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Seasonal variation in surface soil pollen taxa over 12 months in three English mature woodlands

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Abstract

This study aimed to determine whether or not a seasonal, or even monthly, pattern of pollen accumulation could be found in surface soil. Three mature woodlands in the English Midlands were selected and soil samples were taken from the same area, each month, for 12 months. Pollen types were recorded and compared across the woodlands. Pollen condition was assessed for seasonal differences in Bannam's woodland. Two of the woodlands (Windmill Naps and Wirehill) had pollen assemblages that were dominated heavily by *Quercus* pollen but the third (Bannam's) was more balanced. Each pollen type found was present in almost every month and winter was, for some types, a time of increased pollen. No clear, consistent patterns were visible in the results across the woodlands or for each pollen type. Although some pollen types showed an increase in levels during their flowering season, this was only consistent across all three woodlands for grasses (Poaceae). The condition of the pollen grains remained similar across the 12 months with a small (up to 12%) increase in fresh pollen in May. This study concludes that for damp woodland soil in the English Midlands, a clear, seasonal or monthly pattern cannot be determined.

Keywords: *pollen assemblage, forensic palynology, pollen grain condition*

Seasonal variation of pollen in the atmosphere is well documented (Sofiev & Bergmann 2013) but little is known about this for surface soil. Pollen is always found in surface soil samples taken from any time of year (Adams-Groom 2015), but would it be fair to hypothesise that there would be seasonal differences relating to pollen seasons, particularly during the spring? This study is one of several focussing on pollen in surface soil samples with a view to increasing and quantifying information for forensic palynologists.

Pollen is useful as a trace forensic tool because it is microscopic, robust and morphologically diverse between the taxa (Walsh & Horrocks 2008; Adams-Groom 2012). Seasonal information can be useful in determining when an incident may have occurred. In some cases, it has been possible to determine a season for a victim's last exposure to the air by looking at pollen breathed into the nasal turbinates before death (Brown 2006; Wiltshire & Black 2006). Montali et al. (2006) sought to determine a 'crime pollen

calendar' and studied pollen deposition from the air onto bodies over the course of a year and were able to determine three seasons. Bruce and Dettmann (1996) analysed samples from Australian surface soil by season and found variation for several pollen types. Andersen (1974) conducted a five-year study into seasonality of pollen deposition into Tauber traps, although the results cannot be directly compared with the soil substrate where various taphonomic processes take place (Havinga 1984; Spicer 1991; Van Mourik 2003).

There are many factors that can determine the presence and amount of pollen in the soil at any given time. These include pollen rain onto a location from external sources due to season and climate (Hjelmroos 1991; Jackson & Kearsley 1998), pollination strategies and pollen productivity of local plants (Molina et al. 1996; Broström et al. 2008), plant height, local habitat structure and flowering age of trees (Matthias et al. 2012), sedi-

mentation and impaction of pollen on other surfaces, as well as soil type, condition and processes (Fægri & Iversen 1992; Traverse 1994; Adams-Groom 2015).

In the UK, mature woodland tends to be a fairly stable habitat (Proctor 2013) and therefore a good location in which to examine pollen assemblages over time. It also tends to be a frequent habitat for post-murder body deposition. Moreover, damp woodland soils usually contain plenty of palynomorphs, which can readily adhere to footwear and other items of forensic interest. When obtaining control samples from a crime scene, it is often only the top few millimetres of soil that are retrieved for comparative analysis, since this is the layer most likely to be collected on a suspect's footwear or clothing and therefore likely to offer the best match (Adams-Groom 2012).

This study aimed to determine whether or not seasonal, or even monthly differences could be

detected in pollen profiles of surface soil samples taken in woodland over a 12-month period.

Material and methods

Sampling sites

Three mature woodlands were chosen for the study, all located in the Midlands of the UK: Wirehill Wood, Bannam's Wood and Windmill Naps Wood (Figure 1). These particular woodlands were chosen because they were all Sites of Special Scientific Interest (SSSI) meaning that detailed vegetation data was readily available for them and all had easy public access. For each woodland, an area of approximately 5–8 m² was chosen, based on access to uncovered surface soil. Within each area, a number of smaller sampling locations were chosen, which comprised of fairly evenly-spaced patches of exposed soil. The samples were

