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Comparison of two pollen counting methods of slides from a hirst type volumetric trap

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Abstract

Two of the most frequently used methods of pollen counting on slides from Hirst type traps are evaluated in this paper: the transverse traverse method and the longitudinal traverse method. The study was carried out during June–July 1996 and 1997 on slides from a trap at Worcester, UK. Three pollen types were selected for this purpose: Poaceae, Urticaceae and *Quercus*. The statistical results show that the daily concentrations followed similar trends ($p < 0.01$, R-values between 0.78–0.96) with both methods during the two years, although the counts were slightly higher using the longitudinal traverses method. Significant differences were observed, however, when the distribution of the concentrations during 24 hour sampling periods was considered. For more detailed analysis, the daily counts obtained with both methods were correlated with the total number of pollen grains for the taxon over the whole slide, in two different situations: high and low concentrations of pollen in the atmosphere. In the case of high concentrations, the counts for all three taxa with both methods are significantly correlated with the total pollen count. In the samples with low concentrations, the Poaceae and Urticaceae counts with both methods are significantly correlated with the total counts, but none of *Quercus* counts are. Consideration of the results indicates that both methods give a reasonable approximation to the count derived from the slide as a whole. More studies need be done to explore the comparability of counting methods in order to work towards a Universal Methodology in Aeropalynology.

Introduction

One of the main aims in Aeropalynology is to produce daily results of pollen counts to be used in forecasting and to be given out by the media. In this context it is important that the count is produced quickly each day so that the analyst reads only a small sample from the total area of the slide. Most of the European teams use one of the following four methods. These are the random sampling method (Makinen, 1980), the transverse traverses method (Emberlin et al., 1994), the longitudinal traverses, including continuous fields readings (Dominguez et al., 1991) and tangential field readings (Mandrioli, 1990). All of them produce the daily count expressed in pollen grains/m3 of air, but this may not be enough to allow comparisons to be made between the results obtained in the various aerobiological laboratories. One of the main disadvantages of all of the methods is the small proportion of the whole slide analysed, usually between 10–13% of the total surface.

The choice of the counting method to be used by a team has been based mostly on historical and traditional reasons and it is difficult to change to another method once one has been applied. For this reason few comparative studies have been done. Kapyla and Penttinen (1981) evaluated the error and efficiency of some counting methods in relation to the aims of doing the count. They concluded that the random fields method is good at estimating the daily mean concen-

