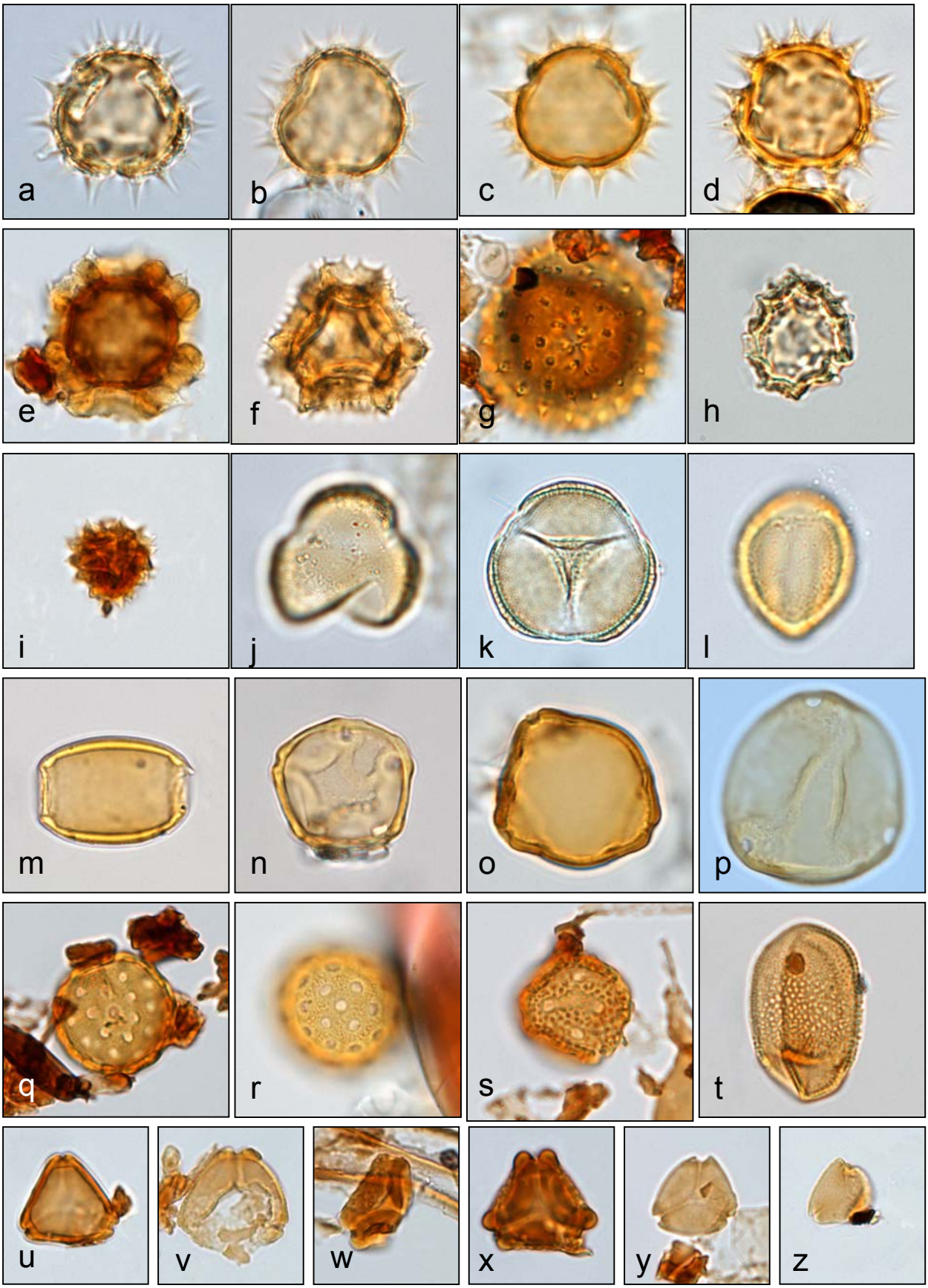


Plate 4

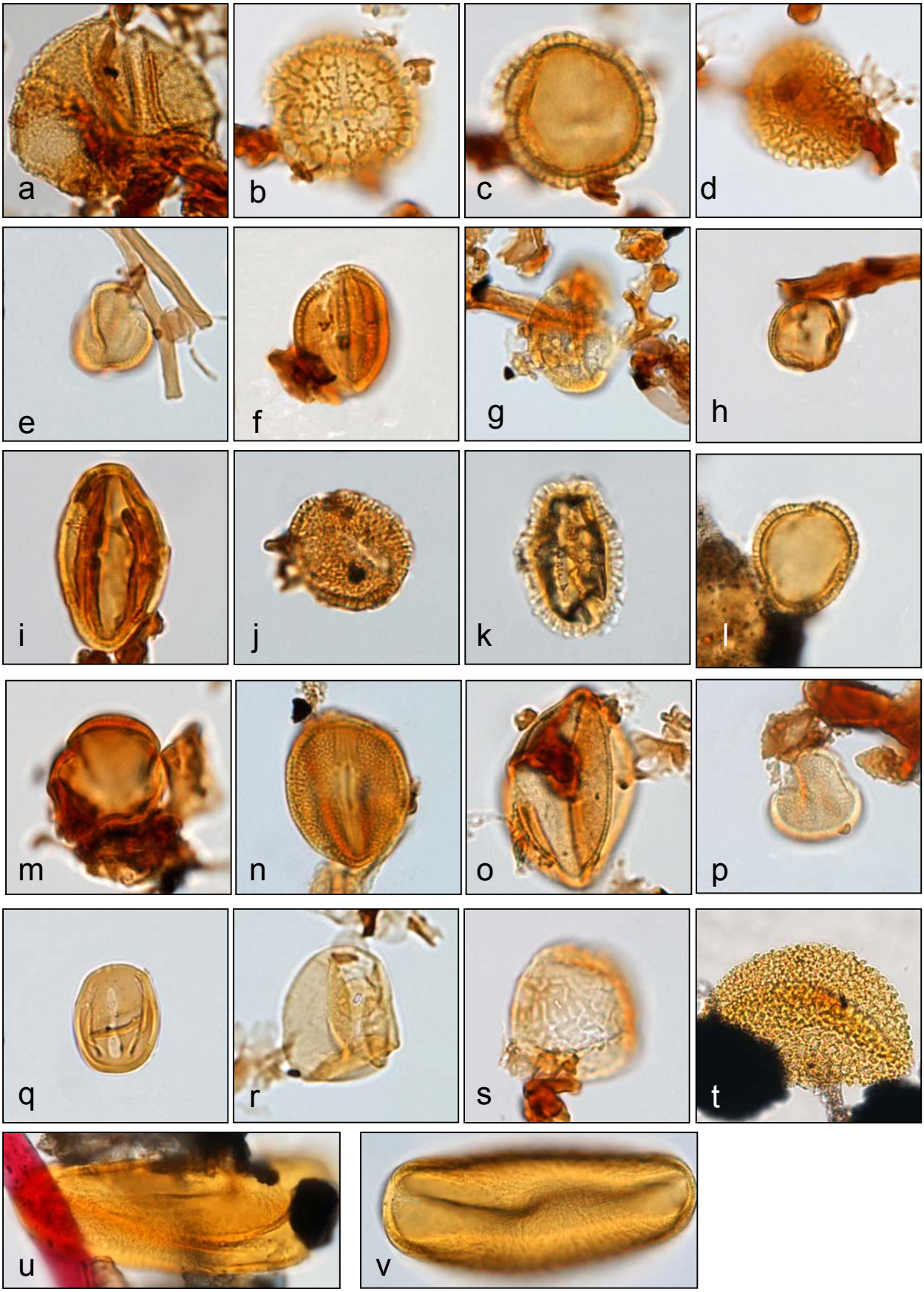