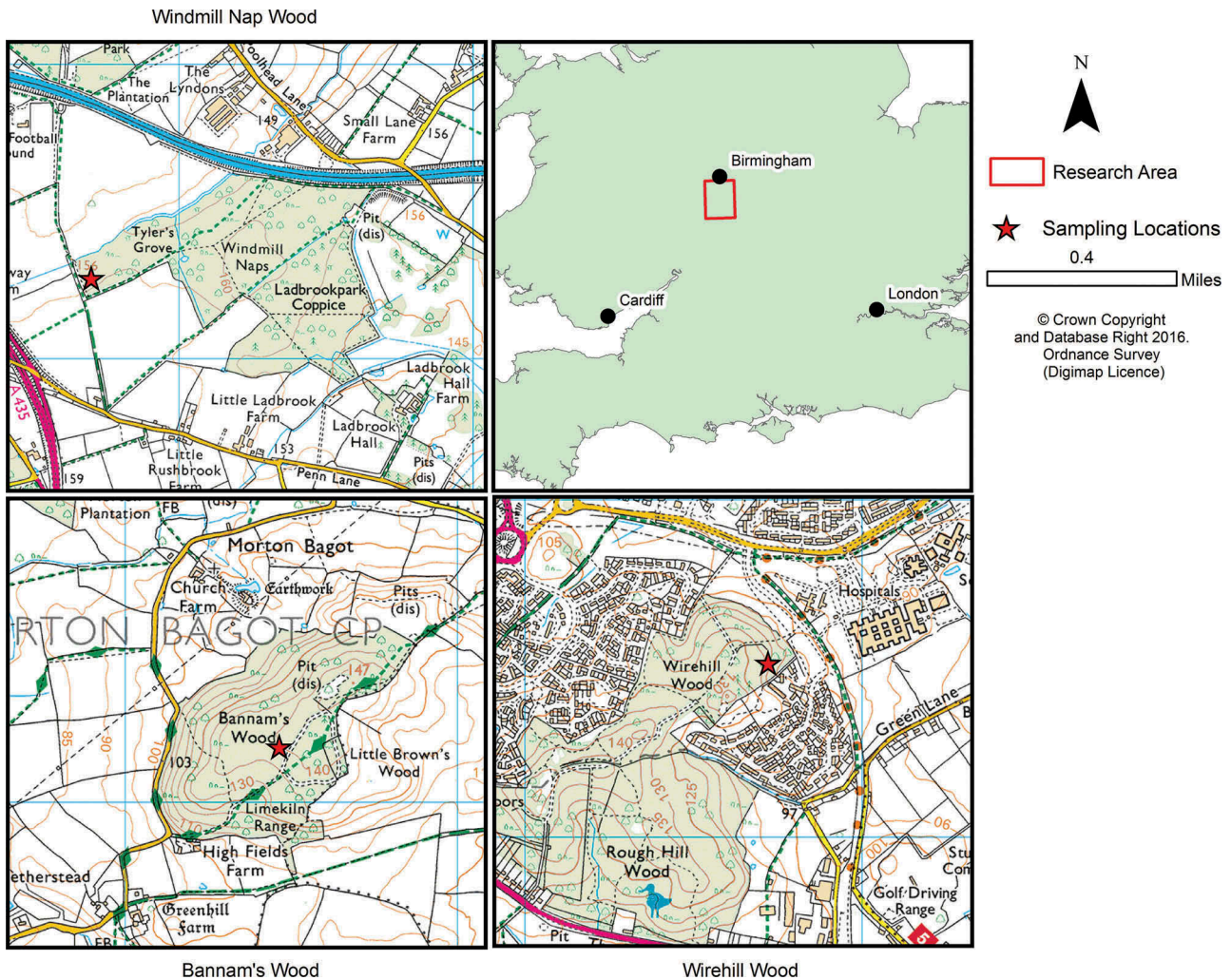


Figure 1. Map to show location of the three woods found in the Midlands of the UK and individual maps of the woods showing sampling location and surrounding habitat.

