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Sampling in aerobiology. Differences between traverses along the length of the slide in Hirst sporetraps

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Abstract

Two years of data from four longitudinal traverses along each day's slide prepared from a continuously running Burkard sporetrap have been analyzed statistically. Using the Friedman test, a statistically significant difference was found between the four traverses, with a greater than 7% loss of pollen grains in the two outer traverses in relation to the inner. Four slides were then selected for more detailed analysis, using 18 longitudinal traverses with a 1-mm separation from the upper to the lower edge of the Melinex tape. There was found to be a progressive decline from the centre to the outside, and more than 4% of pollen grains were found outside the typical 14 mm width of the impaction orifice. There was no correlation between pollen grain size and the decline in counts from the centre to the outside. For the complete data set, there was a general rise in the diversity of pollen types with increasing sample counts, but above about 1000 pollen grains per sample there were no more than 27 pollen types found, often even fewer. A discussion is presented of whether four traverses really should be a fixing sample size, or whether it might be better to fix the total pollen count beginning with a traverse in the middle of the slide and ending with a variable number of traverses when that count is reached.

Keywords: Aerobiology; Methodology; Sampling error; Burkard sporetrap

1. Introduction

Hirst sporetraps (Hirst, 1952) sample airborne particles by sucking in air at a flow rate of 10 l per min (14.4 cubic meters per day). This air impacts against an adhesive surface on a 19 mm width Melinex tape that is moved at 2 mm per h by a clockwork mechanism. Thus, in a day the air intake has impacted on an adhesive surface of 48 mm × 19 mm = 912 mm^2, and in theory any airborne particles must have adhered to that surface, although it is well known that efficiency is lower than 100%. The air flows in through a rectangular orifice of 14 mm × 2 mm. Ignoring possible lateral loss, therefore, airborne particles in the 14.4 cubic meters are captured on a surface of 14 mm × 48 mm = 672 mm^2.

To obtain the total count of particles adhered to the Melinex tape surface we might begin at one corner and finish at the opposite. This would provide highly accurate results, but would be very time consuming. To achieve a reduction in time with a minimal loss of information, several sampling methods have been proposed, all of them being based on decreasing the sampling surface, and assuming that particles would be uniformly distributed.

Different sampling methods have been proposed according to whether one wants to obtain an hourly value of pollen concentration or a daily one. Essentially there are three sampling methods: longitudinal traverses (along the length of the slide) between the shorter edges of the slide; transverse traverses between the longer edges of the slide; or microscope fields chosen randomly or systematically located (Ogden et al., 1974; Domínguez, 1994).

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The Spanish Aerobiology Network (REA) recommends using four longitudinal traverses in the centre of the slide (Domínguez, 1995), scanning all the 48 mm of the day's tape. Thus, using a 400 × microscope magnification with a field diameter of 0.45 mm, each traverse samples an area of $0.45 \times 48 = 21.6$ mm^2, i.e. 3.2% of the total theoretical surface (672 mm^2), and equivalent to 0.46 cubic meters of air. Four traverses sample 1.86 cubic meters of air or 12.9% of the total intake. It should be noted that this percentage would be 9.5% if we were to use the total Melinex surface available in the above estimate.

Aerobiological methods using Hirst sporetraps have been reviewed by several authors. Käpylä (1989) surveys the effectiveness of adhesives and mounting media. Rantio-Lehtimäki et al. (1991a,b) and Galan et al. (1995) review the dependence of pollen grain concentrations on the height at which the sporetrap is placed. The aerodynamic efficiency of the apparatus has been discussed by the designer, Hirst (1952), as well as by Bhat and Rajasab (1989) and Mandrioli (1994). Pedersen and Moseholm (1993) study the precision of the daily pollen count as made by different persons counting. Domínguez (1994) gives a general review of the methodology. Finally, Käpylä and Penttinen (1981), after evaluating counting methods, conclude that twelve transverse traverses, systematically each two hours, are sufficient to estimate the daily mean concentration.

The aim of the present work is to attempt to answer the following questions: Are the four longitudinal traverses equivalent? Are four longitudinal traverses sufficient, or are they excessive? Is there the same accuracy for all days in the year? What kind of information is lost in sampling?

2. Materials and methods

Data from 1993–1995 were used (13 May 1993 to 12 May 1995, 718 days total, with 12 days' data lost because of two power failures). The sampling device was a Burkard sporetrap placed in the Agrarian Engineering School of Extremadura University, Badajoz (SW Spain).

