# Variations in Pollen and Fungal Air Spora:

**An analysis of 30 years of monitoring for the clinical assessment of patients in the Western Cape.**

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## Abstract

Background and aims
Pollen and fungal spore concentrations in the atmosphere of Cape Town have been monitored since 1984 in two areas of Cape Town. Volumetric spore traps were used to monitor the air spora that trigger allergic disease in susceptible individuals. A pollen count was produced for the diagnosis and treatment of patients attending respiratory clinics at the academic hospitals but the findings of the different aerobiological monitoring areas have never been compared. We considered that more than one aerobiological area should be monitored to produce a representative pollen count for the most densely populated areas of Cape Town.

Methods
The pollen taxa and fungal spore genera collected from the two aerobiological zones, now named the West Coast and Valkenberg Aerobiomes were defined. Eight of the air spora were selected for detailed comparison. The relative abundance and seasons of Poaceae, or grasses, the tree pollen taxa Cupressaceae, *Platanus* and *Quercus* and the fungal spores: *Alternaria*, *Cladosporium*, *Epicoccum* and *Pleospora* were evaluated and compared in each aerobiome. Differences in the annual distribution and seasonal limits of the air spora in the two aerobiomes were found using statistical techniques.

Results
Significant differences were found between the tree pollen loads in the different aerobiomes. Spring pollinating trees were the most prevalent pollen taxa in the Valkenberg Aerobiome with short flowering seasons that spanned six weeks (August-September) for *Platanus* and *Quercus* but eight weeks (July-September) for Cupressaceae. The grass season was longer (September-March) in both aerobiomes and grasses flowered earlier at the inland site. Poaceae dominated the annual pollen catch at the coastal aerobiological zone. *Parietaria* was the only weed taxon with significant concentrations. *Pleospora* showed a seasonal trend peaking in mid to late winter at the West Coast. No comparable peaks for *Pleospora* were seen from the Valkenberg sites. *Cladosporium* concentrations were low and seldom breached the significant threshold of 3,000 spores/$m^{-3}$ in either of the aerobiomes. The influence of meteorological parameters on *Cladosporium* and temperature on Poaceae was explored. A table was designed that clarified the ranges for *Cladosporium* and a formula was adapted for predicting the start of the grass season. Significant decreases in the Poaceae concentrations in both aerobiomes were observed and discussed with reference to Global Warming.

Conclusions
The differences in the pollen spectra and seasonality of the selected allergenic air spora indicate that both aerobiomes should be monitored concurrently for patients who live and work in these different microclimates. Pollen profiles for skin, blood and specific IgE testing panels should be reassessed to include Cupressaceae, *Parietaria*, *Myrica*, *Pleospora* and basidiospores. When patients are recruited for clinical drug trials, their place of residence or work should be within the realm of the aerobiome that is being monitored. Current pollen

monitoring programmes should be consulted for immunisation regimes to grass and tree pollen. These findings will be applied to the diagnosis and prescription of immunotherapy in clinical practice.

Declaration of Authorship

I declare that this thesis has been composed solely by myself and that it has not been submitted in whole or in part in any previous application for a degree. Except where it is stated by reference or acknowledgement, the work presented is entirely my own.

Date.....26/06/2018 Signed by..... Signed by candidate

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# Abbreviations

| | |
|---|---|
| **AAAAI** | The American Academy of Allergy, Asthma and Immunology |
| **AFI** | Annual Fungal Index |
| **AGI** | Annual Grass Index |
| **AIU** | Allergy Immunology Unit |
| **APAWG** | Asia Pacific Working Group |
| **API** | Annual Poaceae Index |
| **API** | Annual Poaceae Index |
| **ARIA** | Allergic Rhinits and its Impact on Asthma |
| **BCE** | Before the Common Era |
| **CI** | Confidence Interval |
| **CFSF** | Cape Flats Sand Fynbos |
| **CMA** | Cape Metropolitan Area |
| **$CO_2$** | Carbon Dioxide |
| **DOY** | Day of year |
| **EAACI** | European Academy for Allergy and Clinical Immunology |
| **EAN** | European Aeroallergen Network |
| **GAM** | Generalised Additive Model |
| **IgE** | Immunoglobulin E |
| **Km** | Kilometre |
| **REA** | Red Espanôla de Aerobiologique (Spanish Pollen Network) |
| **RH** | Relative Humidity |
| **RNSA** | Réseau National de Survellance Aérobiologique |

| | |
|---|---|
| **RXH** | Red Cross Hospital |
| **SAWS** | South African Weather Service |
| **SAAO** | South African Astronomical Observatory |
| **SANBI** | South African National Biodiversity Institute |
| **SCIT** | Subcutaneous Immunotherapy |
| **SE** | Standard Error |
| **SFI** | Seasonal Fungal Index |
| **SLIT** | Sublingual Immunotherapy |
| **TBV** | Table View |
| **TPI** | Total Pollen Index |
| **TRUP** | Two Rivers Urban Park |
| **UCT** | University of Cape Town |
| **WAO** | World Allergy Organization |

## A Glossary of Common Names of Allergenic plants

| Scientific name | Plant | Common name |
|---|---|---|
| Arctotheca populifolia | weed | sand pumpkin |
| Avena barbata | grass | wild oats |
| Briza maxima/minor | grass | *klokkies*/bells |
| Cupressaceae | tree | cypress |
| Cynodon dactylon | grass | Bermuda grass |
| Dimorphotheca pluvialis | weed | daisy |
| Erica sp | weed/herb | heather |
| Lolium perenne | grass | ryegrass |
| Lagurus ovatus | grass | bunnytail |
| Myrtaceae | tree | gum |
| Oleaceae | tree | olive |
| Parietaria | weed/herb | Pellitory-of-the-wall |
| Pennisetum clandestinum | grass | *kikuyu* |
| Pinus | tree | pine |
| Plantago lanceolate | weed | English Plantain |
| Poa annua | grass | winter grass |
| Podocarpus | tree | yellowwood |
| Platanaceae | tree | plane |
| Protea sp | weed/herb | Protea |
| Quercus | tree | oak |
| Restio | weed/herb | reed |
| Salix | tree | willow |
| Stenotaphrum secundatum | grass | Buffalo grass |
| Taraxacum officinale | weed | dandelion |
| Typhus | weed/herb | bulrush |

Chapter 1

# Introduction

## 1.1 Scope and structure of the thesis

The subject of this thesis is the comparison of the atmospheric pollen and fungal spores, or air spora, from four pollen sampling sites in Cape Town to obtain a representative pollen count for clinicians who treat allergic disease. Four pollen sampling sites in Cape Town suburbs operated at varying times from 1984-2010. Pollen counts from these sampling sites provided a detailed report of the daily pollen and fungal spore (air spora) concentrations in the ambient air. Hirst type volumetric spore traps were used to trap pollen from the atmosphere at each sampling site.

The air spora from three pollen sampling sites, Rondebosch, Table View and Observatory were all monitored for more than three years during the years 1984-2010. The Mowbray pollen sampling site operated for only 18 months, so no long-term data were available for this site. The air spora data from the Rondebosch, Table View and Observatory pollen sampling sites were compared. Eight aeroallergens: Poaceae*, Cupressus, Platanus*, *Quercus Alternaria*, *Cladosporium Epicoccum*, and *Pleospora* were selected from the individual databases, based on their prevalence from the sampling data as well as their known allergenicity (Velasco-Jiménez et al. 2013; Sadys et al.2016, Inal et al. 2008). The concentrations and seasonal differences of these aeroallergens from the different locations in Cape Town were compared. The influence of weather on Poaceae and *Cladosporium* concentrations was examined. These two aeroallergens were chosen as they are known to produce positive skin tests. They also generated specific IgE in patients with allergies in a previous study in the Cape Town area (Potter et al. 1991).

## 1.2 The research in context

The pollen count provides clinicians with the identity and seasonal patterns of the individual pollen taxa that may trigger symptoms of allergic disease in sensitized individuals. These diseases include: Seasonal or intermittent rhinitis (hay fever), allergic conjunctivitis (ocular allergy) and asthma (Inal et al. 2008, Wheatley 2015). Allergists who have access to reliable pollen counts in a specific geographic area are advantaged. They are better able to select the correct aeroallergen skin prick, blood or specific IgE testing panels for patients. In addition, the pollen flowering seasons of allergenic pollen were defined.

Grass pollen immunotherapy is frequently performed by allergists. It is therefore essential to know the start and end dates of the grass pollinating season so that pollen immunotherapy by the sublingual route (SLIT) or subcutaneous immunotherapy (SCIT) to tree or grass pollen (Jutel et al, 2015) can be implemented in a clinical setting outside the specific tree and grass pollinating seasons. This is necessary for reasons of safety (Maloney et al. 2014) and the adjustment of dosing schedules. Standardised clinical trials with allergen specific immunotherapy are essential (Canonica et al. 2007). The WAO has published a position paper on the practise of Sub-lingual immunotherapy (Canonica et al. 2009) and recommendations for appropriate sublingual immunotherapy clinical trials have been published (Casale 2009). Equally strict measures must be taken to ensure that the grass seasonal limitations are clearly defined.

Weather directly influences the levels of pollen and fungal spores in the atmosphere (Barnes et al. 2001, Grinn-Gofron 2011, Green 2004). Meteorological parameters known to influence pollen and fungal spore concentrations in the atmosphere include: Temperature, relative humidity, wind speed and direction and rainfall (Grinn-Gofron et al. 2018, Puc 2011).

## 1.3 The research problem and objectives

There are no long-term (i.e. greater than three continuous years) published studies that compare regional pollen and fungal spore data sourced from Cape Town pollen sampling sites. The inland and coastal suburbs of the Greater Cape Town Metropolitan area, where the pollen sampling sites are situated, are topographically different with varying vegetation types (Manning and Goldblatt, 2012). The pollen taxa trapped at the site reflects the vegetation surrounding the site Therefore a thorough knowledge of the vegetation and land use of the area surrounding the sampling site is essential. The topography, climate and meteorological parameters must be carefully examined. Although it is generally accepted that the area within a 30-km radius of each sampling site (Eng *et al*, 2002, Kiotseridis *et al*, 2013) would fall within the range of that particular sampling site, this premise may not hold true if there is great variability of the geographical features within a relatively small area, which would favour the growth of different plant species (Katelaris 2004). We compared the pollen and fungal spore findings from the sites to see if one sampling site could adequately provide a pollen count that could be used for patients attending the paediatric and adult allergy clinics of the two academic hospitals, Red Cross War Memorial Children's Hospital and Groote Schuur Hospital.

