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Publisher: Taylor & Francis

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Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

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Available online: 03 Sep 2009

To cite this article: Valentina Razmovski, Timothy O'meara, Mervi Hjelmroos, Guy Marks & Euan Tovey (1998): Adhesive tapes as capturing surfaces in Burkard sampling, *Grana*, 37:5, 305-310

To link to this article: <http://dx.doi.org/10.1080/00173139809362683>

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Adhesive tapes as capturing surfaces in Burkard sampling

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Razmovski, V., O'Meara, T., Hjelmroos, M., Marks, G. & Tovey, E. 1998. Adhesive tapes as capturing surfaces in Burkard sampling – Grana 37: 305–310. ISSN 0017-3134.

Although pressure-sensitive adhesive tapes appear to offer many advantages over traditional adhesives in sampling with a Hirst-type spore trap, their particle capturing efficiency has never been documented. In this paper, the particle capture efficiency of four pressure-sensitive adhesive tapes was compared to the standard vaseline adhesive in the Burkard 7-day volumetric spore trap. The capture efficiency was assessed at flow rates of 8 and 10 l min⁻¹ over a 16 week period with each tape run alongside the standard. Image analysis was used to count and measure the surface area of individual inorganic and organic particles collected onto each tape. Approximately 230,000 individual particles were analysed, the majority of particles collected on both standard and pressure-sensitive adhesive tapes had a surface area less than 150 µm² (approximately diameter 14 µm). Capture efficiency was expressed as a percentage of the counts on the standard and classified by particle size and flow rate. For particles <1000 µm² (<36 µm in diameter), the best adhesive tape captured 247.6% at 8 l min⁻¹ and 132.5% at 10 l min⁻¹. For particles ≥1000 µm², the best adhesive tape captured 86.9% at 8 l min⁻¹ and 69.8% at 10 l min⁻¹. In addition to image analysis, manual counts of pollen grains and *Alternaria*-like spore were performed over a four day period for each adhesive and parallel standard at the flow rate of 10 l min⁻¹. On the adhesive tapes, pollen grains <20 µm, and pollen grains >20 µm with heavy ornamentation and/or free standing exine elements were captured best. For the best adhesive tape, the capturing efficiency compared to the standard vaseline was 83% for all pollen grains and 60% for *Alternaria*-like spores.

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(Manuscript accepted 15 March 1999)

Adhesives as the capture media for aerobiological sampling date back to 1873 where Blackley used a sticky solution containing glycerin to measure the amount of pollen in the atmosphere (Waite 1995). In 1952, Hirst assessed the spore content of the air by means of vaseline-coated microscope slides in his automatic volumetric spore trap (Hirst 1952).

The Hirst-Burkard 7-day volumetric spore trap is the most widely utilised sampler within aerobiology and phytopathology. At present, a variety of adhesives are employed in the sampler, depending on location and temperature-related climatic conditions (Comtois & Mandrioli 1997). Vaseline, which is hardened by adding paraffin, is still the most common adhesive for the spore trap. The main disadvantage is its sensitivity to temperature and humidity changes, causing variation in its capturing and retention efficiency. Also, its method of application, which is largely operator dependent, produces variation in film thickness.

Käpylä (1989) suggested a mixture of vaseline, silicone oil and paraffin as the best adhesive. Recently, silicone fluid was recommended as the capture media in future sampling (Comtois & Mandrioli 1997, Galan & Dominguez-Vilches 1997). However, the optical quality of this adhesive is poor, applying a uniform adhesive layer is difficult and the solution is toxic (Comtois & Mandrioli 1997).

Pressure-sensitive adhesive tapes used in this study appear to offer a number of advantages over previously used media for aerobiological sampling. They: (1) do not require manual

application of an adhesive solution to a melinex type backing material and therefore some of the variations in layer thickness and non-uniform adhesion are eliminated; (2) are permanent adhesives and thus remain sticky over prolonged periods of time; (3) remain adhesive over a wide temperature range (–30–120°C) and therefore may be suited to a wide range of environments where aerobiological samples are collected; (4) are chemically stable and non-toxic; (5) are transparent and therefore optically good for light microscopy; (6) are compatible with immunostaining for the detection eluted of allergens from airborne particles (Tovey et al. 1998), and (7) are compatible with aqueous mounting media.