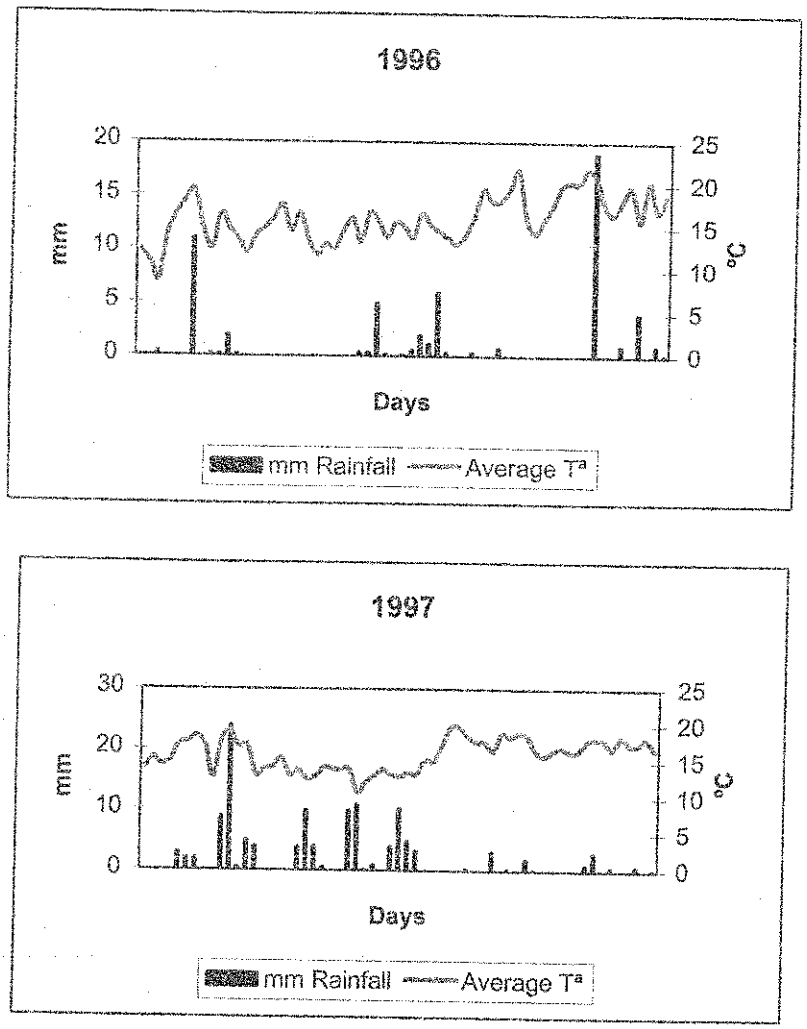

Figure 1. Weather conditions in Worcester during June and July 1996 and 1997.

Table 1. Total pollen counts for Poaceae, Urticaceae and *Quercus* obtained with the transverse traverses (t.t.) and the longitudinal traverses (l.t.) method in June–July 1996 and 1997. Data expressed as cumulative counts (the sum of daily averages in pollen grains/m3).

	Poac t.t.	Poac l.t.	Urti t.t.	Urti l.t.	Quercus t.t.	Quercus l.t.
1996	3786	4959	2814	3907	783	1356
1997	2200	2846	1079	1768	150	240

Table 2. Kolmogorov-Smirnov's test for normality applied to the daily pollen concentrations obtained with both methods.

	Transverse traverses		Longitudinal traverses	
	Max.D	P <	Max.D	P <
Poaceae 1996	0.160	0.01	0.153	0.01
Urticaceae 1996	0.224	0.01	0.142	0.01
Quercus 1996	0.341	0.01	0.335	0.01
Poaceae 1997	0.214	0.01	0.236	0.01
Urticaceae 1997	0.184	0.01	0.275	0.01
Quercus 1997	0.389	0.01	0.369	0.01

tration, but the transverse traverses method provides a good estimate of the short term concentrations and diurnal variations. Comtois et al. (1999) also analysed the accuracy of different counting methods on twelve pollen types. They counted the pollen grains over

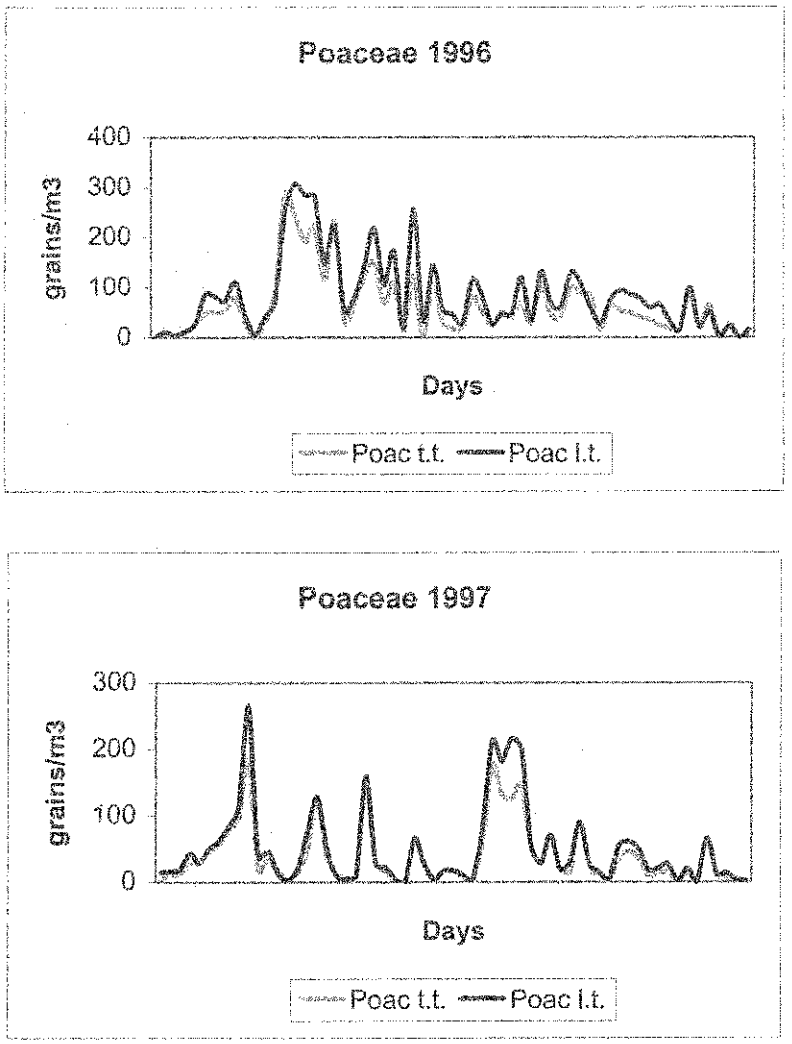

Figure 2. Daily Poaceae pollen counts in Worcester during June and July 1996 and 1997. The data are expressed as pollen grains/m3 of air.

