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Towards a ‘‘crime pollen calendar’’—Pollen analysis on corpses throughout one year

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Abstract

A palynological study was carried out on 28 corpses brought in one year (June 2003–May 2004) to the morgue of the Institute of Legal Medicine of Parma (Northern Italy). This preliminary research focuses on the date of death, which was known for all corpses examined. Pollen sampling and analyses were made with the first aim of comparing the pollen grains found on corpses with those diffused in the atmosphere in the region in the same season as the known date of death. Eyebrows, hair-line near the forehead, facial skin and nasal cavities were sampled. Most of the corpses had trapped pollen grains, with the exception of two December corpses. All pollen grains were found with cytoplasm and in a good state of preservation. In this way, a series of reference data was collected for the area where the deaths occurred, and we examined whether pollen grains on corpses could be an index of the season of death. To verify this hypothesis, the pollen analyses were compared with data reported in the airborne pollen calendars of Parma and the region around. Pollen calendars record pollen types and their concentrations in the air, month by month. The quantity of pollen recorded on corpses did not prove to be directly related to the quantity of pollen in the air. But qualitatively, many pollen types which are seasonal markers were found on corpses. Main corpse/air discrepancies were also observed due to the great influence that the local environmental conditions of the death scene have in determining the pollen trapped by a corpse. Qualitative plus quantitative pollen data from corpses appeared helpful in indicating the season of death. A preliminary sketch of a ‘‘crime pollen calendar’’ in a synthetic graphic form was made by grouping the corpse pollen records into three main seasons: A, winter/spring; B, spring/summer; C, summer/autumn. Trends match the general seasonal trend of pollen types in the air.

Keywords: Forensic science; Pollen; Corpses; Season; Pollen calendar

1. Introduction

Palynology has been used frequently to identify scenes of crime using pollen to reconstruct the plant context where a crime occurred, and sometimes to check the suspected season of death or to find out when a crime had been executed [1–6]. In other words, the two main questions the forensic palynologist is called on to answer are ‘when?’ and ‘where?’ a crime occurred. In this paper, our aim will be to focus on ‘when?’ and to investigate the problem of recognising the season of death. Details of the sites and areas under investigation will be largely left out, and only reported to clarify certain results.

Pollen by its own nature is a seasonal marker and thus it can be a useful record when the season of death is unknown [1,7]. The airborne pollen grains for each month depend on the flora and vegetation of the area, the flowering season of plant species and meteorological conditions which vary from year to year.

Pollen grains, widely dispersed in the atmosphere, constitute the pollen rain, a reflection of the regional vegetation producing the pollen [8,9]. Wind-pollinated plants, which produce great quantities of pollen and release them into the atmosphere, are the most heavily represented in the pollen rain. Zoophilous plants are less prevalent, because they produce less pollen as a consequence of their better effectiveness in their pollination process. Their pollen grains may be present but infrequent in the atmosphere, and are usually found in great numbers only near mother plants [9]. Airborne pollen grains eventually fall out or are rained out of the atmosphere and then deposited on

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the ground. The pollen rain can be studied by using artificial traps for airborne pollen or by analysing moss cushions or other terrestrial and aquatic surfaces [8,9, p. 16]. In this paper, the term 'pollen rain' is used in the sense of the quality and quantity of airborne pollen grains observed by means of microscopic analyses for a given area, rather than the 'expected production and dispersal patterns of spores and pollen for any given area' according to the definition proposed by Bryant et al. [1,10].

As surface pollen samples usually represent up to five years accumulation [9] or more, they contain a mixture of pollen produced over several months. They are not useful as seasonal pollen markers. But pollen grains which are released into the atmosphere soon after flowering are closely linked to each season. These can be easily recognised by pollen calendars based on the readings of daily slides containing the airborne pollen captured by artificial traps [11]. Such pollen calendars can indicate 'when, where and how' pollen grains are present in the atmosphere. Pollen calendars of a site show the month by month composition and concentration (pollen/m^3 of air) of the airborne pollen rain at the site and its surroundings with pollen tables and graphs. Primarily depending on the plant cover, pollen calendars may be similar in the same phytogeographical region or very different in different regions [11,12]. Italy constitutes a peninsula containing very different climatic regions, resulting in high plant biodiversity (about 6000 species according to the Flora d'Italia; [13]). More than 70 aerobiological stations over the country form a monitoring network, the Aeroallergen Italian Network coordinated by the Aerobiological Italian Association (AIA) [11]. The network collects daily data on airborne pollen, focusing on those which are known to be allergenic and can be harmful for human health. It supplies detailed information on the local pollen rains and regional-scale differences. Together, the pollen calendars show that every year there are general pollen seasons, and that these seasons are represented by characteristic main pollen types according to flowering periods. So, in Northern Italy, Cupressaceae, *Corylus* and *Alnus* are prevalent in the winter pollen rain; *Ulmus* and *Populus* are mostly found at the passage from winter to spring; *Ostrya*, *Betula*, *Acer*, *Quercus* and *Platanus* are typical of the spring season; *Pinus*, *Castanea* and Gramineae are more common in the late spring and early summer; Urticaceae, Cheno-Amaranthaceae and Compositae are the main elements of the summer and autumn pollen rains [11,14–17].

A preliminary pollen study on 19 corpses of people who died in the period 'December 2002–March 2003', with known date of death, showed that pollen grains of the season of death are present on corpses (for example, *Alnus* and Cupressaceae in February, and *Populus* in March) and that sometimes such pollen grains prevail (for example, *Corylus* in February; [18]). Therefore, pollen present on corpses appears helpful for detecting the season of death; corpses can trap it from pollen rain in the last few days before death [7,18–20]. So, we repeated the observations throughout a year with the aim of verifying how much pollen of the season of death can be recorded on corpses, and if pollen grains from corpses can be a reliable guide to the season of death.

In reality, most variables involved in criminal cases are frequently unknown, particularly those concerning the victims of murder who have acted as pollen traps. In the complex framework of many crimes, it is difficult to disentangle seasonal pollen from the more general regional and yearly pollen. Reference tools can sometimes be helpful to palynologists working in forensic investigation. Airborne pollen calendars can help to compare pollen on corpses with pollen in the air during the time of death. However, they can have intrinsic problems and thus can be of limited use for forensic purposes [18,20, and unpublished data]. For example, it must be taken into account that artificial spore-traps are generally positioned above ground level where people walk, and they more easily capture the light, medium to small pollen grains with smooth exine, which are present at higher levels [21]. Moreover, a monitoring station is a single pollen-sampling point and caution should be used in interpreting pollen conditions over a wider area [22]. Rare pollen grains can also be lost during routine slide readings [23].

