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# Comparison of Counting Methods for the Study of Air-borne Pollen with Special Reference to *Broussonetia* Pollen

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**ABSTRACT:** This study compares the accuracy of various pollen counting methods in order to find an optimum sample size for the study of air-borne pollen obtained from Burkard seven-day volumetric recording trap in Taiwan. Pollen species counts and estimates of pollen quantity obtained using various sample sizes are compared. The coefficients of variance of various sample sizes and diurnal variations in pollen concentration are also analyzed. The results show that a minimum sampling of one transverse traverse per hour or every two hours from trapping tape is suitable for the estimation of the total pollen quantity collected in a day and for the study of diurnal variations in pollen concentration at a collecting station.

**KEY WORDS: Air-borne pollen, Counting method, Pollen concentration.**

## INTRODUCTION

In flowering seasons, large amounts of anemophilous pollen grains are released and suspended in the air. Inhalation of some of such air-borne pollen can induce allergic reactions in the respiratory systems of some humans (Banik and Chanda, 1992; Subiza *et al.*, 1991). The pollen calendar thus provides important information for those who are allergic to pollen. In Northern Europe, expected concentrations of air-borne pollen (by species) are forecasted everyday to remind those with allergies to be careful (Spieksma, 1980). In Taiwan, some species of air-borne pollen, such as Bermuda grass (*Cynodon dactylon*) (Han *et al.*, 1993; Su *et al.*, 1986) and rice (*Oryza sativa*) (Tsai *et al.*, 1990), have been proven to be allergenic via a series of tests and studies (which included skin, immunoblot, and radioallergosorbent testing). Therefore, it is becoming increasingly necessary for the people of Taiwan to gain a good understanding of the local pollen calendar.

For the purposes of research, samples of air-borne pollen are usually collected by a trapping instrument. In many countries, the Burkard seven-day volumetric recording trap is used most frequently (Hasnain *et al.*, 1995; Ong *et al.*, 1995; Lacey and McCartney, 1994; Pessi and Pulkkinen, 1994) because of its high efficiency of collection. However, in the various aeropalynological studies carried out in Taiwan over the last thirty years (Chao *et al.*, 1962; Chen, 1984; Chen and Chien, 1986; Chen *et al.*, 1972; Chen and Huang, 1980; Huang and Chung, 1973; Tsou and Huang, 1982; Wang, 1973), this instrument has never been used.

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Before using this instrument for the study of air-borne pollen in Taiwan, optimum methods should be established. In addition, the observation and identification of the pollen grains contained on a trapped tape is laborious and time consuming. It is impor-tant to find an appropriate counting method, especially when the samples collected are large.

There are three counting methods commonly used to analyze samples of air-borne pollen which have been collected via a Burkard seven-day volumetric recording trap (Käpylä and Penttine, 1981). The first, a systematic sampling method, uses lengthwise traverses as sampling units. It is generally used by aeropalynologists in the USA and Sweden (Käpylä and Penttine, 1981). The second one, also a systematic sampling method, uses transverse traverses as sampling units. It is commonly used by European and British aeropalynologists (Emberlin *et al.*, 1994). The third one, generally used by Finnish aeropalynologists (Pessi and Pulkkinen, 1994), is a random sampling method which uses microscope fields as observation units.

During the collection of air-borne pollen by a Burkard seven-day volumetric recording trap, pollen grains are not evenly distributed on the trapping tape (Käpylä and Penttine, 1981). The pollen grains are typically concentrated more in the center of the trapping tape than on the margins. For this reason, the counting method used is a critical component of the quantitative as well as the qualitative study of the collected samples. Different results are obtained from using each of the above-mentioned three counting methods. In general, the transverse traverse counting method is the most suitable, since it results in the lowest margin of error (Käpylä and Penttine, 1981). Therefore the transverse traverse counting method is employed in this study to determine the suitable (or minimum) number of traverses which should be used in quantitative and qualitative studies of air-borne pollen.