Plate 5

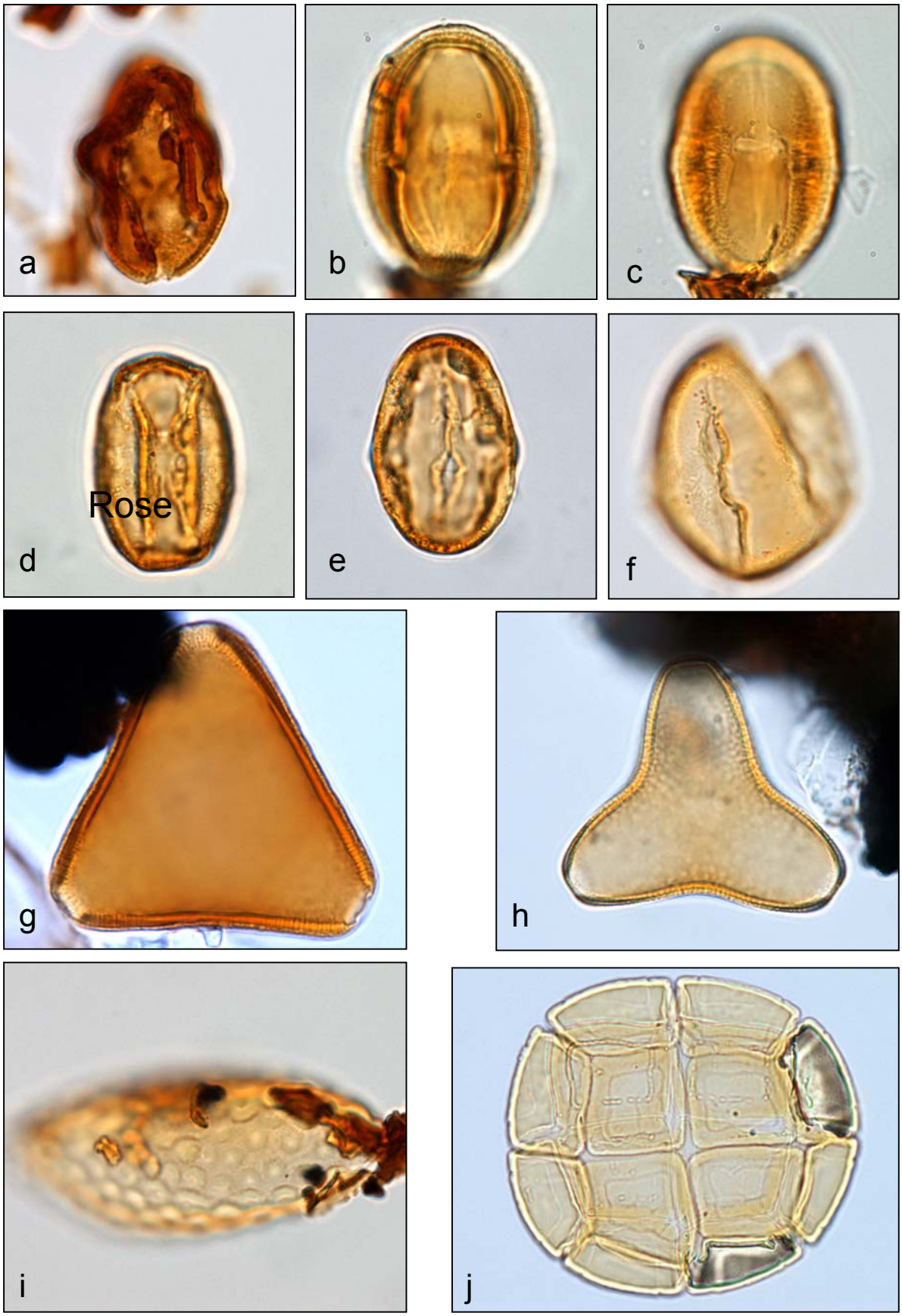
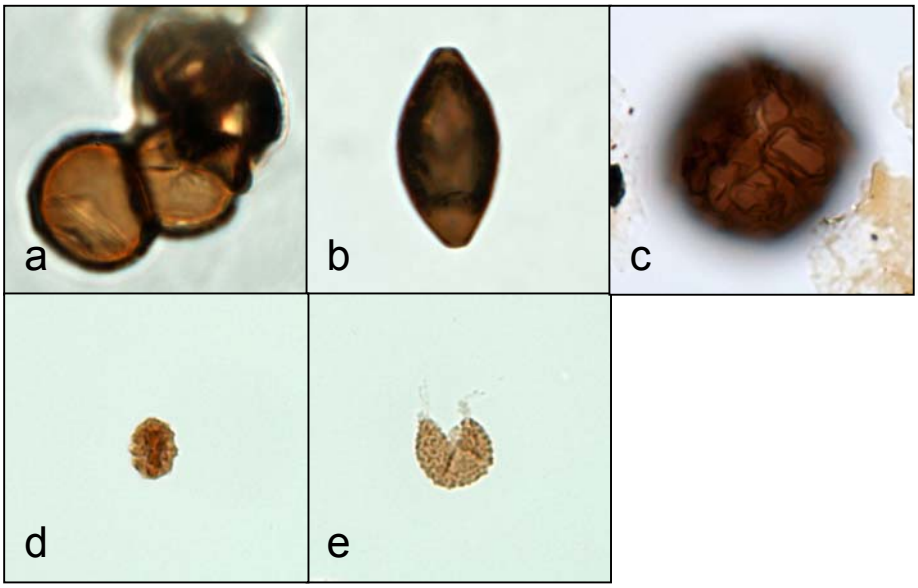


