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Reliability of measures of spores of *Alternaria* and pollen concentrations in air over two towns in rural Australia

Multiple sites for Burkard sampling

TERESA Z. MITAKAKIS and PETER A. MCGEE

Mitakakis, TZ. & McGee, PA. 2000. Reliability of measures of spores of *Alternaria* and pollen concentrations in air over two towns in rural Australia. Multiple sites for Burkard sampling. – Grana 39: 141–145. ISSN 0017-3134.

We examined the reliability of measurements from a single Burkard volumetric trap to represent the distribution of airborne concentrations of spores of *Alternaria* and pollen across two towns in rural Australia. Each town was sampled with three traps, sited 2.0 to 4.9 km apart, simultaneously. Substantial intra-class correlation coefficients (ICC) were observed between all three sites (ICC = 0.52, 95% CI 0.30–0.71 to 0.76, 95% CI 0.61–0.87) when counts of pollen and *Alternaria* spores were relatively high. The correlation was poor when counts were low. Highly variable distributions of cypress pollen were found to be location dependent.

We further compared two central lengthwise microscopic traverses of Burkard trap samples collected daily over one year. Correlation was strong for *Alternaria* spores (ICC = 0.95, 95% CI 0.94–0.96), grass pollen (ICC = 0.94, 95% CI 0.91–0.96) and total non-grass pollen (ICC = 0.91, 95% CI 0.89–0.93). We conclude that a single central traverse of a Burkard trap sample collected at one location provides an acceptable measure of the concentrations of spores of *Alternaria* and grass pollen across the two rural towns when counts are relatively high. The measure is less reliable when counts are low.

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INTRODUCTION

In aerobiological research, data on pollen and fungal spore concentrations are commonly collected from a single site and it is assumed that the collection represents a much larger region. However, the distribution of particles borne in ambient air may vary over short distances. Concentrations of fungal spores and pollen are dependent on the distance from the major sources, prevailing wind directions and local topography. If a major source of particles is close to the sampling trap, concentrations of particles are likely to be higher than if the trap was distant. If prevailing winds are predominantly unidirectional, particles will spread in a concentrated pattern from the source. Particle release coinciding with unidirectional wind may result in an uneven spread of particles across a town. Local topography may exacerbate any effect of wind direction. Ridges or valleys may cause particles to by-pass or concentrate at a single trap. Counts of pollen and spores from a single trap may not reflect the pattern across an entire town, especially when counts, on average, are low.

This preliminary investigation aimed to determine the reliability of data from a single sampler to estimate concentrations of pollen and fungal spores in the air over two rural towns in New South Wales, Australia. We chose to focus on spores of the fungus *Alternaria*, and grass and cypress pollen, because of their importance in asthma in

rural New South Wales (Peat et al. 1995, Bass et al. 1991). We anticipated that sources of spores of *Alternaria* and grass pollen would be predominantly from grasslands and crops surrounding the towns. Possible sources of cypress pollen were individual trees in gardens within the towns, and large populations of the native White cypress pine, *Callitris glaucophylla*, in native bushland distant to the towns.

In addition, we examined the reliability of using a single traverse count to assess tape samples from a Burkard trap. Other studies have applied a wide array of methods such as 'hourly' microscopic counts along width traverses (Hjelmroos 1993), or 1 to 4 lengthwise traverses (Rutherford et al. 1997, Palmas & Cosentino 1990, Munuera Giner, 1995). To produce a reliable count, Kapyła & Penttinen (1981) recommended twelve transverse traverses, and Molina, et al. (1996) two central longitudinal traverses. These approaches are impractical for extensive studies of a range of particles. The reliability of the single longitudinal traverse counting method can be tested by comparing two different traverses along the slide.

METHODS AND MATERIALS

Sampling locations

The towns in rural New South Wales, Australia, were chosen for examination because of high prevalences of atopy to allergens of

Alternaria have been recorded in resident children (Peat et al. 1995). The town of Moree (population 10,000) is positioned at a latitude of 29°S, and 293 km inland. Moree is exceptionally flat. The second town, Wagga Wagga (population 50,000) is positioned 550 km south at 35°S, 267 km inland. Wagga Wagga has a low ridge extending north-east to south-west on the eastern side of the town. The housing of these towns is mostly single story. Both towns are surrounded by crop and pasture production and have a temperate climate.

Placement of volumetric traps

Following Australian standards (AS 2922-1987 Ambient air -guide for the siting of sampling units, Standards Australia), siting a trap required a multistorey building having a flat, open and accessible roof, approximately 15 metres above ground. Sampling sites in both towns were restricted primarily by the lack of multistorey buildings, and by the requirement for such buildings to be adequately distanced to represent the area of the town. All sites were above house-roof level, distanced to represent the distribution of the residential area of the town, and positioned on the highest building meeting all other criteria.