To our knowledge no detailed comparisons between pollen on corpses and in the atmosphere in the same month have been made so far. We therefore attempt such comparisons, and investigate the possibility of producing a special pollen calendar which consists of pollen data from corpses. This "crime pollen calendar" aims to supply information about the different typologies of pollen grains trapped by corpses depending on the season. Towards this goal, our first step was to standardise sampling points and methods [18], and next present here a further preliminary step in this research. We examine data obtained from corpses of persons who died over a whole year through a systematic pollen sampling. The period is June 2003–May 2004 and the area is Parma, a city in the Emilia Romagna region in Northern Italy (Fig. 1), home of Italy's RIS Scientific Police Force.

2. Method

The study deals with corpses taken to the morgue of the Institute of Legal Medicine—University of Parma in the period June 2003–May 2004. Altogether 28 corpses, from 1 to 4 per month, suitable for pollen analyses were examined (Table 1). Corpses were directly transported to the morgue soon after

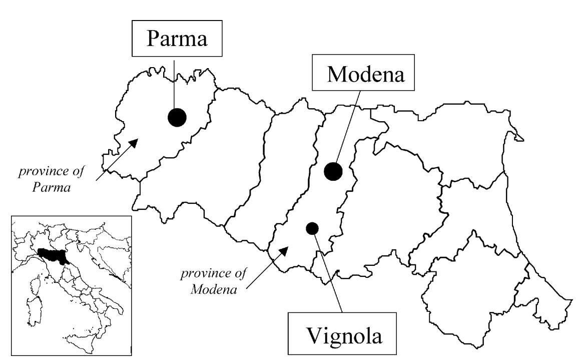

Fig. 1. Location map of the Emilia Romagna region (in black) in Northern Italy, and position within Emilia Romagna of the three monitoring stations quoted in the text.

Table 1
Notes on deaths (places and months) and sampling points on corpses studied in the period June 2003–May 2004

Case	Notes on the death				Sampling on corpses							
	In/outdoor	Site	Town/city	Month	Date	Face	Nasal cavity		Eyebrow		Hair	No. of slides
							Right	Left	Right	Left		
1	Out	Near a river	Pontetaro	June	6/12/2003				x		x	2
2	In	Hospital	Parma		6/13/2003				x		x	2
3	Out	Unknown	Salsomaggiore		6/16/2003				x	x	x	3
4	Out	Unknown	Zibello		6/18/2003				x		x	2
5	In	Home	Parma	July	7/4/2003	x	x	x	x	x	x	6
6	Out	Courtyard	Fornovo		7/9/2003	x	x	x	x	x	x	6
7	Out	Garden	Parma		7/14/2003	x	x		x	x	x	5
8	In	Home	Parma	August	8/4/2003	x	x	x	x	x	x	6
9	In	Home	Parma		8/5/2003		x	x	x	x	x	5
10	Out	Near a river	Salsomaggiore		8/11/2003	x	x	x	x	x	x	6
11	In	Home	Fidenza		8/28/2003	x	x	x	x	x	x	6
12	In	Home	Corniglio	September	9/8/2003	x	x	x	x	x	x	6
13	Out	Farmyard	Colorno		9/16/2003	x	x	x	x	x	x	6
14	In	Home	Bore	October	10/16/2003	x	x	x	x	x	x	6
15	In	Jail	Parma		10/30/2003	x	x	x	x	x	x	6
16	In	Hospital	Parma	November	11/2/2003	x	x	x	x	x	x	6
17	In	Hospital	Parma		11/26/2003	x	x	x	x	x	x	6
18	In	Home	Parma	December	12/19/2003		x	x				2
19	In	Hospital	Parma		12/22/2003		x	x				2
20	In	Home	Parma	January	1/14/2004	x	x	x	x	x	x	6
21	Unknown	Unknown	Unknown		1/22/2004	x	x	x	x	x	x	6
22	Out	Wood	Albareto	February	2/14/2004	x	x	x	x	x	x	6
23	Out	Motorway	Parma		2/18/2004	x	x	x	x	x	x	6
24	In	Home	Parma	March	3/2/2004	x	x	x	x	x	x	6
25	In	Home	Parma		3/12/2004	x	x	x	x	x	x	6
26	In	Home	Parma		3/26/2004		x	x	x	x	x	5
27	In	Home	Parma	April	4/12/2004	x	x	x	x	x	x	6
28	Out	Building site	Colorno	May	5/8/2004	x	x	x	x	x	x	6

death. This allowed us to exclude the possibility that more recent pollen grains were trapped by corpses. The people died indoors, i.e. at home, in jail or hospital, in 61% of the cases, and outdoors, i.e. in a wood, garden or building site, in 36% of the cases. In one case, it was unknown if the death occurred inside or outside. No details on the state of cadavers (dressed, covered, and so on) and the environmental setting were communicated to physicians so this information could not be obtained by palynologists.

At the morgue, to avoid any contamination, a series of safety measures, basic to pollen sampling, were taken. These included thorough cleaning of hands, use of disposable gloves, disposable spatulas and brushes, new slides, cleaned tools, and closed doors and windows [20]. Possible airborne contamination by pollen and spores present in the laboratory was checked by exposing slides covered with silicon oil or glycerine and water (50% of volume) during the sampling phases. No pollen was located.

As the aim of this study was to verify if pollen grains of the season of death were present on corpses, we decided to take samples only from facial skin, nasal cavities, eyebrows and hair-base (only the base near the forehead). These parts are more exposed and suitable to trap pollen from the air, and they are usually washed quite frequently, daily or weekly, by everyone. Thus, they should better reflect the pollen rain of the time of death [18,20]. Pollen grains were directly mounted on slides avoiding any chemical treatment which could destroy the cytoplasm and intine. Pollen from the nose was obtained by sampling within the nasal cavities with spatulas, and the slides were haematoxylin-eosin stained. Some pollen were mounted in glycerol jelly and stained with basic fuchsine, according to the methods routinely adopted in aeropalynology [11,24]; they were smeared with silicon oil immediately before the sampling. Overall, 142 slides were examined, from 2 to 6 per case according to the condition of the corpses (Table 1).