Plate 6



APPENDIX 1. Percent occurrence of all pollen types identified in the Control Soil and Evidentiary Clothing samples.

	Control Samples						Evidentiary 1						Evidentiary 2						Evidentiary 3					
	Soil Scene			Soil Local			T-Shirt			Jeans			T-Shirt			Jeans			T-Shirt			Jeans		
Samples	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Palynomorphs																								
***Acacia* spp**	0	0	0	0	0	0	0	0	1	0	0	0	0	*	1	0	*	0	0	0	2	0	*	0
Araucaria heterophylla	1	2	0	1	*	0	0	3	2	1	0	2	3	*	0	1	0	0	1	3	8	3	5	1
Asplenium australasicum	1	1	2	4	1	1	11	18	14	13	21	25	5	3	7	9	3	59	12	2	5	12	8	5
Asteraceae sp. 1	1	1	3	0	2	0	2	2	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0
Asteraceae sp. 2	7	6	8	30	28	25	4	9	6	8	10	4	6	2	2	1	4	3	6	0	2	5	1	1
Asteraceae sp. 3	1	*	*	0	*	3	4	2	3	2	2	2	1	2	0	3	1	2	1	1	0	2	0	0
Asteraceae sp. 4	0	*	0	*	0	0	0	1	*	1	1	1	0	0	0	0	0	0	1	0	0	2	0	0
Asteraceae sp. 5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asteraceae sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
Asteraceae spp	2	0	0	0	0	0	0	0	1	3	1	1	1	0	0	2	7	0	4	1	1	3	4	8
***Banksia/Dryandra* spp**	0	*	0	0	*	0	0	0	0	2	*	1	0	0	0	*	0	*	1	0	0	0	0	0
***Betula* sp.**	*	0	0	1	0	0	0	1	0	0	*	0	0	5	1	0	7	*	1	1	1	5	0	1
Camellia japonica	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Casuarinaceae spp	*	4	0	2	1	0	5	2	2	3	2	2	2	2	5	5	5	7	10	3	10	10	9	9
cf *Fumaria muralis*	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chenopodiaceae sp. 1	2	3	3	2	1	0	4	3	1	0	*	2	1	2	2	3	0	0	0	0	2	1	1	0
Chenopodiaceae spp	1	*	*	3	3	1	1	6	1	0	1	0	1	0	0	0	1	0	1	1	1	*	2	0
Cryptogam spore sp. 1	*	*	*	1	0	1	4	*	0	1	0	1	1	0	*	0	0	0	0	0	1	1	1	0
Cryptogam spore spp	0	0	0	0	0	0	0	1	1	0	0	0	0	2	0		0	0	0	0	0	0	1	0
Cyathea cooperi	*	*	0	1	0	1	0	1	0	0	0	2	3	1	2	*	1	3	0	3	1	*	0	1
Cyperaceae spp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Gardenia jasminoides	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
Harpephyllum caffrum	7	1	8	3	3	7	3	10	9	6	4	14	2	4	5	3	*	0	3	5	5	4	4	5
***Hebe* Autumn glory**	0	0	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Jasminum* sp.**	1	1	0	2	0	0	0	0	0	0	0	0	2	0	0	2	*	0	0	0	0	1	*	0