Moree is approximately 4 km in diameter. One Burkard trap was placed centrally in the town, 11 m above ground on the second storey roof of the Moree Community hospital. The second site was 1 km north on the second storey roof of the local radio station, 8 m above ground, and the third site was 2.2 km south-southwest on the roof of the local Bureau of Meteorology Station, 5.5 m above ground. The radio and meteorological stations are located 3.1 km apart. All sampling sites were within the residential area.

Wagga Wagga is approximately 6 km in diameter. One Burkard trap was placed centrally in the town, 12.5 m above ground on the third storey roof of the Wagga Wagga Base hospital. The second Burkard trap was sited 2 km north of the hospital on the roof of the town's cinema complex 18 m above ground, and the third 3 km southwest of the hospital on the roof of a squash centre, 6 m above ground. The cinema and squash centre are located 4.9 km apart. All sampling sites were located on the western side of the ridge.

Sample collection and analysis

Three 7-day Burkard volumetric traps (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, U.K.), were set up simultaneously. In Moree, air particles were sampled between 20th October and 16th of November, 1998 (27 days), and Wagga Wagga between 16th July to 13th August, 1998 (28 days). In Wagga Wagga, particles in the air were not sampled on the 6th of August due to exceptionally wet weather, preventing change of the tapes for one day. The dates chosen for sampling were based on timing of concurrent research.

Spores and pollen were trapped onto adhesive tape (Mylcote 111, Dow Corning, on melinex tape), which was subsequently cut into segments relating to 24 hour periods. The segments were mounted onto slides, stained with Calberla's stain, then viewed by light microscopy at a

magnification of 250X for spores of *Alternaria*, grass pollen, cypress pollen and total pollen, along a full lengthwise traverse. Spores and pollen were expressed as number per cubic metre of air.

Reliability of counts from a single lengthwise traverse was calculated by counting two traverses of 365 slides collected at the hospital site, Wagga Wagga, between 25th June, 1997 to 24th June, 1998. Traverses were counted along the midline of the tape being approximately 6 mm from one side of the particle deposition, and 3 mm from the midline, toward one side of the tape.

Statistical analysis

Intraclass correlation coefficients (ICC) were calculated using square root transformed data between sites (Bland & Altman 1996), and between the two traverse counts of slides (Deyo et al. 1991). For traverse counts, *Alternaria* and non-grass pollens were calculated for the entire period, whilst grass pollen data were examined for the season when grasses were flowering, September to December, to remove the bias of paired zero values.

ICC represents the proportion of all variation (between days and between sites on a given day) that is attributable to variation between days. The more reliable the measurement on a given day, that is the less variation between sites on a day, the higher the value of ICC. Those values whose 95% confidence interval excludes zero are significantly different from zero at $p=0.05$.

RESULTS

Comparison of samples across town

Moree

Daily counts reached a maximum of 917 *Alternaria* spores/m³, 260 grass pollen grains/m³, 422 cypress pollen grains/m³ and 563 total pollen grains/m³ (Fig. 1). Counts of all particles sampled at the meteorology site were consistently lower than the other sites, yet the pattern of distribution of counts was similar. Data from the three sites for all particles were strongly correlated (Table I).

Wagga Wagga

Daily counts of spores of *Alternaria* at the three sites were less than 17 spores/m³, and 6 grass pollen grains/m³ (Fig. 2). Cypress pollen reached a maximum of 423 grains/m³ and total pollen reached 596 grains/m³. Counts were highly variable between the three sites, and were poorly to moderately correlated (Table I).

Slide traverses

Daily counts of the two traverses examined for *Alternaria* spores and grass pollen were similar (0.6% and 0.7% annual difference respectively between the traverses), while for total pollen the annual count was 17% higher in the side traverse. The average difference between daily *Alternaria* counts was 0.36 spores. The intra-class correlation coefficients for the