Microscopic examination was accomplished by light microscope (400× and 1000× magnifications) in the Palynological Laboratory of Modena. Identifications were made with the help of a reference slide collection plus atlases and keys [8,25–29]. The pollen flora was named following the botanical nomenclature of the Flora d'Italia [13] (for example, Compositae instead of Asteraceae, and Umbelliferae for Apiaceae). Results were filed in an electronic database. The number of pollen grains and fungi spores and hyphae recorded

Table 2
Palynological analyses of corpses: number of records (pollen grains, plus fungi in brackets) per sampling point and per case and number of pollen types, per case and total

Case	Notes on the death		Pollen (and fungi) recorded							
	In/outdoor	Month	No. of records							No. of types
			Face	Nasal cavity		Eyebrow		Hair	Total	
				Right	Left	Right	Left			
1	Out	June				5		7	12	3
2	In					0		8	8	3
3	Out					26	0	18	44	6
4	Out					3		0	3	2
5	In	July	7 (8)	0	0	10 (5)	7	0	24 (13)	4 (2)
6	Out		50	0	0	12	14	19	95	12
7	Out		3	27 (2)		15 (7)	7	14 (6)	66 (15)	8 (2)
8	In	August	14	0	0	4	7	4	29	4
9	In			0	0	2	5	4	11	6
10	Out		0	0	0	0	0	4	4	1
11	In		32	0	0	0	0	0	32	6
12	In	September	8	2 (2)	0	10 (6)	3	3	26 (8)	7 (2)
13	Out		2	3 (5)	0	1 (3)	1	3 (1)	10 (9)	4 (2)
14	In	October	0	0	0	0	1 (4)	1	2 (4)	1 (2)
15	In		0	0 (1)	0	0	1 (2)	1	2 (3)	1 (2)
16	In	November	3	0	0	4	0	3	10	4
17	In		0 (1)	0	0	2 (1)	3	2 (1)	7 (3)	3 (2)
18	In	December		0	0				0	–
19	In			0	0				0	–
20	In	January	2	1	0	3	4	2	12	6
21	Unknown		2	0	0	1	1	4	8	3
22	Out	February	5	3	2	3	1	0	14	5
23	Out		1	0	0	4	1	1	7	4
24	In	March	0	0	0	2	0	0	2	1
25	In		6	0	0	1	0	0	7	2
26	In			0	0	1	0	1	2	7
27	In	April	0	5	2	4	6	0	17	3
28	Out	May	1	0	0	0	0	2	3	2
Total records			136 (9)	45 (10)		175 (28)		101 (8)	457 (55)	34 (2)

from each sampling point per corpse is shown in Table 2, and per month in Table 3. Table 3 also shows the percentage yearly pollen spectrum calculated on a pollen sum including all recorded pollen grains. Pollen taxa were subdivided into three ‘‘seasonal groups’’ using the three airborne pollen seasons suggested by Accorsi et al. [15]. Each pollen taxon was assigned to one or more seasonal groups according to its main ‘‘flowering/presence in the air’’ period in the region, based on aerobiological data from more than 10 years of monitoring readings. Seasonal pollen groups (i.e., the ‘expected’ A–C pollen season in Table 3) are: A, winter/spring pollen (from January to the first half of April, with peaks in March-first half of April); B, spring/summer pollen (from the second half of April to July, with peaks in the second half of April–May); C, summer/autumn pollen (from August to December, with peaks in July–September).

Pollen data from corpses was compared with the airborne pollen data of the monitoring station of Parma run by the local Health Agency, and with data of other two monitoring stations of the Emilia Romagna region, in Vignola and in Modena, run by our laboratory ([14,17,30,31 and unpublished data; Fig. 1). The stations of Parma and Vignola are part of the Aeroallergen Italian Network. The stations of Vignola and Modena read all pollen types recorded, including very rare types, as they are run also with a scientific aim [15]. The pollen concentrations reported in the paper, if not otherwise stated, refer to the Parma station. According to the method of this network, pollen is removed from the air by volumetric seven-day Hirst-type traps (aspiration method). Comparison of pollen data from corpses and air concerned both the variety of pollen types and their abundance (Fig. 2). The abundance of selected types was compared by the non-parametric Spearman’s Rank correlation coefficient method (Table 4).

3. Results

Pollen and fungi were recorded from 26 corpses (Table 2). The two corpses with no records were those of persons who

Table 3
Palynological analyses of corpses: monthly and yearly spectra and sums of the three seasonal groups (A–C)

Percentage pollen spectra		Expected season	Monthly spectra												Yearly spectrum
Pollen families	Pollen types		B		C					A			B		
			June'03	July	August	September	October	November	December	January'04	February	March	April	May	
Betulaceae	Alnus	A	1.5						–	15.0	9.5	9.1			1.5
Cannabaceae	Humulus	C		0.5	13.2				–						2.4
Cheno/Amaranthaceae	Cheno/Amaranthaceae	C			9.2	22.2			–						3.3
Compositae	Ambrosia	C				2.8			–						0.2
	Artemisia	C	4.5	1.6	1.3				–						1.5
	Xanthium cf.	C	1.5		3.9			5.9	–						1.1
	Asteroideae indiff.	C			2.6			11.8	–						0.9
Corylaceae	Carpinus	A							–			9.1			0.2
	Corylus	A							–	15.0	42.9	18.2			3.1
Cruciferae	Cruciferae	B/A						17.6	–						0.7
Cupressaceae	Juniperus type	A/C	1.5	1.1	1.3				–	10.0		9.1			1.5
Euphorbiaceae	Mercurialis	C					50.0	41.2	–	25.0	4.8	9.1			3.5
Fagaceae	Castanea	B	13.4	0.5					–						2.2
	Quercus	A		3.8		2.8			–						1.8
Gramineae	Gramineae—wild group	B/A/C	46.3	33.0	35.5	13.9	50.0	5.9	–	30.0	14.3		35.3	66.7	31.5
Juglandaceae	Juglans	A		1.6		16.7			–		4.8		47.1		3.9
Leguminoseae	Medicago	B/A		4.3	10.5				–						3.5
	Robinia cf.	B		2.2					–						0.9
	Leguminosae indiff.	B/A		3.8	1.3				–					33.3	2.0
Oleaceae	Fraxinus	A							–			9.1			0.2
Papaveraceae	Papaver rhoeas type	B		0.5					–						0.2
Pinaceae	Picea	B		1.1					–						0.4
	Pinus	B	3.0	0.5	6.6	27.8			–			9.1			4.2
	Pinaceae indiff.	B/A/C							–		9.5				0.4
Plantaginaceae	Plantago	C		7.0	1.3				–						3.1
Platanaceae	Platanus	B							–		9.5				0.4
Polygonaceae	Rumex	C	3.0	5.9	1.3				–						3.1
Ranunculaceae	Ranunculus type	B/A/C		2.7					–						1.1
Salicaceae	Populus	A		3.2					–	5.0		18.2			2.0
Tiliaceae	Tilia	B	9.0	13.5					–						6.8
Ulmaceae	Ulmus	A							–			9.1			0.2
Umbelliferae	Umbelliferae	C		0.5	1.3				–						0.4
Urticaceae	Parietaria cf.	C	16.4	12.4	7.9	13.9			–						9.8
	Urtica dioica cf.	C			2.6			17.6	–		4.8		17.6		2.0
Monthly pollen sum			67	185	76	36	4	17	0	20	21	11	17	3	457
Pollen types			10	20	15	7	2	6	–	6	8	9	3	2	34