### 1.4. Specific questions

By using the two continuous long-term time series, namely the sites in Rondebosch and Table View between 1997-2002 and the sites in Observatory and Table View between 2010 and 2014, the following questions were asked:

1. Are pollen and fungal spore counts from the coastal and inland areas significantly different?
2. Are the dominant pollen taxa similar at the different sites, when pollen indices are compared?
3. If significant differences are found in the pollen count, are there major difference in the tree, grass and weed catch or fungal spore genera?
4. Were there differences between the start and end dates of the grass season and the tree season at the coast and inland?
5. Were peak pollen days seen on the same day, or different days at the coastal pollen site Table View and inland, at the Rondebosch or Observatory sites?
6. Were peak days for fungal spores (overall load or individual genera) recorded on different days at the coastal and inland sites?
7. Were there peak days at the coast that did not occur inland and vice versa for any of the pollen taxa or fungal spore genera such as *Pleospora* in Table View?
8. Why are *Cladosporium* levels so low in the Cape, when *Cladosporium* sensitisation levels are similar to those of other temperate areas?
9. Is the suggested *Cladosporium* threshold (Bagni, Davies & Mallea et al 1977) concentration soundly reasoned or do high wind speeds, >2 m s disperse *Cladosporium* spores before they can be trapped?

10. Are selected weather variables such as temperature, relative humidity, rainfall and wind speed and direction positively or negatively associated with peaks or changes in any of the pollen or fungal levels?
11. Can changes in the sampling loads by category and individual pollen taxa over the whole length of the time series at the Rondebosch and Table View sites be demonstrated?
12. Has the average temperature in the Western Cape as measured between 1980 and 2014 changed and can this be correlated with the observed decline in grass or tree pollen levels? Land use, or an increase in buildings is a big factor in decreasing grass pollen. If a decrease in temperatures and concomitant declining pollen levels is demonstrated, might this phenomenon indicate the consequences of Climate Change?
13. When the cut-off levels of a specific pollen taxon such as grass are needed for selecting consequent trial dates for research, or investigating therapeutic treatment for pollinosis such as intranasal steroids or antihistamines, should the geographic locations of the sampling sites be matched to the areas where the patients live and work?

### 1.5 Aims and objectives

Anticipated outcomes for the study were:

(i) To define the diversity of the major allergenic airborne pollen taxa in Cape Town.

(ii) To identify trends in the pollinating season and shifts in the start, peak and end dates of the major allergenic pollen taxa.

(iii) To rank the major allergenic pollen in order of prevalence.

(iv) To examine the influence of temperature on the concentrations of grass pollen in the atmosphere.

(v) To rank the major allergenic fungal spores in order of prevalence.

(vi) To identify seasonal trends in the annual fungal spore load for the major fungal spore aeroallergens.

(vii) To investigate and examine the low *Cladosporium* concentrations in the Cape and to see if there are significant associations with meteorological parameters.

## 1.6 Research design

Discussions with my supervisor and Head of Department in 2009, identified the need to compare the data from the pollen sampling sites I had operated in Cape Town in the suburbs of Rondebosch, Table View, Mowbray and Observatory from 1984-2010. The findings from the pollen sampling were helpful in choosing the optimal time for therapy for allergy sufferers, for the timing and design of antihistamine research studies and for patient recruitment from the Cape Town area. A comparison of the air spora trapped at the sites would show whether one or more pollen sampling sites are needed for Cape Town for clinical practice. The pollen sampling site at Table View was the only operational site in 2009. We decided to reinstate the Observatory pollen sampling site and run it concurrently with the Table View spore trap to obtain pollen sampling records for direct comparison. The Observatory and Table View sampling sites were operated concurrently for the years 2010-2014.

Archived daily pollen and fungal spore concentrations were available from the Cape Town sampling sites at Rondebosch, Table View, Mowbray and Observatory from 1984-2012. The pollen and fungal spore data sets from these three sites were accessed, collated and compared.

*Specific inclusion criteria related to the data*

(i) The pollen sampling sites had to be in the Western Cape Metropolitan area.

(ii) Sites were selected for comparison where a minimum of four years of continuous data were available at a coastal site and inland site

(iii) Sites were selected where sufficiently populated spreadsheets with data that had been captured during the sampling years existed.

(iv) Data sets near the beginning and the end of the collection period were selected to compare air spora concentrations from the entire 30-year data set.

(v) Datasets from sites within range of weather masts where meteorological data were available were selected for comparison.

*Data analysis*

The 30-year database was examined and analysed throughout the study but two data sets were chosen for specific comparison, where possible, as each contained the best populated years and these were the following:

*Comparison I: 1997-2002*

Continuous data sets from an inland site, at Rondebosch and the coastal site at Table View (TBV) were chosen for the first long term time series analysis, using the air spora sampling years 1997-2002.

*Comparison II: 2011-2014*

Continuous data sets from an inland site, Observatory (SAAO) and the coastal site at Table View (TBV) were chosen for the second long-term time series analysis using the air spora sampling years 2011-2014.

*Weather and the air spora*

The influence of weather on Poaceae pollen and *Cladosporium* concentrations and their associations with selected meteorological parameters was examined in the inland and coastal study areas for the years 2011-2014. Seasonal variations in Poaceae and Cladosporium concentrations were identified.

## 1.7 Research Methods

*Pollen Analysis*

Pollen taxa at the different sampling sites were compared in terms of prevalence, seasonal start, peak and end dates and duration. The composition of each sampling site was assessed. The total pollen load was divided into the components: Tree, grass and weed pollen.

The concentration and seasonal profiles of Poaceae (grass) and the trees Cupressaceae, *Platanus* and *Quercus* were compared.

*Analysis of fungal spores*

Fungal spore loads at the sampling sites were compared by assessing the seasonal peaks and concentrations of *Alternaria*, *Cladosporium*, *Epicoccum* and *Pleospora*. The Annual Fungal Index (AFI) for each site was found and the prevalence of each of these fungal genera was calculated.

## 1.8 Application of the research

A comparative analysis of the long Cape Town database is crucial to the understanding of how a representative pollen count should be produced. A representative pollen count would be a count that could be used in allergy clinics for patients who live in coastal and inland areas. The decision to use one or more pollen sampling sites has important implications for future pollen sampling studies in the area and the continuation of pollen monitoring in the

Cape Town. The allergist or clinician needs to know the dates of the start and end of the pollen season to implement immunotherapy regimes for allergic patients appropriately. The prediction of pollen seasons and the forecasting of high pollen concentrations is the ultimate goal for pollen sampling. (de Weger et al. 2014). Identifying seasonal pollen flowering patterns would establish the groundwork for the prediction of pollen seasons and high concentrations for allergenic trees and grasses in Cape Town, for clinicians who manage allergic disease at academic hospitals and in private practice.

**1.9 Chapter Outline**

- Chapter 1 introduces the thesis, explains the research problem and lists the aims and objectives.
- Chapter 2 deals with a review of the relevant literature from the Northern and Southern Hemispheres and notes the lack of published data from African pollen sampling studies.
- Chapter 3 discusses the project methods, pollen sampling techniques and statistical analyses in the thesis.
- Chapter 4 describes the establishment of the pollen sampling sites in Cape Town and describes the topography, climate and vegetation of the areas surrounding the sites.
- Chapter 5 focuses on the findings. The air spora are mapped and the concentrations and seasonal parameters of the four pollen taxa: Poaceae, *Cupressus*, *Platanus* and *Quercus* and four fungal spore genera: *Alternaria*, *Cladosporium*, *Epicoccum* and *Pleospora* are compared.
- Chapter 6 examines the influence of weather on Poaceae and Cladosporium identifying positive and negative associations with meteorological parameters.
- Chapter 7 discusses the novel findings and the strengths and weaknesses of the research and ends with conclusions and recommendations for future research.

# Literature Review

## 2.1 Introduction

Pollen and fungal spore concentrations in the atmosphere guide clinicians in the diagnosis and treatment of their patients, who are sensitised to these aeroallergens. (Baena-Cagnani, 2001) Asthma, allergic rhinitis and conjunctivitis can be triggered by grass, tree or weed pollen, or fungal aeroallergens. Seasonal or intermittent allergic rhinitis usually occurs in spring and is often the response to a pollen allergen (D'Amato et al. 2007).

The fungal spore aeroallergens, particularly *Alternaria* and *Cladosporium,* increase in response to specific meteorological conditions and their seasonal peaks commonly occur in spring and autumn (Sabariego et al. 2000; Stennet & Beggs, 2004; Recio, et al. 2012). However, growth requirements of fungal spore aeroallergens differ, so peaks may occur at other times during the year. Stepalska observes that there is great variation in the seasonal peaks of fungal aeroallergens (Stepalska and Wolek, 2005). Seasonal peaks for fungal spores are not as clearly defined as the seasonal peaks that have been defined for pollen.

