

Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: <http://www.tandfonline.com/loi/sgra20>

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To cite this article: José María Maya Manzano , Santiago Fernández Rodríguez , Inmaculada Silva Palacios, Ángela Gonzalo Garijo & Rafael Tormo Molina (2017): Comparison between two adhesives (silicone and petroleum jelly) in Hirst pollen traps in a controlled environment, Grana

To link to this article: <http://dx.doi.org/10.1080/00173134.2017.1319973>



Published online: 25 May 2017.



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Comparison between two adhesives (silicone and petroleum jelly) in Hirst pollen traps in a controlled environment

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Abstract

Aerobiological sampling through volumetric pollen traps requires the use of an appropriate adhesive, whose selection is a critical factor in these studies. This study compares the differences between two adhesives that have been widely used in aerobiological studies with the aim of providing a persuasive argument for using petroleum jelly as a viable alternative to silicone diluted with the banned substance carbon tetrachloride. The study was performed using Hirst-type pollen traps in an indoor controlled environment to avoid interference attributed to outdoor meteorological parameters. Furthermore, a gravimetric study was conducted simultaneously as a control. Four different pollen types with different pollen sizes and similar flowering seasons were studied (*Acer*, *Platanus*, *Quercus* and *Rumex*). Differences between pollen traps were minimised by interchanging the drums in each pollen trap every 24 hours. Gravimetric results showed homogeneous airborne pollen distribution in the study. No statistically significant differences were found between the results for the sample adhesives for *Quercus* and *Platanus*, whereas significant differences were observed for *Rumex* and *Acer*. In the case of *Acer*, more pollen grains were recorded using the petroleum jelly, while for *Rumex*, more pollen grains were recorded with silicone. After this experiment we consider that petroleum jelly can be used as a viable alternative adhesive to the banned silicone diluted with carbon tetrachloride.

Keywords: aerobiology, aerobiological sampling, methodology, adhesive, evaluation, closed room

Airborne particles (mainly fungal spores and pollen grains) are an important cause of respiratory diseases (D'Amato et al. 2007). Knowledge of air content and its concentration is essential for allergists and allergic patients (Héguy et al. 2008). Hirst-type volumetric pollen traps are routinely used (Hirst 1952) to capture airborne particles in aerobiological surveys (Aylor 1993; Mullins & Emberlin 1997). Measured particles attach to a transparent strip (i.e. Melinex) inside this volumetric trap that is coated with an adhesive. The use of silicone with carbon tetrachloride (CCl₄) has been proposed as an adhesive (Hogan 1971), and advised by the Spanish Aerobiological Network

(Galán et al. 2007). However, this solvent was prohibited by the Registration Evaluation Authorisation of Chemicals (REACH) because it is a carcinogenic agent (Manibusan et al. 2007; National Centre for Biotechnology Information [NCBI] 2015). The primary effects of carbon tetrachloride in humans affect the liver, kidneys and the central nervous system. The symptoms in humans following acute (short-term) inhalation and oral exposure to carbon tetrachloride include headache, weakness, lethargy, nausea and vomiting. The United States Environmental Protection Agency (EPA) has classified carbon tetrachloride as a Group B2 probable human carcinogen (Agency

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(Received 30 March 2016; accepted 8 April 2017)

for Toxic Substances and Disease Registry [ATSDR] 2005). Moreover, silicone seems to hamper the swelling and staining of the grains (Käpylä 1989). Petroleum jelly appears in the literature as frequently used in Hirst pollen traps (Comtois & Mandrioli 1997; Galán & Domínguez-Vilches 1997; Hasnain et al. 2007; Galán et al. 2014).