Table 3 (*Continued*)

Percentage pollen spectra		Expected season	Monthly spectra												Yearly spectrum
Pollen families	Pollen types		B		C					A			B		
			June'03	July	August	September	October	November	December	January'04	February	March	April	May	
Monthly percentage sum of seasonal pollen groups		A	*3.0*	*9.7*	*1.3*	*19.4*			–	*45.0*	*57.1*	*81.8*	*47.1*		*14.4*
		B	*71.6*	*62.2*	*53.9*	*41.7*	*50.0*	*23.5*	–	*30.0*	*33.3*	*9.1*	*35.3*	*100.0*	*54.3*
		C	*25.4*	*28.1*	*44.7*	*38.9*	*50.0*	*76.5*	–	*25.0*	*9.5*	*9.1*	*17.6*		*31.3*
Fungi															
	Alternaria			6.1		15.1	36.4	10.0	–						5.3
	Epicoccum			7.0		17.0	27.3	5.0	–						5.5

The 'expected' season (A–C) corresponds to flowering seasons as determined by the airborne pollen monitoring station of Parma as well as by other monitoring stations of the Emilia Romagna region [15]. Pollen types belonging to more than one group were calculated with the first.

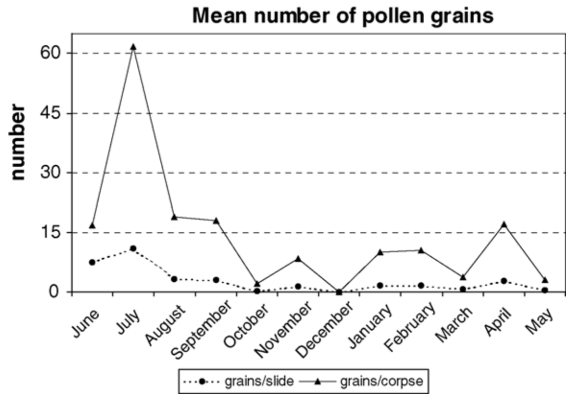

Fig. 2. Pollen on corpses: monthly trends of the mean number of pollen grains recorded per corpse and per slides.

died in December. A total of 457 pollen grains and 55 fungi (spores and hyphae) were recorded.

The number of pollen grains recorded can be considered from different points of view:

(a) *per slide*: altogether, the number per slide varies from 0 in 44% of slides, to 50 in case no. 6 of July, from the face (Table 2). In the months, the mean number per slide ranges from minimum 0 in December to maximum 11 in July (Fig. 2).
(b) *per corpse*: altogether, the number per corpse varies from 0, in case nos. 18 and 19 of December, to 95, in case no. 6 of July (Table 2) and the mean number per corpse/month from 0 in December to 65 in July. The mean number of pollen per corpse and per slide have very similar trends (Fig. 2) since the variation in the total amount of pollen recorded on corpses corresponds to the variation in pollen content of the slides.
(c) *per sampling point*: pollen grains were recorded in all the four sampling points, more frequently in the hair-base, eyebrows and facial skin. Eyebrows captured the highest number of pollen grains (175 in 22 corpses; Table 2). Nasal cavities gave the lowest and most discontinuous results.

3.1. Preservation

All the pollen grains observed in the slides contained their cytoplasm and intine, i.e. they were 'fresh'; no empty exines were recorded. The state of preservation was generally very good, and only few folded grains were found. Also, they did not present clear signs of chemical or biological deterioration of cytoplasm or exine.

3.2. Pollen abundance

Pollen abundance in corpses and air are compared in Fig. 3. The number of grains (monthly mean per slide) and the airborne pollen concentration (monthly sum) show quite similar trends in six months from July to January, while from February to June trends are not consistent. The highest pollen contents on corpses were observed in June and July, two months with very

Table 4
Comparison between pollen on corpses and in air: quantitative data (pollen amount) and qualitative/quantitative data (Spearman's correlation of the main pollen types in each month; HC, high correlation; LC, low correlation; icf, insufficient common families)

