Grana 40 (4–5): 230–239, 2001

The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia

TERESA Z. MITAKAKIS, ALAN CLIFT and PETER A. McGEE

Mitakakis, T. Z., Clift, A. & McGee, P. A. 2001. The effect of local cropping activities and weather on the airborne concentration of allergenic *Alternaria* spores in rural Australia. – Grana 40 (4–5): 230–239. ISSN 0017-3134.

Atopy to the fungus *Alternaria* is strongly associated with respiratory disease. The prevalences of asthma and of allergy to *Alternaria* are high amongst children living in rural towns of south-eastern Australia. In such towns, airborne allergenic spores have been proposed to arise from nearby crops, but this has not been tested and crops are unlikely to be the only sources of *Alternaria*. We sought to identify sources and factors that influence concentrations of spores of *Alternaria* detected in rural towns. Over two years, we sampled spores in two towns (Wagga Wagga and Moree, New South Wales, Australia), in nearby wheat and cotton crops during harvesting and control periods, in a cotton gin and a grain shed. *Alternaria* was present in both towns throughout the study, and above the crops, at the gin and grain shed. Daily and annual concentrations were amongst the highest recorded worldwide and peaks persisted for six months in Wagga Wagga and ten months in Moree. Crop maturation affected the spore load in the air more than the actual days of harvest. Regression analysis showed that the overall spore concentrations above towns correlated with those above crops. Variables of rainfall and maximum temperature correlated with concentrations in both towns, and additionally wind direction in Wagga Wagga. In conclusion, crops and produce handling released spores into the air that reached nearby rural towns, with peaks in spore concentrations following warm temperatures and recent rainfall.

Teresa Z. Mitakakis, School of Biological Sciences A12, University of Sydney; Alan Clift, Dept. of Crop Sciences, University of Sydney; Peter A. McGee, School of Biological Sciences A12, University of Sydney, Sydney, NSW 2006, Australia.
E-mail: teresam@med.usyd.edu.au

(*Manuscript received 13 February 2001; accepted 6 July 2001*)

Atopy to allergens of the fungus *Alternaria* is strongly associated with allergic disease, particularly with asthma (Halonen et al. 1997). In inland, rural towns of New South Wales (NSW), Australia, approximately 1 in 10 children have asthma and 1 in 7 children are allergic to the fungus *Alternaria* (Peat et al. 1995). The management of asthma in inland NSW may be more effective if the biology of *Alternaria* were better understood.

Alternaria is found widespread in plants, colonising all stages of plant growth (Farr et al. 1989). Geographically, the fungus is distributed worldwide but is particularly prevalent in dry climates, such as in inland NSW. In rural areas, the allergenic conidial spores of the fungus are thought to be released from crops. Respiratory symptoms have been recorded to coincide with ripening and harvest of cereals (Harries et al. 1985, Hensley et al. 1988) and with increased airborne concentrations of *Alternaria* spores (O'Hollaren et al. 1991). Crop harvests have also been reported to coincide with increased concentrations of *Alternaria* spores (Sreeramulu & Ramalingam 1963). As harvesting aerially disseminates plant waste, spores may be scattered into wind permitting dispersal to nearby towns.

While crops may be a source of spores of *Alternaria*, it is unlikely that they are the only source. Sites where crop produce is stored and processed are contributors to airborne *Alternaria* spores (Lacey 1971, Hill et al. 1984, Beard et al. 1996), with transport from the field to storage facilities another potential source. Once spores are airborne, weather can further influence concentrations of *Alternaria* spores (Herrero & Zaldivar 1997, Angulo-Romero et al. 1999).

The aim of this research was to examine the effects of local crop harvesting, produce handling and of weather on the concentration of airborne spores of *Alternaria* found in inland rural towns of NSW, Australia.

MATERIALS AND METHODS

Overview

The annual distribution of the daily concentration of spores in Wagga Wagga and Moree, NSW, were recorded along with spore concentrations in nearby cotton and wheat crops and concentrations within a grain shed and a cotton gin. The towns were chosen on the basis of previous epidemiological studies of asthma and allergy (Peat & Woolcock 1991, Peat et al. 1995).

Study sites

The town of Moree (population 10,000) is positioned at a latitude of 29°S, and 300 km inland (Fig. 1). Moree is exceptionally flat. The

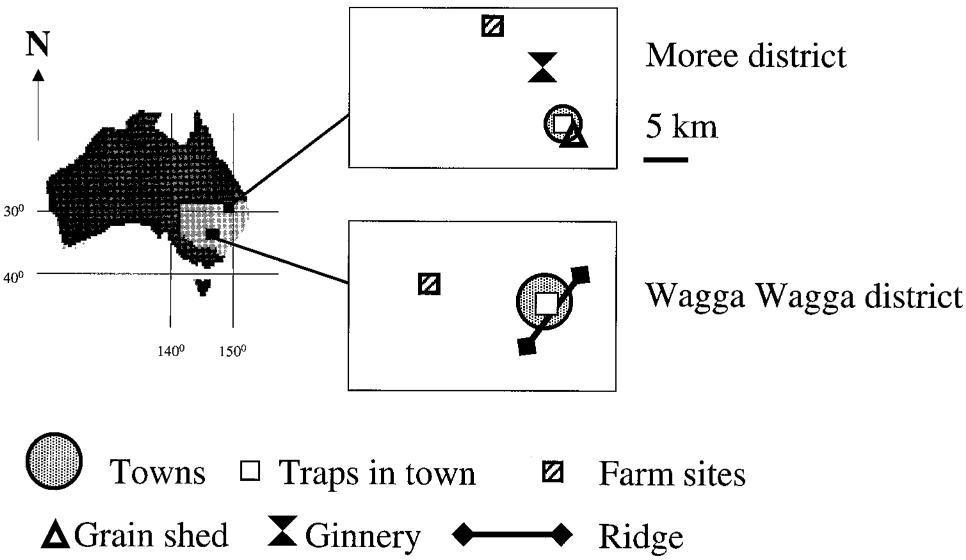

Fig. 1. Location of trap sampling sites, gin and silo relative to towns of Wagga Wagga and Moree in New South Wales, Australia.

crops commonly grown in the district include wheat, barley and early-sorghum crops which are harvested in summer, and cotton and late-sorghum which are harvested in autumn. Moree has a large silo and holding sheds in which grains and cotton seed are stored for most of the year. Two cotton gins are located within 20 km northwest of the town.