whole slides and then made sub-samples selecting a number of transverse or longitudinal traverses or a number of random microscope fields. The results showed that all the counting methods make an error and that this error depends on the sample size.

Two of the most frequently used methods of pollen counting on slides from Hirst type traps are evaluated in the study reported in this paper: the transverse traverse method (used by Pollen UK-British Federation of Aerobiology) and the longitudinal traverse method (used by the Spanish Aerobiology Network, REA).

Materials and methods

The samples were obtained by a Hirst type volumetric trap placed on a roof top at University College Worcester, in Worcester, UK, during June–July 1996 and 1997. The three most abundant pollen types during the considered period have been selected: Poaceae, Urticaceae and *Quercus*. The weather conditions (precipitation and temperature) have been considered to explain the differences in pollen concentrations between 1996 and 1997: warm and dry in 1996 and cold and wet in 1997.

Once the samples were mounted, they were analysed using both counting methods. The technique used by Pollen UK takes twelve transverse traverses

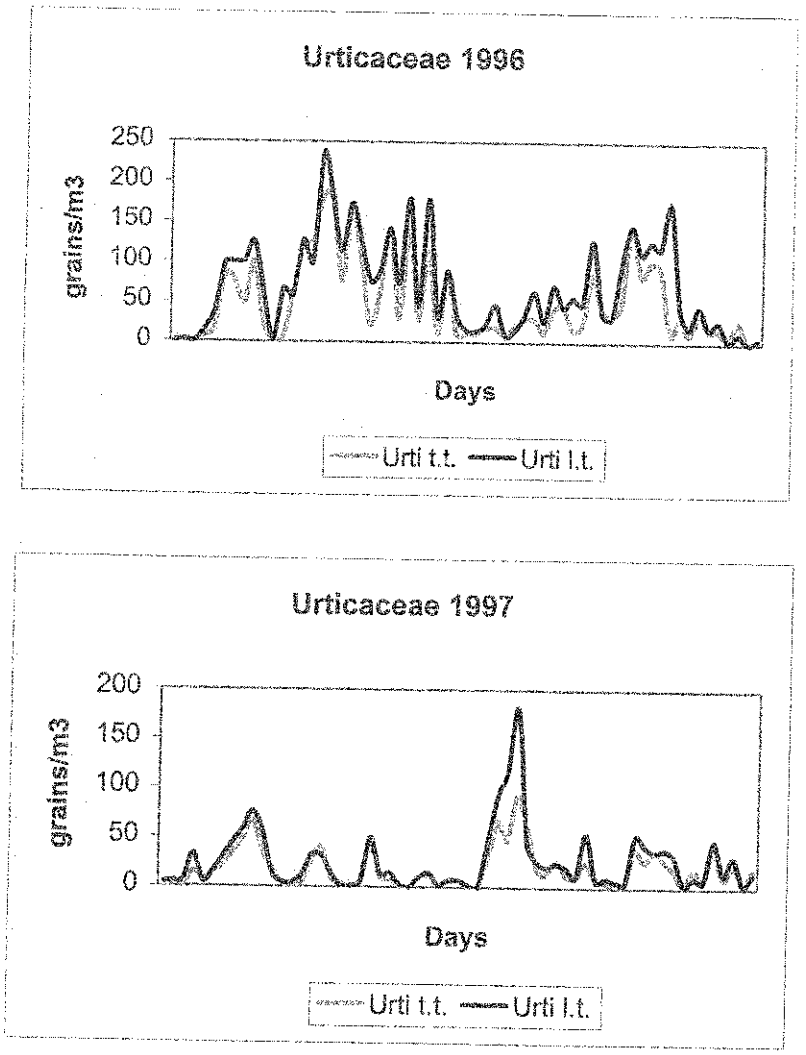

Figure 3. Daily Urticaceae pollen counts in Worcester during June and July 1996 and 1997. The data are expressed in pollen grains/m3 of air.