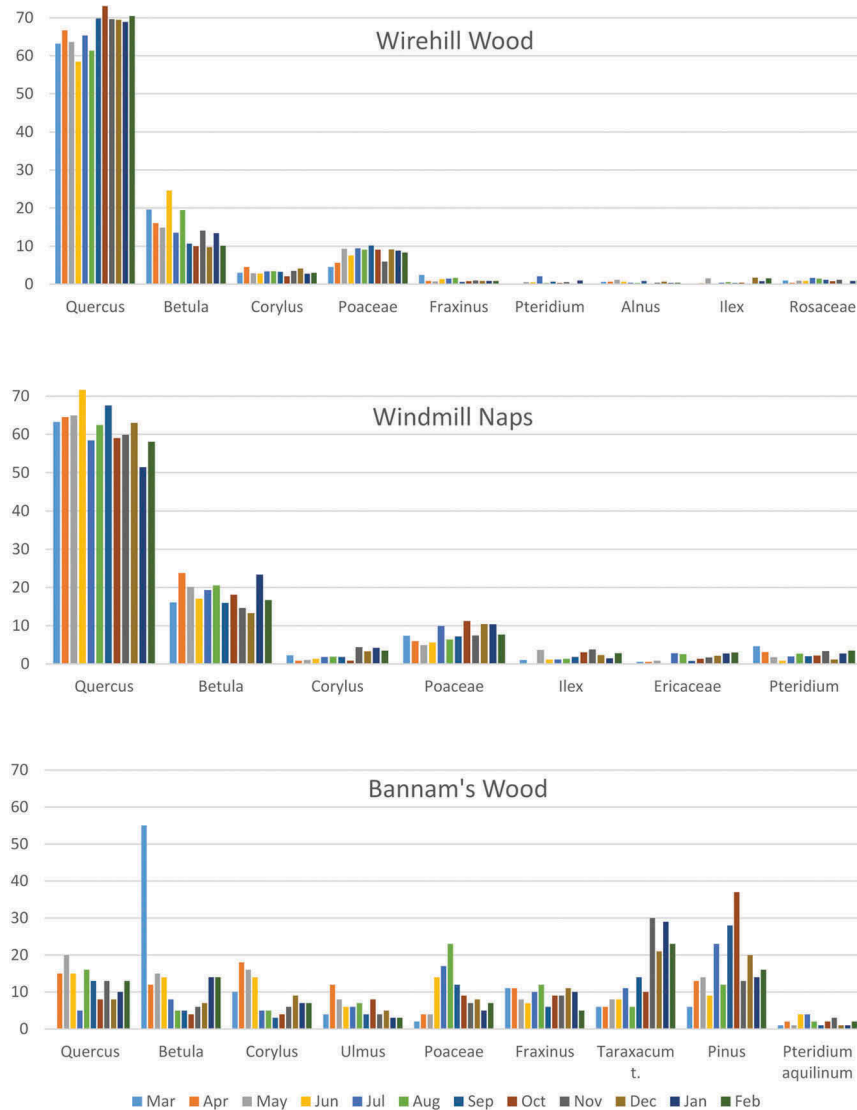


Figure 2. Pollen percentages (y-axes) for selected taxa, per month, for each woodland.

collected in the same way, in the same patch each month. However, the soil was collected from a different point within each patch, otherwise the previous month's sampling would have affected the pollen concentrations of the current month's sample. The samples were collected from areas that were as undisturbed as possible, but all three woodlands were busy with dog walkers and contained plenty of animal inhabitants.

The collection of the soil was done using flat-tipped tweezers to collect material from the top 1–2 mm of the surface until 1 cm³ had been obtained for each sample. The tweezers were marked to the 2 mm level to ensure the same depth of collection each time. If the surface of the soil was hard, the top 1–2 mm would be scraped to loosen the soil first. Three samples were taken once a month, from each woodland, for 12 months, from March to February, and at the same time of day (± 3 hours). Weather conditions and other factors, which could have had an effect on the

sample were noted for each sampling day, including average wind speed, high and low temperature, the number of days rainfall in between each sample collection and the consistency of the soil.

Wirehill Wood, grid reference (GR): SP 052640. — Located near Redditch, in Worcestershire, Wirehill Wood lies adjacent to a large hospital with housing estates on three sides and further woodland to the south and its network of paths is used frequently by walkers. It covers 17.44 ha and is an ancient woodland with vegetation varying from dense wooded pockets with abundant undergrowth to more openly spaced trees with little ground cover, as well as areas of marshland. The vegetation is dominated by sessile oak (*Quercus petraea*), downy birch (*Betula pubescens* Ehrh.) and silver birch (*B. pendula* Roth.) with more scattered presence of hazel (*Corylus avellana* L.), ash (*Fraxinus excelsior* L.), rowan (*Sorbus aucuparia* L.), lime (*Tilia cordata* Mill.

and *T. platyphyllos* Scop.), wych elm (*Ulmus glabra* L.), field maple (*Acer campestre* L.), aspen (*Populus tremula* L.), dogwood (*Cornus sanguinea* L.), privet (*Ligustrum vulgare* L.) and alder (*Alnus glutinosa* [L.] Gaertn.). The undergrowth is predominantly bracken (*Pteridium aquilinum* [L.] Kuehn.), bramble (*Rubus fruticosus* L. agg.) and bilberry (*Vaccinium myrtillus* L.) with bluebell (*Hymenophyllum wilsonii* [L.] Chouard ex Rothm.) carpeting patches of the wood. The soil is slightly acid, base-rich loamy or clayey (CSAI 2011).

