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Santiago Fernández Rodríguez ^a, Rafael Tormo Molina ^a, Inmaculada Silva Palacios ^b & Ángela Gonzalo Garijo ^c

^a Departamento de Biología Vegetal, Ecología y Ciencias de la Tierra, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Spain

^b Departamento de Física Aplicada, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Badajoz, Spain

^c Sección de Alergología, Hospital Infanta Cristina, Badajoz, Spain

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Two sampling methods for the Petri dish detection of airborne fungi

SANTIAGO FERNÁNDEZ RODRÍGUEZ¹, RAFAEL TORMO MOLINA¹,
INMACULADA SILVA PALACIOS² & ÁNGELA GONZALO GARIJO³

¹*Departamento de Biología Vegetal, Ecología y Ciencias de la Tierra, Facultad de Ciencias, Universidad de Extremadura, Badajoz, Spain,* ²*Departamento de Física Aplicada, Escuela de Ingenierías Agrarias, Universidad de Extremadura, Badajoz, Spain,* ³*Sección de Alergología, Hospital Infanta Cristina, Badajoz, Spain*

Abstract

Burkard personal samplers for Petri dishes use two air throughput devices, either with a sieve or with a funnel, for monitoring of airborne fungi concentration. These two different methods have been compared in a comparative study. The two personal traps used for comparison were run simultaneously, one equipped with a sieve and one with a funnel only. Possible correlations with rainfall, temperature, wind speed, and relative humidity were studied. The average concentrations were 74 and 339 colony forming units/m³ (a factor of 4.6 difference) for the funnel and sieve devices, respectively. More than 70 taxa of airborne fungi were identified. The four most important genera in decreasing order of concentration were *Cladosporium*, *Alternaria*, *Penicillium*, and *Aspergillus*, the last two present in similar concentration. A significant negative correlation with wind speed was detected. Statistical analysis (ANOVA) revealed significant differences between the two sampler types except for *Alternaria*. In conclusion, the sampler with a sieve provides more colonies than the same sampler with a funnel. Both kinds of samplers are affected by wind speed, with the capture of airborne fungi propagules being reduced as the speed increases.

Keywords: *Mycology, airborne fungi, viable sampling, Burkard personal sampler*

A great variety of samplers and methods have been used to monitor airborne fungi and other bio-aerosols. Since their efficiency depends on many factors, it is custom to distinguish between sampling, collection, and biological efficiencies (Nevalainen et al., 1992). With so many conditions involved, it is not easy to rigorously compare samplers. Continuous sampling of airborne fungi employs non-viable substrates in stationary spore traps, but sampling using viable media is possible using personal or portable samplers, although these can, of course, also be used for non-viable sampling. Personal samplers are usable for periods ranging from just a few minutes to a full working shift (Kenny et al., 1998). Although one might expect stationary samplers and personal samplers in the same place to give similar results (Tormo et al., 2010), there are often differences because the spectrum of fungi found with the latter is more closely associated with soil or vegetation borne mycoflora and hyphal fragments than

that collected by stationary spore traps in the same geographic region (Green et al., 2006).

The designers of personal bio-aerosol samplers have had to respond to the many different requirements of their customers – for instance, the trapping of small biological particles such as bacteria and viruses, which are mainly of interest to microbiologists, or even PM-05 dust, and the survival of these captured microorganisms (Thompson et al., 1994); sampling for phytopathological monitoring (McCartney et al., 1997); or use in extreme environments (Marshall, 1997). It is well accepted that, with such a wide variety of samplers, there is a need for standardised and recommended methods and procedures (Macher & Willeke, 1992; Henningson & Ahlberg, 1994).

The Burkard Manufacturing Co. Ltd, Rickmansworth, UK, provides two personal volumetric air samplers based on the same motor, one for non-viable airborne particles captured on an

adhesive-covered microscope slide, and another, the Burkard Personal Culture (BPC) sampler, designed to accommodate a Petri dish of 9 cm in diameter. The airflow rate allowed by these samplers is 10 litres per minute for the former, and 20 litres per minute for the latter (Jensen & Schafer, 1994). The latter is equipped with a 100-holes sieve plate and a removable conical funnel that can be mounted over the sieve.