Wagga Wagga (population 50,000) is positioned 550 km south of Moree at 35°S, 270 km inland. Wagga Wagga has a ridge extending north-east to south-west on the eastern side of the town. Cereal grains and canola are grown in the district which are harvested in summer. The town has one silo complex located centrally within the town. Both towns have a temperate climate.

Spore sampling

The concentration of spores in air was measured using a Burkard volumetric spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK), which is a continuous air sampling device that provides daily concentrations (Hirst 1953). Traps were calibrated weekly to maintain a flow rate of 10 L/min. Particles were collected onto silicone grease (Molycote 111, Dow Corning, UK) which has greater particle collection efficiency than other adhesives and is stable over a range of temperatures (Solomon et al. 1980, Galan & Dominguez-Vilches 1997). Weekly tapes were cut into seven segments each relating to a 24 hour period. The segments were mounted onto microscope slides, stained with Calberla's stain for pollen identification (data not presented), then viewed by light microscopy at a magnification of 250X for spores of *Alternaria* along a full lengthwise traverse. The repeatability of the counting technique was substantial (Mitakakis & McGee 2000). Data were expressed as number of spores per cubic metre of air (Käpylä & Penttinen 1981).

Spore traps were positioned in accordance with Australian standards (AS 2922-1987 Ambient air-guide for the siting of sampling units, Standards Australia). In Moree, a spore trap was placed centrally in town, 11 metres above ground on the second storey roof of the Moree Community hospital. The hospital is surrounded by grassland, the town's main road and a river. Most of the population of the town live within 3 km of the hospital. The trap collected spores from air between 8 May, 1997 to 10 May, 1999.

In Wagga Wagga, a trap was placed centrally in town 12.5 metres above ground on a third storey roof of the Wagga Wagga Base hospital. The hospital is located in a dense residential area, with most residents of the town living within 6 km. The trap collected spores between 25 June, 1997 to 1 August, 1999. Sampling at a single location was representative of the distribution of *Alternaria* across both towns (Mitakakis & McGee 2000). While median values are appropriate for these data, means are also presented for direct comparison to other studies.

To sample crops, traps were sited just above the crop canopy to reduce sampling ambient spores that may have travelled some distance. To reduce errors arising from environmental and field characteristics (Rotem 1988), two traps were used simultaneously.

As the prevailing wind direction in Moree is from the north, a farm was selected (Auscott Limited) positioned 20 km north-northwest of Moree, where both cotton and wheat were grown. In each sampling period, two traps were located 0.5 to 1 km apart within the chosen field, with the exception of the first cotton harvesting period during which three traps were used. Each trap was positioned with the orifice of the trap approximately 1.4 metres above ground. Motors were powered by 12V batteries supplemented by solar panelling.

In Moree, spores in the air above a cotton crop were sampled during the harvesting season for three consecutive seasons. In 1997, spores were sampled over 28 days (18 April–15 May; crop harvested 16–24 April). In 1998, spores were sampled over 18 days (21 April–7 May; crop harvested on 21, 27 and 28 April, interrupted by rain). In 1999, spores were sampled for 32 days (24 March–25 April; harvested 11–14 April; slashed 19–20 April). Spores were not sampled between 14–19 April due to illness of the attendant.

To examine the effect of crop maturation and harvesting, spores were sampled for 33 days, as a "control period", in a recently sown wheat field in late winter (20 August–23 September, 1997). Spores were later sampled from the air above the canopy of mature wheat in the same field over 40 days in late spring (22 October–2 December; harvested 12–14 November).

As the prevailing winds in Wagga Wagga are from the northwest, a private farm was selected where wheat was grown, located 10 km to the northwest. Spore traps were located in adjacent fields, approximately 0.5 km apart, and were positioned with the orifice of the sampler approximately 1.3 metres above ground. The motors were powered by 12V batteries supplemented by solar panelling.

In 1997, spores were sampled from air over 43 days above immature wheat in winter as a control period (25 June–8 August). Spores were later sampled from the air above the canopy of mature wheat in the same field over 27 days in late spring (9 November–6 December; harvested 29 November–1 December).

Spore sampling in grain shed and gin

In Moree, grains are stored and cotton is ginned (mechanically separated into fibre, seed and trash) for most of the year. As daily variation of spore concentrations was expected to be low in these environments due to similar day to day activities and protection from the elements, spores were sampled for a short period. One trap was set up inside a grain shed for two weeks during the wheat harvesting season (GrainCorp Co.; 20 November–4 December, 1998). The shed holds up to 110,000 tonnes of seed. Wheat grain and cotton seed were stored at the time of sampling. The trap was placed so that the orifice was one metre above grain.

Two traps were sited outside a gin for seven weeks during cotton ginning (7 May–25 June, 1998; Namoi Cotton Cooperative). One trap was positioned where cotton bales entered the ginning process. The second trap was placed four metres above ground on the roof of a unit that filtered trash ("dust room"), eight metres from where the trash was being deposited into the back of an open truck. The gin was operated 24 hours a day, however sampling was sporadic as the gin was intermittently shut down to repair faults.

Isolation of *Alternaria* from plant materials

Plant samples were collected from crops in which the traps were sited to determine if *Alternaria* was present in the tissues. Wheat leaves were collected during the control periods at both towns. Leaf tissues were surface sterilised (Johnston & Booth 1983) then incubated on potato dextrose agar at room temperature up to 14 days. Wheat collected from the grain holding shed at Moree was plated onto potato dextrose agar then incubated at room temperature up to 14 days.

Mature cotton bolls collected in a defoliated cotton field separated into fibres and bracts, harvested cotton bolls collected from 20 cm within a cotton module awaiting processing separated into fibre and trash, and ginned cotton fibre were collected. All tissue samples were plated onto V8 juice agar and incubated at room temperature up to 14 days and examined for growth of *Alternaria*.

Meteorological data

Daily weather data were obtained from the Bureau of Meteorology, Sydney, for both towns for the duration that traps were operated. These were 9 am and 3 pm measures of ground temperature, relative humidity, cloud cover, wind direction and velocity, and daily measures of rainfall to 9 am, minimum and maximum temperature.