	Control Samples						Evidentiary 1						Evidentiary 2						Evidentiary 3					
	Soil Scene			Soil Local			T-Shirt			Jeans			T-Shirt			Jeans			T-Shirt			Jeans		
Samples	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Palynomorphs																								
Liliaceae sp. 1	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liliaceae spp	2	4	2	*	3	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Liquidambar styraciflua	0	0	0	1	*	0	2	2	3	0	1	2	7	5	10	6	7	5	8	17	11	16	15	4
Monocolpate sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	*	0	1	0	0	0	1	0	1	0
Monocolpate sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	*	0	0	1	0	0	0	0	1	0	0
Monocolpate sp. 2	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Monocolpate spp	0	0	0	0	0	0	0	*	0	0	1	1	0	0	0	0	1	0	0	0	1	1	0	0
Myrtaceae sp. 1	6	5	2	4	1	3	4	2	2	5	4	6	4	20	4	6	23	9	3	9	4	17	15	24
Myrtaceae sp. 2	5	9	11	7	11	6	7	4	8	5	7	5	6	5	4	1	5	3	0	0	3	0	0	1
Myrtaceae sp. 3	6	11	10	4	3	7	10	11	4	8	3	4	5	2	2	8	5	1	4	9	5	5	10	7
Myrtaceae spp	41	39	26	26	28	23	25	23	29	29	27	29	23	17	20	28	36	18	28	67	35	41	31	58
Nephrolepis cordifolia	2	2	1	18	23	20	4	5	1	1	1	2	*	2	2	6	4	3	1	2	1	4	4	1
***Pelargonium* spp**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0
Philodendron selloum	4	2	1	2	1	1	1	2	1	3	2	0	5	4	3	2	1	0	0	1	0	0	0	1
Pinus radiata	2	2	3	5	1	2	4	3	5	1	*	2	4	8	7	4	3	6	11	5	7	5	2	6
Poaceae spp	66	73	80	51	62	60	65	62	67	65	72	57	80	48	75	65	38	40	71	35	59	38	53	46
***Proteaceae* spp**	1	1	0	0	0	0	*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus suber	4	4	3	6	4	1	2	2	5	3	1	2	7	41	15	4	3	11	4	3	2	*	2	0
***Restionaceae* sp.**	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Romula rosea	0	0	0	0	0	0	1	0	0	0	*	0	0	0	0	0	0	0	1	0	1	o	1	0
***Rosa* sp**	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 1	1	0	1	0	0	0	0	*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 3	2	*	1	0	*	*	1	1	*	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 4	1	1	1	*	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 5	0	1	0	*	0	0	0	2	0	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0

	Control Samples						Evidentiary 1						Evidentiary 2						Evidentiary 3					
	Soil Scene			Soil Local			T-Shirt			Jeans			T-Shirt			Jeans			T-Shirt			Jeans		
Samples	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Palynomorphs																								
Tricolporate sp. 6	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricolporate sp. 7	2	0	4	3	2	1	4	*	1	2	2	1	5	1	1	2	9	3	1	0	1	0	5	2
Tricolporate sp. 8	0	*	0	0	1	0	2	*	1	0	2	5	0	0	0	1	0	0	0	0	0	0	0	0
Tricolporate sp. 9	0	0	0	0	1	2	0	1	1	6	1	3	0	*	1	2	0	0	0	0	1	0	0	0
Tricolporate sp. 10	0	0	0	0	0	2	1	2	5	2	3	0	4	3	3	6	2	1	4	0	3	1	3	2
Tricolporate sp. 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	8	0	0	0	0
Tricolporate spp	4	0	4	*	4	4	4	4	3	1	5	0	2	4	5	9	10	4	3	7	2	1	7	2
Triporate spp	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Triporate sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0
Typha* sp. cf. *muralis	0	0	*	2	*	*	1	1	2	3	3	1	0	0	0	1	0	0	1	0	0	*	0	0
Gen *et* sp. indet.	25	25	22	15	12	22	18	9	17	18	16	13	17	10	19	19	18	18	17	13	21	18	13	13
Gen *et* sp. indet. 1	0	0	0	3	1	2	6	4	3	3	1	4	2	3	2	1	2	3	0	2	1	2	0	0
Fungal spores	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*
***Lycopodium* spike**	6	5	5	6	10	10	1	1	4	2	3	6	157	102	292	173	82	147	3361	2685	2154	828	2037	1570

APPENDIX 2.

Percent occurrence of all pollen types identified in the Background Clothing samples.