Slides were counted by scanning four longitudinal traverses beginning in the centre and separated by 1 mm. Raw counts of pollen grains were used in all calculations. Total pollen grain counts per month (for the 24 months) and per traverse (b1, b2, b3 and b4) were calculated. The latter were used to calculate the percentage loss, P, in the outer traverses relative to the inner two:

$$P = 100*[(b2 + b3) - (b1 + b4)]/(b2 + b3)$$

To test whether the differences between the four traverses are due to chance or have statistical significance, we used the Friedman test computed by the program 3S of the BMDP package, that perform also a multiple comparisons test between pair of traverses. This is a non-parametric test that follows a chi-square-like distribution, such that the higher the result, the greater the difference between the samples being compared. The significance level is given by a likelihood value P, which is the probability that the value calculated would be less than the value expected for the chi-square function, so that a value of $P < 0.05$ represents a significant difference.

There were 24 pollen types that summed more than 500 pollen grains over the whole study period. Using this selection of pollen types only, in order to avoid errors originated by a low frequency of pollen grains, the difference between outer and inner traverses was recalculated, as well as the Friedman test for the counts per traverse in the months that these types were present.

To study the transversal distribution of the pollen grains on the Melinex tape, we selected four days with a high concentration of pollen grains, and scanned 18 longitudinal traverses separated by 1 mm, beginning at 0.5 mm from the edge of the tape. The mean values of the percentages for the four slides of each of the 18 traverses were fitted to a polynomial using the polynomial regression program 5R of the BMDP package.

To check whether there is any correlation between the differences between traverses and pollen grain size, we calculated the Pearson correlation coefficient between pollen grain size (using both the polar and equatorial axes) and the percentage loss (P). For this we selected only the pollen types that have an evident uniformity in size (that contain one or only a few taxa), and then measured 25 pollen grains on slides mounted in the same conditions as the sporetrap samples, and mean of both axes were used.

3. Results

Monthly total amounts of pollen grains separated into the four traverses are listed in Table 1. In the table, there is a column with the percentage loss each month and the total percentage loss (P). Fig. 1 shows the same data accumulatively by months. There is a clear difference between the outer and the inner traverses, the total loss from inner to outer being 7.07%. This loss occurs in 21 of the 24 months, reaching more than 19%, although in the other 3 months the situation is inverted and there is a gain.

The results of the Friedman test comparing the four traverses ($f = 23.55$, $P = 0.000$) show that the differences between them are statistically significant, and are not caused by chance. Table 2 gives the results from all possible paired comparisons. There are no differences

Table 1
Number of pollen grains counted by month and by traverse, b1 and b4, outer traverses; b2 and b3, inner traverses

Month	b1	b2	b3	b4	Total	P
5/93	1948	2646	2563	2256	9413	19.29
6/93	3181	3544	3600	3402	13727	7.85
7/93	1498	1642	1528	1444	6112	7.19
8/93	403	434	399	371	1607	7.08
9/93	308	311	318	292	1229	4.61
10/93	98	109	113	85	405	17.57
11/93	115	144	151	133	543	15.93
12/93	277	300	319	297	1193	7.27
1/94	362	371	457	372	1562	11.35
2/94	1382	1374	1253	1453	5462	−7.92
3/94	2836	2934	3206	3199	12 175	1.71
4/94	2857	2989	3064	2710	11 620	8.03
5/94	5580	5469	4981	5162	21 192	−2.79
6/94	3553	3796	3681	3290	14 320	8.48
7/94	913	925	860	858	3556	0.78
8/94	299	306	305	239	1149	11.95
9/94	257	270	248	224	999	7.14
10/94	153	144	138	150	585	−7.45
11/94	166	174	150	157	647	0.31
12/94	251	296	279	280	1106	7.65
1/95	512	561	520	482	2075	8.05
2/95	1232	1339	1417	1194	5182	11.97
3/95	3322	3724	3501	2930	13 477	13.47
4/95	7476	8030	8090	7251	30 847	8.64
5/95	2518	2541	2220	1798	9077	9.34
Total	41 497	44 373	43 361	40 029	169 260	7.08

$P = 100*((b2+b3)-(b1+b4))/(b2+b3)$.

between traverses 1 and 4, or traverses 2 and 3, whereas there are significant differences comparing the outer traverses with the inner, except for 1 with 3 (the critical value for a 0.05 significance level is 2.64, so that if the ZSTAT test is greater than this value it means that the two columns compared are statistically different and they are marked with two asterisks).