The starting dates of the seasonal aeroallergens are colloquially known as 'start dates' in most studies. It is useful to identify the start date of the major pollen allergens like Poaceae and *Betula*. Allergologists refer to the start dates in the treatment of allergy and asthma. Medication may protect sensitized patients in advance of the season, in regions where start dates for grass aeroallergens can be predicted (Beggs et al. 2015). The flow chart in Figure 2.1 below shows the aspects of pollen and fungal spore sampling that are relevant to the thesis and are discussed in this review of the literature

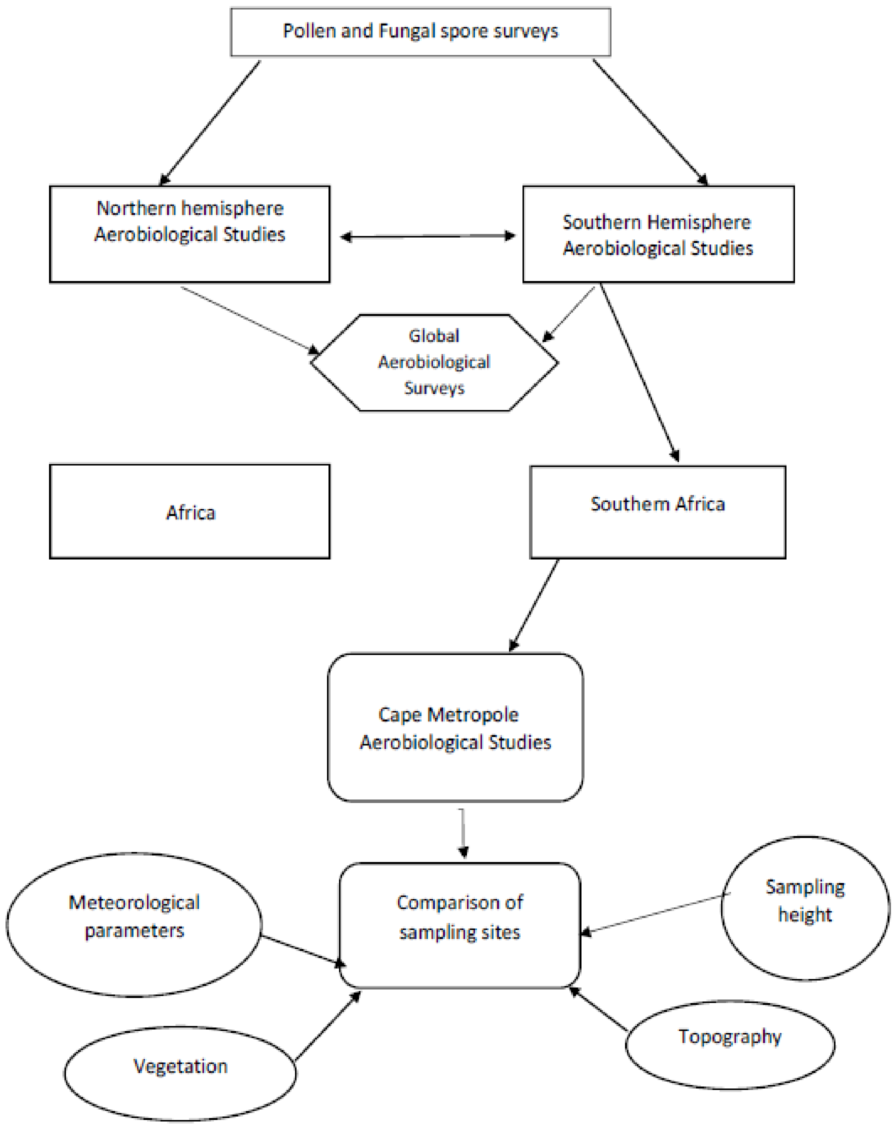



Figure 2.1 Flow chart of the aspects of aerobiology considered in the study.

## 2.2 Northern hemisphere aerobiological surveys

Early aerobiological studies, in Europe and North America, have provided a sound basis for current aerobiological research. Oren Durham pioneered pollen sampling in the United States when he designed the Durham gravimetric spore trap in 1925 (Singh and Mathur, 2012). Air spora trapped with Durham spore traps were reported per square centimetre of air and Durham spore traps are still in use today (Erkara, 2009). These samplers are simple, portable and inexpensive.

Hirst improved on the spore trap design by producing the Hirst automatic seven day recording volumetric spore trap, which operates unattended for seven days. When the Hirst spore trap came into use, the trapping surface was exposed to a known volume of air. Therefore, pollen and fungal spores could be quantified per cubic metre of air per 24-hour period. The hour of capture of the air spora could also be deduced (Hirst, 1952).

The microbiologist Philip Gregory, studied bioaerosols and their individual aerodynamics, tracking their routes, as they fell through the air in flight paths dictated by their shapes. (Gregory 1973). Hirst and Gregory collaborated in an aerobiological study, assessing frequencies of the air spora at the Plant Pathology experimental station, Rothamsted. The investigators compared sporing patterns with sunshine hours, rainfall, temperature and relative humidity. They compared data collected at 2 metres and at 24 metres above ground at the same site, during the peak pollen season and found the spore trap placed at 24 metres above the ground trapped air spora in the range 68% to 81% of the air spora collected at the lower sampling height (Gregory & Hirst, 1957). Hyde and Williams pioneered air spora sampling in Cardiff (Hyde & Williams 1944). These early forefathers of aerobiology set high standards for subsequent aerobiological research.

Aerobiology and the relationship between allergology and seasonal pollen was investigated from 1942 in Sweden. Bringveld et al. investigated the influence of meteorological factors on *Betula* (birch tree) pollen to predict start dates. (Bringveld, Engstrom & Nilsson 1982). These researchers extended the elegant research of Hyde & Williams (Ibid) and Davies in the United Kingdom who studied grass seasonality. (Davies & Smith 1974).

The presence of fungal spores was measured and spores were found in the atmosphere up to 1,000 metres above ground, but the comparison of different areas within a city or region was not widely investigated in early studies (Nilsson, 1973). The Natural History Museum in Stockholm houses a valuable reference centre for the identification of modern pollen grains (personal experience-visit to the Natural History Museum 1990). This includes the collection of more than 25,000 pollen grains and fungal spores, mounted and collected by Professor Erdtman, a pioneer of pollen analysis, whose beautiful drawings of pollen grains are held in a collection that is accessible to visiting researchers to this day (Edlund, 2014). Professor Gunnar Erdtman founded *Grana*, an international journal of Palynology and Aerobiology in 1954 (Nilsson & Praglowski 1978).

**2.3 Northern Hemisphere long- term pollen sampling**

As early as 1988 an inventory was drawn up listing long term pollen sampling sites in Europe (Nilsson 1988). Long term pollen surveys from Europe (Spieksma et al. 1995; Emberlin, et al. 1999, Gioulekas, et al. 2004) are invaluable, as they assist in the construction of pollen calendars from the sampled areas, which are usually within cities. These studies profiled regional pollen and fungal spores and provided seasonal information for clinical use. Formulae using archived data, current data, meteorological parameters and phenological plant models are still used to forecast the start and peak dates for major pollen allergens, for the clinicians who treat allergic disease (Eggen et al. 2013).

*European pollen networks*

Owing to the high density of the population in Europe and the relative proximity of many of her cities, multiple European pollen monitoring stations exist. France operates at least 70 Hirst-type spore traps, forming the Réseau National de Surveillance Aérobiologique (RNSA) pollen network (Rieux, Personnaz & Thibaudon 2008; 43). Spain operates the Spanish Red Española de Aerobiología (REA) network, (Soldevilla, et al. 2007) and there are many more such pollen networks in Europe. There are larger pollen networks such as the European Aeroallergen Network (EAN) pollen database in Vienna. This network houses pollen information from more than 600 pollen counting stations throughout Europe. It is an interactive website for aerobiologists, scientists, allergologists and the general public. (Jäger, 2000).

*Pollen networks in North America*

A similar pollen reporting network exists in North America. The American Academy of Allergy, Asthma and Immunology (AAAAI) collates data from more than 80 counting stations in North America and two in Canada. Current pollen and fungal spore reports are available online. Predicting significant pollen levels is routinely carried out and the website is used by allergologists, health care professionals and the public (Portnoy & Barnes 2004).

**2.4 Southern Hemisphere long-term pollen sampling**

The prevalence of pollen and fungal spore monitoring in the Southern Hemisphere is vastly different to that of the Northern Hemisphere. On continents such as Australia and New Zealand, Africa and South America, there are fewer pollen sampling sites and studies, when compared to countries in the Northern Hemisphere. Haberle collated data from pollen sampling sites in Australian cities (Haberle et al. 2014). A national pollen network is currently being formed in Australia.  Pollen sensitive patients will be able to access up to date information about the levels of aeroallergens in the atmosphere via an App (www.pollenforecast.com.au). In the Southern Hemisphere, a network for pollen information titled the Asia Pacific Aeroallergen Working Group (APAWG) is in place. It is affiliated to the World Allergy Organization (WAO) (Pawankar et al. 2011). APAWG was initiated by Professor Katelaris. Editor Dr Hasnaian and Assistant Editor, Professor Katelaris also produced the newsletter of Athe Asia Pacific Working Group for the support of emerging societies and to encourage research of the aeroallergens responsible for patient sensitization tand the development of allergic disease such as asthma and rhinitis. There was also a need to alert their clinical colleagues to the role of outdoor and indoor aeroallergens. http://www.worldallergy.org/enews/0209/

*Australia*

Comprehensive long-term studies have been carried out in most of the major Australian cities. In Sydney (Katelaris & Burke 2003, Stennet & Beggs, 2004) Melbourne (Ong, Singh & Knox, 1995, de Morton et al, 2011), Brisbane (Green, Dettman & Rutherford et al, 2004) Darwin (Johnston, Hanigan and Bowman 2009, Stevensen, Haberle, Johnston et al, 2007) and Tasmania (Tng, Hopfl, Haberle et al, 2010), long term pollen records have been applied to research such as grass pollen concentration predictions and pollen concentrations at Olympic Game Venues.

*India*

In India, pollen sampling has been carried out in different areas of the subcontinent and pollen sampling records exist for Calcutta (Mandal, Chakraborty & Roy et al, 2008), Delhi (Singh, Pandit and Dahiya, 2003), Allahabad (Sahney, Chaurasia, M & Chaurasia, S, 2008), Bangalore (Agashe, S.N. and Alfadil, A.G., 1989), Jabalur (Mishra, Singh & Oommachan, 2002) and Madras City (Satheeshkumar and Vittal, 1998) for example. An overview of 50 years of Indian aerobiological surveys is a comprehensive guide to pollen sampling in India (Singh & Dahiya, 2008). More specific research includes the work of Agashe et al who studied Casuarina pollen in tne atmosphere and its significance as an aeroallergen (Agashe et al, 1994).

*Japan*

Aerobiologists in Japan have studied the pollen of Japanese cedar (Wang, Kurihara, Kiryu et al 2008). The relationship between Japanese cedar pollinosis and air pollutants deposited on the pollen was significantly different relative to the number of motor vehicles in the study areas. Diesel exhaust vehicles were seen to increase in the study area (Hirotaka, Shunkichi, & Kazunori 1996). A meta-analysis showed that pollinosis to cedar pollen had increased but the results were variable for age and domicile (Kaneko, Motohashi & Nakamura et al 2005).