Case	Notes on the death		Pollen on corpses and air					
	In/outdoor	Month	Amount (quantitative)		Spearman's correlation (main types in corpses and in air)		Type (qualitative)	
			Total pollen on corpses (number)	Total pollen in air (monthly concentration)			Prevalent on corpse	Prevalent in air
1	Out	June	12	8150 (High)	rs = 0.71 ($N = 8$, $\alpha = 0.05$)—HC	Betulaceae, Compositae, Cupressaceae, Fagaceae, Gramineae, Pinaceae, Polygonaceae, Urticaceae	Gramineae	Fagaceae, Gramineae, Urticaceae
2	In		8				Gramineae	
3	Out		44				Gramineae	
4	Out		3				Gramineae	
5	In	July	24	1990 (Medium)	rs = 0.851 ($N = 9$, $\alpha = 0.01$)—HC	Compositae, Cupressaceae, Fagaceae, Gramineae, Pinaceae, Plantaginaceae, Polygonaceae, Salicaceae, Urticaceae	Gramineae	Gramineae, Urticaceae, Fagaceae
6	Out		95				Gramineae	
7	Out		66				Gramineae	
8	In	August	29	1650 (Medium)	rs = 0.863 ($N = 8$, $\alpha = 0.01$)—HC	Chenopodiaceae, Compositae, Cupressaceae, Gramineae, Pinaceae, Plantaginaceae, Polygonaceae, Urticaceae	Gramineae	Urticaceae, Chenopodiaceae, Gramineae
9	In		11				Gramineae	
10	Out		4				Humulus	
11	In		32				Robinia	
12	In	September	26	1580 (Medium)	rs = 0.4 ($N = 6$, $\alpha = 0.50$)—LC	Chenopodiaceae, Compositae, Fagaceae, Gramineae, Pinaceae, Urticaceae	Chenopodiaceae	Urticaceae, Chenopodiaceae, Gramineae
13	Out		10				Pinus	
14	In	October	2	90 (Very low)	–	–	Gramineae	Pinaceae, Urticaceae, Gramineae
15	In		2				Mercurialis	
16	In	November	10	60 (Very low)	rs = 0.85 ($N = 4$, $\alpha = 0.50$)—LC	Compositae, Euphorbiaceae, Gramineae, Urticaceae	Urtica	Gramineae, Urticaceae, Cupressaceae
17	In		7				Mercurialis	
18	In	December	0	60 (Very low)	–	–	–	Cupressaceae, Gramineae, Urticaceae
19	In		0				–	
20	In	January	12	470 (Low)	No correlation	[Betulaceae, Corylaceae, Cupressaceae, Euphorbiaceae, Gramineae]	Betulaceae	Cupressaceae, Corylaceae, Betulaceae, Gramineae
21	Unknown		8				Gramineae	
22	Out	February	14	152 (Low)	rs = 0.83 ($N = 7$, $\alpha = 0.05$)—HC	Betulaceae, Corylaceae, Euphorbiaceae, Gramineae, Pinaceae, Platanaceae, Urticaceae	Corylus	Corylaceae, Cupressaceae, Gramineae
23	Out		7				Gramineae	
24	In	March	2	11160 (Very high)	No correlation	[Betulaceae, Corylaceae, Cupressaceae, Gramineae, Pinaceae, Salicaceae, Ulmaceae, Oleaceae]	Cupressaceae	Cupressaceae, Salicaceae, Betulaceae
25	In		7				Corylus	
26	In		2				Salicaceae	
27	In	April	17	2680 (Medium)	–	–	Juglandaceae	Gramineae, Platanaceae Corylaceae
28	Out	May	3	15350 (Very high)	–	–	Gramineae	Gramineae, Fagaceae, Pinaceae

Pollen concentration in air is expressed as monthly sum of daily concentration.

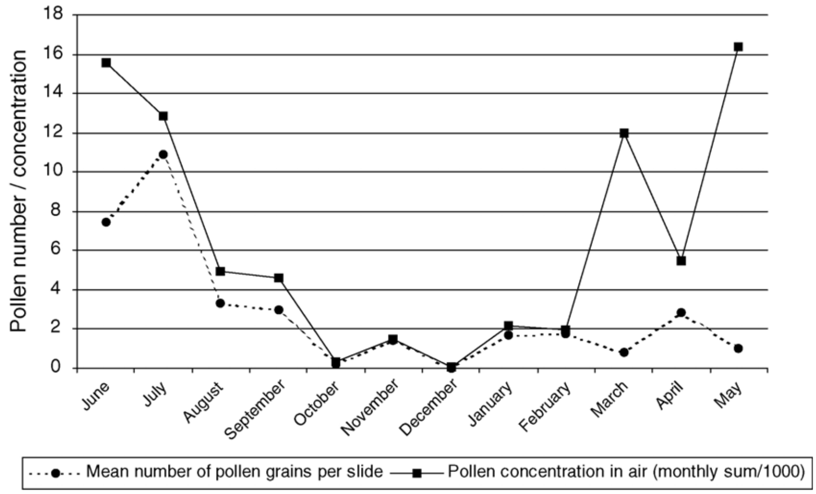

Fig. 3. Pollen on corpses and in the air: monthly trend of the mean number of pollen grains per slide and pollen concentration in the air of Parma. The latter is expressed as monthly sum of daily concentrations (p/m^3/24 h-monthly sum)/1000.

high pollen concentrations in the air, showing in these cases a good correspondence between corpses and air. However, a similar correspondence was not confirmed in March and May which have very low pollen content on corpses and high pollen concentrations in the air.

3.3. Indoor/outdoor

The number of pollen grains on corpses and the indoor or outdoor site of death is reported in Table 2. No significant relationship between indoor or outdoor death and the amount of pollen on corpses was found. A good clear link between an indoor death and a very low amount of pollen on corpses was observed more frequently (for example, case nos. 2 and 9) than a good link between an outdoor death and quantity of pollen on corpses (for example, case no. 6). It was lower than 5 pollen grains per corpse in 30% of outdoor deaths (Table 4).

3.4. Floristic list

The total pollen flora consists of 34 types belonging to 24 families (Table 3). Besides December with no pollen records, the shortest lists (two types) were in October and May, and the longest was in July (20 types). Fungi consisted of two types, *Epicoccum* and *Alternaria*, and were present from July to November. Many pollen types found on corpses are also recorded by the aerobiological monitoring stations in the air of the same month [16,17]. They are: from June to May as shown in Table 3: *Alnus* in June; *Castanea* and *Tilia* in June and July; *Rumex* and *Artemisia* from June to August; *Papaver* in July; *Humulus*, *Medicago* and *Plantago* in July and August; *Pinus* and *Parietaria* from June to September; Chenopodiaceae and Umbelliferae in August and September; *Ambrosia* in September; *Alnus* and Corylaceae from January to March; *Carpinus*, *Fraxinus* and *Ulmus* in March. Pollen grains of *Alnus* and Cupressaceae, included in the *Juniperus* type, are known to be present in the air in more than one season according to the different species and genera (respectively, *Alnus* cf. *viridis* in summer and *A.* cf. *glutinosa* in winter; some species of *Juniperus* in summer, and *Calocedrus*, *Cupressus* and other species of *Juniperus* in winter and spring; [32–34]). The three ''seasonal pollen groups'' match the three main aerobiological seasons (Fig. 4; Table 3).

Nevertheless, in a number of cases some pollen types recorded on corpses were not reported in the monthly pollen calendars of Parma. For example, on corpses there were found *Juglans* and *Robinia* in July (case no. 7), *Juglans* in February and September (case nos. 12 and 23). Also found were *Quercus* in July and September, *Populus* in July and January, *Platanus* in February and *Pinus* in March; these pollen grains were trapped by corpses outside the flowering seasons of the plants in the region, and more generally in Northern Italy [13].

