

Pollen capture media: a comparative study¹

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Abstract

Research laboratories, part of international aerobiological networks are often using different collection media on their exposed tape (or slides). However, their relative pollen capture efficiency has rarely been tested. A single Hirst-trap was used, in Bologna, in February and March 1993, for this experiment. Melinex tapes were divided longitudinally in two equal parts, so that two different media could be tested simultaneously. The tested media were: gelatine/glycerine, hexane/vaseline, silicone/carbon tetrachloride, and paraffin. Each comparisons were repeated on 4 different days. At the time of the sampling, the dominant airborne particles were Cupressaceae and *Cladosporium*. The differences between the different media were non-significant (mean F of different days of 1.720, mean probability of 0.241 (min. of 0.077, max. of 0.356)). The highest probabilities of a significative difference all involved glycerine/gelatine either in comparison with paraffin (0.76), with silicone (0.75), or with vaseline (0.75), while the lowest was for the couple silicone–vaseline (0.59). In general, even if these differences were non-significant, gelatine underestimated spore counts in comparison to silicone, but overestimated them if compared to vaseline or paraffin. However, vaseline looked more efficient then silicone for spore capture. For pollen counts, the same ratios were found, except for the couple silicone–gelatine, which has given similar results. The lowest differences were found with *Alternaria* and *Dreschlera*, while the highest were found with *Cladosporium* and *Epicoccum*. The relationship between spore diameter and differences between media was highly significant ($\tau = -0.786$, $P = 0.027$). This was not the case for pollen, with *Populus* and *Alnus* giving the highest differences, and *Betula* and *Ulmus* the lowest ($\tau = 0.048$, $P = 0.873$). © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

The population under investigation in Aerobiology, i.e. the microbiology of the atmosphere, is invisible to the naked eye. Aerobiologists are studying an invisible particles landscape. This is why our sampling is divided into two major steps: firstly, we draw a known volume of air, and secondly we capture the particles contained in this volume. This capture is done on media that

should be, at the same time efficient for a large array of particles size, but also apt for direct microscopic examination. This is when the third sampling of aerobiology begins: sampling a representative portion of this media surface.

It can be seen that the capture media surface constitutes, physically and conceptually, the end of Aerobiology. After being captured, particles that used to be airborne are deposited and treated (i.e. counted and identified). It is therefore one of the fundamental interfaces of our technical know-how.

If, at the time of the extended gravimetric sampling networks, managed by Durham in North America in the 40's (and even after the Rotorod type sampler introduction under the patronage of the American As-

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¹ “Si les recherches des microbes de l'air acquièrent un développement mérité...(il faut)...spécifier, avec la plus grande rigueur...les qualités des liquides nutritifs...de façon qu'il soit toujours facile de rapporter les résultats à UNE liqueur type adoptée d'un commun accord”. — P. Miquel, 1883.

sociation of Allergy and Clinical Immunology (AAACI), a simple, but uniform, methodology was used; in the first decades after the development of the Hirst-design spore trap, Aerobiology became an individual endeavour. Everyone was managing his own station, under his own terms. Aerobiology was, more than ever, a 'local' science.

However, in the second half of the past decade, integrated national networks became the norm, first in Italy and Spain, and then in England and France and questioning arose when, with the creation of the European data bank in Vienna, we faced the prospect of comparing results obtained with different capture media. Are these data comparable? What type of error is attached to these comparisons?

In fact, this questioning is part of a larger one dealing with methods and errors in Aerobiology. There are multiple sources of errors in Aerobiology. Some we have to live with, such as the different settings (topography, surrounding vegetation) of each station. But others, such as the one related to the reading of the slides (Comtois et al., 1996) or the capture medium selection and application are easier to standardize or to control for.

Kapyla (1989) commented on the scarcity of test studies on mounting media for aerobiological preparations, and on the importance of considering adhesive efficiency in different temperature and on the possible effects of rain and drizzle. For Rotorod samplers, Solomon et al. (1980) have shown that the selection of the capture media could influence the results, but without clear differences, and Gagnon and Comtois (1992) have suggested that the capture differences between two rods of the same sampler could be related to the differential application of the same medium. Kapyla (1989), in search of an optimum adhesive has tested silicone based compounds and proposed a mixture of vaseline, silicone and paraffin as the best adhesive for Hirst-type samplers. However, it never became a standard medium, probably because, contrary to his conclusions, it is not so easy to prepare and to apply.