Statistical analysis

Pearson correlation coefficients were calculated between *Alternaria* concentrations from the crop and hospital sites using Analyse-It for Microsoft Excel (Analyse-It Software Ltd., Leeds, UK.). As the distribution of data was skewed, data were log transformed. A probability of less than 0.05 was considered significant.

The effects of weather and farming activities on spore concentrations in both towns were evaluated using regression modules in STATISTICA (version 5.5, Statsoft Inc., Tulsa, OK). Meteorological data were assessed using a step-wise multiple regression analysis using the first year of data and validated against the second year of data. The increase in variation explained in the model by including cropping activities (spore concentrations detected above crops) was determined using the subset of data from towns for which cropping information was available.

RESULTS

Concentrations of spores in towns

Moree

– The peak period for *Alternaria* spores lasted from early spring to late autumn (Fig. 2). In both years the concentrations were lowest between mid-June to mid-September. As such, the peak period lasted approximately 10 months of each year. The daily concentrations ranged from 0 to 1,334 (median 78, mean 132) spores/m^3, with the highest peak on the 5 March, 1999. Monthly totals ranged from 550 to 10,552 spores/m^3. The annual total beginning 8 May, was 41,273 spores/m^3 in 1997/98 compared to 52,904 spores/m^3 in 1998/99.

Wagga Wagga

– The peak period for *Alternaria* spores lasted from late spring to summer (Fig. 3). The peak spore period in 1998/99 was longer than in 1997/1998. The daily concentrations ranged from 0 to 1,271 (median 27, mean 73) spores/m^3, with the highest peak on the 15 November, 1998. Monthly totals ranged from 53 to 8,034 spores/m^3. The annual total in 1997/98 was 21,372 spores/m^3 compared to 30,660 spores/m^3 in 1998/99.

Concentrations of spores above crops

Spores of *Alternaria* were detected in the air above all crops (Fig. 4). In Moree, concentrations above wheat seedlings ranged from 0–128 (median 26) spores/m^3 air (Fig. 4 *a*). During the harvesting season of wheat, concentrations ranged from 5–745 (median 71) spores/m^3 air (Fig. 4 *b*). During the harvesting season of cotton, concentrations ranged from 8.5–837 (median 88) spores/m^3 air in 1997; 2.3–1,424 (median 104) spores/m^3 air in 1998 and 7.5–4,035 (median 408) spores/m^3 air in 1999 (Fig. 4 *c*, *d* & *e*). Mean concentrations increased three fold during the wheat harvesting season over the control, and 4 to 15 fold during the cotton harvesting seasons.

In Wagga Wagga, the concentrations of spores detected in air sampled above wheat seedlings ranged from 0–23 (median 1.5) spores/m^3 air (Fig. 4 *f*), and from 17–218 (median 80) spores/m^3 air during the wheat harvesting season (Fig. 4 *g*), an increase of the mean concentration by 20 fold.

Concentrations of spores above the cotton crop during harvesting dates in 1997 did not increase compared to days preceding harvesting, yet increased two weeks post-harvest (Fig. 4 *c*). In 1998, concentrations increased slightly with dates of harvesting yet these concentrations were lower than 4 to 6 days post harvest (Fig. 4 *d*). The two traps detected very different results between 2–4 May. The crop surrounding trap A, but not trap B, had been slashed, a process which removes plant stems harvested of cotton from the soil. Concentrations detected during the cotton harvesting season in 1999 showed a slight peak of spores during harvesting but the slashing period was not sampled (Fig. 4 *f*).

Above wheat outside Wagga Wagga, spore concentrations

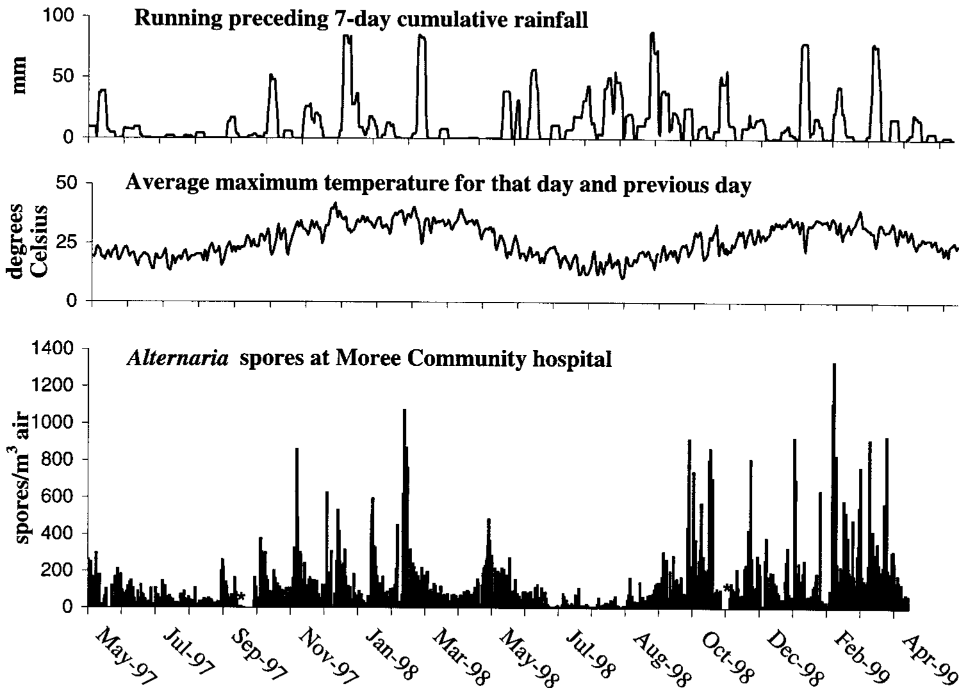

Fig. 2. Annual distribution of weather, crop and town *Alternaria* spore concentrations for Moree between May, 1997 and May, 1999. * – Data missing due to mechanical failure of trap; c – control sampling period; wh – wheat sampling period; ch – cotton harvesting period.