Although pressure sensitive adhesives tapes appear to be highly suitable for aerobiological sampling, the particle capture efficiency of these tapes has not been determined. The aim of this study was to assess the particle capture properties of commercially available pressure-sensitive adhesives tapes by comparison to the standard vaseline/paraffin/toluene mixture in the Hirst-Burkard 7-day volumetric spore trap.

MATERIALS AND METHOD

The standard adhesive which consisted of a mixture of vaseline and paraffin in toluene (9:1:100) was brushed onto a 1 cm strip of Melinex tape (supplied by Burkard Ltd., Rickmansworth Hertfordshire, England). The excess was wiped off with tissue paper.

The four adhesive tapes (1 cm wide) were cut equally and combined, one at a time, as one strip around the drum, parallel to the standard adhesive according to the method of K  p  l   (1989). Thus, the drum was divided vertically into two sides with one side covered with the standard vaseline based adhesive and the other covered with the adhesive tapes. This allowed comparison of two different capture surfaces for each sample collected. Tape No. 1 (supplied by Adhesives Research Inc., Glenrock, Pennsylvania), Tape No. 2 and No. 3 (supplied by JAC, Australia) were water-based acrylic pressure-sensitive adhesives. Tape No. 4 (supplied by Avery Dennison, Painesville, Ohio) was a solvent-based pressure sensitive adhesive. The sampling was conducted during the spring and early summer months (September to December) in a western suburb of Sydney, Australia. The 7-day volumetric Hirst-Burkard spore trap (Burkard Ltd.) was placed on the ground and operated at a suction volume of 101 min^{-1} for 14 weeks and 81 min^{-1} for 2 weeks.

The lower flow rate was used to reduce particle bounce on the adhesive tapes, which was considered to be a potential problem the harder pressure sensitive adhesive tapes. Although a 7-day sampler was used, the tapes were changed every Monday, Wednesday, and Friday, which permitted continuous assessment of the samples. This resulted in paired strips of tape that corresponded to 12 (21 mm) or 18 (36 mm) hour sampling periods.

For microscopy and image analysis, the standard and adhesive tape strips corresponding to the same sampling period were aligned exactly and affixed with double-sided sticky tape in parallel onto microscope slides. The slides were then mounted in a mixture of glycerol gelatine in water (3:1:7). A standard light microscope (Olympus BH2, Olympus, Japan) was used for all analysis. A microscope field ($100\times$) was scanned for the adhesive tape, followed by an identical time-paired field for the standard. This was repeated 3 more times so that for each sampling period of 12 to 18 hours, 4 time-paired fields were analysed for each adhesive tape and standard.

Image analysis was used to count the number and determine the surface area of all individual particles. The particles counted using this method of analysis include all particles that could be differentiated from the background, thus both inorganic (mineral dusts) and organic particles (pollen grains, spores, amorphous organic particles) are included. For counting and sizing the particles in each microscope field, a high resolution video camera (1000×1000 square pixels at 256 gray levels; Pulnix Progressive Scan TM-1001, U.S.A.) and particle counting program under $100\times$ magnification was used.

The particle-counting program was developed in-house, based on the WIT image processing software (Logical Vision, Coreco Inc., Canada). The output gave the number of particles within a particular area distribution; the range was set at $0\text{--}2000\text{ }\mu\text{m}^2$, with $50\text{ }\mu\text{m}^2$ intervals. For all size categories, each field on the adhesive tape was compared to the corresponding time-paired field on the standard. The results were recorded as a percentage of the standard for each size category.