In order to see what could be the effects of the choice of a particular sampling medium on aerobiological results, a study was undertaken which intended to compare paired samples of the most common media used nowadays in aerobiological studies.

2. Methods

The experimental setting was the one proposed by Kapyla (1989), where Melinex tapes are cut into two halves, which are smeared with different adhesives. The methodological difference was that while Kapyla used a controlled indoor experimental design, we used his proposed protocol under a natural sampling protocol, i.e.

the regular use of a outdoor 7-day spore trap. This set-up allows us to compare two media without any sampler to sampler efficiency difference. Four media were compared, two at a time, for 4 days each pair, for a total of 24 days of sampling. The compared media were:

2.1. Glycerine/gelatine (12.5%)

This medium was proposed at the time of gravimetric sampling (Durham, 1946), and was in current use in Canada. The adhesive film thickness is from 100 to 150 μm at the time of its application, and decrease to 20 to 30 μm 3 days after, at low (< 50%) humidity. It has a constant adhesivity from 5 to 35°C. The greatest advantage of this adhesive is its optical quality. Its main disadvantage is its low melting point.

2.2. Silicone fluid/carbon tetrachloride (2%)

This medium was proposed by Hogan (1971), and is the standard adhesive for both the AIA and REA networks in Italy and Spain. It is recommended for Lanzoni VPPS samplers. The adhesive film thickness is < 3 μm , and show a constant adhesivity from – 10 to 125°C. Its main advantage is constant adhesivity for all temperatures normally encountered in aerobiological sampling. Its disadvantage is the carcinogenic properties of the initial solution.

2.3. Vaseline (petrolatum white)/hexane (10%)

Petrolatum white was first suggested for Rotorod (Perkins, 1957) and volumetric spore traps (Hirst, 1952), and is recommended for the Burkard 7-day spore trap. This is the medium in current use in Mexico. The thickness of the adhesive is of 10–20 μm , and shows a constant adhesivity from 20 to 45°C. The melting point of pure vaseline is 55°C. Its main disadvantages are the loss of adhesivity at low temperatures and the high variation in film application by different operators.

2.4. Paraffin

Paraffin is not a standard substance. Therefore, its melting range varies from 48 to 62°C. It is currently use in the UK.

The sampler used for our experiment was a Burkard 7-day spore trap located at the old FISBAT-CNR building in Bologna, at a height of 20 m. It was in operation in the 3 last weeks of February and first week of March of 1993, i.e. at the time of spring trees pollination. Pollen and spores were counted and identified on two longitudinal lines for each half Melinex tape, at 400 $\times$. This counting procedure allows the comparison of both intra-medium and inter-media count variability.