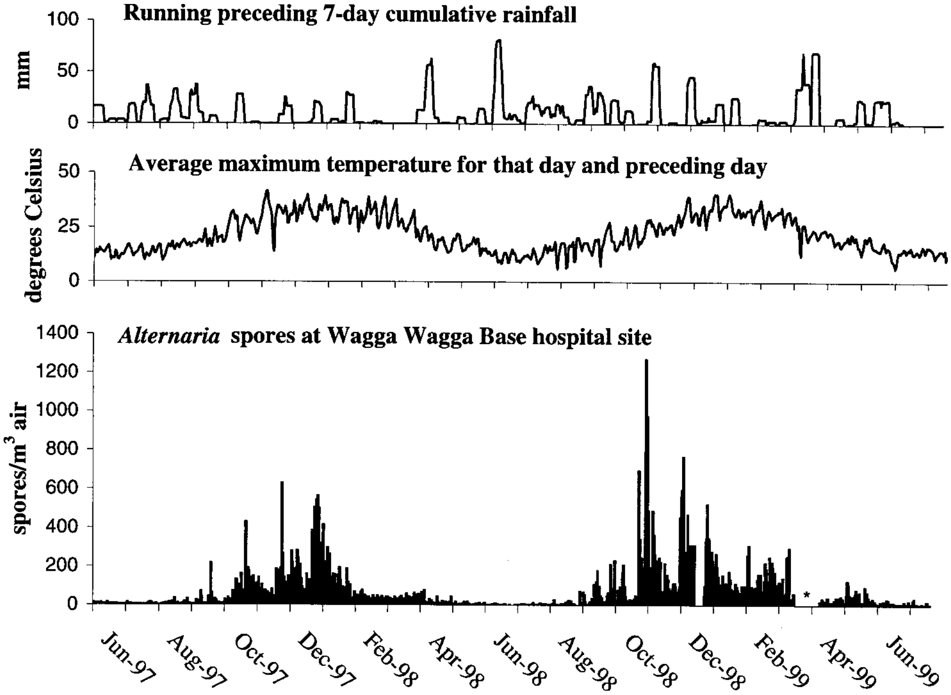

Fig. 3. Annual distribution of weather, crop and town concentrations for Wagga Wagga between June, 1997 to July, 1999. * – Data missing due to mechanical failure of trap; c – control sampling period; wh – wheat sampling period; ch – cotton harvesting period.

did not increase during the dates of harvest. Higher concentrations were detected both prior to and after harvest. The range of spore concentrations was much reduced compared to those in Moree.

When the seven individual crop sampling periods were considered, only the cotton harvest of 1999, when concentrations were lagged one day, significantly correlated with concentrations detected in town (Table I).

Spore concentrations in grain shed and gin

Alternaria was sampled from air at the gin and the grain shed. The excessive particle deposition on all tapes collected prevented an accurate estimation of the concentration of spores at both sources. Only the top layer of collected particles could be viewed, from which plant and fungal tissue were observed, including large numbers of spores of *Alternaria*.

***Alternaria* isolated from plant tissues**

Alternaria was isolated from all samples of plant tissues collected: wheat grains, young wheat leaves, wheat grain from the grain holding shed and grain truck, cotton bolls and bracts from field, harvested, stored and ginned cotton bolls, and stored and ginned trash.

Weather

Annual weather patterns differed substantially in both towns between years (Figs. 2 & 3). Most notably, excessive rainfall between July and September 1998 caused severe flooding in the Moree district. Both towns had approximately fifty percent more rainfall in 1998/99 than the preceding year (1998/99:1997/98 – Wagga Wagga 668: 468 mm; Moree 840: 525 mm). Maximum daily temperature ranged annually between 10 and 42°C in Moree, and 4 to 43°C in Wagga Wagga.

Regression modelling

In Wagga Wagga for the year 1997–98, the spore concentration was significantly dependent on the total rainfall in the 7 preceding days, the maximum temperature of the preceding and present day, the product of rain and maximum temperature, and the wind direction at 9 am (Table II).

By adding concentrations above crops as an additional variable, the multiple correlation coefficient (MCC) increased from 0.382 to 0.461 for the crop sampling dates, with proportionally fewer outliers (concentrations unexplained by the model). When these dates were analysed without crop data, the MCC decreased from 0.461 to 0.423 with a large increase in the number of outliers. Outliers that were explained by the addition of crop data were high concentrations.

When the variables which fit the 1997–98 concentrations were applied to concentrations detected in Wagga Wagga in 1998–99 (no crop data available), the MCC was moderate at 0.456.

Three of the same variables (rainfall in the preceding 7 days, the mean maximum temperature of the preceding and present day, and the product of rain and maximum temperature) fitted concentrations detected in Moree during 1997–98 well (MCC = 0.408, Table III). Wind direction was not significantly related, nor same day crop concentrations. By adding the concentrations above crops lagged one day as a variable, the MCC increased to 0.590, with fewer outliers. For the same crop sampling dates, by removing crop sampling data from the model, the MCC dropped to 0.500 with a substantial increase in the number of outliers.

In the second year, the model did not fit well to the annual distribution of spore data (MCC = 0.16). The only additional variable to independently and significantly correlate with the spore concentration was a product of rainfall (rainfall squared), which increased the MCC to 0.18. For the dates where crops were sampled, the MCC rose substantially to 0.515, compared to 0.492 when the crop concentrations were not included.

DISCUSSION

The sources and influences of airborne *Alternaria* spores in a rural setting have not been previously examined in detail. This study described the annual distribution of airborne spores of *Alternaria* in two rural towns, identified crops, gins and grain storage as sources, found crop harvesting and slashing dispersed spores into the air, and that rainfall and the maximum temperature were critical to the total concentration of spores in the air.

The duration of peak spore concentrations of *Alternaria* during spring and summer, extending through to autumn in Moree, was unusually long compared to elsewhere. In nearly all other countries where *Alternaria* has been examined, peak spore concentrations were detected during one or two of the same seasons found here (Pawsey 1964, Beaumont et al. 1985, Horst et al. 1990, Palmas & Cosentino 1990, Srivastava & Wadhwani 1992, Hjelmroos 1993, Halwagy 1994, Angulo-Romero et al. 1999). Additionally, the quantities of *Alternaria* detected in these towns were much higher than detected in southern Australia (Mitakakis et al. 1997), Kuwait (Halwagy 1994), Jordan (Shaheen 1992), South Africa (Potter et al. 1991), France (Horst et al. 1990), Italy (Palmas & Cosentino 1990), Spain (Munuera Giner & Carrion Garcia 1995) and India (Srivastava & Wadhwani 1992). Spores of *Alternaria* have only been detected in similar quantities in Canada (Li & Kendrick 1995) and elsewhere further to the north in Australia (Rutherford et al. 1997). The high concentrations for long periods of the year is likely to be responsible for the high levels of sensitisation to *Alternaria* in children living in Wagga Wagga and Moree (Peat et al. 1995).