The sampling area was located about 50 m from the main entrance on the eastern side of the wood and on an east-facing slope. The sampling area had a lightly-used earth path running through it. The area was one of the more open sections of the wood with limited ground cover, much leaf litter and a few bluebells. The canopy was dominated by large oak trees and smaller hazel. Within a 5-m-ring beyond the sampling area were bramble, hawthorn (*Crataegus monogyna* L.), spruce (*Picea sitchensis* [Bong.] Carriere), birch, holly (*Ilex aquifolium* L.), bracken and honeysuckle (*Lonicera periclymenum* L.).

Bannam's Wood, GR: SP 114642. — Located near Morton Bagot, in Warwickshire, Bannam's Wood is 30.6 ha and is surrounded by arable and pasture fields. Oilseed rape (*Brassica napus* L.) grew in three fields to the west during the sampling period. Popular with walkers, the wood is fairly dense throughout with some areas having a higher concentration of undergrowth than others. The wood is dominated by ash, pedunculate oak (*Quercus robur* L.), field maple, hazel and hawthorn. Other trees found throughout the wood include wych elm, blackthorn (*Prunus spinosa* L.), dogwood (*Cornus sanguinea* L.), privet and European larch (*Larix decidua* Mill.). The undergrowth is comprised of dog's mercury (*Mercurialis perennis* L.), sanicle (*Sanicula europaea* L.), woodruff (*Galium odoratum* [L.] Scop.), wild strawberry (*Fragaria vesca* L.), bramble, bracken,

bluebell, green hellebore (*Helleborus viridis* L.), carline thistle (*Carlina vulgaris* L.), yellow-wort (*Blackstonia perfoliata* [L.] Huds.) and woolly thistle (*Cirsium eriophorum* [L.] Scop.). There is also an area of pine trees (*Pinus* spp.) within the woods. The soil is lime-rich and clayey with impeded drainage (CSAI 2011).

The sampling area was a small clearing located near to the centre of the woodland with a path running through the centre of it and then dividing into two. The sampling area contained a wide range of vegetation of both trees and herbaceous plants. However, on a return visit post-sampling in the spring, to note the vegetation in detail, the area was found to have been largely cleared and was mostly bare, disturbed ground. Therefore, only the vegetation description available from Natural England was available and it is possible that some taxa had arrived/departed since their survey was made.

Windmill Naps, GR: SP 093724. — Located south of Birmingham, near junction 3 of the M42 and the villages of Portway and Wood End, Windmill Naps Wood is 35.5 ha in size. The motorway borders the north of the wood, a golf club lies to the east and arable and pasture fields to the south and west. Trees found throughout the wood include sessile oak, pedunculate oak, hazel, silver birch, downy birch, rowan, holly, crab apple (*Malus sylvestris* [L.] Mill.), alder buckthorn (*Frangula alnus* Mill.), alder (*Alnus* spp.), ash and wild service tree (*Sorbus torminalis* [L.] Crantz). The undergrowth is dominated by bramble and bracken but there is also bluebell, wavy hair-grass (*Deschampsia flexuosa* [L.] Trin.), honeysuckle, creeping soft-grass (*Holcus mollis* L.), wood sage (*Teucrium scorodonia* L.), male-fern (*Dryopteris filix-mas* [L.] Schott.), common cow-wheat (*Melampyrum pratense* L.) and purple moor-grass (*Molinia caerulea* [L.] Moench.). In one area of the wood, ornamental conifers and rhododendron can be found. The soil is seasonally wet, acid loamy and clayey.

Table I. Flowering periods (and sporulating period for *Pteridium aquilinum* [Lacey & McCartney 1994]), for taxa identifiable to genus or species, compared to the peak pollen/spore month at each woodland.

Taxon	Flowering season	Peak month for pollen type per woodland		
		Wirehill	Windmill Nap's	Bannam's
<i>Alnus</i>	February–April	May	N/A	N/A
<i>Betula</i>	March–May	June	April	March
<i>Corylus</i>	January–April	April	November	April
<i>Fraxinus</i>	March–May	March	N/A	August
<i>Ilex aquifolium</i>	May–June	December	May	N/A
<i>Quercus</i>	April–May	October	June	May
<i>Pinus</i>	April–June	N/A	N/A	October
Poaceae	May–August	September	October	August
<i>Pteridium aquilinum</i>	July–October	July	March	June & July
<i>Ulmus</i>	February–April	N/A	N/A	April

Note: N/A, information not available.