Manual count of pollen grains and *Alternaria*-like spores were undertaken over a four day period for each of the reviewed tapes at the flow rate of 101 min^{-1} . As general sampling is undertaken at a suction rate of 101 min^{-1} , the capture efficiency of these particles was not investigated at 81 min^{-1} . Pollen grains were identified to either species or genera level; for fungal spores, only *Alternaria*-like spores were recorded. This method of spore counting allowed us to cover all the obtuse shaped smooth spores with a beak within a wide size range and included spores of the following species: *Alternaria*, *Bipolaris*, *Calostibella*, *Ceratoporum*, *Chaetoconis*, *Exerohilum*, and *Exosporium*. 12 transverse bands were read for each day at the magnification of $400\times$. The counts were expressed as pollen grains/spores $\text{m}^{-3}\text{ 24hrs}^{-1}$.

Statistical analysis

Four pairs of adhesive tape and standard, representing the four paired fields, were compared on 17 occasions for each of four adhesive types. The flow rate was 101 min^{-1} on 13 occasions and

81 min^{-1} on four occasions. Particles on each surface were allocated to one of 14 size categories and counted.

Particle counts were skewed and the variance of counts increased with the mean. The distribution of counts by size category for each type of adhesive tape or standard was described as the median of the mean of four replicates for each tape or standard.

A log transformation (offset by one) was used to normalise the distribution and stabilise the variance. The offset of one was required because of zero values. Log transformation was used in preference to square root transformation to allow interpretation of the back-transformed coefficient of repeatability.

The repeatability of total particle counts (summed over all particle sizes) for each type of adhesive tape and the standard adhesive was assessed among each set of replicates. This analysis was based on 17 sets of four replicates for each adhesive. For each adhesive tape or standard, the within tape standard deviation of log counts (Chinn 1991) were derived from an analysis of variance. The coefficient of repeatability (Bland & Altman 1986), the range within which 95% of pairs of repeated observations would be expected to lie, was derived from the within-tape standard deviation and anti-logged to give a fold-range (Chinn 1991).

The particle capture efficiency between each type of adhesive tape and its adjacent standard was calculated as the anti-log of the difference between the log-transformed counts. The relation between this efficiency (expressed as adhesive as % of standard) and particle size and flow rate was examined graphically (Fig. 1). This graph revealed differences in efficiency for particles larger or smaller than $1000\text{ }\mu\text{m}^2$ ($36\text{ }\mu\text{m}$ in diameter).

The effects of adhesive type, flow rate, and particle size ($\geq$ or $< 1000\text{ }\mu\text{m}^2$) on the difference in log counts between the adhesive tape and the standard were tested by analysis of variance. Mean levels of efficiency (adhesive tape as % of standard, Table II) were estimated from this analysis of variance by anti-log transformation of the least squares means for each of these characteristics (PROC GLM, SAS, SAS Institute).

RESULTS

Image analysis of absolute particle counts

There were 231,805 observations, which included both inorganic and organic particles that were counted, and sized using image analysis software. Median counts for each type of adhesive tape and each standard, for each particle size category are shown in Table I. The highest counts were observed for the smallest size categories. The particle counts on adhesive tapes, as a proportion of counts on the standard, were highest for small particles on adhesive Tape No. 4 and lowest for larger particles on adhesive Tape No. 1 and No. 3. For Tape No. 2 and No. 4, the over-estimate of the standard for smaller particle size was much greater at 81 min^{-1} than 101 min^{-1} (Fig. 1 and Table II).

Pollen and spore analysis

The results of the manual pollen and spore counts are shown in Figs. 2 and 3 respectively. The most frequent occurring airborne pollen grains during the sampling period were *Olea*, *Poaceae* and different species of *Eucalyptus*; the number of fern spores found was moderate. Single pollen grains of other taxa were found sporadically. For more detailed information on the pollen and spore distribution in the Sydney area, see Bass and Morgan (1997). The warm and humid weather conditions created an excellent living environ-

Table I. Median particle counts (non transformed) by particle size for each adhesive tape and for the standard.

The median for each tape and adhesive was collected over the entire 17 sampling periods. In order to account for differences over time, adhesive Tape No. 1 should be compared with standard 1, Tape No. 2 with standard 2 etc.