	Background Clothing					
	T-Shirt			Jeans		
Samples	1	2	3	1	2	3
Palynomorphs						
***Acacia* spp**	2	4	0	*	3	*
Araucaria heterophylla	182	5	35	198	43	25
Asteraceae sp. 1	*	0	0	0	0	0
Asteraceae spp	1	4	3	1	20	7
***Banksia/Dryandra* spp**	*	0	0	0	0	0
***Betula* sp.**	*	0	0	0	0	0
Casuarinaceae spp	4	3	9	1	2	0
Chenopodiaceae spp	0	0	0	1	5	20
Cryptogam spore spp	0	0	0	0	0	2
Cyathea cooperi	*	1	0	0	0	0
***Grevillea* (Robyn Gordon)**	0	0	0	*	0	0
Harpephyllum caffrum	0	1	3	0	0	0
cf. Harpephyllum caffrum	0	0	3	0	0	*
Liquidambar styraciflua	10	135	104	10	99	70
Monocolpate sp. 2	0	0	0	0	0	1
Monocolpate spp	11	0	0	4	0	0
Myrtaceae spp	10	52	59	20	53	38
Nephrolepis cordifolia	1	1	1	0	0	0
Pinus radiata	42	28	7	44	25	26
Poaceae sp. 1	*	0	0	0	0	7
Poaceae spp	2	15	10	1	23	21
***Stirlingia* spp**	0	0	0	*	0	0
Tricolporate sp. 1	2	9	0	*	9	9
Tricolporate sp. 2	0	24	0	12	8	1
Tricolporate sp. 3	0	0	13	0	0	21
Tricolporate sp. 4	12	0	0	0	0	0
Gen *et* sp. indet.	21	18	53	8	10	52
***Lycopodium* spike**	110	1367	1016	164	634	367

APPENDIX 3.

Percent occurrence of all pollen types identified in the Background Compost samples.

	Background Compost		
Samples	Replicate 1		Replicate 2
Palynomorphs			
Aspleniaceae	0		2
Casuarinaceae spp	*		4
Chenopodaceae spp	2		*
Asteraceae spp	5		4
Nephrolepis cordifolia	1		
Poaceae spp	55		80
Cryptogam spore spp	*		*
Monocolpate spp	2		0
Myrtaceae sp. 1	45		34
Myrtaceae sp. 2	5		9
Pinus radiata	3		1
Cyathea cooperi	3		0
Tricolporate sp. 1	2		2
Tricolporate sp. 2	26		19
Gen *et* sp. indet.	51		45

APPENDIX 4.

1 Untransformed data for the Control Soil and Evidentiary Clothing replicate samples to be used in statistical analysis.

PALYNOMORPHS	*Araucaria heterophylla*	*Asplenium australasicum*	Asteraceae sp. 1	Asteraceae sp. 3	Asteraceae sp. 4	Asteraceae spp	Casuarinaceae spp	Chenopodiaceae sp. 1	Chenopodiaceae spp	Cryptogam spore sp. 1	*Cyathea cooperi*
SAMPLE											
CONTROL SOIL SAMPLES											
SCENE Rep. 1	1	1	1	1	0	10	0	2	1	0	0
SCENE Rep. 2	2	1	1	0	0	6	4	3	0	0	0
SCENE Rep 2.	0	2	3	0	0	8	0	3	0	0	0
LOCAL Rep1.	1	4	0	0	0	30	2	2	3	1	1
LOCAL Rep 2.	0	1	2	0	0	28	1	1	3	0	0
LOCAL Rep 3.	0	1	0	3	0	25	0	0	1	1	1
EVIDENTIARY SAMPLES – EVIDENTIARY 1											
T-shirt, Rep 1	0	11	2	4	0	4	5	4	1	4	0
T-shirt, Rep 2	3	18	2	2	1	9	2	3	6	0	1
T-shirt, Rep 3	2	14	0	3	0	7	2	1	1	0	0
Jeans, Rep 1	1	13	0	2	1	11	3	0	0	1	0
Jeans, Rep 2	0	21	1	2	1	11	2	0	1	0	0
Jeans, Rep 3	2	25	1	2	1	5	2	2	0	1	2
EVIDENTIARY SAMPLES – EVIDENTIARY 2											
T-shirt, Rep 1	3	5	0	1	0	7	2	1	1	1	3
T-shirt, Rep 2	0	3	0	2	0	2	2	2	0	0	1

T-shirt, Rep 3	0	7	1	0	0	2	5	2	0	0	2
Jeans, Rep 1	1	9	0	3	0	3	5	3	0	0	0
Jeans, Rep 2	0	3	1	1	0	11	5	0	1	0	1
Jeans, Rep 3	0	59	0	2	0	3	7	0	0	0	3
EVIDENTIARY SAMPLES – EVIDENTIARY 3											
T-shirt, Rep 1	1	12	0	1	1	10	10	0	1	0	0
T-shirt, Rep 2	3	2	0	1	0	2	3	0	1	0	3
T-shirt, Rep 3	8	5	0	0	0	3	10	2	1	1	1
Jeans, Rep 1	3	12	0	2	2	8	10	1	0	1	0
Jeans, Rep 2	5	8	0	0	0	5	9	1	2	1	0
Jeans, Rep 3	1	5	0	0	0	11	9	0	0	0	1
BACKGROUND CLOTHING SAMPLES											
T-shirt, Rep 1	121	0	0	0	0	1	3	0	0	0	0
T-shirt, Rep 2	3	0	0	0	0	3	2	0	0	0	1
T-shirt, Rep 3	23	0	0	0	0	2	6	0	0	0	0
Jeans, Rep 1	132	0	0	0	0	1	1	0	1	0	0
Jeans, Rep 2	29	0	0	0	0	13	1	0	3	0	0
Jeans, Rep 3	17	0	0	0	0	5	0	0	13	0	0
BACKGROUND COMPOST SAMPLES											
Sample 1	0	0	0	0	0	5	0	0	2	0	3
Sample 2	0	2	0	0	0	4	4	0	0	0	0

