Prevalence and distribution of *Alternaria* allergens in rural New South Wales, Australia

Teresa Zinovia Mitakakis

The University of Sydney
Faculty of Science

A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy

November, 2000

Declaration

I hereby declare that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

T.Z. Mitakakis

Abstract

In rural inland, south-eastern Australia, allergy to the fungus *Alternaria* is prevalent and an important risk factor for asthma. The aim of the thesis was to investigate the distribution and factors influencing allergens of *Alternaria* in the air. As airborne allergenic spores were thought to arise from harvesting of nearby crops, two towns with different agricultural practices were studied. Moree has two crop harvesting periods in summer and autumn whilst Wagga Wagga has one harvesting period in summer.

Over two years, air was sampled daily in Wagga Wagga and Moree using Burkard traps. The reliability of measurements from a single site to represent the distribution of airborne concentrations of spores across each town was examined using data from three traps simultaneously, sited 2.0 to 4.9 km apart, over four weeks. Substantial intra-class correlation coefficients (ICC) were observed between the three sampling sites across both towns (ICC=0.52, 95% CI 0.30-0.71 to 0.76, 95% CI 0.61-0.87) when counts of *Alternaria* spores were relatively high. The correlation was poor when counts were low. Of more than 365 trap tapes examined, the two microscopic traverses strongly correlated for counts of *Alternaria* spores (ICC=0.95, 95% CI 0.94-0.96). *Alternaria* was detected in both towns throughout the two year period with peaks in spore concentrations reflecting the season of crop harvesting in each region.

Individual exposure to spores was examined. Thirty three subjects (adults and children from nine families) wore nasal air samplers and personal air samplers both inside and outside their homes. The effects of activity, location, age on the inhalation of *Alternaria* spores and variation between individuals in the same environment were determined. Every subject inhaled *Alternaria* spores. Personal exposure to *Alternaria* in the home environment varied substantially between subjects. Levels of fungal spores inhaled were higher during periods of activity than during rest, and higher while subjects were

outdoors than indoors. During outdoor activity, the number of *Alternaria* spores inhaled ranged from 4 to 794 (median 11) spores/hr.

Sources of airborne spores was investigated by sampling air above wheat and cotton crops near the towns during harvesting and non-harvesting periods, in a grain and cotton seed storage shed, and a cotton gin. Substantially higher concentrations were detected above crops during harvesting periods compared to non-harvesting periods. Peaks were associated with harvesting and other activities where plants were manipulated. By regression analysis spore concentrations in both towns were modelled against those detected above crops and with weather variables. Only one crop sampling period (cotton harvest) independently correlated with concentrations in town. Analysis combining all data showed concentrations of spores above crops correlated with spore concentrations in the town when lagged by one day. Variables of rainfall and maximum temperature influenced concentrations in both towns, and wind direction in Wagga Wagga alone.

Parents of asthmatic children were asked by questionnaire in which locations symptoms were provoked. Asthma was reported to be exacerbated at grain farms and with disturbance of local vegetation in town and home gardens. Nasal sampling confirmed that activities that disturbed dust or vegetation increased the inhalation of spores.

The factors that release allergen from spores were determined in a modified Halogen immunoassay. Approximately 60% of spores released allergen, and the proportion was influenced by isolate, nutrient availability, viability, and not influenced by sunlight or culture age up to 21 days. Germinating the spores significantly increased the proportion that released total allergen and Alt a 1 ($p<0.0001$). Alt a 1 appears to be a minor contributor to the total allergen released from spores except when spores have germinated.

Conclusions: People living in inland rural regions of Australia are exposed to substantial quantities of allergenic spores of *Alternaria*. Exposure is a highly personal event and is largely determined by disturbance of local vegetation releasing spores such as from nearby crops by wind, harvesting, slashing, transport and processing of produce, and from within town and home gardens. Most spores inhaled are likely to be allergenic, with potency potentially increasing with viability.

Acknowledgments

I need to thank Peter McGee firstly for attending a conference at Melbourne University where we first met, and where I found out about the project. I appreciate the hard work he has had as my supervisor to bend my mind, for being readily accessible for advice and critiquing, and for the support during early difficulties with my working environment in a medical department, and for being a friend. I hope that I have done a little mind bending in return!

Next I would like to express enormous gratitude and pleasure of working side by side with Sara Downs. Not only is she a role model to me of a brilliant and incredibly dedicated and competent scientist, she is a fantastic person who was fun to share an office with or a flat on our field trips or life's ups and downs, or a decent bottle of red. I value our friendship. Sara produced the table presented in Appendices 4.1 and 4.2.

I was incredibly lucky to meet and work with really lovely people at Wagga Wagga and Moree. Rob Hibbard and Tony Day helped me set up and run the Burkard traps all over Wagga and were generous, kind, interested and meticulous. A good few fishing tales and stories of advice were exchanged. In Moree, Flo Smith, Colin Rice and Lewis Macey were also wonderful. They not only looked after the Burkard at the Hospital but also assisted at the gin and silo and pretty much whenever they could, including the childhood respiratory testing that forms Sara's thesis. Thank you all and Sara and I miss you.

Thanks also go to Rachel Ward and Harvey Gaynor at Auscott and Don Rooke at the Bureau of Meteorology, Moree for looking after more Burkard traps, and for tolerating repeat sampling periods. I'd like to thank John Armstrong for permission to sample at Graincorp, and to Shane Towns for permission to sample at Namoi Cotton Cooperative gin.