*South Korea*

Pollen monitoring studies have been undertaken in the Korean Peninsula, (Park, Kim, H, & Kim KR et al 2008), in Seoul, South Korea (Oh, Lee, HB & Lee, HR 1998) and in Ulsan (Jung & Choi, 2013) are examples.

,

*South America*

In South America, airborne pollen concentrations of cities such as Santiago, Chile (Villegas & Nolla 2001) Medellin, Columbia (Guarin, et al. 2015) have been described.

*Buenos Aires and Cape Town*

Pollen records from Argentina are especially interesting as the capital, Buenos Aires is situated on the same latitude, as Cape Town, at 34° S. Like Cape Town, aerobiological recors show the pollen peak occurs in October, while a minor peak for the weed Myrica is seen in March. However, the climate is far more humid than that of Cape Town and fern spores and mosses abound. In Buenos Aires, Tauber traps were used for sampling so comparative abundance of the pollen analysed from the traps in the two cities cannot be compared. Protea, Podocarps, Poaceae, *Celtis* spp and *Ulmus* were identified which is commensurate with the pollen seen in Cape Town. However, some seasonal peaks, such as Poaceae differed substantially and were consistently later in the calendar year in Buenos Aires than in Cape Town by approximately six weeks. (Majas, & Romero 1992). The later peak for Poaceae was also seen in Patagonia, although the main pollen peak occurred in October and San Carlos de Bariloche, Patagonia, Argentina (Bianchi & Olabuenaga, 2006).

**2.5 Pollen sampling in Africa**

On the African continent, researchers in various disciplines in Nigeria have studied the air spora. In a survey of the modern pollen rain in terms of variety, abundance and seasonality, differences in sampling height were examined at Nsukka (Njokuocha 2006). At Ota, in south-western Nigeria, an unusual Harmattan dust was analysed by palynologists (Adeonipekun & John, 2011), while air spora from Anyigba, in the eastern district of Nigeria were mapped (Essien et al. 2014). Again, in Nigeria, air spora of Lagos were monitored to determine their abundance and seasonality (Adeniyi et al. 2014). Tauber traps, rather than Hirst-type volumetric spore traps, were used in these studies.

**2.6 Pollen Sampling in South Africa**

Pollinosis and the regional differences in pollen and fungal spore levels in the atmosphere were first observed in South Africa when grass levels were quantified using a Durham gravity spore trap (Ordman 1947). Cupressaceae pollen from cypress trees which pollinated from May to October in Johannesburg, ahead of the remainder of the spring pollinating trees was seen in an early aerobiological study (Ordman, 1963). Ordman observed that that the grass flowering season in the winter rainfall area of the Cape occurred before the Johannesburg grass season. Ordman’s descriptions of the Cape tree and grass flowering seasons were important observations, but were not supported by aerobiological surveys (Ordman, 1947). Nonetheless, Ordman’s careful observations were proved to be accurate when aerobiological surveys were subsequently undertaken in the two cities (Berman 2013).

Air spora studies have been used to measure the pollen concentrations and fungal spore loads in South African cities in Gauteng (Cadman 1990) and in KwaZulu Natal (Cadman & Dames 1993, Dames & Cadman 1994, Vismer et al. 1995), but just two short studies extended the knowledge of the atmospheric air spora in Cape Town (Hawke 1989; Cadman & Dames, 1996). Hawke and Meadows (1989) conceded that their analysis the of air spora and the influence of meteorological parameters spanned the very short period of five weeks during winter, when pollen and fungal spore levels are usually low. This mid-winter period and brief sampling was not representative of fungal spore loads measured over one year. Despite the short sampling time, they posited that higher air spora levels were associated with low wind speeds and that wind speeds greater than 7 m/s adversely affected trapping efficiency. This hypothesis might explain why some small particles are absent from the catch in the air spora studies of the Cape Metropolitan Area (Berman 2013).

### 2.7 Accurate regional pollen concentrations

Katelaris emphasizes that the correct siting of the spore trap for aerobiological studies, or pollen sampling, is extremely important because the air spora findings must accurately represent the city or the area, for which the study is designed (Katelaris, Burke and Byth, 2004). The aim of any aerobiological survey should be to use the minimum number of spore traps that will accurately measure the largest possible area. This is advisable, as pollen sampling is expensive and time consuming.  The correct positioning of the pollen sampler in the study area is crucial to the production of a representative pollen count (Soldevilla et al., 2007). Regional pollen or fungal spore levels, identified from aerobiological surveys using Hirst type volumetric spore traps, have been correlated with seasonal symptoms such as allergic rhinitis in clinics around the world (Potter et al. 1991; Bass et al. 2000; Bousquet et al. 2005; Dursun et al. 2008; Johnston, Hanigan & Bowman 2009).

#### 2.7.1 Sampling Sites in different locations within a city

The data from one sampling site only, might not adequately represent the pollen and fungal spore profiles for the sampled area when pollen concentrations are required for clinical trials. Katelaris points out that the data from a single spore trap in a city may be adequate for public information purposes. However, the same findings may be inadequate for a clinical drug trial where direct individual patient exposure to pollen may differ, with varying pollen concentrations and seasonal cycles in different suburbs of a city (Katelaris, Burke & Blythe 2004).

In some aerobiological studies designed for diagnostic and therapeutic use, one site was considered to be sufficient (Potter et al. 1991, Arobba et al. 2000, Mardones et al, 2013). A four-year study of aeroallergens at Red Cross Children's Hospital in Rondebosch was correlated with the clinical and immune responses of 209 paediatric clinic patients. The pollen and fungal spore concentrations were measured at the Red Cross Children's Hospital sampling site in Rondebosch. Major aeroallergens from the sampling study were compared with the specific IgE responses of outpatients from clinics with symptoms of upper respiratory infection and lower respiratory symptoms, suggestive of an allergic basis to rhinitis and asthma. The immune responses in atopic patients with environmental allergens correlated with their pollen and fungal spore exposure. The findings showed that one third of the 209 children with suspected allergic disease had specific IgE responses to the major sensitising environmental allergens as follows: *Dermatophagoides pteronyssinus* 73%, South African grasses 38%, tree pollens 22.4%, flower and weed pollens 19.6%. Specific IgE responses to the major fungal spores were *Alternaria* 18.6%, *Cladosporium* 8.1% and *Epicoccum* 8.1%. Daily pollen concentrations from one sampling site were used for patients who lived within a 30-km radius of the sampling site.

Fernández-Rodriguez addressed the issue of the placement of sampling sites when he examined the effect of distance on the pollen measurements in a city in south west Iberia (Fernández-Rodriguez et al. 2014). This study showed a correlation between the daily values in sites 2.9 km apart, but mean pollen concentrations for allergenic pollen ranged from little or no difference to statistically significant differences in the three-year study. These differences could not be explained by association with meteorological variables and were attributed to different vegetation types.

Recent studies have demonstrated that in some cities, one sampling site alone cannot accurately supply pollen measurements for the area it serves (Weinberger, 2015). Weinberger demonstrated that there was significant spatial variability for tree pollen in New York city by collating data for one pollen season. Tauber spore traps were set up at 45 sites within the city. Cumulative tree pollen counts in the study showed significant variability and ranged from 2 942 to 17 460 grains per $cm^2$ when sited in different locations within the suburbs of New York city, New York.

Analysis of the data from pollen networks has been useful in assessing the spatial variation of selected allergenic pollen taxa (Emberlin and Norris-Hill 1991). Frenz reported that data could be extrapolated from one site to another within a city, when he compared sampling data from sites 5.6 km apart. He added the caveat that the extrapolation of data from one site to a wider area, should be used cautiously. He noted that differences between the two sites within the city of St Paul, Minnesota, increased when the pollen count was more than 100 grains/$m^3$ (Frenz et al, 1997). However, this deduction was based on data collected during only one pollen season and may not be a consistent finding when data collected over several years are analysed.

Cadman & Dames measured pollen and fungal spores at two sites in Cape Town at Kirstenbosch and Parow in 1993, using Burkard volumetric spore traps (Cadman & Dames, 1996). Cadman found significant differences in the pollen totals between the two sites, with a cumulative annual total of 2034 pollen grains at the Kirstenbosch site as compared to 648 at Parow. The annual grass index at Parow was 337, whereas the annual grass catch at Kirstenbosch was a mere 120 grains. A low incidence of indigenous, or fynbos pollen (Manning & Goldblatt 2012:6) was seen at both sites, even though Kirstenbosch is a National Botanic Garden and home to a plethora of indigenous plants. This is not an unexpected finding as fynbo*s* pollen rarely belongs to the wind pollinated group. Most of the fynbos taxa are pollinated by insects, birds or small mammals (Cowling & Bond 1991; Bond 1994,) Cadman suggests that it is preferable to use several sites when air spora sampling is undertaken in a city with the complexities of Cape Town. Kirstenbosch is situated on the lower slopes of the eastern slopes of the Table Mountain, and it is unlikely that grass pollen counts in that part of Cape Town would be representative of grass levels in most of the Greater Cape Metropolitan area.  Kirstenbosch National Botanic Garden is sheltered from the prevailing south-easterly summer wind (http://www.sanbi.org/gardens/kirstenbosch) so the transport of grass pollen to this site is likely to be limited. Cadman describes the Parow site as a 'typical suburban environment' (Cadman & Dames 1996: 80). These findings differed from pollen counts measured in Parow in 2003 (Berman: unpublished data) probably due to the difference in the sites that were chosen.

### 2.7.2 Exposure Threshold Concentrations and Scales

It has been difficult to set practical concentration thresholds for pollen and fungal spore aeroallergens. The frequencies and ranges of the aeroallergens in the hemispheres, countries and regions have differed (Galan et al, 1995, Green et al, 2004). Burge developed a scale using centile ranges from long term concentrations of pollen and fungal spores (Burge 1992). The scale gave ranges for trees, grasses, weeds and fungal spores which were labelled as 'low' 'medium' and 'high.' For grasses, the threshold between low and moderate is given as 5 grass pollen grains per $m^{-3}$ where low grass counts were defined as 0-5 grains per $m^{-3}$. The scale was based on results from 51 pollen stations in the United States of America.

Burge's scale was not adopted by all the pollen stations. In Washington for example, Burge's scale was considered inappropriate for the heavily treed area in that city. Kosisky developed a scale based on the 12-year database from the Washington pollen stations with different ranges (Kosisky et al. 2011). Burge's scale was considered the most appropriate scale for the Cape Metropolitan Area study and has been used in that region for the past two decades.