Table 3 shows the 24 pollen types selected and the results of the Friedman test comparing differences between traverses. Only two of the types, *Cupressaceae* and *Plantanus hispanica*, present a negative difference

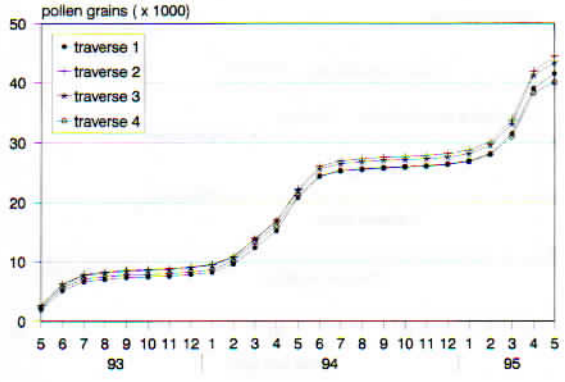

Fig. 1. Accumulative representation of total pollen grains counted by traverse during the study period.

Table 2
Results of multiple comparisons using the Friedman test on all possible pairs of the four traverses (see text for ZSTAT and **).

			ZSTAT
Traverse b1	—	Traverse b2	3.35**
Traverse b1	—	Traverse b3	2.12
Traverse b1	—	Traverse b4	1.01
Traverse b2	—	Traverse b3	1.23
Traverse b2	—	Traverse b4	4.36**
Traverse b3	—	Traverse b4	3.13**

between inner and outer traverses; the rest are positive with values up to 34% (*Ulmus* sp.). Nevertheless the results of the Friedman test show that, except for *Poaceae*, *Plantago* sp, *Eucalyptus camaldulensis*, *Castanea sativa* and *Ulmus* sp., the differences are due to chance.

Fig. 2 shows the percentage distribution of pollen grains found in the 18 longitudinal traverses for the four selected days, as well as the curve from the polynomial regression using the mean of the four 1-day curves. There is a loss of pollen grains from the centre to the outside of the tape. The loss increases exponentially towards the outer edges, and even more than 4% of the pollen grains appear outside the 14 mm typical width of the sporetrap input orifice. There is a slight shift of the centre towards the right, as the maximum should in theory be between traverses 9 and 10. Taking traverse number 10 as the centre, the two neighbouring traverses at 1 mm to each side show a 1.3% loss of pollen grains, at 2 mm the loss is 5.5% and at 3 mm it is 13.8%.

Only 11 pollen types of the 24 selected as having more than 500 pollen grains in total (Table 3) had the required size uniformity to be used in the calculations of the relation between size and percentage loss, assuming that size could affects the pollen distribution over the adhesive surface. These pollen types selected were: *Olea europaea*, *Cupressaceae*, *Eucalyptus camaldulensis*, *Urticaceae p.p.*, *Morus sp.*, *Urtica membranacea*, *Typha sp.*, *Platanus hispanica*, *Populus sp.*, *Castanea sativa* and *Alnus glutinosa*. There was no correlation using either the polar axis or the equatorial axis sizes. Fig. 3 shows the results using the polar axis size.

The diversity, i.e. the number of pollen types found per day, is affected by the sample size: the smaller the pollen grain count, the fewer the number of pollen types. In Fig. 4 one sees that the number of pollen grains per day reaches 3000 in spring (35 days surpass 1000 pollen grains, the maximum being on 30 Mar 95 with 3333 pollen grains). The number of pollen types per day reaches a maximum of 27 on two days in June (9 June 94 and 17 June 93); 51 days surpass 20 pollen types, although the days highest in pollen types were not highest in pollen grains, with only 10 of these 51 days having more than 1000 pollen grains. In general

Table 3
Total number of pollen grains by traverse using the 24 selected pollen types