PALYNOMORPHS	***Gardenia jasminoides***	***Harpephyllum caffrum***	***Hebe* Autumn Glory**	***Jasminum* sp.**	**Liliaceae spp**	***Liquidambar styraciflua***	**Myrtaceae spp**	*Nephrolepis cordifolia*	*Philodendron selloum*
SAMPLE									
CONTROL SOIL SAMPLES									
SCENE Rep. 1	0	7	0	1	2	0	58	2	4
SCENE Rep. 2	0	1	0	1	6	0	64	2	2
SCENE Rep 2.	0	8	2	0	2	0	49	1	1
LOCAL Rep1.	0	3	1	2	0	1	41	18	2
LOCAL Rep 2.	1	3	0	0	3	0	43	23	1
LOCAL Rep 3.	0	7	0	0	3	0	39	20	1
EVIDENTIARY SAMPLES – EVIDENTIARY 1									
T-shirt, Rep 1	0	3	0	0	0	2	46	4	1
T-shirt, Rep 2	1	10	0	0	0	2	40	5	2
T-shirt, Rep 3	0	9	0	0	0	3	43	1	1
Jeans, Rep 1	0	6	1	0	1	0	47	1	3
Jeans, Rep 2	1	4	0	0	0	1	41	1	2
Jeans, Rep 3	0	14	0	0	0	2	44	2	0
EVIDENTIARY SAMPLES – EVIDENTIARY 2									
T-shirt, Rep 1	0	2	0	2	0	7	38	0	5
T-shirt, Rep 2	0	4	0	0	0	5	44	2	4
T-shirt, Rep 3	0	5	0	0	0	10	30	2	3
Jeans, Rep 1	0	3	0	2	0	6	43	6	2
Jeans, Rep 2	0	0	0	0	1	7	69	4	1

Jeans, Rep 3	0	0	0	0	0	5	31	3	0
EVIDENTIARY SAMPLES – EVIDENTIARY 3									
T-shirt, Rep 1	0	3	0	0	0	8	35	1	0
T-shirt, Rep 2	0	5	0	0	0	17	85	2	1
T-shirt, Rep 3	0	5	0	0	0	11	47	1	0
Jeans, Rep 1	1	4	0	1	0	16	63	4	0
Jeans, Rep 2	0	4	0	0	0	15	56	4	0
Jeans, Rep 3	0	5	0	0	0	4	90	1	1
BACKGROUND CLOTHING SAMPLES									
T-shirt, Rep 1	0	0	0	0	0	7	7	1	0
T-shirt, Rep 2	0	1	0	0	0	90	35	1	0
T-shirt, Rep 3	0	2	0	0	0	69	39	1	0
Jeans, Rep 1	0	0	0	0	0	7	13	0	0
Jeans, Rep 2	0	0	0	0	0	66	35	0	0
Jeans, Rep 3	0	0	0	0	0	47	25	0	0
BACKGROUND COMPOST SAMPLES									
Sample 1	0	0	0	0	0	0	50	1	0
Sample 2	0	0	0	0	0	0	43	0	0

PALYNOMORPHS	*Pinus radiata*	Poaceae spp	Proteaceae spp	*Quercus suber*	Tricolporate sp. 3	Tricolporate sp. 7	Tricolporate sp. 8	Tricolporate sp. 9	Tricolporate spp	Gen. *et* sp indet.
SAMPLE										
CONTROL SOIL SAMPLES										
SCENE Rep. 1	2	66	1	4	2	2	0	0	7	25
SCENE Rep. 2	2	73	1	4	0	0	0	0	2	25
SCENE Rep 2.	3	80	0	3	1	4	0	0	5	25
LOCAL Rep1.	5	51	0	6	0	3	0	0	0	23
LOCAL Rep 2.	1	62	0	4	0	2	1	1	6	13
LOCAL Rep 3.	2	60	0	1	0	1	0	2	6	26
EVIDENTIARY SAMPLES – EVIDENTIARY 1										
T-shirt, Rep 1	4	65	0	2	1	4	2	0	5	26
T-shirt, Rep 2	3	62	0	2	1	0	0	1	8	16
T-shirt, Rep 3	5	67	0	5	0	1	1	1	9	24
Jeans, Rep 1	1	65	0	3	0	2	0	6	5	27
Jeans, Rep 2	0	72	0	1	1	2	2	1	11	21
Jeans, Rep 3	2	57	0	2	1	1	5	3	1	23
EVIDENTIARY SAMPLES – EVIDENTIARY 2										
T-shirt, Rep 1	4	80	0	7	0	5	0	0	6	20
T-shirt, Rep 2	8	48	0	41	0	1	0	0	7	24
T-shirt, Rep 3	7	75	0	15	0	1	0	1	8	24
Jeans, Rep 1	4	65	0	4	0	2	1	2	15	21
Jeans, Rep 2	3	38	0	3	0	9	0	0	12	30

Jeans, Rep 3	6	40	0	11	0	3	0	0	6	21
EVIDENTIARY SAMPLES – EVIDENTIARY 3										
T-shirt, Rep 1	11	71	0	4	0	1	0	0	8	22
T-shirt, Rep 2	5	35	0	3	0	0	0	0	16	16
T-shirt, Rep 3	7	59	0	2	0	1	0	1	5	30
Jeans, Rep 1	5	38	0	0	0	0	0	0	2	27
Jeans, Rep 2	2	53	0	2	0	5	0	0	10	18
Jeans, Rep 3	6	46	0	0	0	2	0	0	4	14
BACKGROUND CLOTHING SAMPLES										
T-shirt, Rep 1	28	1	0	0	0	0	0	0	9	22
T-shirt, Rep 2	19	10	0	0	0	0	0	0	22	13
T-shirt, Rep 3	5	7	0	0	0	0	0	0	9	37
Jeans, Rep 1	29	1	0	0	0	0	0	0	8	7
Jeans, Rep 2	17	15	0	0	0	0	0	0	11	10
Jeans, Rep 3	17	19	0	0	0	0	0	0	21	36
BACKGROUND COMPOST SAMPLES										
Sample 1	3	55	0	0	0	0	0	0	28	53
Sample 2	1	80	0	0	0	0	0	0	21	45

APPENDIX 4 Cont.