## MATERIALS AND METHODS

A Burkard seven-day volumetric recording trap was used to collect air-borne pollen on March 24 and April 8, 1993. The pollen was collected as follows: Melinex tape coated with Gelvotal solution (a mixture of 35 g Gelvotal, 100 ml distilled water, 50 ml glycerol and 2 g phenol) was placed on a rotating drum which rotated the tape past a 2 mm x 14 mm orifice (at a rate of 48 mm per 24 hours) to expose it to the air. After being exposed, the tape was cut into 48 mm sections. The sections were mounted on slides by Entellan (Merck, Germany) for microscopic observation. Pollen species identification were observed under a Leitz Diaplan microscope with ocular lens 12.5x and objective lens 40x. The pollen grains were counted using the transverse traverse method. A 48 mm section of the exposed tape can be divided into 96 traverses (four traverses correspond to a 1-hour sample). In order to determine the minimum number of transverse traverses which should be used for the quantitative and qualitative study of air-borne pollen, samples of 1 (interval = 24 hours), 2 (interval = 12 hours), 4 (interval = 6 hours), 6 (interval = 4 hours), 8 (interval = 3 hours), 12 (interval = 2 hours), 24 (interval = 1 hour), 48 (interval = 30 minutes), 72 (interval = 20 minutes) and 96 traverses per day were used. The percentage difference between these species counts obtained via the various sample sizes and the total pollen species actually counted in the 96 transverse traverses was calculated.

For quantitative analysis, only *Broussonetia* pollen grains were counted, since this genus was most numerous during the collecting period. The pollen contained in all 96 transverse traverses was first counted. Next, the pollen grains contained in the various sample sizes mentioned above were counted, then corrected by a factor to obtain an estimate of the pollen contained in all 96 transverse traverses (sample size x factor = estimated quantity of pollen grains in 96 transverse traverses). The percentage difference between these estimated pollen quantities and the pollen quantity actually counted in the 96 transverse traverses was then calculated.

The coefficients of variance for sample sizes of 4, 6, 8, 12, and 24 transverse traverses per day were calculated not only to determine the optimum sample size (the lower the magnitude of the coefficient, the better) but also to indicate the evenness of pollen distribution on the trapping tape. The method for finding the coefficient of variance for a sample size of 24 traverses is used as an example: The pollen grains in the first of four transverse traverses in each hour were counted , summed and then multiplied by a factor to attain an estimate of the total count. The same was done with the second, the third and fourth traverses from each hour. The data obtained were then compared to obtain the coefficient of variance (c. v. = $\sigma/\mu$; $\sigma$ = standard deviation, $\mu$ = population average).

The diurnal variation of *Broussonetia* pollen grains was analyzed using the March 24 tape only. The changes in pollen concentration which occurred over the course of the day were tracked using sample sizes of 96, 24, 12 and 8. The minimum sample size which could be used to accurately track the variation in pollen concentration throughout the day was determined.

## RESULTS AND DISCUSSION

The results of the qualitative analysis of the tape were as follows: The number of pollen species observed increased as the size of the sample increased. The number of species counted in all 96 transverse traverses was regarded as the maximum species count, or 100 %. When 12 or more traverses were observed, the number of species observed was greater than 90 % of actual count in both the March 24 and April 8 samples (Fig. 1). This suggests that for the qualitative study of air-borne pollen, a minimum sample of 12 transverse traverses per day should be used.