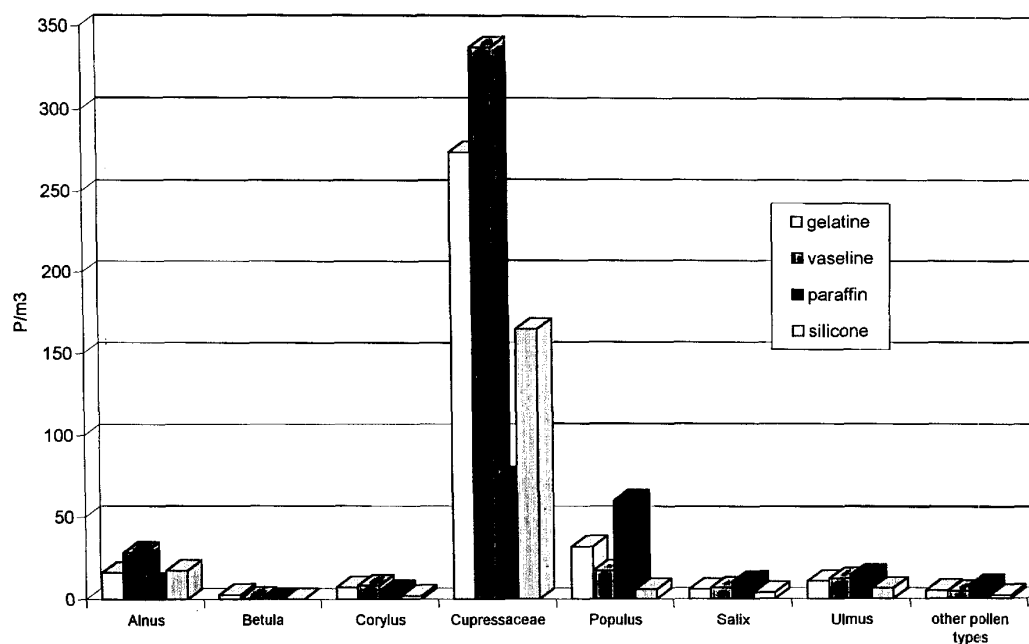


Fig. 1. Average pollen counts for the most important taxa segregated for all four media.

3. Results

At the time of sampling, the most abundant airborne pollen in Bologna were Cupressaceae (79%), *Populus* (8%), *Alnus* (6%), *Ulmus* (3%), *Salix* (2%) and *Corylus* (2%). The most abundant spores were *Cladosporium* (93%), Ascospores (4%) and Basidiospores (3%). Counts for total pollen and total spores averaged respectively 301/m³ and 306/m³.

What was the effect of transversally changing the capture media on the longitudinal count results? On average, for all identified species, the difference was of three pollen grains/line or of four spores/line. This represent an error of 13 and 12%, respectively. However, maximum absolute differences could be as high as 236, or of 35.5%. Minimum values were of course 0. Highest inter-media differences were found between vaseline/hexane and glycerine/gelatine media, for both pollen counts and spore counts. Smallest maximum differences were encountered between glycerine/gelatine and silicone/carbon tetrachloride (a maximum difference of 30, i.e. of 10.1%).

Overall, average specific differences for pollen types varied between 0.2% (for *Corylus*) to 19% (for *Populus*) (*Salix*: 2%; Cupressaceae: 12%; *Ulmus*: 13%; *Alnus*: 14%). For spores, specific differences varied between 5% (for *Cladosporium*) to 22% (for Basidiospores) (Ascospores: 12%; *Alternaria*: 20%). These specific differ-

ences are represented in Fig. 1 and Fig. 2; while overall differences are presented in Fig. 3.

However, it must be realized that there is also differences found between longitudinal counts on the same medium, i.e. of two proximal longitudinal lines. On average, these were of two pollen grains/line or of one spore/line. The largest intra-medium pollen count differences were found for paraffin (6%); the smallest one for silicone/CTC (0.5%) (vaseline/hexane: 1%; glycerine/gelatine: 2%). For spores, the difference was smaller and varied between 0.2 and 2.5%.

Average specific differences for the same medium could be as high as 33% (for both *Corylus* and *Populus*) and as low as 0.4% (for Cupressaceae) (*Alnus*: 5%; *Salix*: 10%; *Ulmus*: 18%). Spores specific intra-medium differences were from <1% (*Cladosporium*) to 33% (Ascospores) (Basidiospores: 20%).

An analysis of variance allowed us to test the significance of the inter-media differences as compared to the intra-medium differences (Table 1).

It can be seen that for all comparisons, the differences are not significant. No interaction was found between species and media in the ANOVA. The A*B probability varied from 0.472 to 0.996.

Paired comparisons were all non-significant. However, the highest probabilities of a significant difference, using a non-parametric rank test, all involved glycerine/gelatine either in comparison with paraffin (0.76), with silicone (0.75), or with vaseline (0.75), while