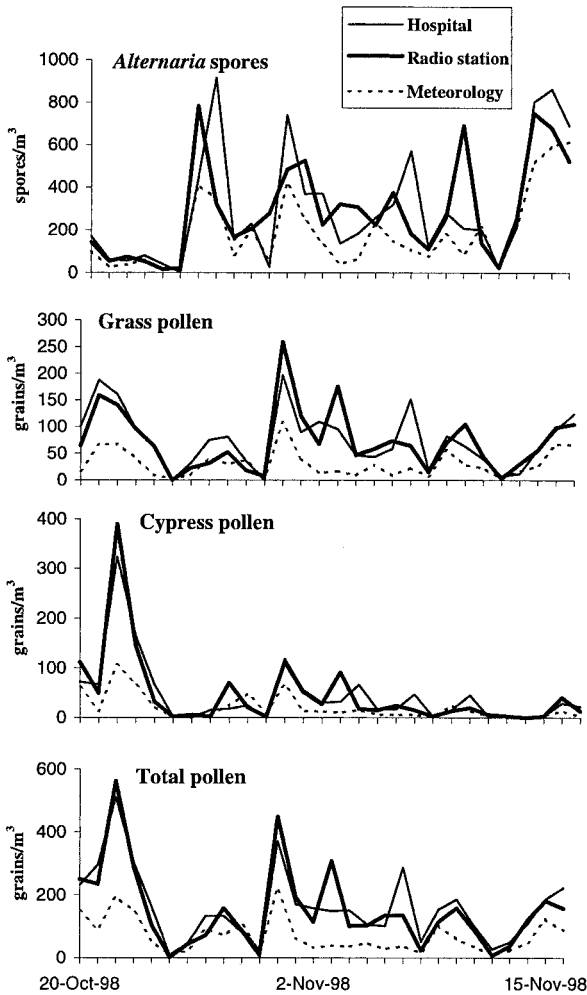


Fig. 1. Spore and pollen counts for the three sites in Moree.

two traverse counts were strong; ICC=0.95, 95% CI 0.94–0.96 for *Alternaria* (Fig. 3), ICC=0.94, 95% CI 0.91–0.96 for grass pollen and ICC=0.92, 95% CI 0.89–0.93 for total non-grass pollen.

DISCUSSION

This study has raised a number of issues regarding the reliability of air sampling at a single site to represent particle distribution across a region. Reliability was found to be dependent on concentration of particle type, season and location under investigation.

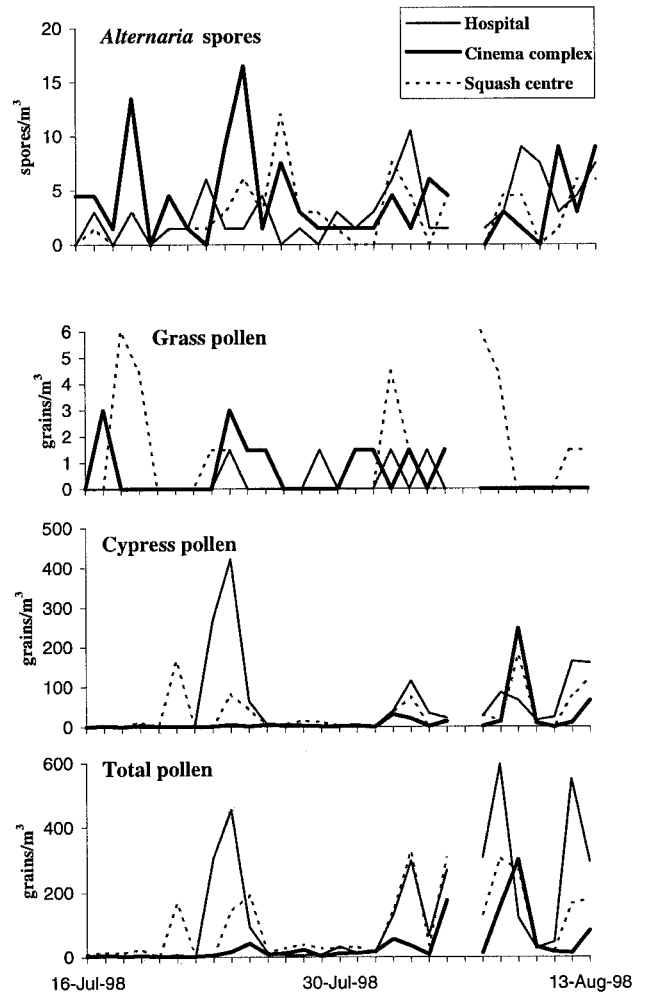


Fig. 2. Spore and pollen counts for the three sites in Wagga Wagga.

When sampling spores of *Alternaria* and grass pollen, a reliable estimate of airborne levels across the town was gained from a single sampler when the particle concentrations were high, as shown by the data collected across Moree. The poor correlations observed at Wagga Wagga for grass pollen and *Alternaria* spores may be attributed to the low counts detected. While variable, all counts were relatively low indicating a 'low season' for those particles, a point of importance to clinicians and patients.