3.5. Spearman's rank correlation coefficient

A number of pollen types were present both on corpses and in the airborne calendar of the same month. From these a selection of pollen types per month were compared with Spearman's ranks to verify if there was a correlation between their abundance in the two sources (Table 4). Pollen on corpses

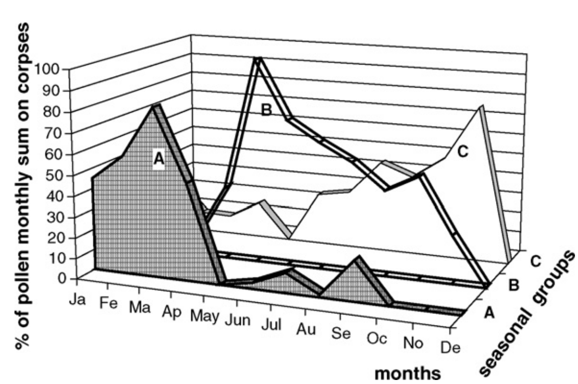

Fig. 4. Pollen calendar based on pollen found on corpses. Monthly trend of the three seasonal pollen groups (percentage values): (A) winter/spring; (B) spring/summer; (C) summer/autumn seasonal group.

and in the air were highly correlated in four months, July, August, June (where 9 or 8 pollen families were compared—$N = 9$ or 8), and February ($N = 7$). In September ($N = 6$) and November ($N = 4$) data was poorly correlated. In January and March there was no correlation. In the other months, data from corpses was not sufficient.

4. Discussion

In most cases, the corpses act as traps of pollen from the environment the people had been in. But, as is well known, many variables influence the effectiveness of such pollen traps as well as the pollen spectrum of a corpse.

Interestingly, the case of the two December corpses which did not trap any pollen can help to illustrate some of these variables. On one hand, the setting of the death (indoors) and the points of sampling (only nasal cavities) were unfavourable for pollen trapping. On the other hand, even outdoors very few pollen grains should be present in the air and deposited on the ground in December. In the first series of observations that included two corpses from December 2002 [18], pollen grains were not found (only one *Epicoccum* spore was observed in nasal cavities). It must be recalled that each year in Italy, December has always had the lowest monthly concentration of pollen in the air, and there are many days with no pollen in the air at all [16]. There is perhaps a link between the amount of pollen spread in the environment during a particular month and the probability of finding pollen on corpses.

In fact, the highest quantity of pollen on corpses was recorded in June and July (case nos. 3, 6 and 7), which are among the months with the highest pollen concentration in the air both for the year and in general for the region [15,16,31]. However, the hypothetical link observed in December between the lack of pollen on corpses and the scarcity of pollen in the air, or the high levels on corpses and in the air in June and July, was not confirmed when the data was considered over the whole year. So, in June for example very low counts were present (case no. 4), and in August (from case nos. 8 to 11, with five to six slides each; Tables 1 and 2) high and low pollen counts were found on different corpses.

Pollen counts on corpses are normally very low in comparison with pollen concentrations in the air. Air concentrations range from very few to many thousands of grains/m^3 of air depending on the pollination process of the various plant species [9,11,16]. To explain the corpse/air discrepancies, there are reasons both intrinsic to the nature of corpses and extrinsic caused by the environment. Intrinsic reasons include the fact that corpses are not so efficient pollen samplers as artificial spore traps. They are passive and capture pollen by chance from the air, while an artificial spore trap is active and continuously samples a great amount of outdoor air every day. Moreover, the healthy state of a person's mucosae [35,36], the use of sticky cosmetics on face and hair [20], the presence of sweat, individual differences in eyebrow thickness and the personal washing frequency are all factors which affect the chances of capturing and keeping pollen. As for environmental variables, e.g. type of flora or weather conditions, there are as many combinations as there are scenes of crime. Low pollen levels were found on corpses from the months of March and May, which commonly have very high pollen concentrations in the air reflecting the high number of species which are in flower [16,30,31]. The three March corpses (case nos. 24–26) were from people who died at home, and this fact presumably explains their lower capacity for diminished trapping of pollen. However, in May (case no. 28), the person died on a building site. In June and August too, a low amount of pollen was found on people who died outdoors (nos. 4 and 10 with only three to four grains per corpse). So, no correspondence was established between indoor/outdoor death and number of pollen grains on corpses. In forensic cases, pollen could raise questions about the context and circumstances of death. An explanation could be that the person died indoors and the corpse was taken outdoors shortly before being discovered, or that it was raining so that the air was virtually cleaned of pollen [15,16]. Careful investigation is necessary to ascertain if particular conditions prevailed at the time of death. The data suggests that in the months with high pollen levels, from March to August, these high levels were not reflected in corpses. During these months, pollen grains are more frequent in the environment, but the quantity on corpses is influenced by the site of death and particular conditions of death. It is necessary to study many more cases in order to interpret correctly the level of pollen and explore the variables which influence this parameter.

Unlike quantitative data, qualitative data showed quite a good correspondence between the pollen types on corpses and those recorded by the aerobiological monitoring stations in the air of the same month. This suggests that a link between the types of pollen on corpses and the season of the death may exist. It may be that the floristic list is a better guide to the season of death than the amount of pollen recorded on a corpse.

It is known that pollen trapped by corpses is not always, or cannot be at all, a seasonal marker. In fact, it can be picked up from anywhere the people went or from anything they had contact with [1,6]. Moreover, the pollen grains on corpses cannot be unequivocally assigned to pollen from the air or to pollen deposited on objects or the ground. In surficial deposits the pollen rain is stratified over many years, the more recent towards the top, the more ancient deeper in the deposit and pollen of different months is mixed at the same level [8,9].

When investigating the season of death, it is crucial to disentangle which pollen belongs to the previous years and which was produced in recent months. 'Old' and 'new' pollen grains can coexist in the same substratum and the same environment, and we have to be sure that all the pollen found on corpses is 'new' to establish the season of death. Though pollen grains from the air are more likely to be 'new', they may also have been deposited in a few days and then picked up by corpses. The question of whether pollen grains are trapped from the air, objects or the ground is not relevant for our topic. The real question is whether the pollen found on the corpses was 'new', whether it was part of the seasonal pollen rain at the time of death.