Cropping activities contributed to the concentration of spores detected in both towns. *Alternaria* was isolated both as endophytic and saprophytic from tissues collected from the crops and crop products, and spores were found in air above crops. The concentration of spores was higher above crops during maturation and harvest than when the crops were immature. Overall, the range of concentrations above crops reflected the range of concentrations detected in the respective towns. The lack of correlation between crop and town for single periods was not surprising given that harvesting on all the farms in the district would contribute to the spores in the air above the town. However, a strong correlation was evident when data for all harvests were combined.

While large increases in spore concentrations above crops during harvest were detected by others (Abdel-Hafez et al. 1990, Lacey 1973, Sreeramulu & Ramalingam 1963), such

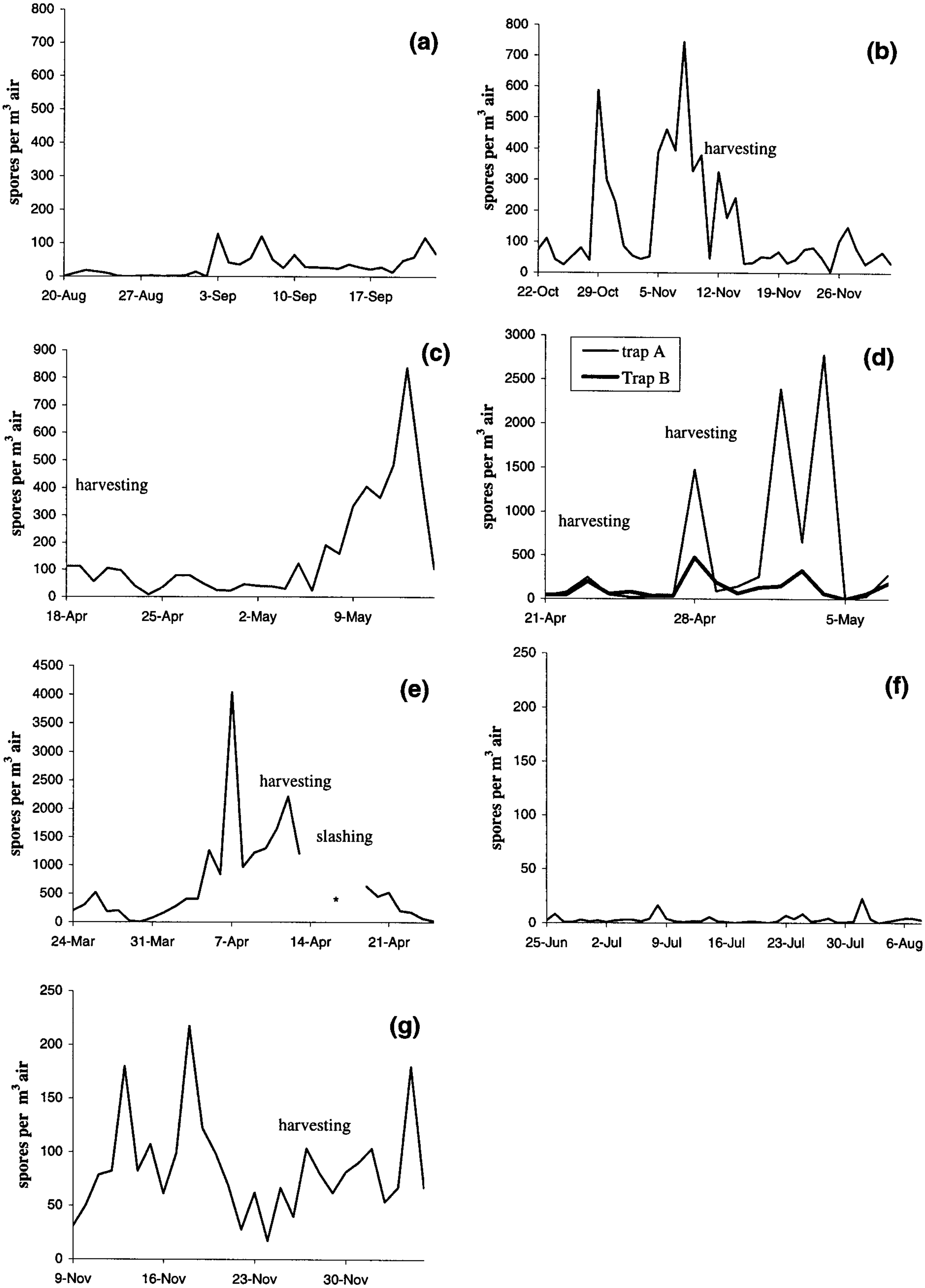

Fig. 4. *(For legend see page 236).*

Table I. *Pearson correlation coefficients between log transformed counts of Alternaria spore at crop and hospital sites.*
* – denotes significance <0.05.

Sampling period	Same day correlation with hospital	p-value	One day lag correlation to hospital	p-value	Number of days (no lag, lag)
Moree					
cotton harvest 1997	– 0.57	0.14	– 0.49	0.26	8, 7
wheat control 1997	0.08	0.66	0.25	0.16	35, 34
wheat harvest 1997	0.12	0.44	0.08	0.62	41, 40
cotton harvest 1998	0.02	0.95	– 0.10	0.70	16, 15
cotton harvest 1999	0.22	0.25	0.48	0.011*	29, 28
Wagga Wagga					
wheat control 1997	– 0.06	0.69	– 0.04	0.80	45, 44
wheat harvest 1997	– 0.04	0.87	– 0.05	0.82	21, 20

Table II. *Multiple regression analysis of weather variables and crop counts against counts of Alternaria in Wagga Wagga.*
*MCC – multiple correlation coefficient.