I would like to thank Euan Tovey for his support and co-supervision, and for employing me in the fourth year to look at allergens. Thankyou for giving me confidence and experience in this area and for your teaching style and lateral thinking. Thanks also for the financial support to travel to Perugia. Thanks to Tim O'Meara for advice and morale boosting.

I would like to thank Guy Marks for his project advice, statistical assistance and personal advice. Thank you Wei Xuan for statistical advice and for performing the statistics found in Chapter 3. Thanks to Alan Clift for performing the regression analysis found in Chapter 4 and for tales of the middle east. Thanks to Gavin Greenoak for use of the solar simulator. Thanks to Prof. Portnoy and associates for providing antibodies used in chapter 5. Thank you David Guest for the use of your microscope in Melbourne. Thank you Mr Jason Sercombe, Ms Sara Downs, Ms Sue Downie and Dr George Car for the assistance with data collection in Chapter 3. Also to NH&MRC and the Asthma Foundation of NSW for a wage.

Lastly, big purrs and chin rubs to my two cats.

Publications

Accepted articles

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

Abstract

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.

2. **Mitakakis, T.,** Tovey, E, Xuan, W. and Marks, G. Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia. *Clinical Experimental Allergy* 2000; 30: 1733-1739.

Abstract

Background: In inland NSW, Australia, allergic sensitisation to the fungi *Alternaria* and *Cladosporium* and to pollen is common and an important risk factor for asthma.

Objective: We report the results of a series of experiments designed to assess the nature of personal exposure to these airborne allergenic particles. We have tested the effect of exposure conditions and level of activity on measurements of the personal exposure.

Method: Personal Air Samplers (PAS) and Nasal Air Samplers (NAS) were employed. NAS are fitted just inside the nose and collect inhaled particles by impaction, while the PAS use a pump-operated filter with constant air flow (2 litres/min). Thirty three subjects (adults and children) used both NAS and PAS simultaneously for four one hour periods during which they performed activities or rested, both inside and outside their homes. Samples were analysed by light microscopy. *Alternaria* spores, *Cladosporium* spores, grass pollen and non-grass pollen were counted.

Results: Both samplers detected substantial variation in exposure between subjects. Between members of the same household, the intra-house correlation coefficient ranged from <0 to 0.38. Levels of pollen and fungal spores inhaled were higher during periods of activity than during rest, and higher while subjects were outdoors than indoors. During the active outdoor period, the number of *Alternaria* spores inhaled ranged from 4 to 794 (median 11) spores/hr, *Cladosporium* from 0 to 396 (median 4) spores/hr, grass pollen from 0 to 81 (median 1) grains/hr and non-grass pollen from 0 to 72 (median 5) grains/hr.

Conclusion: This is the first study to quantify individual inhaled levels of allergenic fungal spores and pollen under normal domestic circumstances. Exposure can be substantial and highly variable between individuals. The amount of particles inhaled relates both to location of the individual and activity being performed, independent of age group.

3. Mitakakis, T.Z., Barnes, CS, Tovey, E. Spore germination increases allergen release from *Alternaria*. *Journal of Allergy & Clinical Immunology* 2001;107:388-90.

Abstract

Allergen released from individual spores of the fungus *Alternaria* has not been investigated. Germination of spores has been suggested to increase allergen release. This study examined allergen released from individual spores, and the effect of germination on allergen availability. Allergen release was determined with the Halogen™ immunoassay, using serum IgE from *Alternaria* sensitised subjects and three Alt a 1 specific antibodies. Not all spores released allergen. Germinating the spores significantly increased the proportion that released allergen ($p<0.0001$ for all antibodies). Alt a 1 may be a minor contributor to the total allergen released from spores except when spores have germinated. How these results reflect the allergen content of spores in air that people inhale requires investigation.

Manuscripts submitted or under preparation

4. **Mitakakis, T.Z.,** Clift, A., Mc Gee, P. Crops, weather and local sources influence airborne concentrations of allergenic *Alternaria* spores in two rural towns of Australia. Submitted to *Grana* (under review May, 2001).

5. **Mitakakis, T.Z.**, O'Meara, T.J., Tovey, ER. Allergen release from spores of *Alternaria* is not affected by simulated sunlight. *Atmospheric Environment* (to be submitted June, 2001).

Collaborative papers

6. Downs, S.H., Marks, G.B., **Mitakakis, T.Z.,** Lëuppi, J.D., Car, N.G. & Peat J.K. Having lived on a farm and protection against allergic diseases in Australia. *Clinical Experimental Allergy* 2001; 31:570-575.

7. Downs, S.H., **Mitakakis, T.Z.,** Marks, G.B., Car, N.G., Belosouva, E.G., Lëuppi, J.D., Xuan, W., Downie, S.R., Tobias, A. & Peat J.K. Clinical relevance of *Alternaria* sensitisation and exposure in children. *American Journal of Respiratory & Critical Care Medicine* (In press, May 2001).

8. Downs, S.H., **Mitakakis, T.Z.,** Car, N.G., Girgis, S.T., Belosouva, E.G., Peat J.K. & Marks, G.B. Airborne concentrations of *Alternaria* spores and grass pollen and emergency hospital attendance for asthma. *American Journal of Epidemiology* (submitted November, 2000).

Table of Contents

Chapter 1

Chapter 2

List of Tables

List of Figures

List of Appendices