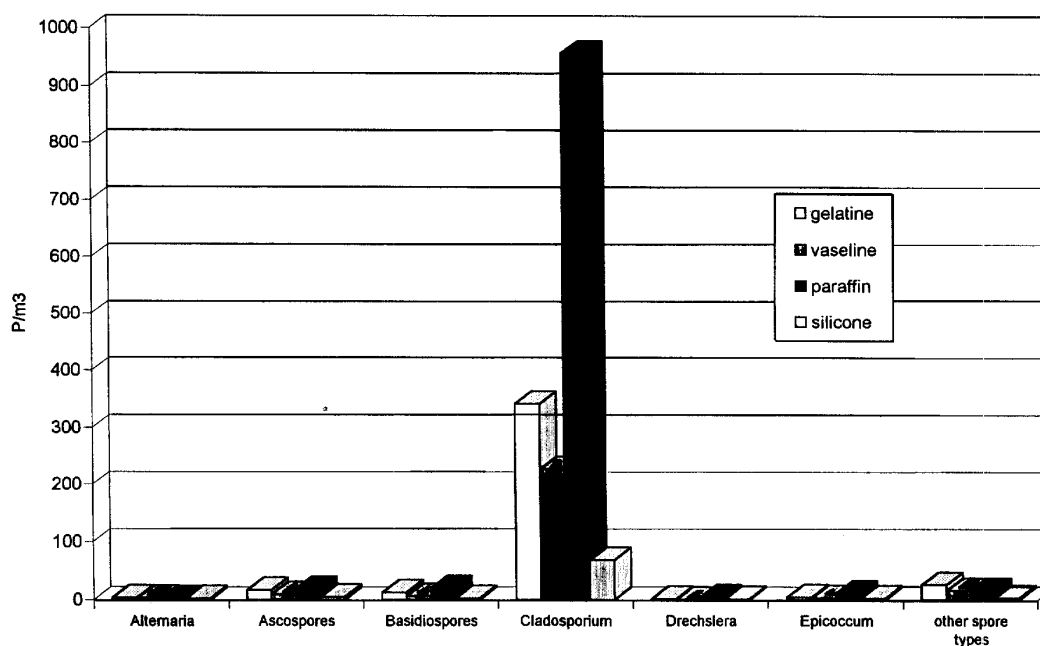


Fig. 2. Average spores counts for the most important taxa segregated for all four media.

the lowest was for the couple silicone–vaseline (0.59). In general, even if these differences were non-significant, gelatine underestimated spores counts in comparison to silicone, but overestimated them if compared to vaseline or paraffin. However, vaseline looked more efficient than silicone for spores capture. For pollen counts, the same ratios were found, except for the couple silicone–gelatine, which has given similar results. The lowest differences were found with *Alternaria* and *Drechslera*, while the highest were found with *Cladosporium* and *Epicoccum* (see Fig. 4).

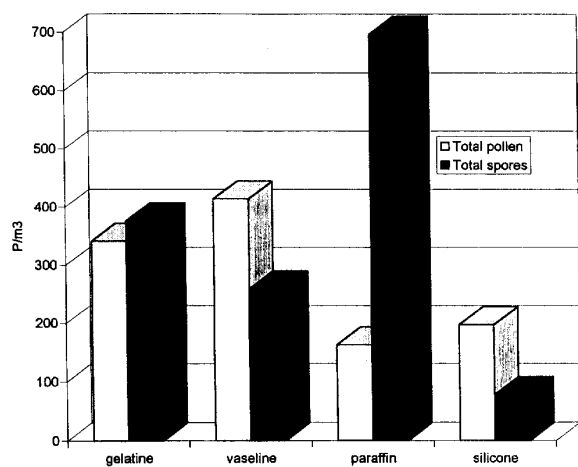


Fig. 3. Average total pollen and spores counts for all four media.

4. Discussion

No overall difference was found between the different tested media, because the error added by changing medium is equivalent to the one found between longitudinal lines of the same media. Therefore, differences in media should not lead to a different interpretation of aerobiological results. These would be, whatever the media, of the same order of magnitude. Similar results were found by Galán and Domínguez-Vilches (1997), for paired comparisons between silicone and vaseline based media.