Pollen type	b1	b2	b3	b4	Total	*P*	*n*	Friedman	*p*
Quercus sp.	11 962	12 879	13 088	11 701	49 630	8.87	23	4.7087	0.1944
Poaceae	7709	8457	8074	7570	31 810	7.57	23	11.8125	0.0081
Olea europaea	3506	3750	3563	3506	14 325	4.12	17	0.9706	0.8084
Cupressaceae	2486	2548	2392	2472	9898	−0.36	23	3.0391	0.3856
Plantago sp.	1946	2106	2066	1839	7957	9.28	23	13.4609	0.0037
Eucalyptus camald.	1343	1435	1301	1280	5359	4.13	24	10.0875	0.0178
Urticaceae p.p.	1108	1111	1220	1163	4602	2.57	23	2.4750	0.4798
Rumex sp.	914	954	962	869	3699	6.94	21	2.1571	0.5404
Amaranthaceae-Chen.	711	759	764	679	2913	8.73	21	5.8143	0.1210
Fraxinus-Phillyrea	553	564	612	568	2297	4.68	13	2.8385	0.4172
Morus sp.	554	626	525	528	2233	5.99	7	0.6429	0.8866
Urtica membrancea	421	442	501	467	1831	5.83	23	1.9956	0.5733
Pinaceae	468	453	452	396	1769	4.53	21	6.3571	0.0955
Typha sp.	288	304	295	271	1158	6.68	12	2.4250	0.4890
Platanus hispanica	275	263	242	237	1017	−1.39	8	0.4875	0.9216
Populus sp.	221	201	194	168	784	1.52	6	0.3500	0.9504
Anthemideae	195	199	206	129	729	20.00	21	6.5857	0.0863
Brassicaceae	176	197	155	136	664	11.36	21	6.7857	0.0791
Cyperaceae	160	159	161	117	597	13.44	18	6.7857	0.0791
Castanea sativa	142	146	151	150	589	1.68	18	11.1167	0.0111
Liguliflorae	118	177	137	142	574	17.20	17	4.2706	0.2337
Salix sp.	119	163	156	121	559	24.76	7	0.7286	0.8665
Ulmus sp.	92	166	166	124	548	34.94	7	9.1286	0.0276
Alnus glutinosa	125	131	126	118	500	5.45	9	0.2700	0.9656
Rest (72 types)	1699	1824	1632	1591	6746	4.80			

P, percentage loss; *n*, number of months analysed; Friedman, results of Friedman test; *p*, probability.

there is a positive correlation between the number of pollen grains and the number of pollen types per day, so that apparently as more pollen grains are counted more pollen types are found. But this is not always the case. When 1000 pollen grains are reached, instead of an increase in pollen types one finds fewer. The number of pollen types increases in a logarithmic way rather than a linearly one with number of pollen grains, so that ever more effort is necessary to find a new pollen type (Fig. 5).

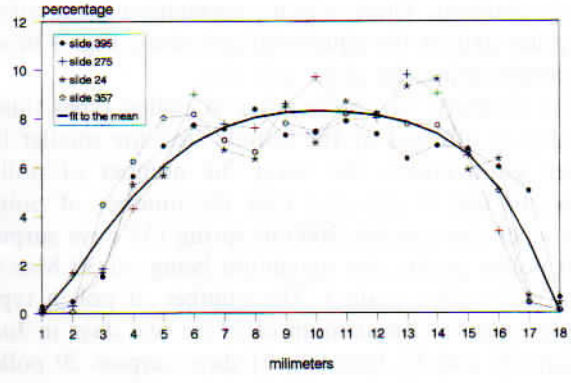

Fig. 2. Total pollen grains counted in 18 longitudinal traverses of four slides. Number of the slide means the day numbers from the beginning. Solid line is the fit to the mean of the four 1-day curves.

4. Discussion

Traditionally, the air volume has been taken as the criterion in aerobiological volumetric sampling. But the amount of particles in that air volume is what is really being measured, and that varies greatly depending on many factors, such as sporetrap location or the season of the year.

Assuming that all airborne particles during a day's intake (14.4 cubic meters) have adhered to the Melinex tape, and since only a subset of them will be counted, we must calculate the most suitable sampling size. In finite populations the sampling size as a function of the

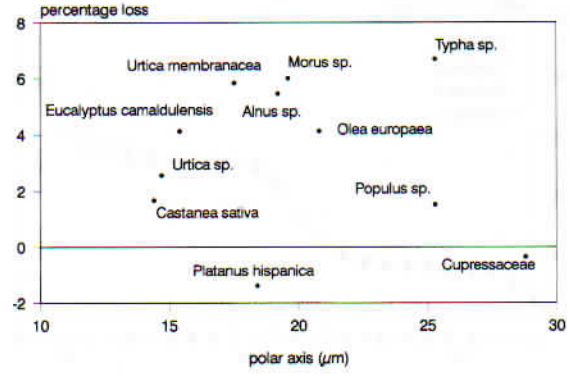

Fig. 3. Scatter diagram comparing polar axis size and the percentage loss in 11 selected pollen types.