In this study, as in the previous study [18], all the pollen grains from corpses were found fresh and in a good state of preservation. By fresh pollen we mean that either the pollen grains were not exposed to the atmosphere too long before they were trapped by the corpses, or they were not deposited on the ground in previous years. In other words, they looked 'new'. In fact, it is well known that pollen preserve cytoplasm for a variable time after its release from the mother plant depending on the environmental conditions, but in natural circumstances this time seems to be not longer than few months. In fact, in the slides from airborne pollen monitoring, which are routinely coloured with fuchsine [11], the pollen grains present in the air each month are largely produced by plants that flower in that month. They mainly contain cytoplasm, and only a few exceptions of pollen emitted empty from anthers have been sometimes observed (cultivated Rosaceae [37] and *Corylus* [38]). Occasionally, isolated pollen grains are observed with intact cytoplasm three to four months after flowering of the producing species, but in these cases the grains generally were darker in colour, often had less cytoplasm and were markedly different in appearance (for example, *Taxus*, *Cedrus*, and unpublished data [39,40]). Sometimes, empty pollen grains from plants which had not flowered are observed, thus suggesting that pollen can return to the atmosphere after settling. If pollen grains are maintained in an absolutely dry state, they usually preserve their cytoplasm. In the *exiccata* of our reference pollen collection, properly dry conserved, we observed that pollen grains directly collected from flowers can preserve cytoplasm for many years. But, when pollen grains are deposited on the ground surface, the changes in the weather, including rain, linked to the change of seasons cause cytoplasm fragmentation of pollen and spores [41], also including the emission of cytoplasm particles, and facilitate their emptying. Moreover, alternate dry and wet conditions can damage the exines [42]. Experimental research on pollen from birch, ryegrass and other grass species, has shown that pollen grains produced during the flowering period remaining on open anthers, if wetted, can germinate or can rupture within minutes, and their fragmented cytoplasm are emitted through their pore regions [43]. We conclude that the presence of cytoplasm, and the general good appearance of pollen is a reliable indication that it is not 'old' and is in fact from the current year.

It is worth emphasising that forensic sampling for 'new' pollen is made more efficient by sampling only at selected points on the corpses. Eyebrows, the hair-base, facial surfaces and nasal cavities are points that are well exposed and usually frequently washed. If the whole hair, or clothes and shoes are included in the sampling, pollen from before the date of death is present, and may be prevalent. In fact, two pollen grains without cytoplasm were collected from clothes by sampling directly with slides with a sticky surface of silicon oil [18]. They were both from outside the season of death. Pollen from *Ailanthus*, a tree which commonly flowers in summer (June–July; [13]), was found on the trousers of a person who died in January, and pollen from *Artemisia*, with many species which also flower in summer, was found on a woollen cap of a person who died in February. Moreover, chemical treatments used to extract and concentrate pollen from substrates, eliminate the cytoplasm and remove the possibility of collecting the information about the state of preservation of the pollen grains.

To continue searching for an answer to the question if pollen on corpses is a seasonal marker for the time of death, we also observe that the pollen types found on corpses frequently belonged to plants that were in flower at the time of death. The

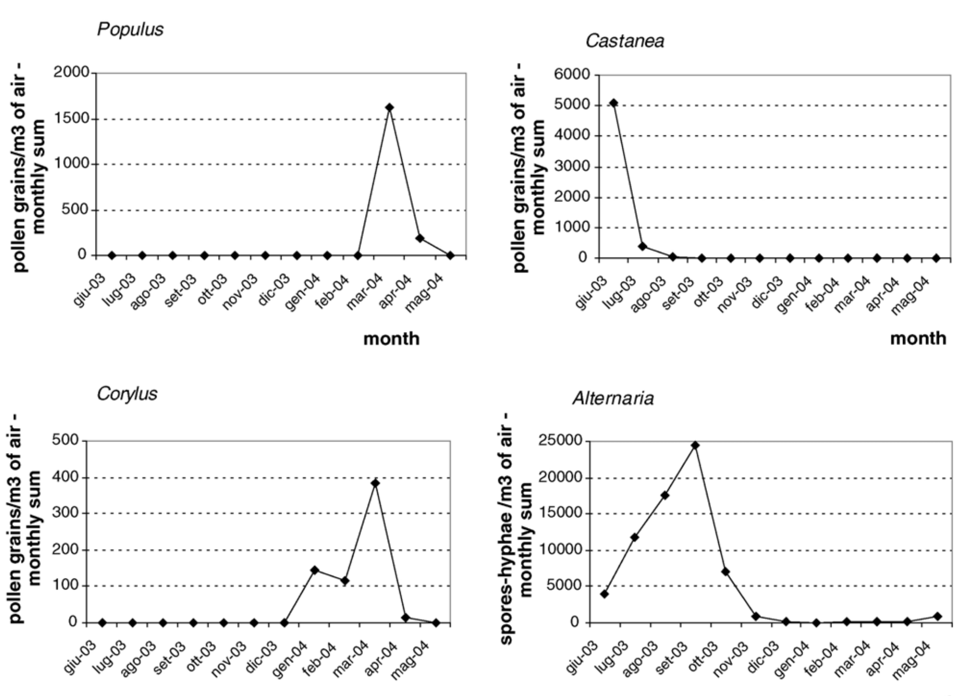

Fig. 5. Pollen and fungi in the air (monitoring station of Parma): total monthly pollen sum in the period June 2003–May 2004 of *Populus*, *Castanea*, *Corylus* and *Alternaria*.

pollen flora from corpses of each month was similar to the airborne pollen flora of the same month, and about 80% of the monthly pollen types recorded on corpses were also present in the air. In some cases, single abundant pollen types point to one/two months where they were found in accordance with their highest concentrations in the air (Fig. 5), e.g. *Populus* (March), *Castanea* (June), *Tilia* (June, July; Fig. 6). *Corylus* was a marker of the period from January to March. Accordingly, in 2002 [18], the pollen of *Corylus* was the prevalent type in February and March when this pollen continues to be frequently recorded in the air [16]. *Alternaria* and other fungi, though frequent in the air throughout the year [17], are most common during the late summer and autumn and may be an index of these seasons in the study area (Fig. 5). However, it must be noted that, similarly to the traditional methods used to obtain correct pollen inferences, a combination of floristic lists is more useful than a single recording. The presence of *Alnus*, *Corylus* and *Ulmus* in the same spectrum clearly indicates March, while the presence of *Alnus* (see above), *Artemisia* and *Rumex* indicates June (Table 3).

An open question still remains whether, in forensic cases, the date of death can be established by pollen when a corpse is found well after death occurs. In these cases, a more recent pollen rain could have been deposited on the corpses in the days they remained exposed, and this could cause confusion. Even if empty pollen grains can be regarded as the more ancient, the presence of cytoplasm only indicates that they are recent, not that they belong to the moment of death. As many different circumstances are encountered, each case must be considered in its context and in an interdisciplinary perspective (for example, the date of death is established by the forensic physician, and then pollen grains could be checked for comparison). However, it must be recalled that generally mixed palynological assemblages can be recognised, or can be suspected, by the presence of different states of preservation and by a floristic list reflecting many months.