### 2.7.3 Sampling height above ground

Retrospective data analyses often compare data from sites that were not originally designed for comparison. Emberlin observed in the 1987-1992 survey that the spore trap sampling heights in the study have a range of 8-32 metres above the ground. The altitude range was equally variable at 21-110 meters above sea level (Emberlin et al. 1994). Similar problems regarding disparate sampling heights for fourteen sampling stations in Australia and New Zealand were noted when comparing grass pollen concentrations (Medek et al 2015). Where spore traps are mounted at comparable heights, as was seen in the sampling sites of Sydney, Australia (Katelaris, Burke and Byth 2004) or in Spain (Infante et al, 1999) comparisons of trapped aeroallergens are more reliable. Räntio-Lehtimaki compared spore traps at ground level and at 15 metres and concluded that tree pollen and basidiospores were less affected than weed and grass pollen. Grass pollen started flowering two weeks earlier at ground level and grass concentrations were more than four-fold higher at ground level for grasses. (Räntio-Lehtimaki et al.1991). This finding does not agree with the 19-32 % difference calculated by Gregory and Hirst (see 2.2) but this may be explained by the difference in wind speed and/or direction in the two studies. Galan recommends that the spore sampler be elevated above the ground for the minimal requirements of sampling but does not recommend a particular height (Galan et al. 2014).

A disadvantage of using retrospective pollen sites where methods were not standardised is that pollen levels are affected by the sampling height of the spore trap. In one study the heights in the analysis varied from 2 to 14 metres above the ground (Haberle et al. 2014:3). The researchers overcame this difficulty by reporting pollen frequencies as a percentage of the Total Pollen Index (TPI) for each site. This large scale ecological study from Australia and New Zealand established the basis for using pollen as a biogenic indicator of Climate Change. This is accomplished by tracking seasonal pollen changes across continents in response to changing climatic conditions.

### 2.7.4 Seasonal dates

The start, peak and end dates and the duration of the pollen season for grass, tree or weed pollen may vary between sites and they may vary at the same location in different years, in response to varying weather patterns. Heavy rainfall and droughts affect pollen concentrations (Suphioglu et al, 1992, Knox 1993, Medek 2015). Fungal spores in the atmosphere respond to changes in weather patterns (Sadys 2016, Grinn-Gofron et al, 2011). It is therefore desirable to measure pollen and fungal spore concentrations over several years and to continually update pollen calendars which may change over time (Medek 2015, Berman 2013).

### 2.7.5 Meteorological parameters and the air spora

Climate dictates the vegetation type in a geographic region (Manning & Goldblatt, 2012: p3) but varying pollen seasons and concentrations are associated with weather. Temperature; $T_{max}$, $T_{mean}$ and $T_{min}$, relative humidity, precipitation and sunshine hours may be correlated with pollen and fungal spore concentrations (Barnes et al, 2001, Grinn-Gofron 2011, Green 2004). Examples of the studies that demonstrated associations between meteorological parameters and air spora are discussed.

*Temperature and the grass pollen season*

Positive and negative associations with pollen taxa and fungal spore genera in global studies have been shown. Grass pollen peak concentrations and cumulative temperature have been positively associated in Northern hemisphere studies in England (Davies and Smith 1974; Emberlin et al. 1999) A positive correlation between the grass season and minimum and maximum temperature was shown in Brisbane, Australia in the Southern Hemisphere (Green et al. 2004).

*Relative Humidity*

*Alternaria* concentrations were shown to be negatively correlated with relative humidity levels in the atmosphere. Although rainfall and increased Relative Humidity (RH) are necessary for sporulation, ideal conditions for the release of *Alternaria* conidia were shown to be high temperature and low RH (Sidel et al, 2015).

*Tree pollen, rainfall and wind direction*

Wind direction was found to be significantly correlated with airborne pollen. *Platanus Quercus* and Cupressaceae concentrations were correlated with land use and wind direction for the years 2008-13. Higher annual *Platanus* pollen concentrations in 2010 were significantly correlated with higher rainfall levels from the previous winter and tree pollen was significantly correlated with wind speed and direction (Rojo et al. 2015).

*Spore plumes and wind speed*

Wind speed was one of the meteorological variables that was associated with large spore plumes in the atmosphere of Tulsa, Oklahoma. *Cladosporium* is known to colonize grain crops and very high levels have been found in crop farming areas when spore plumes that contained large numbers of spores borne by strong winds, formed spikes of fungal spores. These spikes typically occurred at midday and often preceded thunderstorm. Increases in *Alternaria* and *Cladosporium* have been seen when temperature, air pressure wind speed, and dew point levels increased. An association with thunderstorms was seen and the large increases in spore plumes, which increased very quickly from 20,000 to 200,00 spores/$m^3$ constituted a significant exposure risk for residents sensitized to *Cladosporium* (Burch & Levetin 2002).

*Sunshine hours*

Mean temperature and sunshine hours were positively correlated with increased tree, grass and weed pollen in Ankara, Turkey and increased sunshine hours were found to be a significant risk factor for weed pollen (Kizilpinar 2011). In a study comparing grass concentrations in Montreal and Spain, mean temperature and sunshine were positively correlated with grass concentrations and negatively correlated with relative humidity (RH) (Valencia-Barrero, Comtois & Fernandez Gonzalez 2001).

*Rainfall and fungal spores*

Rain generally reduces the bioaerosol content of the air by washing most fungal spores and pollen grains from the atmosphere. Conversely, some fungi, like ascospores and basidiospores, require rain for conidial release (Weber 2003).

### 2.7.6 Cladosporium concentrations

*Cladosporium* is an important aeroallergen and it is frequently the dominant fungal spore in aerobiological surveys in temperate areas (Oliveira et al. 2010). In their global review of fungal spore emissions, Dallafior and Sesartic (2010) found nearly half the Annual Fungal Index to be composed of fungal spores. Atmospheric levels are influenced by temperature, rainfall and wind speed and direction (Recio et al. 2012).

*Cladosporium* belongs to the order, now classified as the Ascomycota, previously named Fungi Imperfecti (Levetin et al. 2016). The fungus colonizes decaying plant material and releases large numbers of conidia or spores, which may be found in the indoor or outdoor air (Recio, et al. 2012). Positive skin prick and specific IgE tests for *Cladosporium* are frequently reported. At Red Cross Children's Hospital in Cape Town, patients with IgE specific responses to *Cladosporium* were shown to have recurrent, seasonal admissions to the hospital's intensive care unit with acute severe asthma attacks (Roux, Smit & Weinberg 1993; p178). *Cladosporium* spores are small and therefore easily respirable, so they reach the lower airways and are known to be important triggers for asthma (Taylor & Jonsson, 2004).

*Cladosporium threshold levels*

The threshold level for triggering symptoms in sensitized individuals has been set at 3000 spores/$m^3$ (Gravesen 1979; 138). The evidence to support this claim is not strong, although this threshold is frequently quoted (Larsen, 1981, Cadman, 1991, Peternel Culig, J. & Hrga, 2004, Thibaudon & Lachasse, 2006) The main basis for setting the 3000 spores/$m^3$ threshold concentration is the premise that allergenicity is directly related to the size of the pollen grain, or fungal spore. The size of the *Cladosporium* spore was compared to the size and diameter of a grass pollen grain and the threshold concentration was calculated accordingly. (Bagni, et al. 1977).

C*ladosporium prevalence*

*Cladosporium* is frequently ranked amongst the top five fungal spore allergens, when tested in allergy clinics (Potter et al. 1991; 82,) and it is the most abundant spore in many sampled countries (Calderón et al. 1997, Fernández-Rodríguezet al. 2014, Sadyś et al. 2015). Positive skin prick and specific IgE tests for *Cladosporium* are frequently reported. At Red Cross Children's Hospital in Cape Town, patients with IgE specific responses to *Cladosporium* were shown to have recurrent, seasonal admissions to the hospital's intensive care unit with acute severe asthma attacks (Roux, Smit & Weinberg 1993; p178). *Cladosporium* spores are small and therefore easily respirable, so they reach the lower airways and are known to be important triggers for asthma (Taylor & Jonsson, 2004).

Furthermore, *Cladosporium* was shown to have strong non-random associations with *Alternaria alternata* and *Aspergillus fumigatus* in a population sample of 2094 allergic adults and children attending outpatient clinics in Cape Town. These strong, clustering, allergen specific IgE associations amongst this group of Ascomycota could not be attributed to cross reactivity. It was observed that 80% of the patients sensitised to Cladosporium were found to be concordantly also sensitised to *Aspergillus fumigatus* and *Alternaria alternata*. (Potter et al, 1991; 151).

*Cladosporium in temperate areas*

*Cladosporium* concentrations are found in both the northern and southern hemispheres, but concentrations are higher in temperate regions (Şakiyan & Inceoğlu, 2003). *Cladosporium* is known to colonize grain crops and very high levels have been found in crop farming areas when spore plumes that contained large numbers of spores borne by strong winds, formed spikes of fungal spores. These spikes typically occur at midday and often precede thunderstorms. The plumes at times contain *Cladosporium* concentrations which exceed 140 000 $m^3$ of air. The sudden large increases in airborne *Cladosporium* spores is of great clinical significance for hay fever sufferers (Burch & Levetin 2002; 109).

*Low Cladosporium levels from global coastal sites*

*Cladosporium* levels have been found to be lower in coastal cities than in cities further inland, within the same vegetation biome and this has been confirmed by comparing the measurements from networks of air sampling stations operating within the same country. When reporting the findings from their networks, which included coastal and inland sampling stations, the concentrations in coastal cities, e.g. Barcelona have been found to be consistently lower than the concentrations sampled in inland cities (Infante 1999. Aira *et al*. 2008).

*Cladosporium* concentrations at different altitudes

It has been shown that *Cladosporium herbarum* spore counts rise incrementally with height above sea level. (Rodriguez-Rajo, Iglesias & Victoria, J. 2005; Jesús Aira et al. 2008). Low *Cladosporium* concentrations were noted at Ahmadi, a suburb of Kuwait, on the Persian Gulf. These low concentrations were attributed to temperatures greater than 29°C, during summer, which exceeded the upper temperature threshold for *Cladosporium* spores. These high temperature ranges also account for the summer lows and winter peaks for *Cladosporium* in the city of Kuwait. Temperatures that fall within the optimum range for this spore are only identified from aerobiological studies in the winter months in this city of high temperatures (Davies 1969; 429).