Other adhesives have also been proposed, such as double-sided self-adhesive tape (3M) (Alcazar & Comtois 1999) and even a handmade mixture proposed by Käpylä (1989). Later on, Alcázar et al. (2003) compared 3M adhesive tape with silicone, and revealed that the tape was less efficient in conditions of low temperature and rain. Razmovski et al. (1998) reported that 3M adhesive tape was less efficient than petroleum jelly adhesives for fungal spores such as *Alternaria* and most types of pollen, as well as for smaller particles, which could be due to these smaller particles being covered by larger particles making them more difficult to see with a microscope. Petroleum jelly only maintains its physical properties up to 45 °C. Therefore, its use during the summer in Spain and other Mediterranean countries has been suggested to be problematic (Comtois & Mandrioli 1997). However, the wet surface of the petroleum jelly may cushion the bounce of particles, allowing the particles to penetrate into the layer (Razmovski et al. 1998).

Silicone with diethyl ether ((C₂H₅)₂O) has also been proposed as an adhesive (Thibaudon et al. 2015), but some chemical properties of this product make it inadvisable because it is a very volatile, highly flammable liquid that is mildly irritating to skin and mucous membranes (NCBI 2015). Moreover, its use is strongly regulated, and it is included in a red list of chemicals frequently used in the illicit manufacture of narcotic drugs and psychotropic substances under international control (INCB 2017).

After the ban of silicone diluted with carbon tetrachloride, looking for an appropriate alternative to this adhesive could be interesting for aerobiologists. Petroleum jelly may be a suitable (and cheap) choice. Petroleum jelly is currently the most commonly used adhesive amongst aerobiologists in Europe (Galán et al. 2014). Our research group uses this adhesive, and we believe that more studies are needed before its use is discarded.

This study compares the differences between two adhesives that have been widely used in aerobiological studies with the aim of providing a persuasive argument for using petroleum jelly as a viable alternative to silicone diluted with carbon tetrachloride. Comparisons among adhesives should take into account environmental influences and differences between samplers (Tormo-Molina et al. 2013). For this reason, the current study was conducted in an

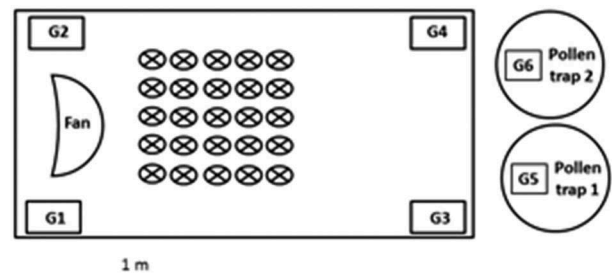


Figure 1. Experimental set-up of the conducted study.

indoor environment and drums were periodically interchanged in order to remove variations between pollen traps. The combination of these two factors provided an opportunity to study the behaviour of two adhesives (petroleum jelly and silicone diluted with carbon tetrachloride) under the most homogeneous and artificial environmental conditions.

Material and methods

Two Hirst-type pollen (Hirst 1952) traps were placed in a closed room, in order to avoid the differences attributed to meteorological conditions, which influence the operation of the samplers and the heterogeneous distribution of pollen on the slides. Intake holes were separated by 45 cm and their flows were measured periodically to verify that they remain constant. Melinex tape (19 mm wide) was placed on the drum and coated with petroleum jelly, previously heated, or with silicone diluted with carbon tetrachloride. To avoid differences between pollen traps (Tormo-Molina et al. 2013), the drums were interchanged between pollen traps every 24 hours. These two adhesives were chosen by the authors because several works had been previously published using them, making it possible to establish comparisons between both adhesives.

Four taxa with a similar onset in their flowering seasons, including trees and herbaceous pollen sources, were chosen for the aerobiological study. Plants of *Rumex bucephalophorus* L. and branches of *Acer negundo* L., *Platanus hispanica* Miller ex Münchh. and *Quercus rotundifolia* Lam. were used in the early pollination state as pollen sources. These plants were maintained during all the period studied; a fan was used to disperse pollen, the table with plants and pollen traps was situated in the centre of the room, lateral walls had glass windows where no direct sunlight reached plants (Figure 1). Plants and branches were homogeneously distributed in water pots on a table (2 m in length). Plants exhibited free release of pollen, which was verified by direct observation. All the branches and inflorescences had opened their flowers during the time the experiment was performed.