2 Untransformed data for the combined Control Soil and Evidentiary Clothing samples to be used in statistical analysis.

PALYNOMORPHS	*Araucaria heterophylla*	*Asplenium australasicum*	Asteraceae sp. 1	Asteraceae sp. 3	Asteraceae sp. 4	Asteraceae spp	Casuarinaceae spp	Chenopodiaceae sp. 1	Chenopodiaceae spp
SAMPLE									
CONTROL SOIL SAMPLES									
SCENE	3	4	5	1	0	24	4	8	1
LOCAL	1	6	2	3	0	83	3	3	7
EVIDENTIARY SAMPLES – EVIDENTIARY 1									
T-shirts	5	43	4	9	1	20	9	8	8
Jeans	3	59	2	6	3	27	7	2	1
EVIDENTIARY SAMPLES – EVIDENTIARY 2									
T-shirts	3	15	1	3	0	11	9	5	1
Jeans	1	71	1	6	0	17	17	3	1
EVIDENTIARY SAMPLES – EVIDENTIARY 3									
T-shirts	12	19	0	2	1	15	23	2	3
Jeans	9	25	0	2	2	24	28	2	2
BACKGROUND CLOTHING SAMPLES									
T-shirts	147	0	0	0	0	6	11	0	0
Jeans	178	0	0	0	0	19	2	0	17
BACKGROUND COMPOST SAMPLES									
Compost Samples	0	3	0	0	0	13.5	6	0	3

PALYNOMORPHS	Cryptogam spore sp. 1	*Cyathea cooperi*	*Gardenia jasminoides*	*Harpephyllum caffrum*	*Hebe* Autumn Glory	*Jasminum* sp.	Liliaceae spp	*Liquidambar styraciflua*	Myrtaceae spp	Nephrolepis cordifolia	*Philodendron selloum*
SAMPLE											
CONTROL SOIL SAMPLES											
SCENE	0	0	0	16	2	2	10	0	171	5	7
LOCAL	2	2	1	13	1	2	6	1	123	61	4
EVIDENTIARY SAMPLES – EVIDENTIARY 1											
T-shirts	4	1	1	22	0	0	0	7	129	10	4
Jeans	2	2	1	24	1	0	1	3	132	4	5
EVIDENTIARY SAMPLES – EVIDENTIARY 2											
T-shirts	1	6	0	11	0	2	0	22	112	4	12
Jeans	0	4	0	3	0	2	1	18	143	13	3
EVIDENTIARY SAMPLES – EVIDENTIARY 3											
T-shirts	1	4	0	13	0	0	0	36	167	4	1
Jeans	2	1	1	13	0	1	0	35	209	9	1
BACKGROUND CLOTHING SAMPLES											
BGT	0	1	0	3	0	0	0	166	81	3	0
BGJ	0	0	0	0	0	0	0	120	73	0	0
BACKGROUND COMPOST SAMPLES											
Compost Samples	0	4.5	0	0	0	0	0	0	139.5	1.5	0

PALYNOMORPHS	*Pinus radiata*	**Poaceae spp**	**Proteaceae spp**	***Quercus suber***	**Tricolporate sp. 3**	**Tricolporate sp. 7**	**Tricolporate sp. 8**	**Tricolporate sp. 9**	**Tricolporate spp**	**Gen. *et* sp indet.**
SAMPLE										
CONTROL SOIL SAMPLES										
SCENE	7	219	2	11	3	6	0	0	14	75
LOCAL	8	173	0	11	0	6	1	3	12	62
EVIDENTIARY SAMPLES – EVIDENTIARY 1										
T-shirts	12	194	0	9	2	5	3	2	22	66
Jeans	3	194	0	6	2	5	7	10	17	71
EVIDENTIARY SAMPLES – EVIDENTIARY 2										
T-shirts	19	203	0	63	0	7	0	1	21	68
Jeans	13	143	0	18	0	14	1	2	33	72
EVIDENTIARY SAMPLES – EVIDENTIARY 3										
T-shirts	23	165	0	9	0	2	0	1	29	68
Jeans	13	137	0	2	0	7	0	0	16	59
BACKGROUND CLOTHING SAMPLES										
T-shirts	52	18	0	0	0	0	0	0	40	72
Jeans	63	35	0	0	0	0	0	0	40	53
BACKGROUND COMPOST SAMPLES										
Compost Samples	6	202.5	0	0	0	0	0	0	73.5	147

APPENDIX 5.