*Cladosporium and the height of the spore trap*

It has already been noted that *Cladoporium* concentrations rise as the height of the city or site above sea level increases, but concentrations were found by some aerobiologists to increase with sampling height at the same location (Gregory & Hirst, 1957). The method used for measuring *Cladosporium* at different altitudes was to mount suction spore traps on light aircraft. The finding was consistent, regardless of the season (Khattab & Levetin, 2008; 532). An assessment of pollen and *Cladosporium* clouds over the oceans surrounding the British Isles showed that the *Cladosporium* cloud with the greatest *Cladosporium* concentration was invariably 300-600 metres higher than the pollen cloud. (Hirst, 1967;375).

*Diurnal periodicity of Cladosporium*

Strong diurnal patterns have been documented for *Cladosporium spp* and peak levels have been found at night (Lin & Li, 1996) in the morning (Troutt & Levetin, 2001) and during the afternoon (O'Connor et al., 2014). There are well documented seasonal peaks for this spore, which are frequently seen during autumn and spring months in temperate areas. (Infante et al., 1999; 21, Recio et al, 2012). Some investigators reported spring-summer peak levels, but low winter counts were found from most studies in temperate climates (Şakiyan & Inceoğlu, 2003).

*Meteorological parameters associated with Cladosporium levels*

*Cladosporium* spores are known to be 'dry air spores' (Peternel *et al*, 2004). It therefore follows that there is great seasonal variation in the *Cladosporium* concentrations from spore traps situated in areas with well-defined seasons. The optimum temperature range for *Cladosporium* is more than 15°C (Hjelmroos, 1993) and less than 29°C (Sabariego, De La Guardia & Alba 2000, Rodriguez-Rajo et al. 2005). Outside of this temperature range, *Cladosporium* conidia start to disappear from the atmosphere. The relationship between meteorological parameters and *Cladosporium* spores has been examined by numerous investigators. They have found a great variation in peak spore concentration and the time of day and even month, when peak concentrations are measured in the atmosphere (Aira *et al*. 2008; Recio *et al*. 2012; Rodriguez-Rajo *et al*. 2005; Peternel *et al*. 2004, Sakiyan *et al*. 2003).

*Rainfall*

It has long been known that rainfall has a 'washing effect' and removes inorganic and organic particles from the atmosphere (Hirst, Stedman & Hurst 1967; 351, Hjelmroos, 1993; 45). Low *Cladosporium* levels were recorded during the spring season when heavy rains occurred, clearly depressing the levels when compared to a year with less rain. (Peternel, 2004; p 305).

**2.8 Global pollen sampling**

The increasing prevalence of asthma and allergy in some countries (Asher et al. 2006, Bousquet et al. 2009) and in specific paediatric age groups (Odhiambo et al. 2009) has piqued interest in assessing the fungal spore and pollen profiles in many cities. Pollen calendars are needed to assess these aeroallergen profiles and seasonality. The large numbers of such studies attest to this. (Mitakakis & Guest 2001, Garcia-Mozo, Mestre & Galan 2007; Rodríguez-de la Cruz et al. 2010, Raza et al 2012).

Beggs warns that pollen calendars from sites operated in isolation may be misleading and that pollen networks are preferable, where data from several pollen monitoring sites are collated. Networks may be used to disseminate information for regional pollen levels, both actual and predicted (Beggs et al. 2015). In areas where networks are unavailable, pollen calendars are a useful first step towards assessing the atmospheric concentrations and seasonal peaks of the local pollen and fungal spore aeroallergens.

Karatzas et al. notes that long term time series from pollen networks within continents have been used for decades (Karatzas, Riga & Smith 2013). Haberle et al. linked pollen networks on a broader scale by entering pollen data from 11 cities in Australasia into a data bank for analysis. The seasonal variations of the major pollen aeroallergens and the local rainfall and temperature were analysed for correlations that would assist with predicting start dates and the seasonal limitations of allergenic pollen (Haberle et al. 2014).

A disadvantage of using retrospective pollen sites where methods were not standardised is that pollen levels are affected by the sampling height of the spore trap. The heights in the analysis varied from 2 to 14 metres above the ground (Haberle et al. 2014:3). The researchers overcame this difficulty by reporting pollen frequencies as a percentage of the Total Pollen Index (TPI) for each site. This large scale ecological study from Australia and New Zealand established the basis for using pollen as a biogenic indicator of Climate Change. This is accomplished by tracking seasonal pollen changes across continents in response to changing climatic conditions.

Buters et al. are constructing a global map of active pollen sampling sites. Selected global sites must comply with standard data collection equipment and methodology and the sites must have been operating continuously for more than four years. The data collected from these global sites will be used for epidemiological pollen allergy research. Global time series of pollen data will assist with predictions of pollen seasons. (Personal communication with Professor Jeroen Buters, Aerobiology and Pollution Interest Group, European Academy for Allergy and Clinical Immunology (EAACI), 2016). The global pollen monitoring map may be seen in Figure 2.2 below.

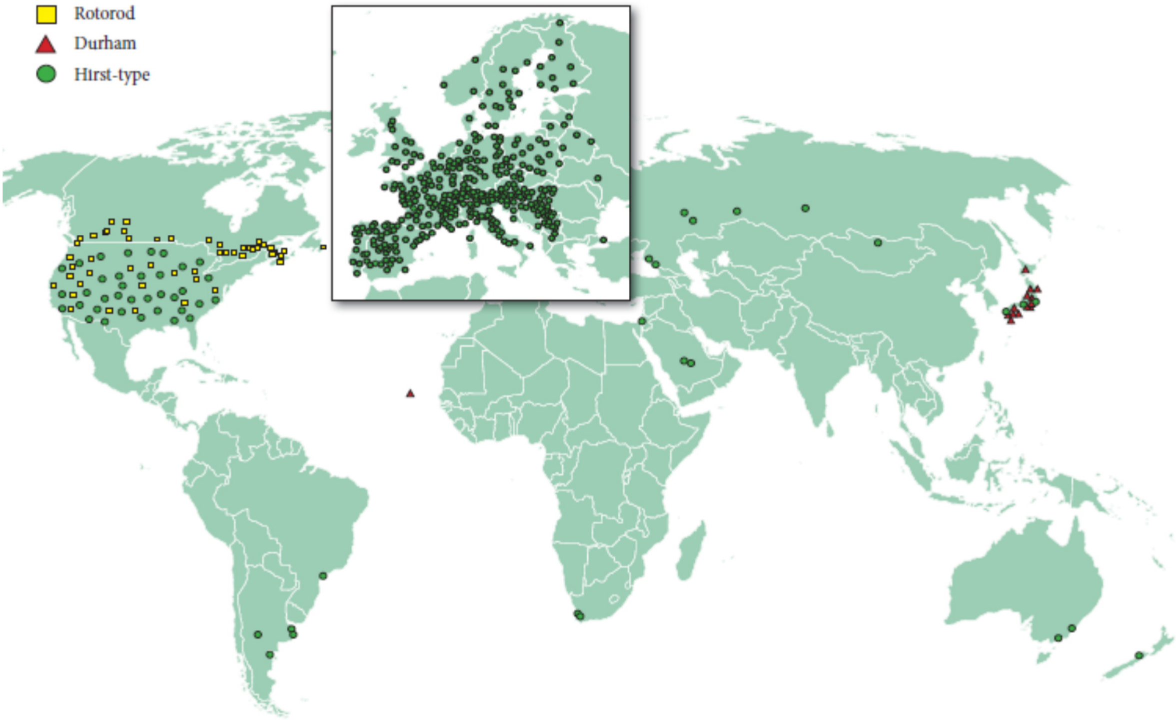



Figure 2.2 Global map of pollen sampling stations courtesy of Professor Jeroen Buters

The criteria for pollen spore traps included in this map are that the equipment and sampling methods must meet international standards and should have been operating for a minimum of four years.

### 2.9 Climate Change and pollen concentrations

Long-term air spora data analyses have been conducted that lend weight to the evidence that supports Climate Change (Levetin & van de Water, 2008, Cecchi et al. 2010; Ariano et al. 2010; Ziska et al. 2011; Ziska et al. 2012). These studies demonstrate changes in pollinating levels that are strongly associated with changing weather patterns and increased $C0_2$ levels. Rogers showed the effect of increased $C0_2$ levels on *Ambrosia artemisiifolia* (Ragweed) in carefully controlled greenhouse and chamber experiments, which were summarised as follows:

*"[Our] study suggests that under future predicted greenhouse gas emissions and associated climate conditions, either an early spring onset or variability in spring onset along with elevated CO2, there will be an overall increase in ragweed pollen production*"
(Rogers et al. 2006: 869).

Climate change effects on pollen start dates have been seen to be variable. Changes in climate patterns are inconsistent and vary in different geographical areas. This will impact dissimilar geographic areas in different ways (Genarro et al. 2015). While Rogers showed that ragweed pollen production was likely to increase due to anthropogenic climate changes, Recio et al contended that grasses in the Mediterranean region of Malaga were experiencing shorter seasons. Start dates were delayed in association with an overall increase of 0.6ºC in temperature since 1970. Paradoxically the Annual Poaceae Index (API) for grass pollen increased (Recio et al. 2010).

Ziello, noting the increase in allergenic pollen taxa across 97 sampling stations in Europe expressed surprise at this finding (Ziello et al. 2012). Weber however, stresses the complexity of the meteorological variables and their associations with bioaerosol levels, such as pollen. noting that while rain and humidity inhibit certain bioaerosols, moisture is also a requirement for their growth (Weber 2003). Higher temperatures might therefore curtail the grass season but not diminish the total grass index. This would explain the apparent contradiction between the findings of these two studies.

**2.10 Summary**

Where weather, vegetation and topography differ significantly within a city, the suggested 30-kilometer radius for a site should be carefully considered. Long term data are advised as extended data collections have a smoothing effect and eliminate unusual seasonal patterns, such as drought or excessive rain. Unusual weather patterns affect the seasonality and concentrations of pollen and fungal aeroallergens. In addition, different pollen and fungal spore aeroallergens have unique associations with meteorological parameters so that unusual weather patterns do not have uniform effects on the different aeroallergen concentrations. Different aeroallergens have their own unique growth requirements and will respond differently to the same meteorological phenomena.