μm^2	Adhesive tape				Standard			
	1	2	3	4	1	2	3	4
50	135	171	148	246	110	112	101	111
100	123	154	127	228	96	98	98	110
150	52	72	51	98	47	51	50	48
200	30	38	24	44	25	26	26	24
250	17	21	13	28	17	18	16	15
300	11	14	10	17	12	12	11	10
350	8	9	5	9	7	8	10	8
400	5	6	4	6	7	6	6	5
500	5	9	5	8	9	9	9	7
750	5	9	5	6	12	9	11	9
1000	2	2	2	2	5	4	5	3
1500	1	1	2	2	5	3	4	3
2000	1	0	1	1	3	2	1	1
2000	1	1	1	1	3	1	2	2
Total	416	510	418	728	338	373	352	353

Efficiency

The efficiency of the adhesive tapes was expressed in terms of the adhesive tape count as a percentage of the standard count. Fig. 1 shows that for all particle sizes greater than $1000 \mu\text{m}^2$ ($36 \mu\text{m}$ in diameter) the adhesive tape tended to underestimate the standard. For smaller particles the direction of the differences varied between tapes and flow rates.

The effects on efficiency depicted in Fig. 1 were examined by analysis of variance. Particle size categories were grouped into large ($\geq 1000 \mu\text{m}^2$) and small ($< 1000 \mu\text{m}^2$). There were significant interactions between flow rate and particle size, flow rate and adhesive tape type, and adhesive tape type and particle size (all $P < 0.0001$). The efficiency between the adhesive tape and standard under each of these conditions is detailed in Table II.

Repeatability

Coefficients of repeatability for total particle counts for the adhesive tapes and the standards were all in the range of 1.9 to 2.4 fold (Table III). This is the range within which 95% of pairs of replicate total particle counts could be expected to lie. Two individual total particle counts which fall within this range (i.e. differ by less than two fold) could not be regarded as significantly different.

The optical quality of all the tape adhesives was superior when compared to the standard vaseline. All the tapes were compatible with the mounting media.

During the collection period the mean daily temperature fluctuated from $+16^\circ\text{C}$ to 27°C and the humidity range was between 46 and 100% with 23 periods of rain noted. The effect of temperature or humidity on particle capture efficiency of the tape was not analysed.

DISCUSSION

Many aerobiological samplers require an adhesive to capture and retain particles on a sampling surface. A number of adhesives have been examined over the years (Comtois & Mandrioli 1997, Galan & Dominguez-Vilches 1997, Kapyla 1989), however there has been no investigation of the capturing ability of pressure-sensitive adhesive tapes. We have found that using pressure sensitive adhesive tapes can result in an apparent improvement in the particle capture efficiency for small inorganic and organic particles ($< 1000 \mu\text{m}^2$, or theoretically, $< 36 \mu\text{m}$ in diameter) as assessed relative to the standard using, image analysis software. For adhesive Tape No. 2 and No. 4 at 101 min^{-1} and for Tape No. 1, No. 2 and No. 4 at 81 min^{-1} , the capture efficiency was higher than the standard. The adhesive tapes may therefore be more efficient for the collection of smaller inorganic and organic airborne particles such as some fungal spores or pauci-micronic particles. For larger inorganic and organic particles

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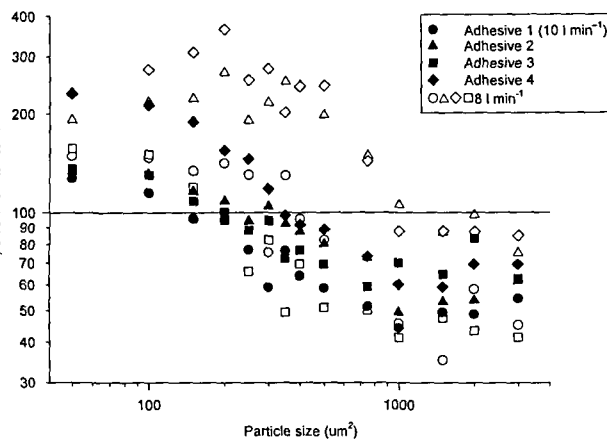


Fig. 1. Agreement between adhesive tape and standard by particle size and flow rate. Counts on the adhesive tape are expressed as percentages of the counts on the corresponding standard.

ment for the fungi and the concentration of fungal spores was high.