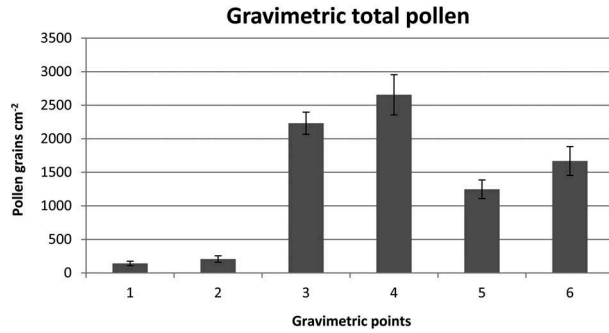


Figure 2. Total pollen grains/cm² recorded at the gravimetric points (see Table I for separated analysis). Standard error of the mean (SEM) appears in each column.

Six gravimetric sampling points, which used petroleum jelly as an adhesive, were located around the sources and near the pollen trap intake holes over the whole sampling period. Thirty optical fields were counted in each gravimetric sample, in the centre of the slide and each one separated by at least another optical field. For each optical field, the numbers of pollen grains for each pollen type were counted, obtaining a total of 720 data (6 slides $\times$ 30 fields $\times$ 4 pollen types). These values were provided in pollen grains/cm². The aim for this sampling was to assess potential asymmetrical distribution of airborne pollen.

To avoid the heterogeneous distribution of pollen on the slides from volumetric samples, only pollen in the centre of the slides was counted, with two longitudinal scans being analysed, according to the methodology of the Spanish Aerobiological Network (Galán et al. 2007) and following the recommendations by Tormo-Molina et al. (1996, 2013). The two reference points to start longitudinal scans, separated 1 mm from each other, were always in the centre of the microscopic slide at 9 mm from the edge of the tape.

Sampling was performed in March 2013 (18 days for *Quercus* and *Rumex*, 14 days for *Acer* and 12 days for *Platanus*). The Wilcoxon-test was applied to analyse differences between pairs of twin gravimetric points (Table I), and between adhesives for each pollen type (Table II) the hourly and daily data from the whole period were used, and four-hourly, eight-hourly and daily averages of concentration in grains/m³ for the four pollen types were analysed. These analyses were performed because hourly data are less robust than daily data as errors in sampling are higher (Comtois et al. 1999; Šikoparija et al. 2011; Buters et al. 2012). Average values and standard error of the mean were calculated. In addition, Spearman's correlation coefficients between the data of the two adhesives were calculated (Table III). An automatic weather station inside the room was used

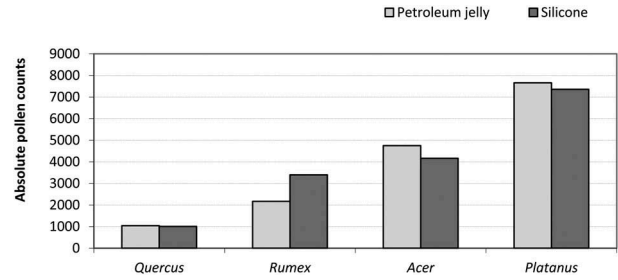


Figure 3. Differences between adhesives in the absolute pollen counts.

for monitoring the meteorological parameters of mean temperature (in °C), relative humidity (%) and sun radiation (in W/m²) hourly.

Results

Mean temperature ranged from 10 °C to 33 °C (average temperature was 18.5 °C), relative humidity from 40% to 84% (average relative humidity of 68.1%), and sun radiation from 0 to 15.8 W/m² (average sun radiation was 2.7 W/m²).