The BPC sampler has been used extensively worldwide, for example in India (Gupta et al., 1993; Sharma et al., 2010), Saudi Arabia (Hasnain et al., 1995, 2004; Al-Suwaine et al., 1999), Canada (Comtois & Marcoux, 1999); Cuba (Aira et al., 2002), Chile (Aira et al., 2003), and Poland (Buczyńska et al., 2007, 2008). These publications, however, have not always provided methodological specifications; and the available data is incomplete (Table I). The use of the air throughput device (sieve or funnel) has only rarely been indicated (Sharma et al., 2010), or whether a correction factor was applied to the raw data (Aira et al., 2002, 2003; Sharma et al., 2010), which is usually indicated when a sieve plate is used. This factor depends significantly on the number and diameter of the sieve holes.

Studies comparing the BPC sampler with other standard samplers show it to provide reliable results (Madelin, 1994; Mehta et al., 1996; Tormo et al., 2010). Nevertheless, it is clear that using a sampler, which includes a sieve in the air flow before the Petri dish, requires some adjustments to be made ready for the statistical probability of more than one colony forming unit (CFU) impacting at each site, and there are tables of these correction factors depending on the number and diameter of holes (Andersen, 1958; Peto & Powell, 1970).

The aim of the present study was to compare the results of one year of simultaneous sampling with two BPC samplers, one using only the sieve plate and the other only the conical funnel. Sampling was approximately weekly to take the seasonal variations and meteorological factors into account that could affect the results.

Table I. Methodological specifications of a choice of recent studies using Burkard Personal Culture (BPC) samplers for Petri dishes (culture medium: MEA, malt extract agar; SDA, Sabouraud dextrose agar).

Publication	Sampling time (minutes)	Culture medium	Height above ground (cm)	Airflow rate (litres/minute)
Aira et al., 2002	5	SDA		10
Aira et al., 2003	2	MEA	100	10
Al-Suwaine et al., 1999		SDA/Czapek Dox Agar		
Buczyńska et al., 2008		MEA		
Comtois & Marcoux, 1999	3–4	SDA/MEA	Variable	15
Hasnain et al., 1995	10	SDA/Czapek Dox Agar	20	10
Hasnain et al., 2004	10	SDA/Czapek Dox Agar	20	10
Sharma et al., 2010	10	Rose Bengal Agar	100	10

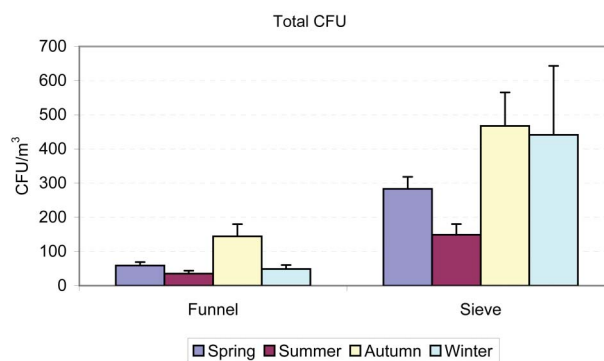


Figure 1. Seasonal total colony forming unit (CFU) concentrations using samplers with a funnel or a sieve device; bars represent standard errors.

Materials and methods

Two BPC samplers for Petri dishes prepared to accommodate a standard Petri dish of 9 cm in diameter were used – one with a funnel-shaped intake device but without a sieve, and the other with a 100-holes sieve plate but without the funnel device. Sampling was done on the terrace roof of a 16 m high building of the Faculty of Sciences, University of Extremadura, Badajoz (southwest Spain).

A total of 50 samples were taken simultaneously with both samplers between March 2009 and March 2010 at an approximately weekly frequency, at 12:00 h UTC (Coordinated Universal Time). A previous study had shown that the concentration of airborne particles is the highest at this time of the day (Tormo et al., 2010). Sabouraud agar with chloramphenicol (SDA) was used as growth medium. After 5–7 days incubation at 27 °C, the colonies on the dishes were identified and counted. Data are expressed as CFU per m³.