Differences were not of the same magnitude for all media. Largest (but still non-significant) inter-media errors were found for vaseline/hexane and glycerine/gelatine comparisons; while the largest intra-medium error was found for paraffin. It seems then that media which are more difficult to apply would, therefore, lead

Table 1
ANOVA of the four tested media for the main represented pollen taxa (>2% of abundance)

Pollen type	Parametric test		Non-parametric Kruskal–Wallis <i>H</i>
	<i>F</i> value	<i>P</i> value	
Total	1.171	0.3516	13.54
<i>Alnus</i>	1.158	0.3564	5.871
Cupressaceae	2.356	0.1103	5.274
<i>Populus</i>	2.744	0.0773	5.171
<i>Salix</i>	1.670	0.2134	6.469
<i>Ulmus</i>	1.218	0.3353	4.753

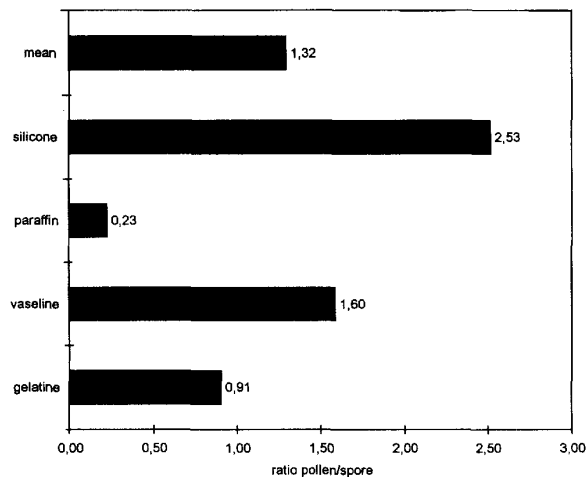


Fig. 4. Relative pollen and spore collection efficiency for all four media.

to more sample to sample or technician to technician variability in efficiency of capture.

As for the relative efficiency of media, it appears that vaseline/hexane will collect more pollen, 48% more than the mean of all media; while paraffin collected 41% less. It is more difficult to separate, for hyaline spores count, the collection efficiency from the observation facility of the media. However, for spores, paraffin seems to collect almost twice the amount of spores than those collected by glycerine/gelatine.

No specific relationship was found between pollen size and the differences between media ($r = 0.148$; $P = 0.7512$), probably because average pollen size varied only between 23 and 31 μm . For spores, with differences in size ranging from 15 to 70 μm , a significant relation ($P = 0.0379$) was found ($r = 0.940$) (Fig. 5). The slope of the relation was also significant and

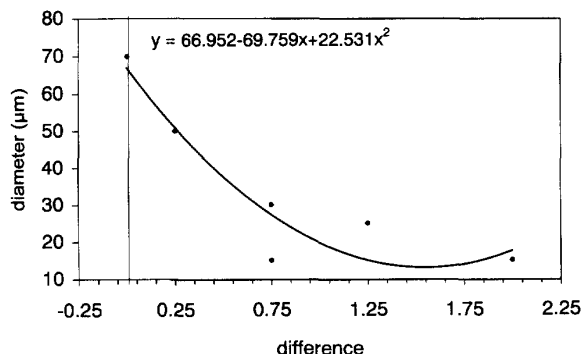


Fig. 5. Relation between spore diameter and mean differences between media.

negative, i.e. that smallest spores brought larger differences.

As for pollen concentration's influence on differences, a significant relation ($P = 0.0451$) was found with the probability of a significant difference between media ($r = 0.821$) ($y = 0.33 + 0.005x$). The slope was weak put positive. Therefore, as mean concentration increases, there is more probability of seeing a significant difference between media.

5. Conclusions

A change of adhesives brings some differences in the pollen counts. However, these differences are of the same order as those found between two longitudinal lines read on the same adhesive. Therefore, a change of adhesive, at least for the ones tested, did not bring a significant change in pollen count, defined as one which would bring a different interpretation of the results.

Although these differences are non-significant, they did add to the general sampling/reading error contained in all aerobiological data. For this reason, and because it is one of the easiest elements to standardize, we still recommend that only one adhesive should be in general use. This will have, as instantaneous effect, a decrease in the general error of aerobiological data. International aerobiological organizations should, therefore, initiate and support such a standard. However, for the moment, results comparisons between media should be allowed, even if they are not the most powerful way of finding biogeographical differences.

Even if it is not the most efficient, its large array constancy in regards to temperature, makes silicone fluid the most evident choice. However, its less than perfect optical quality makes it easier with this adhesive to underestimate spore counts. In addition, because of the toxicity of the solution, and the importance of having a uniform adhesive layer, extreme care should be taken in its application which should be under the guidance of a professional operator.

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