Statistical variables Wagga Wagga		n (days)	Beta	p-value	MCC*	n of outliers >2.5 SD
Total year 1997–1998		363		**0.0000**	**0.3824**	**17**
cumulative 7 day rainfall	**(a)**		– 0.6220	0.0000		
mean 2 day maximum temperature	**(b)**		0.4956	0.0000		
a×b	**(c)**		0.7462	0.0000		
wind direction	**(d)**		0.0896	0.0874		
Crop sampling dates 1997–98		75		**0.0000**	**0.4614**	**1**
cumulative 7 day rainfall	**(a)**		– 0.9066	0.0017		
mean 2 day maximum temperature	**(b)**		0.6122	0.0001		
a×b	**(c)**		0.4575	0.0131		
wind direction	**(d)**		0.3955	0.0612		
same day crop count	**(e)**		0.2545	0.0310		
Crop dates without crop data		75		**0.0000**	**0.4235**	**30**
cumulative 7 day rainfall	**(a)**		– 1.0847	0.0002		
mean 2 day maximum temperature	**(b)**		0.7903	0.0000		
a×b	**(c)**		0.5047	0.0074		
wind direction	**(d)**		0.4694	0.0291		
Total year 1998–1999						
same variables		355		**0.0000**	**0.4564**	**19**
cumulative 7 day rainfall	**(a)**		– 0.3685	0.0002		
mean 2 day maximum temperature	**(b)**		0.3833	0.0000		
a×b	**(c)**		0.7575	0.0000		
wind direction	**(d)**		0.0767	0.0619		
(no data from crops)						

increases were only observed in half the cases of harvesting in this study. That peaks were not always detected may have resulted from a number of causes. Wind direction can change rapidly and dominant wind directions by which the farms were chosen, may not have occurred on the specific days. In addition, harvesting may have been intermittent and thus the concentration of spores would have been diluted by time. Alternatively, given that many of the fields were larger than 200 hectares, movement of harvesting equipment around the field would have affected the collection of spores by a stationary air sampler, as demonstrated during slashing in 1998. Overall, while harvesting did contribute to spores in the air, the maturation of the crop itself would largely determine the concentrations of spores released.

As gins and grain storage facilities are operated for most of the year, continual release of spores into the air from a range of sites around the towns would contribute further to the concentration of spores. The importance of these sources needs to be clarified, especially given that some silos and gins are located within urban areas. The duration of ginning

Fig. 4. Alternaria spore concentrations detected in air sampled above crops using volumetric spore traps. **(a)** Moree control period 1997, sampling in immature wheat, 1997 **(b)** Moree wheat harvest period, 1997 **(c)** Moree cotton harvest period, 1997 **(d)** Moree cotton harvest period, 1998 showing counts from individual traps **(e)** Moree cotton harvest period 1999 **(f)** Wagga Wagga control period, sampling in immature wheat, 1997 **(g)** Wagga Wagga wheat harvest period, 1997. * – Data missing due to illness of trap attendant.

Table III. *Multiple regression analysis of weather variables and crop counts against counts of Alternaria in Moree.*
*MCC – multiple correlation coefficient.

Statistical variables Moree		n (days)	Beta	p-value	MCC*	n of outliers >2.5 SD
Total year 1997–1998 applying Wagga Wagga variables		352		**0.0000**	**0.4076**	**20**
cumulative 7 day rainfall	**(a)**		− 0.9723	0.0234		
mean 2 day maximum temperature	**(b)**		0.1057	0.0000		
a×b	**(c)**		1.524	0.0000		
Crop sampling dates 1997–98		101		**0.0000**	**0.5898**	**4**
cumulative 7 day rainfall	**(a)**		− 1.0350	0.0015		
mean 2 day maximum temperature	**(b)**		0.0781	0.3121		
a×b	**(c)**		1.6030	0.0000		
1 day lag crop data	**(f)**		0.3029	0.0000		
Same dates without crop data		101		**0.0000**	**0.5001**	**40**
cumulative 7 day rainfall	**(a)**		− 1.017	0.0044		
mean 2 day maximum temperature	**(b)**		0.0874	0.0302		
a×b	**(c)**		1.6304	0.0000		
Total year 1998–1999 applying first year variables		359		**0.0000**	**0.16**	**21**
cumulative 7 day rainfall	**(a)**		− 0.1363	0.2576		
mean 2 day maximum temperature	**(b)**		0.3077	0.0000		
a×b	**(c)**		0.3153	0.0087		
Adding additional factor		359		**0.0000**	**0.18**	**21**
cumulative 7 day rainfall	**(a)**		0.1203	0.4198		
mean 2 day maximum temperature	**(b)**		0.2913	0.0000		
a×b	**(c)**		0.7216	0.0001		
a×a	**(g)**		− 0.6682	0.0044		
Crop sampling dates 1998–99		21		**0.0373**	**0.5146**	**1**
cumulative 7 day rainfall	**(a)**		3.1902	0.0372		
mean 2 day maximum temperature	**(b)**		0.4654	0.1094		
a×b	**(c)**		− 14.958	0.0051		
a×a	**(g)**		12.328	0.0059		
1 day lag crop data	**(f)**		− 0.1953	0.4196		
Same dates without crop data		21		**0.0218**	**0.4923**	**1**
cumulative 7 day rainfall	**(a)**		3.0380	0.0414		
mean 2 day maximum temperature	**(b)**		0.4422	0.1202		
a×b	**(c)**		− 14.393	0.0053		
a×a	**(g)**		11.789	0.0063		

and silo activity in Moree and silo activity in Wagga Wagga may in part explain why the annual spore distribution in towns extended beyond the crop maturation and harvesting season.

While only wheat and cotton crops were considered here, harvesting of other nearby types of crops would have contributed to the concentration of spores in the air over the towns. Near Moree, barley and sorghum are also commonly grown and both host *Alternaria* (Hill & Lacey 1983, Ramakrishna et al. 1991, Gonzalez et al. 1997). Barley is harvested immediately prior to wheat, while early and late varieties of sorghum are harvested between March and June. These months correspond to the annual spore season detected in Moree. While we were unable to sample all crop types in the area, *Alternaria* was detected in air sampled alongside an outdoor barley grain pile, and from the grain itself (data not shown). Thus most, if not all crops, in the regions would have contributed to the airborne spores of *Alternaria* detected. While crops contribute some spores, they would not produce all the spores detected. The entire spore season is more likely to be determined by the maturation, death and disturbance of all plants in the region, including other crops and uncultivated vegetation, both outside and within the towns.