The raw data from the sampler with the sieve were corrected according to the equation given by Feller (1968). Meteorological data of the same day of aerobiological sampling were used to check for possible relationships with rainfall, temperature, wind speed, and relative humidity, considering only statistically

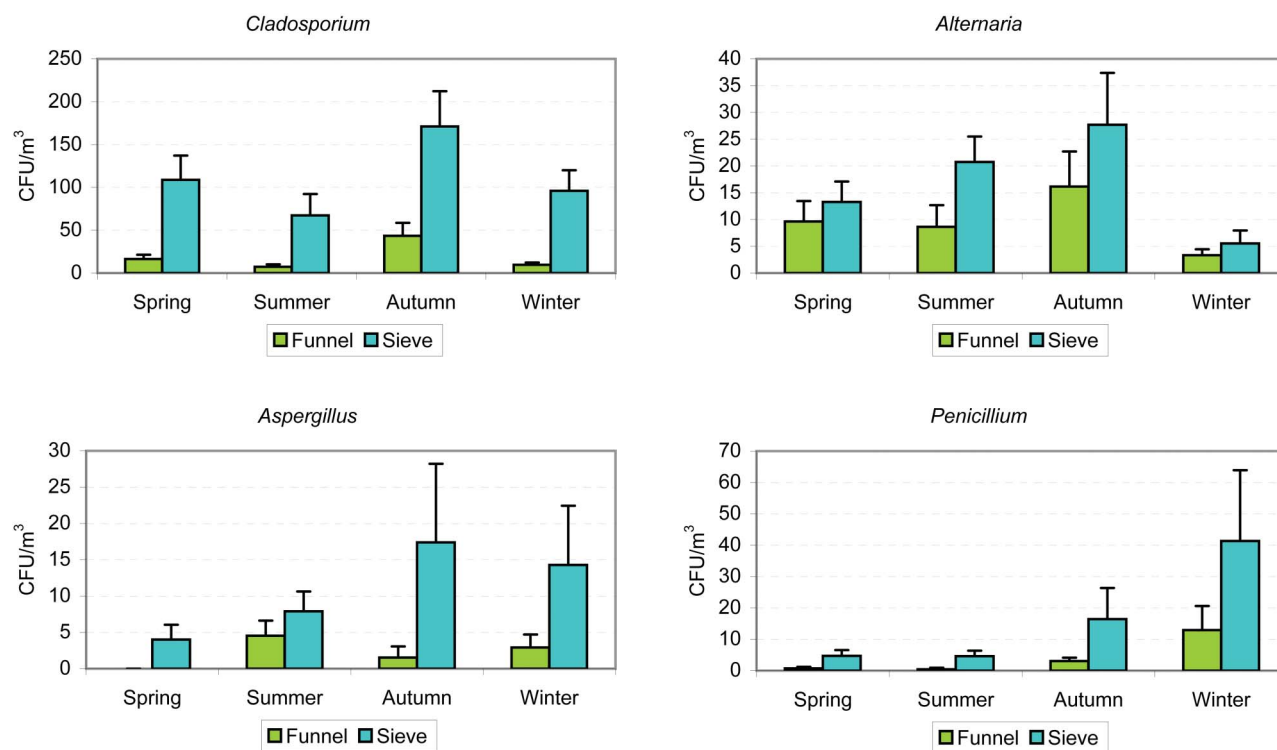


Figure 2. Seasonal colony forming unit (CFU) concentrations of *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium*, using samplers with a funnel or a sieve device; bars represent standard errors.

significant correlations at $p < 0.01$ levels. As a preliminary trial, differences in sampling using the sieve with or without the funnel device were studied by taking five 10-minute continuous samples using the two samplers simultaneously. For the statistical analyses, when the log-transformed data were normally distributed, the parametric analysis of variance (ANOVA) and Pearson correlation coefficient techniques were applied. When normalisation was not possible, the non-parametric Kruskal–Wallis test and Spearman correlation coefficient were used. Data was analysed for the total counts of the four main fungal types *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium* in each sample. Identification of fungi propagules and colonies has been realised from their morphological characteristics using some general and specific references (Ellis, 1971, 1976; Grant

Smith, 1990; Ellis & Ellis, 1997; de Hoog et al., 2001; Klich, 2002; Larone, 2002).

Results

The preliminary comparison of two samplers, both equipped with a sieve, but one with and the other without the funnel device showed no significant differences – the data were normally distributed, and the value of the t -test was 0.220 with a p -value of 0.837 (Table II).

In comparison of the sieve and the funnel samplers, a total of 2759 CFU were counted, the average concentration for the sampler with the funnel device was 74 CFU/m³ and for the sampler with the sieve 339 CFU/m³. In both cases, autumn was the season with the highest average concentrations and summer the season with the lowest. With the funnel-equipped sampler, there were hardly any differences between spring and winter, but there were by far greater concentrations in winter with the sieve-equipped sampler (Figure 1).