1 Bray – Curtis Index transformed data for the Control Soil and Evidentiary Clothing replicate samples.

SS1	0.115																			
SS2	0.12	0.15																		
SS3	0.28	0.315	0.3																	
SL1	0.235	0.29	0.26	0.16																
SL2	0.205	0.285	0.235	0.17	0.12															
SL3	0.18	0.205	0.165	0.27	0.265	0.245														
TSI1	0.22	0.285	0.235	0.275	0.24	0.245	0.18													
TSI2	0.17	0.225	0.18	0.26	0.26	0.235	0.135	0.13												
TSI3	0.145	0.21	0.165	0.265	0.255	0.215	0.14	0.18	0.12											
JI1	0.215	0.26	0.215	0.3	0.26	0.265	0.185	0.14	0.13	0.14										
JI2	0.25	0.28	0.255	0.295	0.33	0.28	0.185	0.16	0.155	0.18	0.19									
JI3	0.215	0.225	0.17	0.265	0.3	0.285	0.21	0.235	0.17	0.22	0.21	0.28								
TS3DW1	0.27	0.32	0.3	0.3	0.365	0.33	0.265	0.315	0.25	0.285	0.335	0.3	0.275							
TS3DW2	0.25	0.25	0.225	0.335	0.35	0.315	0.22	0.265	0.195	0.245	0.245	0.295	0.165	0.22						
TS3DW3	0.205	0.23	0.23	0.255	0.255	0.26	0.12	0.18	0.12	0.17	0.18	0.22	0.18	0.25	0.19					
J3DW1	0.235	0.25	0.295	0.335	0.365	0.33	0.275	0.345	0.31	0.295	0.335	0.365	0.32	0.315	0.355	0.28				
J3DW2	0.435	0.45	0.44	0.4	0.475	0.44	0.34	0.36	0.33	0.375	0.36	0.32	0.35	0.335	0.31	0.33	0.37			
J3DW3	0.23	0.255	0.235	0.295	0.305	0.285	0.19	0.205	0.135	0.18	0.165	0.26	0.165	0.295	0.16	0.165	0.315	0.31		
TSW1	0.325	0.33	0.39	0.42	0.44	0.435	0.375	0.375	0.345	0.385	0.405	0.4	0.37	0.35	0.38	0.315	0.21	0.43	0.38	
TSW2	0.225	0.245	0.225	0.295	0.32	0.265	0.175	0.25	0.195	0.205	0.285	0.23	0.225	0.245	0.2	0.195	0.265	0.36	0.19	0.305

JW1	0.28	0.26	0.335	0.345	0.42	0.365	0.26	0.31	0.28	0.275	0.345	0.295	0.34	0.33	0.35	0.29	0.195	0.355	0.27	0.23
JW2	0.225	0.255	0.27	0.295	0.315	0.32	0.205	0.245	0.225	0.245	0.265	0.26	0.245	0.295	0.275	0.195	0.22	0.36	0.225	0.235
JW3	0.275	0.285	0.325	0.335	0.365	0.37	0.315	0.33	0.31	0.295	0.34	0.35	0.345	0.335	0.375	0.32	0.22	0.41	0.305	0.185
BGTS1	0.79	0.795	0.8	0.8	0.845	0.8	0.77	0.785	0.735	0.79	0.8	0.795	0.745	0.73	0.715	0.735	0.73	0.745	0.695	0.735
BGTS2	0.635	0.645	0.645	0.635	0.645	0.64	0.62	0.59	0.58	0.64	0.615	0.635	0.575	0.575	0.575	0.545	0.565	0.61	0.535	0.47
BGTS3	0.57	0.575	0.58	0.595	0.64	0.575	0.54	0.58	0.53	0.575	0.595	0.59	0.545	0.53	0.53	0.52	0.485	0.58	0.52	0.48
BGJ1	0.835	0.855	0.85	0.845	0.845	0.845	0.825	0.8	0.79	0.85	0.835	0.85	0.78	0.785	0.775	0.79	0.79	0.8	0.745	0.765
BGJ2	0.595	0.635	0.62	0.58	0.58	0.59	0.615	0.555	0.56	0.605	0.575	0.635	0.555	0.585	0.585	0.57	0.53	0.615	0.5	0.5
BGJ3	0.575	0.6	0.59	0.6	0.64	0.58	0.575	0.57	0.545	0.6	0.6	0.61	0.55	0.55	0.525	0.535	0.49	0.575	0.505	0.48
C1	0.27	0.3	0.285	0.375	0.37	0.32	0.3	0.35	0.305	0.31	0.34	0.34	0.345	0.35	0.375	0.3	0.295	0.46	0.355	0.365
C2	0.265	0.235	0.2	0.39	0.345	0.315	0.255	0.33	0.25	0.265	0.25	0.34	0.235	0.355	0.27	0.235	0.33	0.46	0.27	0.41

APPENDIX 5 cont.

2 Bray – Curtis Index transformed data for the combined Control Soil and Evidentiary Clothing samples.

JW1	0.21										
JW2	0.155	0.17									
JW3	0.265	0.23	0.235								
BGTS1	0.69	0.74	0.73	0.79							
BGTS2	0.54	0.545	0.525	0.595	0.685						
BGTS3	0.42	0.45	0.47	0.575	0.605	0.265					
BGJ1	0.745	0.8	0.77	0.81	0.095	0.7	0.67				
BGJ2	0.52	0.525	0.5	0.565	0.59	0.22	0.21	0.575			
BGJ3	0.455	0.49	0.495	0.61	0.595	0.305	0.26	0.64	0.25		
C1	0.27	0.37	0.295	0.38	0.78	0.56	0.51	0.83	0.595	0.445	
C2	0.265	0.395	0.325	0.41	0.78	0.575	0.505	0.84	0.615	0.47	0.155