In our research, more information can be obtained by combining qualitative and quantitative data, as the pollen spectrum (Tables 3 and 4). For example, in the months with high total pollen concentrations in the air, June–August, the abundance of the main pollen types (eight to nine types listed in the seventh column of Table 4) present both in corpses and in

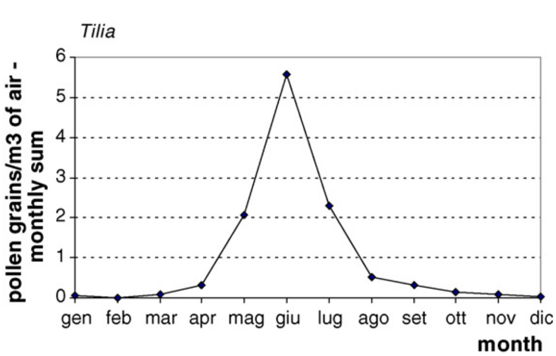

Fig. 6. Pollen in the air (monitoring stations of Modena and Vignola): total monthly sum of *Tilia*. The values are calculated as the mean of 15 years of pollen monitoring and sampling (1990–2004).

the air were highly correlated. Plants in flower and with higher production (anemophilous) are more likely to be present on the corpses as they have the highest pollen concentrations in the air, and abundantly cover the ground surface. Obviously, this probability increases when the plant is in the vicinity of the person and if the person is in contact with the plant just before death. In these conditions, zoophilous plants are also more likely to be trapped. In general, this suggests that the key pollen grains of the monthly pollen rain are likely to be found on corpses and provide useful information about a season/month.

Finally, it must be noted that pollen grains from plants not flower at the known time of death were also recorded. Commonly, these are not expected to be reported in pollen calendars of the relevant months unless, as mentioned above, deteriorated or empty, recycled pollen is recorded. For example, *Juglans* and *Robinia* were found on the facial skin, eyebrows and nasal cavities of a person who died in his garden in July (case no. 7). These trees generally bloom in April–May [13], i.e. one/two months before the date of death, so the pollen probably did not arrive on the person from the air but from contacts with plants, which are very common in the region, or with the ground where pollen was deposited. Again, *Juglans* was found outside the flowering season in two other months. In February (case no. 23), its pollen was on the eyebrows of a person who died in a wood and thus probably was picked it up from the ground. In September (case no. 12), it was found on the eyebrows of a person who died at home, the pollen was probably on the floor where the person fell down, possibly brought in by shoes from outdoors. These are the simplest hypotheses, but they need to be checked by observation of vegetation and analysis of control pollen samples from the sites. These are current techniques in forensic palynology [1,2,6,10,20].

The presence of pollen grains out of season at the time of death is in accordance with results frequently obtained from corpses in forensic cases [6]. In general, sampling is a key phase and must be carried out directly at the scene of crime by the forensic palynologist, because it cannot be repeated and it requires specialist knowledge on how and where to collect samples [2,5,18,20]. The number and typology of sampling points per corpse influenced the results. The more exposed parts thought to be the most useful to check for pollen grains indicating the time of death contained mainly seasonal pollen but with some important exceptions. The number of samples was sometimes too low and needs to be increased and adapted to the seasons. The less pollen in the environment, in the air or on the ground, the more samples must be collected.

Anemophilous pollen is the most useful to indicate the season and it is easily recognised in the pollen calendars. But zoophilous pollen can also be an important seasonal marker even if it is less common in the air than at ground level. Thus, spore traps may not catch them. They can also vanish during routine readings taken by aerobiological network stations [23] or even remain unreported given that the aim of those readings is generally to collect data on the most allergenic and abundant pollen grains. This happened with *Tilia* which was recorded on corpses of people who died in June and July (case nos. 1, 2 and

6). It was not in the pollen calendars of Parma because it is not usually reported by that monitoring station.

5. Conclusions

The number of corpses studied so far is high enough to yield firm conclusions about the link between pollen on corpses and the season of death. But some important indications can be inferred from the data obtained in this year-long investigation and these complement our previous studies [18].

Corpses trap pollen from the ground and from objects and plants with which people have had contact before their death [1,6]. Our data strongly suggested that, if the more exposed parts of the face are analysed, most pollen grains are captured still fresh and that they can quite frequently be trapped from the air or from recent deposits. In ascertaining season of death, the exposed parts of the face seem to be the most useful for obtaining pollen from plants which are in flower. Nevertheless, the corpse/air discrepancies which were found suggested that the principle cannot be generalised.

Quantitative data alone are not a helpful parameter to deduce the season/month of death. In fact, the environment and condition of death can notably influence the amount of pollen on corpses, and the observed discrepancies between pollen abundance on corpses and in the atmosphere are due to the local plant cover. Again, many more cases need to be analysed, carefully with precise information about people and deaths, before the actual level of the parameter ''amount of pollen'' can be estimated. It emerged that the list of pollen types on each corpse is clearly linked to the season of death, especially when the pollen spectrum is considered overall. However, the types which are prevalent in a given case depend more on the death pollen scene than on the general pollen rain of the area.

In this research, the comparison between the pollen recorded on corpses and seasonal pollen rain present in the region was made on the basis of known variables: the date of death, the region and locality of death. Pollen rain composition can be ascertained from pollen calendars from the aerobiological network, if present, and it was found that pollen on corpses largely corresponded to the pollen rain for the region [15, and subsequent]. This shows that there is a close connection between forensic palynology and aerobiology, and the airborne pollen calendars can be a significant reference tool for establishing the season or month when an unknown death happened.

Our data shows that the three ''seasonal pollen groups'' of corpses match the three main aerobiological seasons, based on the regional monitoring stations, indicating that a preliminary ''crime pollen calendar'' was obtained from corpses (Fig. 4; Table 3).

A specific calendar for the Parma region requires much more data, and we are still far from a ''crime pollen calendar'' which would exist without reference to the airborne pollen calendar. But airborne pollen calendar by itself has some important limitations in use. The extreme variability of crime scenes strongly influences which pollen is trapped on corpses, and new research is necessary to examine this problem. The crime and air calendars, used together, appear to be a good tool in interpreting pollen from corpses when the season of death is unknown.

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