In towns, *Alternaria* has been isolated in air above grass particularly during mowing (Sreeramulu 1958, Pawsey 1964, Lacey 1975) and thus spores are likely to be released from parks, sports fields and paddocks which are common in both towns. Inside the home *Alternaria* spores have been isolated in carpeting, bedding and furniture (Schober 1991, Beguin 1994) and in domestic pet fur (Cabanes et al. 1996, Khosravi 1996). *Alternaria* spores were found in and around the homes of Moree residents (Mitakakis et al. 2000), in both gardens and indoors. All these sources of spores would contribute cumulatively toward the total spores to which a person is exposed.

It was not surprising that spore concentrations correlated with the interactions of recent rainfall and warm temperatures, as these factors largely determine spore production and release (Rotem 1988). In Wagga Wagga, which has a distinct ridge running along one side of town, the concentrations were additionally dependent on wind direction. In Moree, which is flat, wind direction was not significantly correlated with spore concentrations. During the second year of sampling at both sites (1998/99), increased annual rainfall, and possibly the observed flooding, was associated with a substantial increase in annual total spore concentrations. An additional rain component slightly improved the model of spore concentrations. The equations presented are the first for *Alternaria* that can be used to predict spore concentration on the following day. By measuring the preceding week's rainfall, the day's maximum temperature, and by adding the next day's predicted maximum temperature, the expected spore concentration can be estimated. Most importantly, by sampling spores above nearby crops, the additional information greatly improved the fit of the model to predict the spore concentrations.

It is unknown why the concentration of spores in Moree correlated with concentrations detected in crops on the previous day, yet in Wagga Wagga they correlated with concentrations of the same day. Possibly the peak of diurnal spore release is later in the day for Moree or that crops were grown more distant from the town than in Wagga Wagga.

The data presented here indicate a possible explanation for the incidence of allergic sensitisation to *Alternaria* in the town of Broken Hill, where the prevalence amongst children is the highest recorded of the seven towns examined in NSW (23.1%, Peat et al. 1995). The region of Broken Hill does not support cropping and allergy to *Alternaria* must arise from alternative sources of the fungus. Broken Hill is located in the arid west of the state, with predominant vegetation outside the town being low, shrubby and sparse, interspersed with ephemeral plants. We would expect the vegetation to be infected with *Alternaria* as the fungus is capable of germinating at a relatively low water activity (Hocking et al. 1994). We speculate that disturbance of vegetation, such as by wind, both within town and nearby, may release sufficient spores of *Alternaria* to induce sensitisation, however additional sources would need to be considered. For example, study of the indoor environment would be necessary as residences and schools typically use evaporative cooling which may have filters contaminated with fungi (Heinmann et al. 1994).

In conclusion, in inland rural communities of NSW, measures to reduce rates of *Alternaria* related allergy and asthma need to consider the array of sources of the fungus that contribute to the airborne spore load.

ACKNOWLEDGEMENTS

We thank the following people for the excellent on site maintenance of the spore traps : Florence Smith, Colin Rice, Lewis Macey and Rachel Webb in Moree, and Tony Day and Rob Hibbard in Wagga Wagga. We are grateful to Mr Harvey Gaynor for permission to use the Auscott Limited farm, to Mr Bruce Harris for the use of his farm and assistance, to Mr John Armstrong for permission to sample at Graincorp Co., and to Mr Shane Towns for permission to sample at Namoi Cotton Cooperative gin. We also thank the NH&MRC for their funding support.

REFERENCES

Abdel-Hafez, S. I. I., Moubasher, A. H., Shoreit, A. A. M. & Ismail, M. A. 1990. Fungal flora associated with combine harvester wheat and sorghum dusts from Egypt. – Journal of Basic Microbiology 30: 467–479.

Angulo-Romero, J., Mediavilla-Molina, A. & Dominguez-Vilches, E. 1999. Conidia of *Alternaria* in the atmosphere of the city of Cordoba, Spain in relation to meteorological parameters. – International Journal of Biometeorology 43: 45–49.

Beard, M. D., Buchan, R. M. Reeves, F. B. & Salasek, M. 1996. Pulmonary reactive components of winter wheat grain dust at Colorado farms and elevators. – Applied Occupational Environmental Hygiene 11: 1409–1416.

Beaumont, F., Kauffman, H. F., van der Mark, T. H., Sluiter H. J. & de Vries, K. 1985. Volumetric aerobiological survey of conidial fungi in the North-East Netherlands. – Allergy 40: 173–180.

Beguin, H. 1995. Mould biodiversity in homes II. Analysis of mattress dust. – Aerobiologia 11: 3–10.

Cabañes, F. J., Abarca, M. L. Bragulat, M. R. & Castellá, G. 1996. Seasonal study of the fungal biota of the fur of dogs. – Mycopathologia 133: 1–7.

Farr D. F., Bills, G. F., Charmuris G. P. & Rossman, A. Y. 1989. Fungi on plants and plant products in the United States. – APS Press, St Paul, MN.

Galan, C. & Dominguez-Vilches, E. 1997. The capture media in aerobiological sampling. – Aerobiologia 13: 155–160.

Gonzalez, H. H. L., Martinez, E. J. & Resnik, S. L. 1997. Fungi associated with sorghum grain from Argentina. – Mycopathologia 139: 35–41.

Halonen M., Stern D. A., Wright A. L., Taussig L. M., Martinez F. D. 1997. *Alternaria* as a major allergen for asthma in children raised in a desert environment. – American Journal of Respiratory & Critical Care Medicine 155: 1356–1361.

Halwagy, M. H. 1994. Fungal airspora of Kuwait City, Kuwait, 1975–1987. – Grana 33: 340–345.

Harries, M. G., Lacey, J. Tee, R. D., Cayley, G. R. & Newman Taylor, A. J. 1985. *Didymella exitialis* and late summer asthma. – Lancet 11: 1063–1066.

Heinmann, S., Beguin, H. & Nolard, N. (1994) Biocontamination in airconditioning. – In: Health implications of fungi in indoor environments Vol. 2 (ed. R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan & E. S. Hoekstra), pp. 179–186. – Elsevier, Amsterdam.