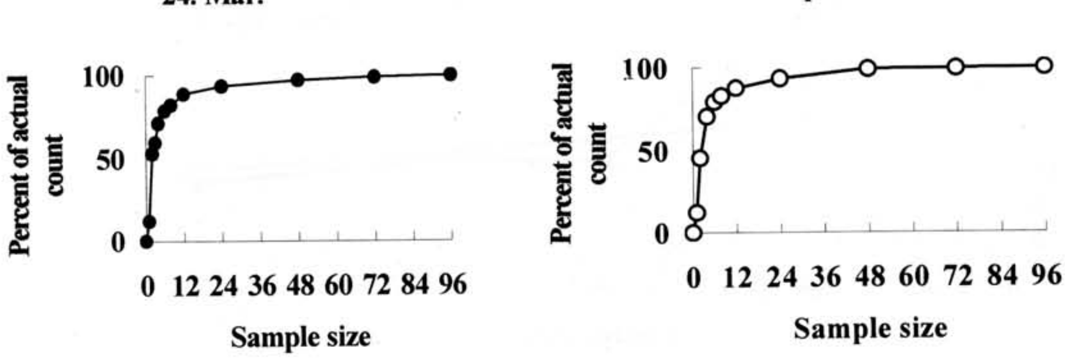


Fig. 1. Pollen Species counts obtained via the various sample sizes as a percentage of the actual pollen species count.

The amount of total pollen grains trapped within a day can be obtained either by counting the pollen grains contained in all 96 transverse traverses (= actual count) or by using a representative sample of traverses to obtain an estimate of the actual count. The estimated count may be different from actual count, and the difference between the two increases as the size of the sample decreases. Fig. 2 shows that the estimated pollen count was close to the actual count (>97 %) when the sample size was greater than 24 for the March 24 tape, and when the sample size was greater than 6 for the April 8 tape. This suggests that a sample size of 24 transverse traverses is most suitable for the estimation of the daily pollen count.

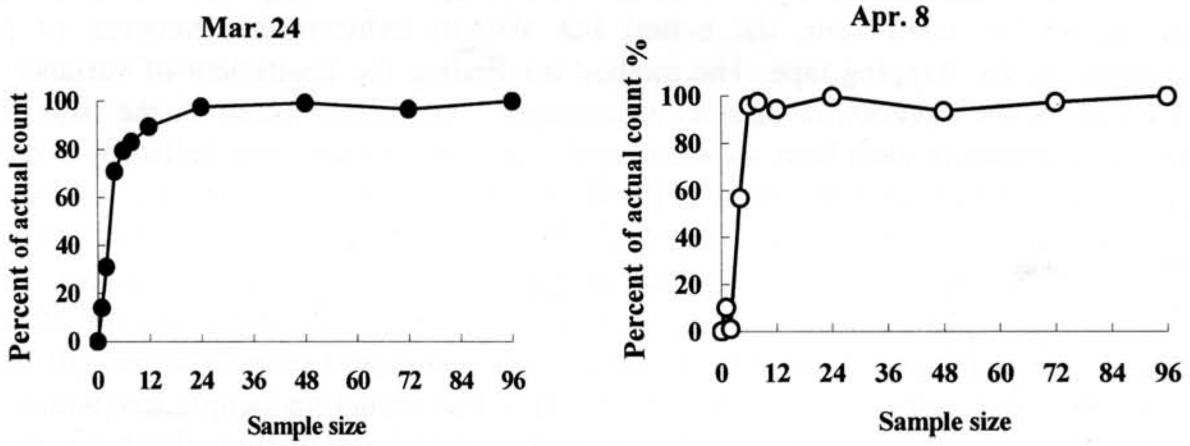


Fig. 2. Pollen grain estimates obtained via the various sample sizes as a percentage of the actual pollen grain count.

The difference between samples was analyzed using the coefficient of variance. It is shown in Fig. 3 that the values of the coefficient of variance decrease, when the sampling sizes increase. The magnitude of change in coefficient values was insignificant for sample sizes larger than 8. The coefficient of variance obtained from sampling one transverse traverse in each hour (*i.e.* sample size = 24) was 8% on March 24 and 10% on April 8. These results suggest that pollen is fairly evenly distributed on the tape over the interval of one hour.