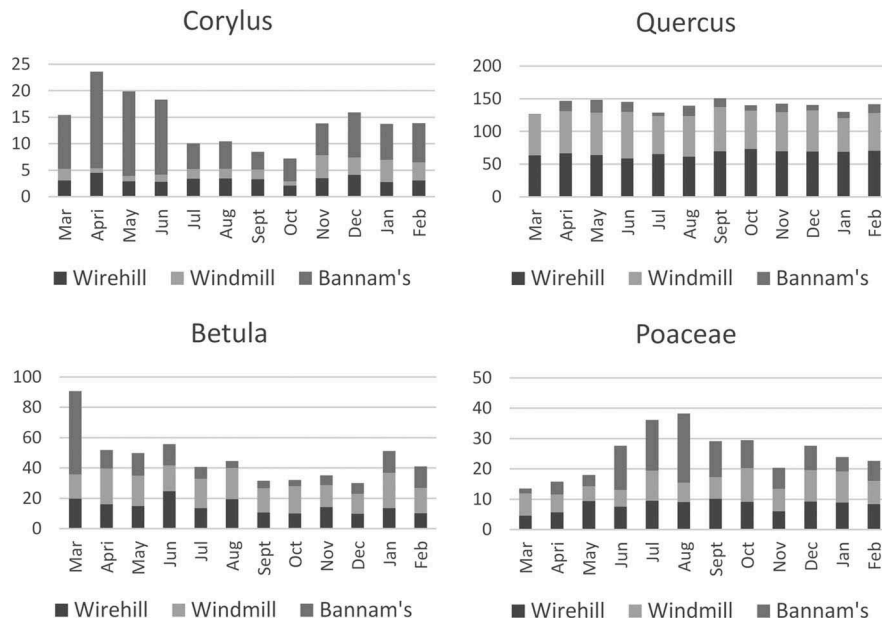


Figure 3. Pollen concentration comparison for *Quercus*, *Betula*, *Corylus* and *Poaceae* across the three woodlands (y = total percentage).

The sampling area was 3 m from the entrance where two paths met in the far southeast corner of the wood on flat ground. The sampling area included path edges and vegetated areas. The sampling area was directly beneath four mature oak trees and bluebells carpeted about half of it. Within the sampling area grew the following: holly, bramble, hazel, grasses, greater plantain (*Plantago major* L.) and frequent bracken. Within 5 m beyond the sampling area grew rowan, birch in high number, hazel, holly, bramble, bracken, grasses, speedwell (*Veronica* sp.), cleavers (*Galium aparine* L.), dandelion (*Taraxacum officinale* Wigg.), dock (*Rumex* sp.), grey willow (*Salix cinerea* L.), nettle (*Urtica dioica* L.), buttercup (*Ranunculus* sp.), thistle (*Cirsium* sp.) and bluebells.

Identification and analysis

Pollen was extracted from the soil matrix using standard techniques of hydroxide digestion, hydrochloric acid treatment, acetolysis and heavy liquid separation (Moore et al. 1991; Brown 2008). The resulting pollen pellets were mounted onto microscope slides and counted using brightfield microscopy at 400 $\times$ –1000 $\times$. For Wirehill, c. 1000 grains were counted per month, for Windmill Naps, c. 600 and for Bannam's wood, c. 500. The higher numbers were required for woods, which were dominated by one pollen type. Counts were then transformed into percentages. Only individual taxa with a total count of more than 100 for the 12 months, for each wood, were included in the

analysis as anything less was deemed too low to show accurate results.

In addition, for Bannam's Wood, a record was taken of the condition of the pollen grains to see if the percentage amount of damaged grains changed over the year. For each month, the condition of 300 grains was recorded as follows: A = good condition; B = poor condition but identifiable; C = damaged beyond recognition. Counts were then transformed into percentages.

The number of days of rainfall and the average wind speed for the periods between each sampling day were compiled. These factors may contribute to the pollen content of the soil during the post-season period (Fægri & Iversen 1992).