The capture efficiency of the adhesive tapes for smaller or medium-sized pollen grains, such as the triangular, smooth *Eucalyptus* sp., and the heavily ornamented, reticulate, spheroid *Olea* pollen was good. For Tape No. 4 (solvent-based adhesive), the capture efficiency of *Eucalyptus* was over 100% (101.5%) when compared to the standard over the 4 day period; the same tape captured 66.7% of *Olea* pollen grains. The big fern spores with perispore were captured reasonably well (58%) on Tape No. 4. None of the adhesive tapes were efficient in capturing smooth, ellipsoidal Poaceae pollen when compared to the standard (20% for Tape No. 4). Overall, the collection efficiency range of the adhesive tapes was 32–83% of the standard, with Tape No. 4 being the most efficient in capturing pollen grains (83% of the standard).

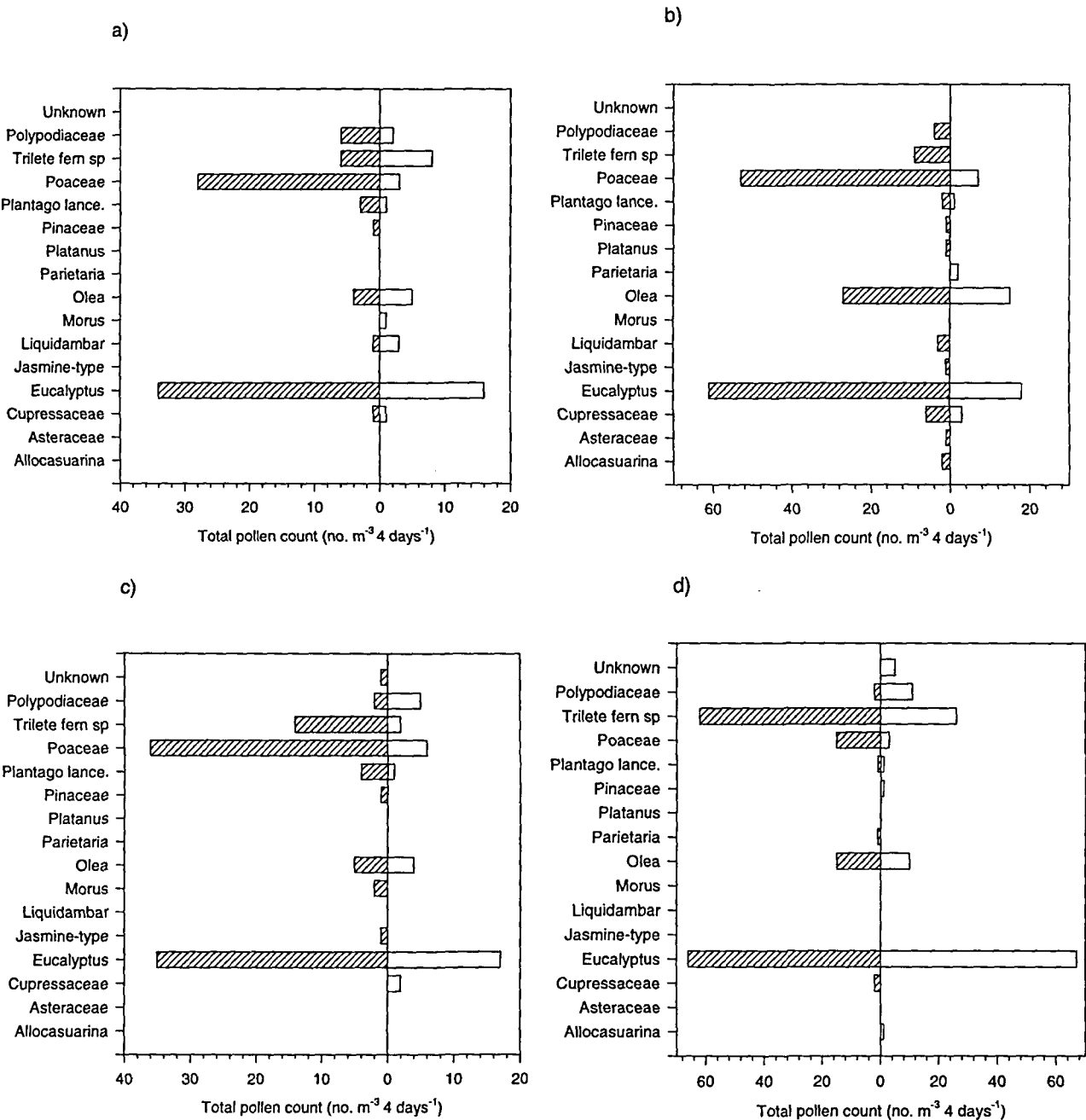


Fig. 2. Total pollen counts (no. of pollen m^{-3}) on the adhesive tapes and standard over a 4 day period: a) Tape No. 1, b) Tape No. 2, c) Tape No. 3, and d) Tape No. 4 ($\square$ Adhesive Tape; hatched Standard).

($\geq 1000 \mu\text{m}^2$) and most pollen grains and *Alternaria*-like fungal spores the adhesive tapes were less efficient than the standard adhesive.

The capture efficiency of adhesives for airborne particles depends on the velocity of the incoming particle, its shape, size, density, composition and the properties of the adhesive (Hinds 1982). The adhesive properties that are most relevant are tackiness and hardness, which affect particle trapping and bounce. Particle bounce has been proposed to limit the efficacy of the adhesive tapes (Ogden et al. 1974). Once a monolayer of collected particles forms on the adhesive tapes,