The gravimetric results showed a homogeneous distribution of total pollen between the three pairs of symmetric recording points. From this analysis, absolute pollen counts for each of the 720 values ranged 0–81 (Figure 2). The highest values were recorded in the corners on the experimental table at gravimetric points 3 and 4, and the lowest at points 1 and 2 (behind the fan). No significant

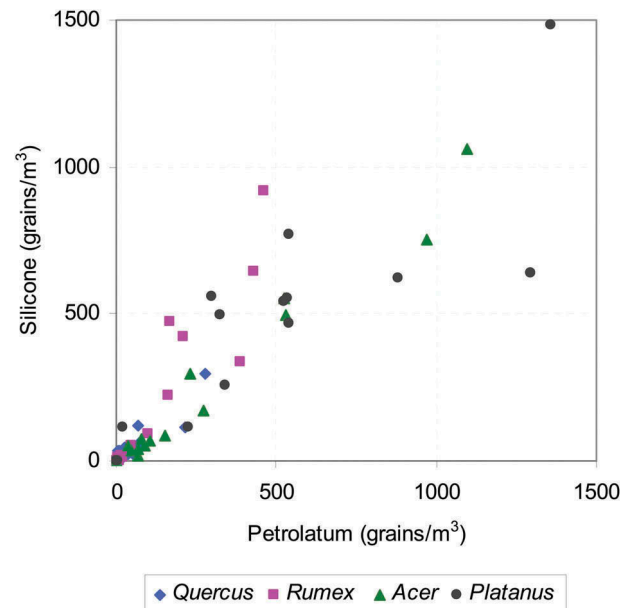


Figure 4. Scatter plot for daily data of *Quercus*, *Platanus*, *Rumex* and *Acer*.

Table I. Wilcoxon signed ranks test between pairs of gravimetric sampling points.

Wilcoxon test		G1–G2	G3–G4	G5–G6
Total	Z	–1.077	–1.028	–1.299
	Sig	0.282	0.304	0.194
<i>Quercus</i>	Z	–1.000	–1.153	–1.781
	Sig	0.317	0.249	0.075
<i>Rumex</i>	Z	–1.633	–1.696	–0.246
	Sig	0.102	0.090	0.805
<i>Platanus</i>	Z	nevc	–1.208	–1.115
	Sig	—	0.227	0.265
<i>Acer</i>	Z	nevc	–1.134	–1.000
	Sig	—	0.257	0.317

Note: Gravimetric sampling points (G1–G6); nevc, not enough valid cases (see Figure 2 for total data representation).

differences were found between pairs of points for the total gravimetric pollen records (Table I). This similarity appeared also for the pollen types separately but for G1–G2 comparisons in two cases there was not enough data to perform the test as pollen records were quite low or absent (Table I). No overloading from the sampling was considered as the number of pollen grains for each optical field was always less than 100 grains.

Similarly, statistical analysis paired by the Wilcoxon-test for hourly data (Table II) did not show significant differences ($p > 0.05$) between the results for the sample adhesives (Figure 3) for *Quercus* and *Platanus*. However, differences were found ($p \leq 0.05$) for *Rumex* and *Acer* (Figure 4). These significant differences appear also with four-hourly and eight-hourly data but only appear in the case of *Acer* for daily data. Moreover, Spearman's rank correlation test was also calculated and pollen average concentrations are shown in Table III, for all the cases correlations were statistically significant ($p < 0.001$). Hourly data distribution for both adhesives are shown in Figure 5.

Discussion

The results of this study showed very low differences between average pollen concentrations. Despite using

the same adhesive, relevant differences in pollen data have been previously reported between pollen traps (Tormo-Molina et al. 2013). Therefore, taking those differences between pollen traps into account is critical for any experiments to test adhesives. The periodic exchange of the drums was used in this study to remove any problem associated with differences in the capture efficiency of the two pollen traps. Another source of error in this type of experiment is that pollen grains are more efficiently recorded in the centre of the adhesive tapes and tend to decrease near the edges (Tormo-Molina et al. 1996). Therefore, results are significant only within the centre of the tape. The current study was also carried out in a controlled indoor environment in order to reduce the influence of meteorological conditions.