Results

Monthly seasonal variation in pollen counts

The monthly pollen percentages for selected taxa, for each of the three woods (Figure 2) demonstrate that useful, distinctive monthly differences cannot be clearly discerned. Each month contained pollen grains from almost every type found in each particular wood. Many taxa exhibited some increase in pollen associated with their flowering period in at least one of the woods, while for others the pollen patterns were less structured (Figure 2, Table I). Winter is not necessarily a time of reduced pollen in the surface soils. For example, *Betula* was just as high in winter as it was in spring at Windmill Naps, while *Corylus* actually showed an increase. Moreover,

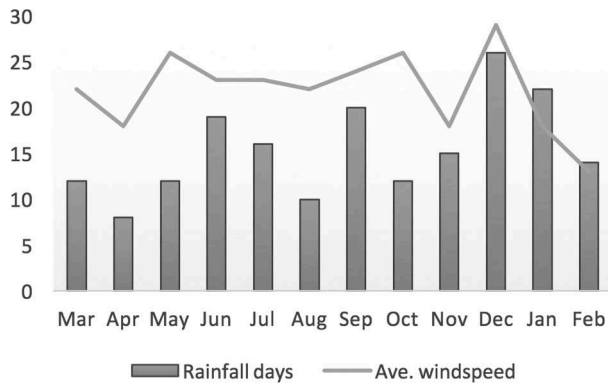


Figure 4. Number of rainfall days and average wind speed (in km/h) for the time periods between each sampling date.

in Bannam's Wood, *Taraxacum*-type was much higher in winter than at other times of year.

Wirehill and Windmill Naps Woods were dominated by oak pollen, while the profile was more balanced in Bannam's wood (Table II). For each pollen type, the pattern across the months was different to the other taxa. The pattern was also different for each individual taxon across the three woods (Figure 3). Grass pollen was the only taxon that showed a general pattern across the three woods, amounts being lowest in the spring and increasing through the months until either late summer or early autumn (Figure 2).

The number of days of rainfall and the average wind speed for the time period between each sampling date both peaked in December (Figure 4). Although there was some elevation in a few pollen types around this time of year, the results were too variable to warrant statistical correlation.

Condition data

The results of the pollen condition percentages per month show that the ratio of A:B:C ran at an average of 14:30:56, respectively (Figure 5). The ratio generally changed little during the summer, autumn and winter months, but there was an increase of 12% above the mean for pollen in good condition (A), compared to pollen damaged beyond recognition (C) in the month of May.

Discussion

This study demonstrates that seasonal differences in woodland soil cannot be clearly determined from a whole pollen assemblage or individual pollen types in a way that could contribute to admissible forensic evidence. Although seasonal differences were found for some pollen types, there was little consistency across the three woodlands and the low concentra-

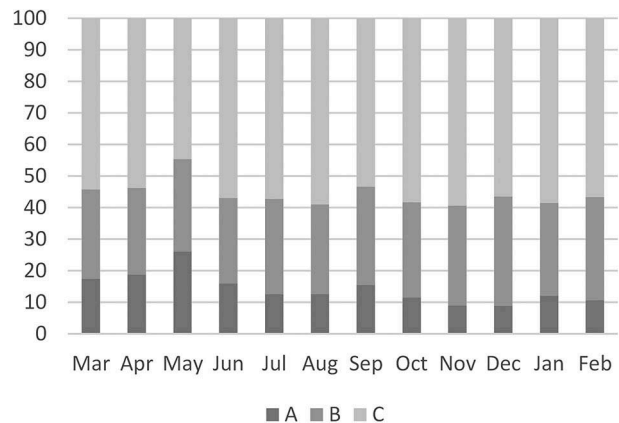


Figure 5. Pollen condition percentages (y-axis) across the 12 month sampling period for Bannam's Wood. A, good condition; B, poor condition but identifiable; C, damaged beyond recognition.

tions of many taxa further concealed patterns. Each month had a different pattern for each wood, even for the same taxon. It is not clear exactly why this should be but it would be the result of various location-specific factors, including local pollen production levels, pollen transportation, meteorological impacts, effects of soil fauna and chemistry and soil condition.