The pattern of distribution of cypress pollen differed at the two towns. The consistency of the cypress pollen counts

Table I. Intra-class correlation coefficient (ICC) and 95% Confidence Interval values comparing pollen and spore counts (square root transformed) at both Moree and Wagga Wagga

Particulate	Moree		Wagga Wagga	
	ICC value	95% CI	ICC value	95% CI
<i>Alternaria</i> spores	0.74	0.58 to 0.86	0.24	0.01 to 0.49
Grass pollen	0.59	0.38 to 0.76	−0.07	−0.23 to 0.17
Cypress pollen	0.76	0.61 to 0.87	0.41	0.17 to 0.63
Total pollen	0.63	0.44 to 0.79	0.52	0.30 to 0.71

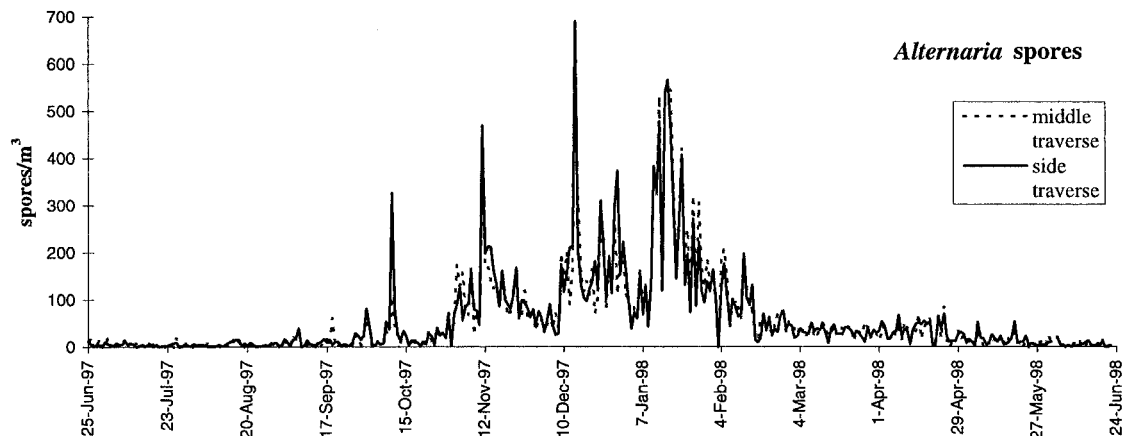


Fig. 3. Two repeat counts of *Alternaria* spores along different traverses of the slides, of air samples collected at the Wagga Wagga Base hospital site.

across Moree suggests that the pollen was well mixed in the ambient air and therefore unlikely to be influenced by localised sources of pollen, sampler height or topography. In Wagga Wagga, the distribution of cypress pollen was highly variable over short distances, despite the range of counts being similar to those in Moree. This variability may be attributed to a number of factors. Localised sources may have influenced counts at the different sites. Wagga Wagga has a greater number of ornamental cypress plants in the residential area than Moree. It is possible that a number of trees were shedding pollen close to the hospital site on the 23rd and 24th of July. The height that the samplers were placed may have additionally influenced the counts detected. Spore and pollen counts decrease with increasing height from ground (Hart et al. 1994) and hence we would expect counts to increase in order of the cinema complex, hospital then squash centre. Pollen counts detected at the cinema complex reflected the height difference, however those at the hospital and squash centre did not. Additionally, while topography of the area may have contributed toward the variability of counts of cypress pollen, the effect is unlikely to be substantial because all sites were on the same side of the town's ridge and had similar topography. In summary, a single sampling site did not accurately represent the distribution of cypress pollen over a residential area, and thus cypress pollen counts from a single site must be viewed with caution. Total pollen in Wagga Wagga must also be viewed with caution as the counts were largely composed of cypress pollen, and to a lesser extent grass pollen.

Other studies have correlated data from different samplers. Substantial correlation were found in pollen when sites were 5.6 km apart (Frenz et al. 1997). Correlations were substantial when counts of grass and total pollen were high (Ong et al. 1995) in traps 8 and 14 km apart. These studies support our suggestion that when a source is distant and particle counts are high, a single trap may represent a reliable measure of particles for a small region.

The reliability of a single traverse as an indication of particle distribution on tapes from Burkard traps has been investigated previously. It has been suggested that twelve transverse traverses (Kapyla & Penttinen 1981), or two

central longitudinal traverses (Molina et al. 1996) are needed to produce a reliable count. In our investigation, the deposition of spores and pollen along two central longitudinal traverses were highly correlated and thus for our conditions a single traverse would be a reliable method to estimate particle deposition.

CONCLUSIONS

Assessment of the sampling procedure is important for clarifying the peak seasons for allergens associated with *Alternaria* spores and pollen grains of grasses and cypresses. During low concentrations of spores and pollen grains, several sampling sites may be necessary to understand the distribution of particles within a town if this information is needed, while only one site is required when the particle count is relatively high. Cypress pollen counts from a single site may not be representative of a larger area, and may require further investigation of the distribution of sources.

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