starting at 9.00 am then at intervals separated by 4 mm, representing two hours travel on the tape. This separation was chosen because the inlet nozzle is 2 mm wide and there would be some spread in particles deposition on either side of the tape beyond this interval. Since the travel of the tape is continuous, a distance of 4 mm separation of transects avoids leaving large areas unsampled. The method used by the Spanish Aerobiology Network (REA) takes four longitudinal traverses of contiguous fields along the length of the slide, starting from the centre and separated by at least 1 mm to avoid oversampling or empty areas. The proportion of the analysed surfaces in the case of this study are 11.25% of the total surface area for the transverse method and 12.85% for the longitudinal method. The width of the traverses in both cases is dependent on the field of view of the microscope objective used and is not a constant with the techniques. In both cases, the daily counts are expressed in pollen grains/m3 of air and they both used 400 magnifications in optical microscopy.

The pollen of the selected taxa were counted over the entire slide for 14 days from 1996 in order to determine which of the two methods gives results that are a closer representation of the count that would be derived from analysing the whole slide. Two different situations were examined, high and low concentrations of pollen grains.

Table 3. Spearman's correlation test between transverse traverses (t.t.) and longitudinal traverses (l.t.) counts for Poaceae, Urticaceae and *Quercus* during 1996 and 1997.

	Valid N	Spearman R	T(N-2)	p
1996				
Grasses t.t.-l.t.	58	0.957	24.70	0.00
Nettles t.t.-l.t.	58	0.861	12.70	0.00
Oak t.t.-l.t.	58	0.780	9.332	0.00
1997				
Grasses t.t.-l.t.	58	0.964	27.52	0.00
Nettles t.t.-l.t.	58	0.945	21.80	0.00
Oak t.t.-l.t.	58	0.809	10.30	0.00

Table 4. Wilcoxon's test results between transverse traverses (t.t.) and longitudinal traverses (l.t.) counts for Poaceae, Urticaceae and *Quercus* during 1996 and 1997.

	N°Case	Rank–	Rank+	Ties	z	2-tail P
1996						
Poac t.t.-l.t.	58	8	47	3	–5.148	0.0000
Urti t.t.-l.t.	58	12	43	3	–5.115	0.0000
Quercus t.t.-l.t.	58	6	31	21	–3.982	0.0000
1997						
Poac t.t.-l.t.	58	9	48	1	–5.597	0.0000
Urti t.t.-l.t.	57	13	39	5	–3.910	0.0001
Quercus t.t.-l.t	45	6	20	32	–2.958	0.0031

The results of the study have been tested for association and correlation by Spearman's Rank , and also for trends and distribution by Wilcoxon's test.

Results

The weather conditions during June-July 1996 and 1997 were rather different (Figure 1). The total precipitation in June–July 1996 was 56.8 mm, while in the same period in 1997 it was 116.2 mm. The distribution of the precipitation was also different. In 1996 the rainfall was above 1 mm on only nine days, while in 1997, the number of days with rainfall above 1 mm increased to 21. The most significant differences in temperature were recorded in the maximum daily readings, with an average in June–July 1996 of 22.13 °C and in June–July 1997 of 20.22 °C. These weather differences resulted in marked contrasts in the pollen records. The counts of the three pollen types considered were significantly higher in 1996 than in 1997 (Table 1). The colder temperatures in 1997 were not only unfavourable for pollen release but also the rainfall depleted concentrations.

Table 5. Spearman's Correlation test results between the total pollen concentrations over the whole slide and the transverse traverses (t.t.) and longitudinal traverses (l.t.) counts in situation of high pollen concentration (13–19 June) and low pollen concentration (25–31 July).

	Valid N	Spearman R	T(N-2)	P
13–19 June, 1996				
Total-Poac t.t	7	0.678	2.065	0.09
Total-Poac l.t.	7	0.821	3.220	0.023
Total-Urti t.t	7	0.964	8.140	0.000
Total-Urti l.t.	7	0.928	5.594	0.002
Total-Quer t.t	7	0.785	2.840	.036
Total-Quer l.t.	7	0.970	—	—
25–31 June, 1996				
Total-Poac t.t	7	0.954	7.199	0.000
Total-Poac l.t.	7	0.954	7.199	0.000
Total-Urti t.t.	6	0.809	3.078	0.027
Total Urti l.t.	6	0.900	4.642	0.005

The daily pollen counts for each taxon according to the different counting method are shown in Figures 2, 3 and 4.