Hensley, M. J., Scicchitano, R. Saunders N. A., Cripps, A. W., Rhuno, J., Sutherland, D. & Clancy, R. L. 1988. Seasonal variation in non-specific bronchial reactivity: a study of wheat workers with a history of wheat associated asthma. – Thorax 43: 103–107.

Herrero, B. & Zaldivar, P. 1997. Effects of meteorological factors on the levels of *Alternaria* and *Cladosporium* spores in the atmosphere of Palencia, 1990–92. – Grana 36: 180–184.

Hill, R. A. & Lacey, J. 1983. The microflora of ripening barley grain and the effects of pre-harvest fungicide application. – Annals of Applied Biology 102: 455–465.

Hill, R. A., Wilson, D. M., Burg, W. R. & Shotwell, O. L. 1984. Viable fungi in corn dust. – Applied and Environmental Microbiology 47: 84–87.

Hirst, J. M. 1953. Changes in atmospheric spore content: diurnal periodicity and the effects of weather. – Transaction of the British Mycological Society 36: 375–392.

Hjelmroos, M. 1993. Relationship between airborne fungal spore presence and weather variables. *Cladosporium* and *Alternaria*. – Grana 32: 40–47.

Hocking A. D., Miscamble F. & Pitt J. I. 1994. Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata* and *Curvularia pallescens*. – Mycological Research 98:91–94.

Horst, M., Hejjaoui, A., Horst, V., Michel, F. & Bousquet, J. 1990. Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. – Journal of Allergy & Clinical Immunology 85: 460–472.

Johnston, A. & Booth, C. 1983. Plant pathologists pocketbook. – Commonwealth Mycological Institute, Kew.

Käpylä, M. & Penttinen, A. 1981. An evaluation of the microscopical counting methods of the tape in Hirst-Burkard pollen and spore trap. – Grana 20: 131–141.

Khosravi, A. R. 1996. Fungal flora of the hair coat of stray cats in Iran. – Mycoses 39: 241–243.

Lacey, J. 1971. The microbiology of moist barley storage in unsealed silos. – Annals of Applied Biology 69: 187–212.

Lacey, J. 1973. Actinomycete and fungus spores in farm air. – Journal of Agricultural Labour 1: 61–78.

Lacey, J. 1975. Airborne spores in pastures. – Transactions of the British Mycological Society 64: 265–281.

Li, D. & Kendrick, B. 1995. A year-round comparison of fungal spores in indoor and outdoor air. – Mycologia 87: 190–195.

Mitakakis, T. Z. & McGee, P. A. 2000. Reliability of measures of spores of *Alternaria* and pollen concentrations in air over two towns in rural Australia. – Grana 39: 141–145.

Mitakakis, T., Ong, E. K., Stevens, A., Guest, D. & Knox, R. B. 1997. Incidence of *Cladosporium*, *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three years. – Aerobiologia 13: 83–90.

Mitakakis, T. Z., Tovey, E. R., Xuan, W. & Marks, G. B. 2000. Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia. – Clinical & Experimental Allergy 30: 1733–1739.

Munuera Giner, M. & Carrion Garcia, J. S. 1995. Daily variations of *Alternaria* spores in the city of Murcia (semi-arid southeastern Spain). – International Journal of Biometeorology 38: 176–179.

O'Hollaren, M. T., Yunginger, J. W., Offord K.P., Somers, M. J., O'Connell, E. J., Ballard, D. J. & Sachs, M. I. 1991. Exposure to aeroallergens as a possible precipitating factor in respiratory arrest in young patients with asthma. – The New England Journal of Medicine 324: 359–363.

Palmas, F. & Cosentino, S. 1990. Comparison between fungal airspore concentration at two different sites in the South of Sardinia. – Grana 29: 87–95.

Pawsey, R. G. 1964. An investigation of the spore population of the air at Nottingham. II. The results obtained with a Hirst spore trap June-July 1956. – Transactions of the British Mycological Society 47: 357–363.

Peat, J. K. & Woolcock, A. J. 1991. Sensitivity to common allergens: relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. – Clinical & Experimental Allergy 21: 573–581.

Peat, J. K., Toelle, B. G., Gray, E. J., Haby, M. M., Belousova, E., Mellis, C. M. & Woolcock, A. J. 1995. Prevalence and severity of childhood asthma and allergic sensitisation in seven climatic regions of New South Wales. – The Medical Journal of Australia 163: 22–26.

Potter, P. C., Berman, D., Toerien, A., Malherbe, D. & Weinberg, E. G. 1991. Clinical significance of aero-allergen identification in the Western Cape. – South African Medical Journal 79: 80–84.

Ramakrishna, N., Lacey, J. & Smith, J. E. 1991. Effect of surface sterilization, fumigation and gamma irradiation on the microflora and germination of barley seeds. – International Journal of Food Microbiology 13: 47–54.

Rotem, J. 1988. Quantitative assessment of inoculum production, dispersal, deposition, survival and infectiousness in airborne diseases. – In: Experimental techniques in plant disease epidemiology (ed. J. Kranz & J. Rotem) pp. 69–83. – Springer, Berlin.

Rutherford, S., Owen, J. A. K. & Simpson, R. W. 1997. Survey of airspora in Brisbane, Queensland, Australia. – Grana 36: 114–121.

Schober, G. 1991. Fungi in carpeting and furniture dust. – Allergy 46: 639–643.

Shaheen, I. 1992. Aeromycology of Amman area, Jordan. – Grana 31: 223–228.

Solomon, W. R., Burge, H. A. & Boise, J. R. 1980. Performance of adhesives for rotating-arm impactors. – Journal of Allergy & Clinical Immunology 65: 467–470.

Sreeramulu, T. 1958. Effect of mowing grass on the concentrations of certain constituents of the air spora. – Current Science 2: 61–63.

Sreeramulu, T. & Ramalingam, A. 1963. Some short-period changes in the atmospheric spore content associated with changes in the weather and other conditions. – Proceedings of the Indian Academy of Science 59B: 154–172.

Srivastava, A. K. & Wadhwani, K. 1992. Dispersion and allergenic manifestations of *Alternaria* airspora. – Grana 31: 61–66.