Local pollen production levels

Quercus was highly represented in the soil in all the woodlands and throughout the year as these trees were dominant in each location. *Quercus* is the main climax vegetation tree in the UK (Proctor 2013) and is wind-pollinated, but also has high deposition rates near to the source trees (Andersen 1974). Pollen production is relatively high in *Quercus* trees as demonstrated by Molina et al. (1996). Moreover, the particularly high *Quercus* levels in Windmill Naps and Wirehill Woods would also have been due to the fact that the sampling areas were dominated by large oak trees, while the immediate sampling area in Bannam's Wood was not dominated by trees of this type. Conversely, in Windmill Naps Wood, for example, despite half of the sampling area being carpeted in bluebells, only ten pollen grains were found compared to 8127 *Quercus* pollen grains. Although approximately 80% of plants are entomophilous or zoophilous in the UK, the vast majority of pollen found in the atmosphere and on the ground comes from anemophilous plants (Adams-Groom 2012). This is clearly the case here, since the anemophilous taxa of *Quercus*, *Betula*, *Corylus*, *Poaceae*, *Pinus* and *Fraxinus* dominate the abundance profile. The more balanced assemblage for

Table II. Abundance of pollen taxa found in the three woodlands.

Latin name	Common name	Wirehill	Windmill Naps	Bannam's
<i>Acer</i> spp.	Maple	—	—	*
<i>Alnus</i> spp.	Alder	**	***	***
<i>Betula</i> spp.	Birch	***	***	***
<i>Carpinus betulus</i> L.	Common Hornbeam	*	—	—
<i>Corylus avellana</i> L.	Common Hazel	***	***	***
<i>Fraxinus excelsior</i> L.	Common Ash	***	**	***
<i>Quercus</i> spp.	Oak	***	***	***
<i>Salix</i> spp.	Willow	*	—	*
<i>Tilia</i> spp.	Lime	*	*	*
<i>Ulmus glabra</i> Huds.	Wych Elm	**	—	***
Cupressaceae	Cypress family	*	—	***
<i>Larix decidua</i> Mill.	European Larch	—	—	*
<i>Picea</i> spp.	Spruce	—	*	*
<i>Pinus</i> spp.	Pine	**	**	***
Ericaceae	Heather family	*	***	*
<i>Hedera helix</i> L.	Ivy	**	**	*
<i>Ilex aquifolium</i> L.	Holly	**	***	*
<i>Lonicera periclymenum</i> L.	Honeysuckle	—	*	—
<i>Sambucus nigra</i> L.	Elder	**	—	*
Apiaceae	Carrot family	*	—	*
<i>Anemone nemorosa</i> L.	Wood anemone	**	—	*
<i>Artemisia vulgaris</i> L.	Mugwort	—	*	—
Asteraceae: <i>Bellis</i> -type	Daisy type	*	*	***
Asteraceae: <i>Taraxacum</i> -type	Dandelion type	**	—	***
Brassicaceae	Cabbage family	*	*	***
<i>Chenopodium</i> spp.	Goosefoot	—	**	**
<i>Galium</i> spp.	Bedstraw	*	—	—
<i>Hyacinthoides non-scripta</i> (L.) Chouard ex Rothm.	Bluebell	*	*	*
<i>Mercurialis perennis</i> L.	Dog's mercury	—	—	***
<i>Plantago</i> spp.	Plantain	*	*	*
<i>Rumex</i> spp.	Dock	*	*	*
Rosaceae	Rose family	***	**	**
Urticaceae	Nettle family	*	*	**
<i>Vicia</i> spp.	Vetch	*	—	—
<i>Iris</i> spp.	Iris	—	*	—
Poaceae	Grass family	***	***	***
<i>Dryopteris filix-mas</i> (L.) Schott	Male fern	—	—	*
<i>Ophioglossum vulgatum</i> L.	Adder's tongue fern	—	—	**
<i>Pteridium aquilinum</i> (L.) Kuhn	Bracken	**	***	***
<i>Polypodium</i> spp.	Polypody	**	**	**
<i>Sphagnum</i> spp.	Sphagnum	—	—	*

***, > 10% of total pollen; **, 3–10%; *, < 3%.

Bannam's Wood is probably due to the fact that the sampling site was in a small clearing, whereas the other two woodland's sampling areas were beneath the canopy where the 'gravity' or 'trunk-space components' were more prevalent (Fægri & Iversen 1992; Matthias et al. 2012).

Pollen transportation

Betula pollen was abundant in all three woods but the trees were not thought to be present in Bannam's Wood, according to the description provided by Natural England, despite the high amount of pollen found there in March. This would suggest that the pollen from this anemophilous tree had blown in from else-

where or represented a long-distance transport event. In a study by Brown (1999), grass pollen had penetrated into the centre of a woodland where it did not grow and comprised 30% of the total pollen level.

Grass (Poaceae) pollen was the only type to show a seasonal pattern, notably in Bannam's Wood and to a lesser extent in the other two woods. Grass pollen, however, is found in most samples from controls and exhibits in the UK and is, therefore, of limited use to the forensic palynologist in this country, conversely often only of interest when found in very low amounts (Adams-Groom 2015). Open habitats are affected differently by meteorological factors as they are more exposed and receive greater amounts of pollen from external sources. However, Horrocks et al.