In the case of the Poaceae (Figure 2), the trends followed by the two methods during the two years are similar, with peaks slightly higher when using the longitudinal traverses method. This fact could be related to the higher percentage of slide counted. The same coincidence between peaks and lows in both years are also evident for the Urticaceae (Figure 3). In both cases, the counts were more different in 1996 than in 1997. For *Quercus*, (Figure 4), more differences are apparent, not only in the trends but also in the concentrations.

Several statistical tests have been applied to study the possible correlation between the partial counts and their relationship to the total counts. As the data did not follow a normal distribution ($p < 0.05$) (Table 2), non-parametric statistics were used. The Spearman's correlation test (Table 3) shows that the correlation between the data in both years is very significant ($p < 0.00$), with higher R-values for Poaceae than for Urticaceae and Quercus. The Wilcoxon's test (Table 4) reveals, however, that there are significant differences in the distribution of the concentrations within the period studied.

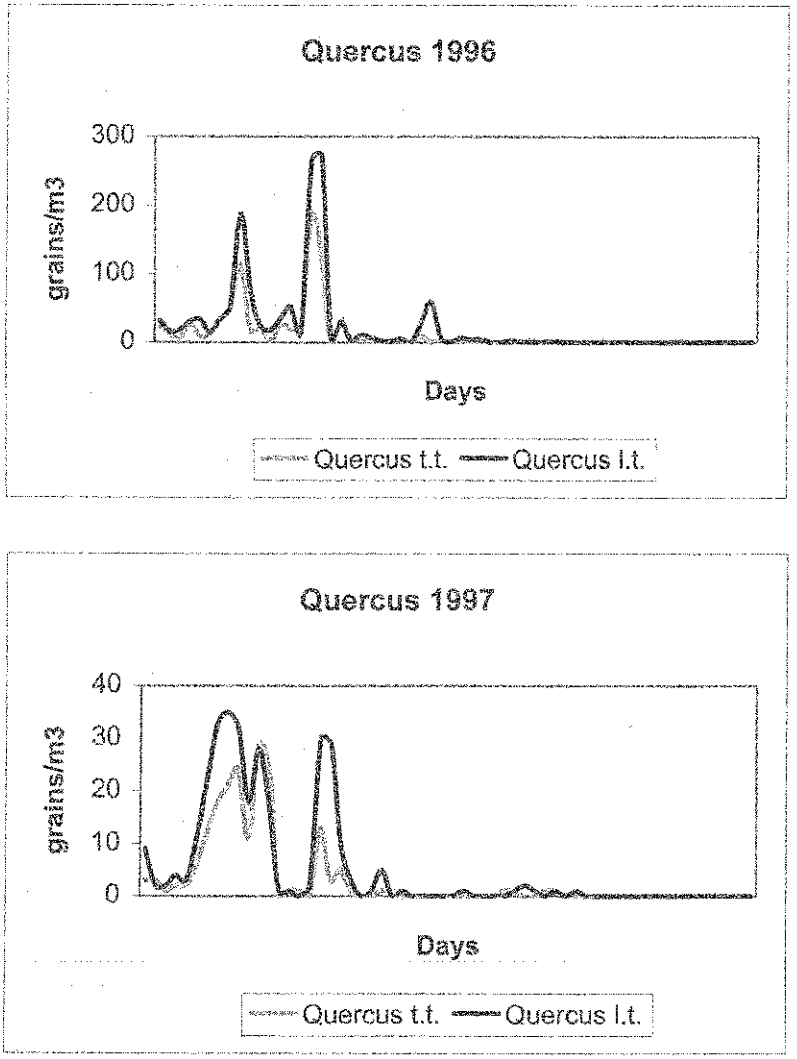

Figure 4. Daily *Quercus* pollen counts in Worcester during June and July 1996 and 1997. The data are expressed as pollen grains/m3 of air.

In order to determine if the counts obtained with both counting methods were representative, Spearman's rank correlation tests were applied between the partial and total counts from examples of slides with high and low concentrations. The results of the non parametric rank correlations on the seven samples (Table 5) indicate that when the presence of pollen grains in the air is maximum (13–19 June, 1996), the four longitudinal traverses method gives results that are better rank correlated with the Poaceae, Urticaceae and *Quercus* total counts than the transverse traverse method. However if the actual figures for the counts are considered then it is apparent that the transverse transect method gives readings that are closer to the counts derived from the total slide more frequently than occurs with the longitudinal method (Table 6).