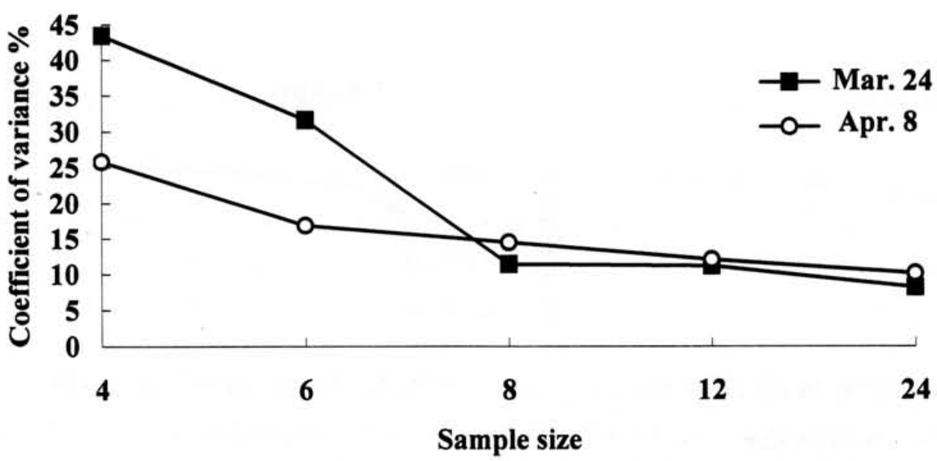


Fig. 3. Coefficients of variance for various sample sizes.

*Broussonetia* was the predominant species of pollen grains encountered during the two sampling periods (March 24 and April 8). In order to determine a suitable time interval between samplings, the fluctuation in the amount of *Broussonetia* pollen grains throughout the day (on March 24 only) was tracked using sample sizes of 8 (interval = 3 hours), 12 (interval = 2 hours), 24 (interval = 1 hour) and 96 traverses. The results are shown in Figure 4. On March 24, there were two main peaks in the concentration of *Broussonetia* pollen grains. Sample sizes of 8 and 12 indicate the occurrence of the two main peaks, but do not show the fluctuation in pollen concentration in detail. A sample size of 24 shows the fluctuations in more detail. This suggests that for the study of the diurnal variation of air-borne pollen a sample size of 24 traverses per day (interval = 1 hour) is more suitable than a sample size of 8 or 12 traverses per day.