particle bounce is possible as the tackiness per unit of surface area decreases (Baron & Willeke 1993). This may effect particle capture on heavily laden adhesive tapes. In contrast, the standard vaseline adhesive provides incoming particles with a "wet" surface that wicks up through the particle deposit by capillary action (Baron & Willeke 1993). An unfortunate consequence of this is overloading and multiple layers of sampled particles, which impedes visibility. This may explain why the adhesive tapes have higher collection efficiency for smaller particles since with vaseline, the smaller particles may become embedded in the vaseline and are

Table II. Particle counts on each type adhesive tape as a percentage of counts on standard, classified by particle size ($1000\ \mu\text{m}^2 \approx 36\ \mu\text{m}$ in diameter) and flow rate.

Bold cells=adhesive tape count significantly lower than standard;
Bold italic cells=adhesive tape count significantly higher than standard.

Adhesive	Large particles ($\geq 36\ \mu\text{m}$)		Small particles ($< 36\ \mu\text{m}$)	
	$u8\ \text{l min}^{-1}$	$10\ \text{l min}^{-1}$	$8\ \text{l min}^{-1}$	$10\ \text{l min}^{-1}$
1	45.3 (36.7–56)	49.0 (43.4–55.4)	108.1 (94.6–123.5)	78.6 (72.8–84.9)
2	90.8 (73.6–112.1)	54.3 (48.1–61.4)	212.7 (186.1–243.0)	100.4 (92.9–108.4)
3	43.4 (35.1–53.5)	69.8 (61.8–78.8)	81.6 (71.4–93.2)	90.6 (83.9–97.9)
4	86.9 (70.4–107.3)	65.7 (58.2–74.2)	247.6 (216.7–282.9)	132.5 (122.7–143.1)

Table III. Repeatability of total particle counts.

Particle summed across all size categories for each collection.

Adhesive/Standard	Within-tape standard deviation (between replicates) in log units	Coefficient of repeatability (fold range)
Tape 1	0.13	2.3
Tape 2	0.13	2.2
Tape 3	0.13	2.3
Tape 4	0.10	1.9
Standard 1	0.14	2.4
Standard 2	0.12	2.1
Standard 3	0.13	2.3
Standard 4	0.14	2.4