When pollen time series are correctly analysed and regional air spora records are kept, predicted and current pollen levels are an invaluable aid to the diagnosis and treatment of allergic disease. Pollen networks are increasingly being used to this end and a global pollen network is being established. This will assist with epidemiological allergic rhinitis and asthma surveys. When pollen network long-term time series are analysed, together with meteorological parameters the start, peak and end dates of major pollen allergens can be predicted.

Seasonal allergic rhinitis may be exacerbated as a result of increased pollen due to Climate Change. A global network of pollen sites is being established to better understand the seasonal patterns and responses of these aeroallergens to changing weather patterns. It is anticipated that this will improve the understanding of the effect of such changes on the symptoms of allergy in patients (Cecchi et al. 2010, Buters 2016).

### 2.11 The thesis in context

The thesis places the air spora of Cape Town at the southern tip of Africa, within the global framework and adds to the findings of Southern Hemisphere countries. The conversations in the global literature and especially the literature from Southern Hemisphere countries like Australia (Katelaris, Burke and Byth 2004, Haberle et al. 2014) have been invaluable as a guide for the study. The parameters used to compare sites and assess the influence of weather in the Greater Cape Metropolitan Area were chosen after studying the extant literature. The selection of the aeroallergens that were chosen for comparison, was influenced by known clinical sensitivity in the study area and by the aeroallergens studied in the Northern and Southern Hemispheres. The statistical methods used for the analyses were comparable with those applied in similar studies (Sadyś, Adams-Groom and Kennedy, 2016, Kiotseridis et al. 2013, Medek et al 2015.)

Chapter 3

# Methods

## 3.1 Introduction

The Methods section is divided into three parts. Part I describes the sampling methods, Part II describes the project design and Part III describes the statistical methods. The project design is illustrated by means of a flow chart. As the plots are presented in Chapter 5, a brief description of the statistical analysis that was used will be repeated.

## 3.2 Part I: Sampling Methods

Hirst type volumetric spore traps (Hirst 1952) were used to measure and identify the air spora trapped at all the Cape Metropolitan air spora monitoring studies at the Rondebosch, Table View, Mowbray and Observatory sites. When microscopes were replaced, the field diameter of the new microscope was measured and the calculation was adjusted accordingly. Sampling techniques were unchanged throughout the 30 years of sampling. The earlier data collections were kept as hard copy, but since 2003 the data have been saved in Excel format, using spreadsheets dedicated to storing the air spora data.

## 3.3 Sampling Site establishment

Each spore trap was sited in an area where the surrounding vegetation was typical of the suburb in which it was placed. The spore traps used for the three clinical studies were all mounted on rooftops. Unobstructed positions were found for each spore trap at the Rondebosch, Mowbray and Observatory sites and the spore traps were raised above the surface of the flat roof on which each trap was mounted. The Table View sampler was an exception and was not mounted on a rooftop. It was positioned with the Air Quality pollutant monitoring instruments at a secure site two metres above the ground and was therefore lower than the other 3 spore traps. All the spore traps were connected to mains (230-250 volts).

Figure 3.1 The Burkard 7 day recording volumetric spore trap

### 3.4 Operation of the Burkard spore trap

The Burkard spore trap aspirates a constant volume of air, calibrated to measure 10 litres per minute through an orifice. The air flows over a specially prepared sticky Melinex, or cellulose strip which is fixed to a drum within the body of the spore trap. The drum moves past the orifice by means of a clockwork mechanism, describing a full revolution or 360° over the period of seven days. A rotating vane on the spore trap constantly directs the orifice into the wind to maximize the trapping of bioaerosols, particularly pollen and fungal spores from the ambient air. The air flow volume is checked each week by means of an air flow meter. All new drums were checked for correct speed and monitored each week.

#### 3.4.1 Preparation of the sampling strip

The spore trap strip was replaced each week at 9 am. The drum was cleaned with alcohol, placed on a stand and secured with a locking nut. The Melinex strip was cut to the correct length and attached to the drum with double sided adhesive tape between the marked, black notches on the drum, taking care that the ends of the strip did not overlap. The strip was coated with Vaseline, or petroleum jelly, which was applied smoothly and evenly with an artist's brush by rotating the drum. The clock mechanism was wound with the key, taking care not to overwind the instrument and checked to make sure the ticking of the clock could be heard. The drum was placed in a covered carrying box and taken to the spore trap. The orifice was cleaned with dental floss and the drum housing was wiped with a Webcol, or alcohol impregnated swab. The drum was then inserted into the drum housing of the spore trap lid and secured with the locking nut, so that the pointer on the spore trap aligned with the red marker on the drum. This ensured that the spore trap orifice was in the correct position to trap spores at the beginning of the Melinex strip. The drum and the marker and marking notches are shown in Figures 3.2a and b below.

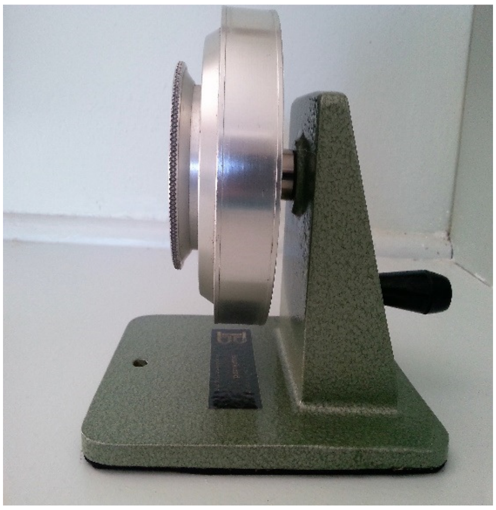


Figure 3.2a Spore trap drum on stand

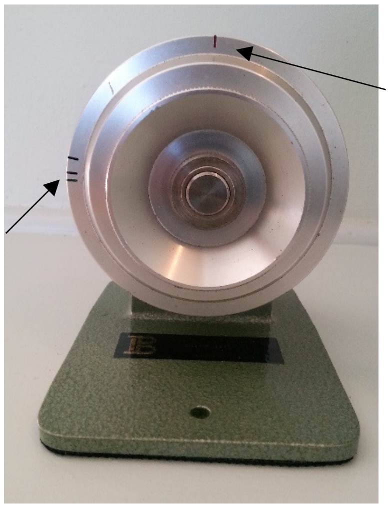


Figure 3.2b Spore trap drum showing marking notches

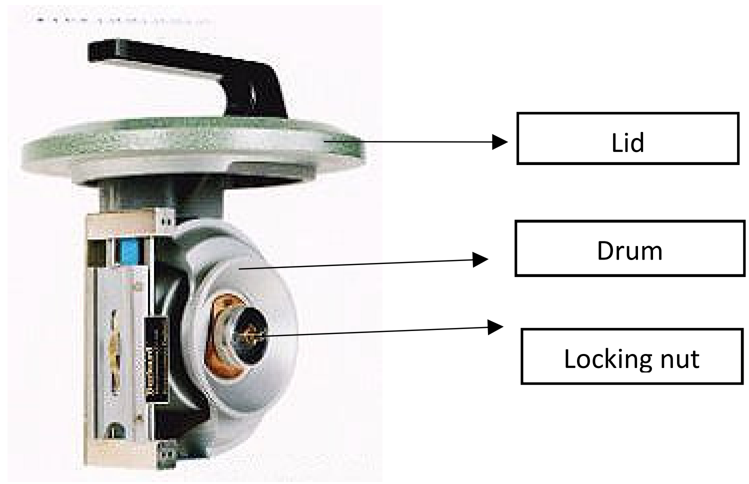



Figure 3.3 Burkard spore trap lid assembly

The locking arm was engaged to fit snugly beneath the lid handle and the air flow was checked with an air flow meter to confirm that the machine was aspirating correctly at 10 litres of air per minute. The air flow was measured to make sure that the flow rate was correct. The calibration of the airflow metre was verified by the Anaesthetic Department technician at Red Cross Children's Hospital.

### 3.4.2 Harvesting the sampling strip

When the seven-day sampling cycle was completed, the air flow was checked and then the drum was exchanged for a drum with a newly prepared strip. The air flow was checked again after the insertion of the new drum. The retrieved drum was taken to the laboratory in a closed carrying case and placed on a drum stand. Seven slides were labelled with the date and site name. The strip was removed, using fine nosed forceps and placed on the Perspex ruler with the starting point of the strip on the left side of the ruler. The strip was trimmed slightly at each end to remove the overlap and sectioned into seven equal strips of 48 *mm* as shown in Figures 3.4a and b below.

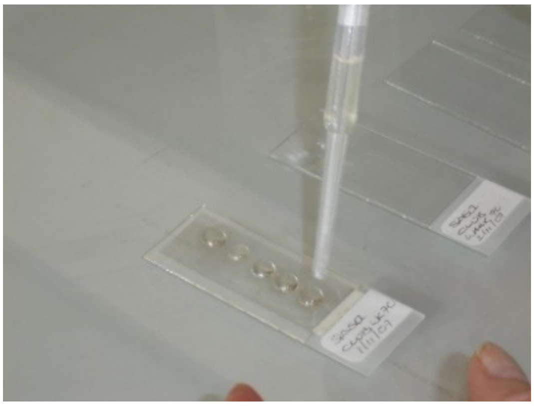


Figure 3.4a Dividing the tape into sections      Figure 3.4b Mounting the slides

### 3.4.3 Mounting the slides

Prepared slides, appropriately labelled, were placed on an absorbent surface. Distilled water was pipetted onto the glass slides as shown. The sectioned strip was carefully rolled onto the slide ensuring that air bubbles were not trapped behind the strip. Three drops of the liquefied glycerine-gelatine mountant (see Appendix A) were added to the strip, as shown in Figure 3.4b above and the coverslip (24 *mm* x 50 *mm*) was placed on top of the strip. The slides were heated very gently and allowed to dry. Excess mountant was carefully removed with a Webcol. Slides were sealed with clear nail varnish.

*Alterations in slide mounting technique*

Minor changes were made to the mounting of slides over the years for practical reasons. Carlberla's solution was the original mountant. From 1994 a gelatin-glycerol mountant was used so that permanent mounts could be made (Rogers & Muilenberg 2001). Gelvatol was originally used to fix the strip to the glass slide but this fixative was exchanged for distilled water in 2010 as water was found to have a better optical density. (See Appendix for mountant reagents and methods).