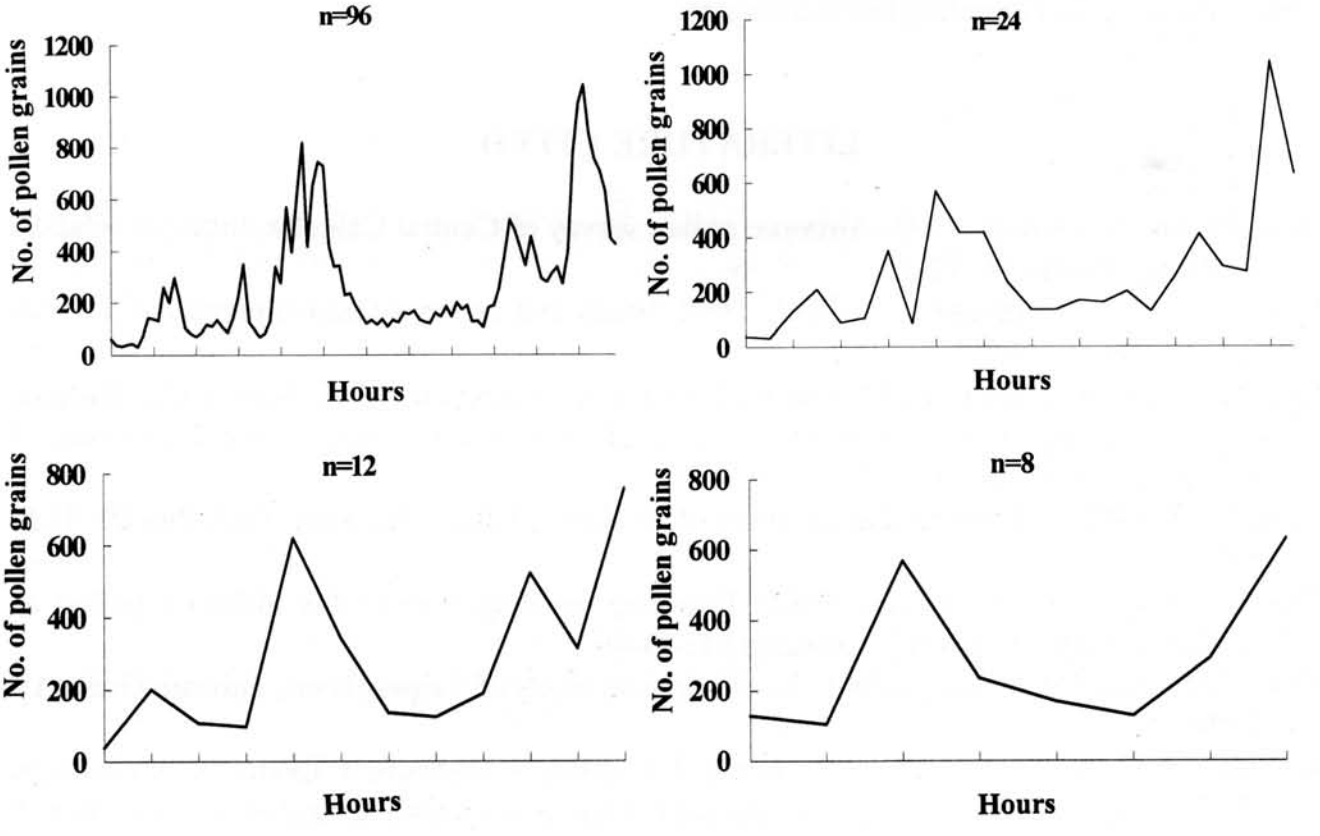


Fig. 4. Diurnal variations in the concentration of *Broussonetia* pollen tracked via sample sizes(n) of 8, 12, 24 and 96.

Various sample sizes have been used previously by those attempting to estimate the daily concentrations of air-borne pollen. These include: a sample size of one transverse traverse for every hour (Hart *et al.*, 1994; Johansen, 1992; Tilak, 1989), one for every two hours (Ong *et al.*, 1995; Emberlin *et al.*, 1994; Johansen, 1992; Lewis *et al.*, 1991) or three for every two hours (Norris-Hill and Emberlin, 1991). Unfortunately no statistical analysis of various sample sizes were undertaken in the above-mentioned investigations. In the present investigation, the effect of sample size on the accuracy of pollen species estimates (Fig. 1) and pollen quantity estimates (Fig. 2) was analyzed for the first time. Only Käpylä *et* *al.* (1981) has estimated the coefficients of variance and diurnal variation, which have also been analyzed in the present study.

The results of the present study indicate that the estimates obtained using a sample size of one transverse traverse per hour and those obtained using a sample size of one transverse traverse every two hours were nearly the same. This indicates that either sample size is good enough for the estimation of daily air-borne pollen concentrations.

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# 空中孢粉計數方法之比較

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## 摘　　要

本文採用英製 Burkard 孢粉收集器收集空中孢粉，比較不同的孢粉計數方法，分析項目包括：孢粉種類數量、孢粉粒預估數量、變異係數和一日間孢粉數量變化曲線，以求得具有代表性的最少觀察面積。結果顯示，在收集有空中孢粉的透明膠帶上，每一小時或每二小時的取樣時間內，以光學顯微鏡物鏡40倍的視野，依玻片短軸的方向觀察並鑑定一行的空中孢粉，可得到良好的評估效果。

關鍵詞：空中花粉，計數方法，花粉密度。

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