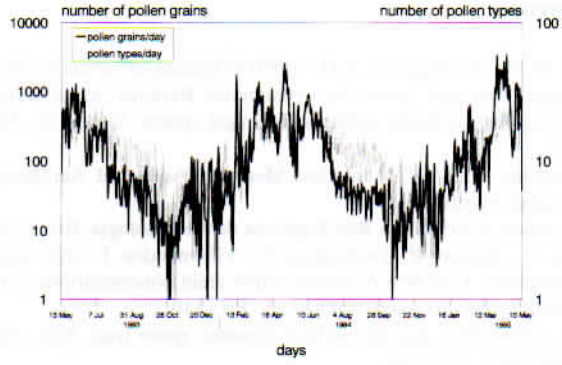

Fig. 4. Daily representation of the number of pollen grains counted and the number of pollen types found on the same day.

percentage can be calculated from the following expression:

$$n = \frac{Z^2pqN}{e^2(N-1) + Z^2pq}$$

where n = sampling size, Z = typified scoring depending upon the accepted confidence level (for 95%, $Z = 1.96$), pq = probability values (0.5 least favourable case), N = population size and e = accepted error (0.05).

The only datum we need to calculate n is the value of N: the population to be sampled is the overall count of pollen grains on the tape surface. We can estimate N since, with four traverses, 12.9% of the population has been sampled, assuming that pollen grains are uniformly distributed. For days with the higher pollen grain counts (up to 3000), the population size would be approximately 24 000 pollen grains (23 256).

The sampling size for that maximal population, assuming a 5% error, will thus be $n = (24\,000 \times 0.9604)/(59.9 + 0.9604) = 378.12$ pollen grains. This best sampling size changes with the size of the population: the smaller the population, the smaller the sampling size. Fig. 6 shows sampling size versus population size, assuming errors of 0.05, 0.04 and 0.03. For a 5% error, the suitable sampling size tends towards 400 pollen grains; with a 4% error towards 600; and with a 3% error 1100 grains, approximately.

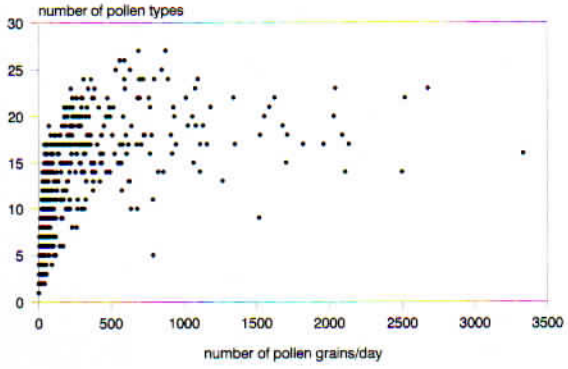

Fig. 5. Pollen types found in relation to pollen grains counted.

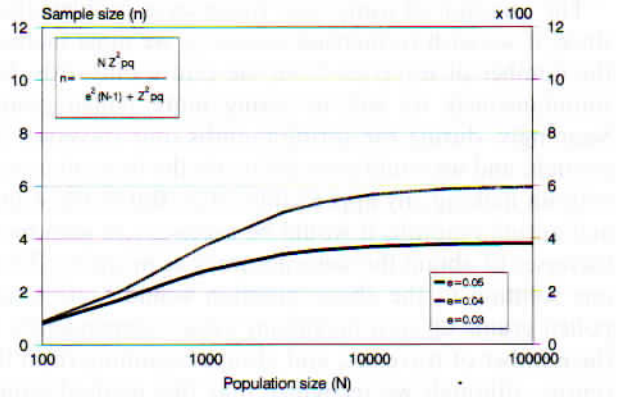

Fig. 6. Best sampling size (n) as function of population size (N), assuming errors of 0.05, 0.04 and 0.03.