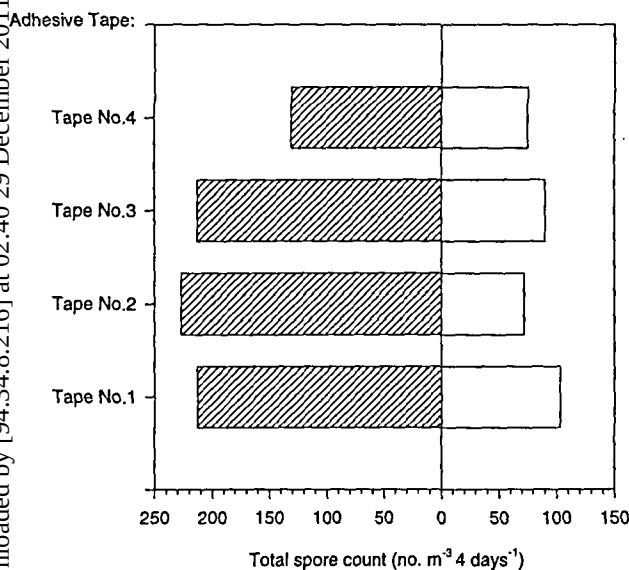


Fig. 3. Total number (spores m^{-3}) of *Alternaria*-like spores captured on the adhesive tapes and standard over a 4 day period ($\square$ Adhesive Tape; hatched Standard).

subsequently covered by incoming larger particles. The smaller particles are thus undetected by the eye and the video camera in the counting program.

Adhesion is dependent on the viscosity and kinetic energy of the sampled particle at the instant it comes into contact with the adhesive (Hinds 1982). The harder of the adhesive or the larger the particle and therefore the greater the incoming velocity, the greater likelihood of bounce occurring. At high velocities, part of the kinetic energy is dispersed and part is converted to rebound energy at the point of impact onto an adhesive. At $10\ \text{l min}^{-1}$, the larger particles strike and rebound more readily from the adhesive tape than the vaseline. Here, the rebound energy exceeds the adhesion energy. The above results indicate that the bounce varies from tape to tape with a range of 49–69.8% of particles captured ($\geq 36\ \mu\text{m}$ in diameter) when compared to the stand-

ard vaseline at $10\ \text{l min}^{-1}$. The wet surface of the vaseline may cushion the bounce, allowing the particles to penetrate into the layer, while the harder adhesive acts as a solid wall, initiating particle bounce. At $8\ \text{l min}^{-1}$, the capture efficiency of larger particles increases significantly for Tape No. 2 and No. 4 by 36.5 and 21.2% respectively. At low velocities, the particle's incoming kinetic energy (and rebound energy) is thus decreased by deforming itself and the adhesive. This would allow more contact time at the particle-adhesive interface and therefore adhesion to take place.

The shape of the particle also influences adhesion onto the adhesive tapes (Hinds 1982). The results for pollen indicate that the more surface area that comes into contact with the tape adhesive (the more projections or ornamentation it has), the greater the likelihood of adhesion taking place.

The repeatability results for the adhesive tapes are similar to the standard. This indicates that applying the vaseline mixture with a brush does not result in greater variations in particle capture when compared to the uniform thickness adhesive tapes. This result has been obtained by applying the same batch of vaseline, by the same operator every time, and it is likely that across sites variations between operators and sites may result in less repeatable counts. Thus for comparisons across sites, such as in the current Europe pollen network or in the US, adhesive tapes may result in more comparable counts.

CONCLUSIONS

The capture surface constitutes an integral part of aerobiological sampling. It is the surface that allows air-borne particles to be captured, quantified and identified. It was found that the capture efficiency of the pressure-sensitive adhesive tapes was dependent upon the size of the particles, flow rate and the type of tape. Using image analysis it was found that pressure-sensitive adhesive tapes were more efficient than the standard vaseline adhesives for the collection of the majority of inorganic and organic airborne particles below $36\ \mu\text{m}$ in diameter. However, the capture efficiency for larger particles and for some pollen grains, especially the smooth and ellipsoid Poaceae species and *Alternaria* - like spores, when

assessed by manual counting was below that of the standard vaseline mixture.

ACKNOWLEDGEMENTS

PhD F Benyon for her instruction on Burkard sampling, Ms N Wheatley and Mr K Searle for the use of their backyard during the sampling period, Mr S Kesteven for his work on the particle-counting program.

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