Petroleum jelly is viscous at room temperature and needs to be smoothed through heating for it to be properly coated on to the tape. This fact was already commented by other authors (Galán & Domínguez-Vilches 1997; Alcazar & Comtois 1999) in whose study the use of silicone was recommended, reporting an easier way to deal with it. However, ease of use does not make petroleum jelly less useful for its aim, and differences found by Galán and Domínguez-Vilches (1997) were small, occasionally significant and mainly depended on the size of the pollen grains collected, regardless of the adhesive used (differences were found interchangeably in favour of one adhesive or another), as was found in our study.

According to results of Galán and Domínguez-Vilches (1997), larger pollen types are more efficiently trapped using petroleum jelly and, in contrast, silicone has been found to be more efficient for trapping smaller pollen types. Comtois and Mandrioli (1997) found that petroleum jelly was more efficient than silicone for captured fungal spores, whilst petroleum jelly produced similar results to silicone for pollen. Due to this fact, and to avoid differences attributed to it, our experiment was carried out taking into account pollen types of different sizes, considering small *Platanus*, medium-sized *Rumex* and *Acer*, and med-

Table II. Wilcoxon signed ranks test between petroleum jelly and silicone adhesives for the different pollen types studied using hourly data, four-hourly data, eight-hourly data and daily data.

	Wilcoxon-test	<i>Quercus</i>	<i>Rumex</i>	<i>Acer</i>	<i>Platanus</i>
Hourly	Z	–0.029	–3.854	–4.187	–0.878
n = 432	Sig	0.977	0.000	0.000	0.38
4-Hourly	Z	–0.013	–2.463	–2.676	–0.335
n = 154	Sig	0.990	0.014	0.007	0.737
8-Hourly	Z	–0.503	–1.968	–2.495	–0.691
n = 54	Sig	0.615	0.049	0.013	0.489
Daily	Z	–0.592	–0.957	–2.166	–0.314
n = 18	Sig	0.554	0.338	0.030	0.754