When pollen counts are low (25–31 July, 1996), the Poaceae results with both methods are significantly correlated with the total counts. In the case of Urticaceae, the results are less representative because one day has a zero total count. Similarly correlations could not be applied for Quercus as pollen occurred on only three slides (Table 6).

Table 6. Total pollen counts and differences with the counts obtained with the transverse traverses and longitudinal traverses methods for Poaceae, Urticaceae and *Quercus* in periods of maximum (13–19 June, 1996) and minimum (25–31 July, 1996) concentrations. The data are expressed as pollen grains/m3 of air.

Date	Total Poaceae	Differ. T.t.	Differ. l.t.	Total Urticace	Differ. t.t.	Differ. l.t.	Total Quercus	Differ. t.t.	Differ. l.t.
13-June	**76**	+12	–7	**60**	+2	–1	**28**	–2	+7
14-June	**220**	+67	+33	**103**	+16	+24	**30**	–10	+22
15-June	**249**	–4	+58	**101**	+26	+1	**18**	+3	–3
16-June	**196**	–5	+88	**164**	+17	+72	**187**	–1	+80
17-June	**273**	–51	+8	**188**	–1	–9	**230**	–103	+42
18-June	**104**	+10	+44	**85**	–9	+28	**9**	–8	–3
19-June	**177**	+54	+24	**121**	+44	+52	**24**	+10	+7
25-July	**78**	+11	+21	**34**	–1	+11	**2**	–2	–2
26-July	**23**	–4	–2	**34**	–18	–16	**0**	0	0
27-July	**55**	+10	–2	**26**	–10	0	**0**	0	0
28-July	**2**	–1	0	**7**	–3	–5	**6**	–5	–5
29-July	**19**	+8	+3	**12**	+15	–1	**3**	–3	–3
30-July	**2**	0	–2	**1**	–1	–1	**0**	0	0
31-July	**9**	0	+6	**3**	+3	+2	**0**	0	0

Discussion

he daily pollen counts obtained with the trans-erse traverse counting method and the longitudinal averses one follow similar trends within the period udied. In both cases differences occur between the timates made from the sampling systems and the unt taken from the whole slide. This is an inevitable nction of subsampling relatively small percentages the slide and can lead to overestimates in some cases underestimates in others. Overall, the two methods counting give close approximations to the count m the entire slide.

It is also well known that the concentrations of llen grains in the air vary throughout the day apyla, 1981; Kapyla, 1984; Galán et al., 1991; rris Hill and Emberlin, 1991; Trigo et al., 1996) it is not possible to define a general daily vari-on pattern due to the influence of particular weather ıditions. Norris Hill and Emberlin, (1991) found t the diurnal variation of Poaceae pollen in the air London on dry days reached a maximum between 18.00 and the 22.00 hours. In the Worcester area ıceae pollen also typically reaches a maximum in afternoon whereas Urticaceae pollen frequently a morning peak. This concentration of pollen ertain periods of day can be well valuated with ı methods and it could explain the similarity in the counts obtained for these two taxa and the high statistical correlation.

Each method has some disadvantages that should be taken into account. In the case of the four longitudinal traverses counting method overestimates can arise from counting only the central regions of the slide where most of the pollen is deposited. Smaller grains and fungal spores can be deposited nearer to the edges in some weather conditions and may be omitted from the count (Tormo et al., 1996). In the case of transverse traverse method, pollen or spores that are deposited within a very short time on the tape, may be missed despite the overlap of sample area beneath the inlet nozzle. Both methods have the advantage of producing results of acceptable accuracy quickly and should be viewed in the context of the overall efficiency of the sampling technique used to capture pollen and spores from the air.

Conclusions

It is difficult to conclude if one of the counting methods considered gives a closer approximation to the count derived from the whole slide. It should be noted that all methods, even the counts taken by reading the entire surface, will give only estimates of the ambient concentrations because the traps are

not 100% efficient. Trapping efficiency can differ with factors such as wind speed and features of the adhesive surface. The amount of air sampled by the traps is itself a relatively small sample of a heterogeneous air flow at one location. The pollen counts should be taken as indicators of concentrations rather than absolute values. It is difficult to decide wether a particular counting method should be recommended and more studies in aerobiological methodology should be done.

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