We can make a monthly estimate of the total amount of pollen grains captured on the tape, and thus the suitable sampling size for that population. Taking a 5% error, we show in Fig. 7 the pollen grain counts throughout the period of study and the suitable sampling size, month by month. One sees that during the spring months the real sampling size was greater than the best sampling size, whereas in winter and autumn we would need supplementary traverses to compensate the loss in accuracy. These differences could change from place to place, as population sizes vary from one station to another (Rantio-Lehtimäki et al., 1991a,b; Galan et al., 1995). Another question is whether one should take a narrower margin of error, in which case one would need to scan more traverses.

Attempting to answer the questions posed in the Introduction, we can conclude the following: obviously the four traverses are not equivalent; there is a clear loss of pollen grains from the centre of the tape towards the edges; also, one must take into account that pollen grains are captured beyond the typical 14 mm tape width, as was shown by Käpylä and Penttinen (1981).

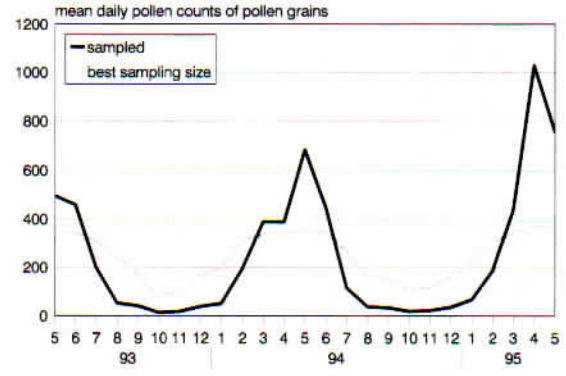

Fig. 7. Monthly representation of the mean daily count of pollen grains and the best sampling size as a function of estimated population size.

The question of using four traverses is problematical since, if we wish to increase accuracy, we must increase the number of traverses from the centre outwards, but simultaneously we will be losing more pollen grains. Seemingly, during the spring months four traverses are enough, and we could even eliminate the two outer ones without making any appreciable error. But in the winter and autumn months it would be necessary to scan more traverses to obtain the same accuracy as in spring. Thus, one solution to the above question would be to count pollen grains up to a maximum value independently of the number of traverses, and always beginning from the centre, although we recognize that this method would involve a loss in methodological uniformity.

Finally, according to the data, the increase in diversity with sampling size is not lineal, but reaches a maximum value beyond which there is no increase in the number of pollen types. Effort invested, therefore, in increasing pollen counts is not reflected in increasing diversity, thus supporting the above idea of counting a fixed number of pollen grains.

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References

Bhat, M.M. and Rajasab, A.H. (1989) Efficiency of vertical cylinder spore trap and seven day volumetric Burkard spore trap in monitoring airborne pollen and fungal spores. Grana 28, 147–153.

Domínguez, E. (1994) Analytical Methods. Advanced Aerobiology Course, Córdoba.

Domínguez, E. (1995) La Red Española de Aerobiología. Rea 1, 3–8.

Galan, C., Alcazar, P., Dominguez, E., Villamandos, F. and García-Pantaleón, F. (1995) Airborne pollen grain concentrations at two different heights. Aerobiologia 11, 105–110.

Hirst, J.M. (1952) An automatic volumetric spore trap. Ann. Appl. Biol. 39(2), 257–265.

Käpylä, M. (1989) Adhesives and mounting media in aerobiological sampling. Grana 28, 215–218.

Käpylä, M. and Penttinen (1981) An evaluation of the microscopical counting methods of the tape in Hirst-Burkard pollen and spore trap. Grana 20, 131–141.

Mandrioli, P. (1994) Sampling Techniques. Advanced Aerobiology Course, Córdoba.

Ogden, E.C., Raynor, G.S., Hayes, J.V., Lewis, D.M. and Haines, J.H. (1974) Manual for Sampling Airborne Pollen. Haffner Press, New York.

Pedersen, B.V. and Moseholm, L. (1993) Precision of the daily pollen count. Identifying sources of variation using variance component models. Aerobiologia 9, 15–26.

Rantio-Lehtimäki, A., Linnea Helander, M. and Pessi, A.-M. (1991a) Circadian periodicity of airborne pollen and spores; significance of sampling height. Aerobiologia 7, 129–135.

Rantio-Lehtimäki, A., Koivikko, A., Kupias, R., Mäkinen, Y. and Pohjola, A. (1991b) Significance of sampling height of airborne particles for aerobiological information. Allergy 46, 68–76.