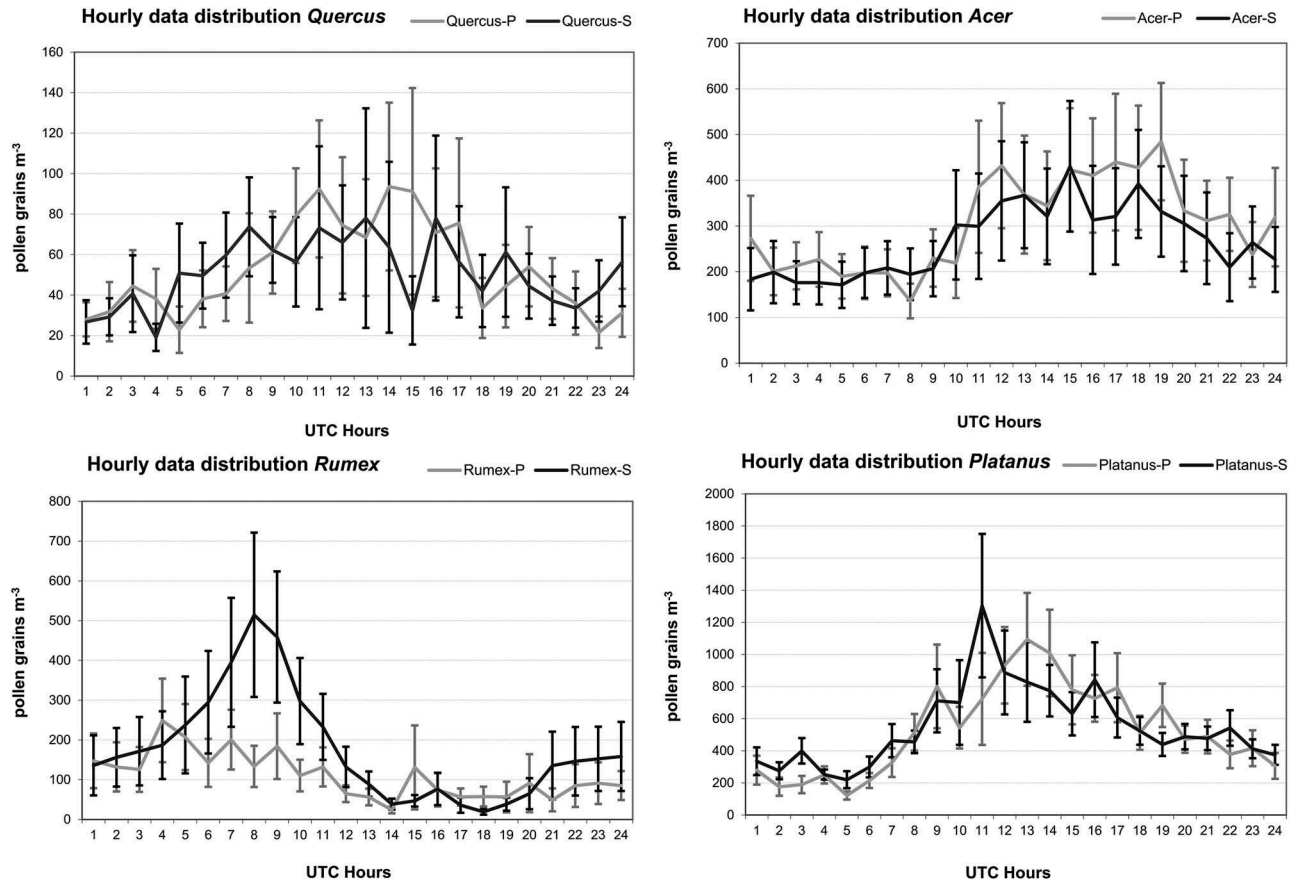


Figure 5. Differences in hourly data distribution between adhesives during the period studied for *Quercus*, *Rumex*, *Acer* and *Platanus*, together with the error of the means.

ium-large sized *Quercus* pollen grains (Trigo et al. 2008). In our work, the differences that could be attributed to different sizes of pollen grains do not seem to have had an impact on the results of our study. The biggest pollen (*Quercus*) and the smallest pollen analysed (*Platanus*) did not show any significant difference between the adhesives, and pollen size differences between *Acer* and *Rumex* are small.

Comtois and Mandrioli (1997) found no significant differences between the adhesives compared; these

differences were of the same order as those that appear between two longitudinal lines read on the same adhesive and so differences in media should not lead to a different interpretation of aerobiological results. Other authors reported that differences up to 30% are acceptable as normal depending on the size of the sample, only by means of resampling the slide (Comtois et al. 1999; Šikoparija et al. 2011; Oteros et al. 2013).

The counting procedure could be another source of error itself; nevertheless, this would be the same for any aerobiological study. Comtois and Mandrioli (1997)

Table III. Mean pollen concentrations (in pollen grains/m³) and standard error of the mean (SEM) in the adhesives during the period studied for *Quercus*, *Rumex*, *Acer* and *Platanus*.

	<i>Quercus</i>		<i>Rumex</i>		<i>Acer</i>		<i>Platanus</i>	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Petroleum jelly	53.1	5.1	110.8	12.0	305.2	23.1	530.1	40.0
Silicone	51.5	5.2	173.4	19.6	267.7	21.1	551.6	41.6
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Pet-Sil	0.459	<0.001	0.733	<0.001	0.791	<0.001	0.475	<0.001

Note: Spearman correlation (*r*) results comparing both adhesives (Pet-Sil) and *p*-values (*p*).

divided the Melinex tapes longitudinally in two equal parts, a method proposed by K  pyl   (1989), so that two different media could be tested simultaneously. This procedure could involve other methodological errors because pollen distribution across the short length of the slide depends on the position over the tape, which shows maximum particle concentration at the centre and decreases towards the edges (Tormo-Molina et al. 1996). Nevertheless, Comtois and Mandrioli (1997) did not mention the amount of distance from the centre to the edge when they counted pollen, although the division of the two adhesives happens in the middle. This comparative methodology was also performed by other authors (Gal  n & Dom  nguez-Vilches 1997), with pollen being recorded from the end of May to the beginning of June.