The log-transformed total concentration data were normally distributed, but the data for the main spore types (*Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium*) were not. Seasonal comparisons using the parametric ANOVA for the total data showed differences for both the funnel device ($F = 8.195$,

Table II. Results of sampling with two Burkard Personal Culture (BPC) samplers for Petri dishes, both with a sieve, one with and the other without the funnel device, values are given in absolute colonies counted.

	With funnel device		Without funnel device	
	Mean	Standard error	Mean	Standard error
Total	75.0	7.2	73.8	8.9
<i>Cladosporium</i>	20.8	3.3	26.6	6.3
<i>Aspergillus</i>	16.2	6.0	14.8	6.0

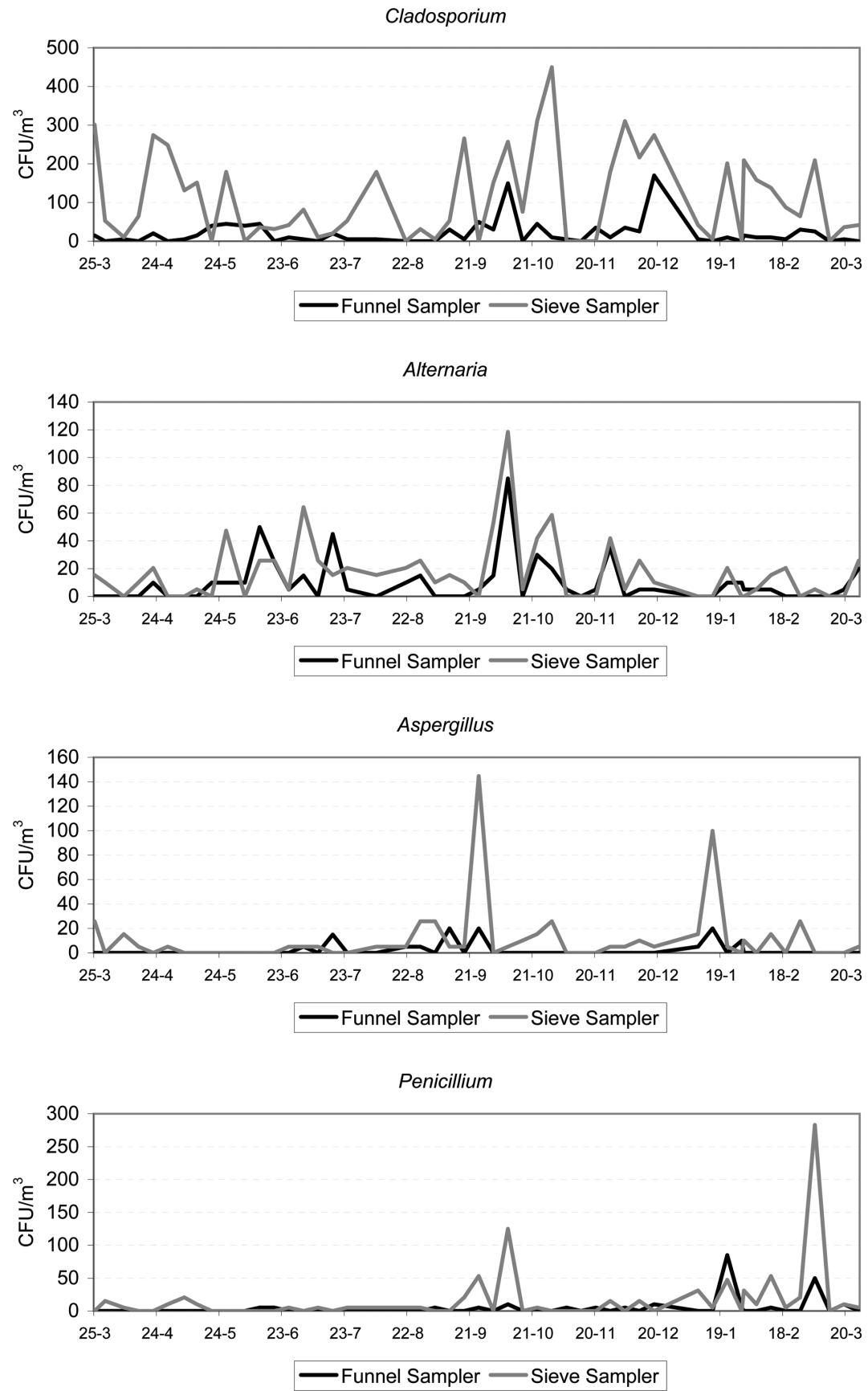


Figure 3. Daily colony forming unit (CFU) concentrations of *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium* as given by the two samplers.