(1998), looking at soil variation in grassland, found no significant difference in a surface soil sample taken after six months compared to those taken earlier.

Arrival of pollen into the soil due to meteorological impacts

For tree taxa in this study, it was noted that there was an increase in soil pollen for some taxa in the autumn and winter. Although the tree taxa in this study do not emit pollen at that time of year in the UK (Emberlin et al. 1990), it has been observed that pollen can be retained on leaves and twigs post-pollination and then be washed off by rainfall into the soil later in the year (Tauber 1967; Andersen 1974). The leaf fall and subsequent deterioration of the leaves, would also allow pollen adhering to them to become incorporated into the soil during the late autumn and winter, particularly when rainfall and wind speed were greater. In this study, it could be seen that both of these factors showed a winter peak so this is likely to be the cause of the increased pollen levels in the soil at that time of year.

Effects of soil fauna, condition and chemistry on pollen in soil

A number of factors lead to pollen degradation, which would have contributed to the high amount of highly degraded grains found in this study. Rich soils often contain high amounts of degraded pollen, particularly those from highly humic conditions (Van Mourik 2003). Observations by the authors have shown that pollen grains that have deposited from the air onto an exhibit are usually in much better condition.

Processes of incorporation of pollen into soil are an important factor regarding presence and condition of pollen in the soil. Differential resistance to decay processes affects the profile, duration and concentration of pollen types in the soil and decay rate and type varies between soil type (Havinga 1984). Initially, in a woodland soil system, pollen is deposited onto the leaf litter horizon 'L', then as the litter decays, the free pollen grains arrive at the horizon F1 where they become incorporated into soil fauna faecal aggregates. Soil fungi may attack these aggregates and some pollen grains become free again. After that, they may become reincorporated, repeatedly in some cases, into further faecal aggregates. In acid soils with low levels of microbiota, the rate of degradation of organic aggregates is low and soil pollen can survive well (Van Mourik 2003). Those that are not re-aggregated are then subject to mineralisation of the exine by bacterial consumption but the rate of this process varies by pollen type (Havinga 1984). The process of embedding, incorporation,

release, re-embedding and re-incorporation promotes damage to the grains. Thus, the pollen grains can survive reasonably well intact for an extremely long time or they may decay completely.

In this study, it was noted that *Pinus*-, *Ulmus*- and *Taraxacum*-types appear to remain identifiable for longer than other types. A 20-year pollen decay study by Havinga (1984) found that *Polypodium*, *Taraxacum*, *Fagus* and *Quercus* were the best survivors in leaf mould soil, whereas *Alnus* and *Corylus* pollen concentrations had decreased to less than 10% after just three years. Therefore, it can be assumed that pollen types that are long-lasting would show less seasonal change in surface soil, whereas those that decay quickly, e.g. *Corylus*, would be more likely to show a seasonal peak when fresh pollen was deposited. However, such a pattern was found only weakly in this study, presumably due to all the other factors affecting pollen in the soil.

Other processes within the soil also cause degradation such as oxidation and other chemical factors (Pearsall 2015). Davidson et al. (1999) demonstrated that pollen grains do not move more than a few centimetres down within the first soil horizon (F1) as this is the region of activity for the soil fauna, particularly earthworms. Horrocks et al. (1998) found no difference in pollen percentages at a depth of 2 cm compared to surface samples taken to 1 mm for a grassland habitat. The woodland soil consistency was generally soft when samples were taken, except in the months of April, August and September, and increased levels of leaf litter and humic material had accumulated on the surface of the soil between October and February. Since worms operate best in moist soil (Garnsey 1994), it can be assumed that a greater level of pollen incorporation from the leaf litter to the surface soil occurred during the wetter periods and this would also have contributed to the elevated autumn pollen levels.

Conclusion

This research suggests that it is not feasible in a forensic context to clearly determine season or month from pollen assemblages retrieved from woodland soil in the British Midlands. The results indicate that whichever time of year samples are taken, they are likely to contain a similar assemblage of pollen types, although the amounts may vary. The percentage of identifiable grains remains similar most of the year with a small increase in fresh pollen in the spring when pollen dispersal is at its peak in this habitat. High levels of pollen deposition and the particular accumulation factors that affect damp woodlands means that there is always plenty of pol-

len in the surface soil. Similar studies on different habitats may show a different result.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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