No statistically significant differences between adhesives were found in our study for the pollen of *Quercus* and *Platanus*. In the case of *Acer*, more pollen grains were recorded using the petroleum jelly, while for *Rumex* more pollen grains were recorded with silicone. Peaks of hourly pollen concentration were similar for both pollen traps showing that there are not significant differences between them. *Rumex* reached maximum values in the early morning, while *Acer* had no clear peak hour and showed higher values during the whole day than during night. Nevertheless, variation in this pattern is so high that it is not possible to justify differences found from this kind of information.

It may be due to its low pollen production (Andersen 1970) and its limited dispersion ability, provoking its deposition shortly after pollen was released. It provokes a continuous pollen record instead of pollen peaks, frequent in the other taxa studied in this work.

Comparison with other studies is hardly possible because *Acer* pollen distribution and its daily pattern have not been studied as much as other pollen types in the literature. It is frequently recorded as an ornamental tree in Mediterranean latitudes, but has limited dispersion ability and pruning during management in the spring further reduces its pollen release (Maya-Manzano et al. 2017). These aspects were avoided in this work because pollen sources were located very near the pollen trap and without any type of management, except watering.

Previous studies carried out with four traps in the same location, but outdoors (Tormo-Molina et al. 2013), showed differences in hourly data even at a few metres distance. This fact is another reason for doing this study indoors, and for trying to increase the study's accuracy by means of changing the drums. Therefore, stochastic turbulence phenomena are less dependent on the pollen trap used in this indoor scenario.

Regarding the gravimetric study, the highest amounts of pollen were recorded at points 3 and 4.

For points 5 and 6 pollen sources were a bit farther than for points 3 and 4 consequently pollen concentration in the air was lower (Figure 2). Obviously, the lowest concentrations were recorded at points 1 and 2, which were located behind the fan and the branches. Consequently, particles were moved from the branches to the remaining points, including the pollen traps.

Differences found in this type of experiments between adhesives or in the pollen counts in any aerobiological study could be attributed, not only to environmental parameters or different adhesives but also to differences between traps (Tormo-Molina et al. 2013). Moreover, there are other intrinsic factors involved in aerobiological sampling (Comtois et al. 1999) such as the number of longitudinal scans that have been completed (  koparija et al. 2011). Certainly, a standardisation of aerobiological methods is needed. However, measurements must be taken into account in comparative studies on adhesives under the same conditions (not only environmental conditions but also evaluating the differences between pollen traps) beforehand to prove if a difference exists and what its nature is.

Conclusion

High pollen concentrations were found in this work under controlled conditions for the pollen types studied, probably due to the indoor environment and the closeness of the pollen sources to the pollen trap. Petroleum jelly and diluted silicone did not show statistically significant differences when used as adhesives in Hirst-type pollen traps for *Quercus* and *Platanus*. In the case of *Rumex*, more pollen grains were recorded with silicone, and for *Acer*, more pollen grains were recorded with petroleum jelly. Those differences could be attributed to different aspects involved in the aerobiological sampling, such as response of adhesives to temperature and inaccuracy of hourly data precision, even when we are studying nearby locations. More studies in different environments are needed to compare the efficiency of both adhesives and to appraise the inherent differences in the efficiency of the pollen traps. Interchanging the drums between traps is a good way of doing this, as is reducing the influence of the meteorological conditions by conducting the study indoors. In any case, and due to the fact that those differences are not recorded only in favour of one of the adhesives, there is no reason to avoid the use of petroleum jelly instead of silicone as an adhesive.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was conducted thanks to the Extremadura Regional Government (Junta de Extremadura), Spain (Project Number PRI06A190 and GR15060 funds), and the European Social Fund (Project number PRI BS10008);

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