$p < 0.001$) and the sieve device ($F = 3.339$, $p = 0.027$). The corresponding seasonal comparisons using the non-parametric Kruskal–Wallis test for the four separate fungus types studied showed significant differences for all except *Cladosporium* with each of the two samplers (Figure 2).

For the daily data, there were significant positive correlations between the two samplers for the total concentrations (Pearson $r = 0.487$, $p < 0.001$) and the concentrations of *Alternaria* (Spearman $r = 0.556$, $p < 0.001$) and *Cladosporium* (Spearman $r = 0.353$, $p = 0.012$), but not for *Aspergillus* or *Penicillium* (Figure 3).

The analysis of correlations with the meteorological data showed only wind speed to be significantly negatively correlated with the total CFU concentration for both samplers, while relative humidity was positively correlated with the total CFU concentration for the sieve sampler only. None of the four fungus types presented correlations separately with any meteorological parameter. The daily total count and *Alternaria* data were positively correlated for both samplers.

Discussion

The Burkard Company's specifications for their BPC (Petri dish) samplers give an air throughput of either 10 or 20 litres per minute. The specification for their personal samplers for slides, with the same motor, gives a nominal air throughput of 10 litres per minute. This could be the reason why many of the published studies we consulted indicate a flow rate of 10 litres per minute, even though the real, flow rate is 20 litres per minute (Jensen & Schafer, 1994). Even after using flow meters acquired from the company, we were unable to completely clarify the problem because the measurements with them ranged fairly widely to a degree up to about 20 litres per minute.

While the BPC sampler seems capable of revealing the seasonal variations in CFU concentration, the actual values of the concentration depend greatly on which device, sieve plate or conical funnel, is applied to the air throughput. As no differences were found whether or not the funnel device was used together with the sieve, the aim of the yearlong trial was to test the relative effectiveness of using the sieve over the funnel. The results clearly showed that using a sieve notably increased the number of detected CFU, in particular, by a factor of more than four.

Other samplers using a sieve to distribute the air-flow over the Petri dish have sieves with more holes:

394 holes in the Samplair AES (Nesa et al., 2001), 400 holes in the Andersen (1958) sampler, 401 holes in the SAS Sampler (Lach, 1985), and 657 holes in the Bio Samp MBS-100 Midore Azen (Shintani et al., 2004). Sieve impaction is based on the aspiration of air through small holes at the top of the sampler. This is a proven technology and the basis of the well-known Anderson principle of air sampling. The impaction speed and the efficiency are functions of the diameter of the holes in the perforated plate and of the speed of the fan.

Application of the corresponding correction factor (Andersen, 1958; Peto & Powell, 1970) increases the total CFU number to be reported exponentially with the number of holes. In particular, if 50 CFU have been counted with a 100-holes sieve then 70 CFU should be reported, and if 90 CFU have been counted then the reported figure should be 235, accordingly. If the number of CFU is equal to the number of holes, however, the correction means reporting an infinite count. In such a case, one needs to reduce the sampling time, although this implies a concomitant reduction in sampling accuracy.

The overall sampling efficiency of an aerosol sampler will clearly be sensitive to wind speed (Chung et al., 1987; Kalatoor et al., 1995) and, indeed, our results confirmed a reduction of the detected CFU with increasing wind speed, whatever device is used in the sampler. This means that an air intake flow rate of 20 litres per minute is perhaps not high enough to avoid air turbulence affecting the sampling. While that flow rate may be suitable for indoor contexts, it would outdoors allow the wind to clearly affect the results.

Conclusion

Sampling with the Burkard personal sample for Petri dishes is most efficient if the sieve plate is used. The efficiency is not increased with the addition of the funnel device, and it is lowest using the funnel device on its own. The measured outdoor fungi concentrations recorded with the sampler occasionally reached more than 2500 CFU/m³, but on average they were around 340 CFU/m³, apparently being highest in autumn and lowest in summer. The highest concentrations of *Cladosporium* and *Alternaria* were recorded in autumn and the lowest in summer and winter, while *Aspergillus* and *Penicillium* occurred in highest concentrations in winter and lowest in spring and summer. Wind speed affected this outdoor sampling with the BPC samplers for Petri dishes, clearly decreasing the measured values of the concentrations.

Acknowledgements

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