### 3.4.4 Calculating the pollen/spore concentration

The conversion of the raw score to the concentration of pollen grains or fungal spores per cubic metre was made as follows: Three longitudinal traverses were read for each slide, along the length of the cellulose strip. The areas selected for reading avoided the margins of the strip where trapping may have been less efficient. This is shown in Figure 3.5 below.

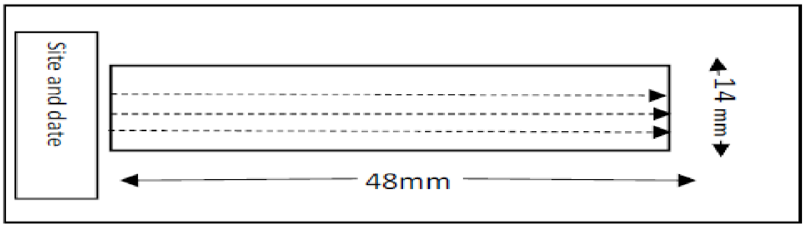



Figure 3.5 Dimensions of the daily strip showing the area read

The number of air spora identified and counted by summing all the pollen taxa and fungal spores counted along the three longitudinal traverses was totalled. This number was the raw score. The raw score was converted to the concentration per cubic metre of air per 24-hour period by applying the Correction Factor. The Correction Factor was calculated by dividing the total area of the strip (48*mm* x 14 *mm*) by the area of the strip that was read, or the sum of the area of the three traverses that were read on each strip (48*mm* x 14*mm* x 0.45*mm* x 3). This calculated figure was in turn divided by the volume of air sampled in a 24-hour period. The volume of air that was sampled or deposited on the strip in a 24-hour period was measured in cubic metres and equalled 14.4 $m^3$ air. (10 litres/minute x 60 mins x 24hrs). This conversion is known as the Correction Factor (CF) (Lacey & West, 2007). The particle counting methods were carried out according to the early work of Spieksma (Spieksma, van den Assem & Collette) 1985.

### 3.4.5 The Correction Factor

The CF was calculated as follows:

$$N \text{ x } \frac{\text{Total area (width of orifice x length of 24-hour strip } mm)}{\text{Area read (Field diameter } mm \text{ x strip length } mm \text{ x 3) x Airflow x Exposure time}}$$

$$\frac{14\ mm \text{ x } 48 mm}{0.45\ mm \text{ x } 48\ mm \text{ x } 3\ x \text{ 10 litres x 60 mins x24hrs}}$$

$$\frac{672\ mm^2}{64.8\ mm^2 \text{ x } 14.4\ m^3}$$

CF = 0.72

Where:

| | | |
|---|---|---|
| *N* | = | the raw score |
| 14 *mm* | = | width of the spore trap orifice |
| 48 *mm* | = | length of the 24-hour section |
| 0.45 *mm* | = | field diameter of the microscope currently in use |
| 3 | = | number of traverses |
| 14.4 $m^3$ | = | volume of air aspirated per 24 hours |

Here exposure refers to exposure time, so that airflow multiplied by exposure gives the total amount of air passing over the strip. The remaining terms correct for the fact that only a proportion of the entire strip for each 24-hour period was counted for grains/spores. With the above numerical input, *N* should be multiplied by 0.72 to express the fungal spore or pollen concentration per cubic metre and was calculated according to the aperture or field diameter of the microscope used. The calculation given above is the one currently in use. We mention that the above calculation assumes that all the grains and spores passing over the strip adhere to the strip and that they do so completely evenly. Both assumptions are of course idealized and they are universally applied to pollen/spore measurements.

## 3.5 Microscopy

The strips were read using a binocular microscope at 400 *x* magnification (Eyepieces 10 *x* Objective 40 *x*). One eyepiece was fitted with a graticule for measuring pollen grains or fungal spores. Each microscope used had a stage micrometer for fixing the position on the strip. The field diameter of the microscope was measured. Three horizontal or longitudinal transects parallel with the direction of movement of the drum, were read for each slide. The aperture of each microscope used throughout this study was measured and the calculation used to convert raw scores to absolute numbers was adjusted accordingly. At the beginning of the first sampling study, pollen atlases and mycology textbooks were consulted for identification, but online pollen guides, atlases and web sites have made this task much easier in recent years.

### 3.5.1 Burge Scale for threshold values

The Burge scale demarcates the threshold concentrations for trees, grasses and weed pollen concentrations in the atmosphere (Burge 1992). The scale divides the concentrations into 'low' 'moderate' and 'high' values and is shown in Table 3.1 below.

**Table 3.1. The Burge Scale for grading pollen thresholds**

| Pollen Category | Low | Moderate | High | Very High |
|---|---|---|---|---|
| Tree | 0-15 | 16-90 | 91-1500 | >1500 |
| Grass | 0-5 | 6-20 | 21-200 | >200 |
| Weed | 0-10 | 10-50 | 51-500 | >500 |
| Fungi/Mould | 0-900 | 900-2500 | 2500-25 000 | >25 000 |

The Burge Scale was selected as the most appropriate measure of pollen thresholds. It was designed by Harriet Burge of the Harvard School of Environmental Science. The academic units in Cape Town participated in clinical trials where the 5-10 grain grass threshold limit they were required to observe was in agreement with the Burge Scale.

### 3.5.2 Reference Slides

Two reference collections of permanently mounted pollen grains were used for the identification of pollen taxa. The first collection was donated by the Botany Department of Universitas (The University of the Orange Free State in South Africa) from the collection of the palynologist, Professor Eduard van Zinderen Bakker. The second collection was obtained from The Department of Allergology at The University of Leiden, courtesy of Dr Frits Spieksma.

### 3.6 Part II: Spreadsheet design

Collaborative studies with palynologists at the University of Cape Town, Department of Environmental Science and The Bernard Price School of Palynology at the University of the Witwatersrand, led to the design of a spreadsheet whereby raw scores were converted to absolute figures using Excel formulae. The Weekly Classification, a weekly sum for each pollen taxon or fungal spore was incorporated into this spreadsheet and the weekly pollen count was divided into the categories: trees, grasses and weeds. These scores were tallied and shown on the Total Summary sheet, together with a brief report for each week, highlighting any significant levels and describing possible health risks (See Appendix for spreadsheet example).

### 3.7 Digitization of the data collection (1984-2014)

In 2013 all archived data were entered into spreadsheets preparatory to analysing the pollen and fungal spore collections from the four sampling sites and the completed spreadsheets were examined.

### 3.8 Part III: Statistical Methods

Maia Lesosky began the statistical analyses and assisted in identifying the prevalence and seasonality of the fungal spores. She displayed weather parameters and spores in tandem and so that a snapshot of the meteorological conditions on a given day could be seen at a glance. The statistical analyses and modelling, listed below were ably and expertly carried out by Dr Birgit Erni, Department of Statistical Sciences, University of Cape Town, who made it possible to simultaneously visualise curves from three different time series simultaneously across unmatched years.

### 3.8.1 Introduction

The multivariate data analysis compared pollen and fungal spore counts from three pollen sampling sites: Rondebosch (RXH), Table View (TBV) and Observatory (SAAO). The sampling sites did not always operate concurrently. The statistical modelling sought to compare the sampling sites by analysing the prevalence and seasons of eight aeroallergens: *Poaceae, Cupressus, Platanus, Quercus, Alternaria, Cladosporium, Epicoccum* and *Pleospora* at the sites. The choice of these aeroallergens was based on their prevalence and/or known allergenicity. Poaceae, *Quercus* and *Platanus* are part of routine skin testing pollen panels in Cape Town Allergy Clinics as are *Alternaria*, *Cladosporium* and *Epicoccum*. Cupressaceae was the dominant pollen at the Observatory site and a strong seasonal spike was seen every year in mid-winter for *Pleospora* at the coastal site of Table View, when fungal spore concentrations are usually low.

For many of the following statistical analyses we used the original count data (before conversion to count per $m^3$), because the pollen and spore counts here exhibit the typical statistical properties of count data (many zeros, a few extremes, skew distributions). The statistical methods developed for such data often require counts as integers, e.g. assuming a negative binomial distribution for the counts. Comparisons of concentrations and seasons used 3D counts which were obtained after the application of the correction factor which converted the counts to concentrations/$m^3$.

The influence of temperature on Poaceae seasons was examined at the inland and coastal sites using weather data obtained from the South African Weather Service (SAWS) data set was analysed for changes over time. Wind roses were plotted from wind soundings, sourced from SAWS (Observatory for SAAO and Yachtclub for Table View) and Chevron Cape Town Refinery, near Table View. The data were analysed using software R (versions 3.4.1, R Core Team 2017). Wind rose plots are based on the code by Clifton. A timeline was drawn to select comparable data sets from the sites and this is shown in Figure 3.6 below.

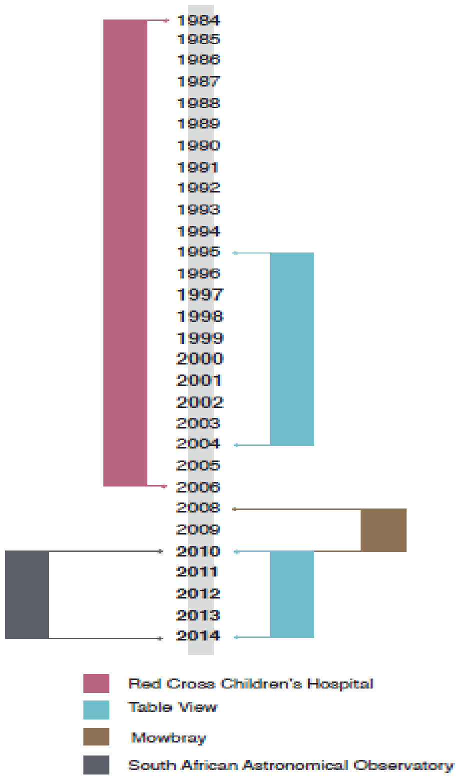



Figure 3.6 The timeline shows the pollen sampling years in Cape Town at Rondebosch: 1984-2006, Mowbray: 2008-2009 and Table View 1995-2004 and 2010-2014 and Observatory 2010-2014.

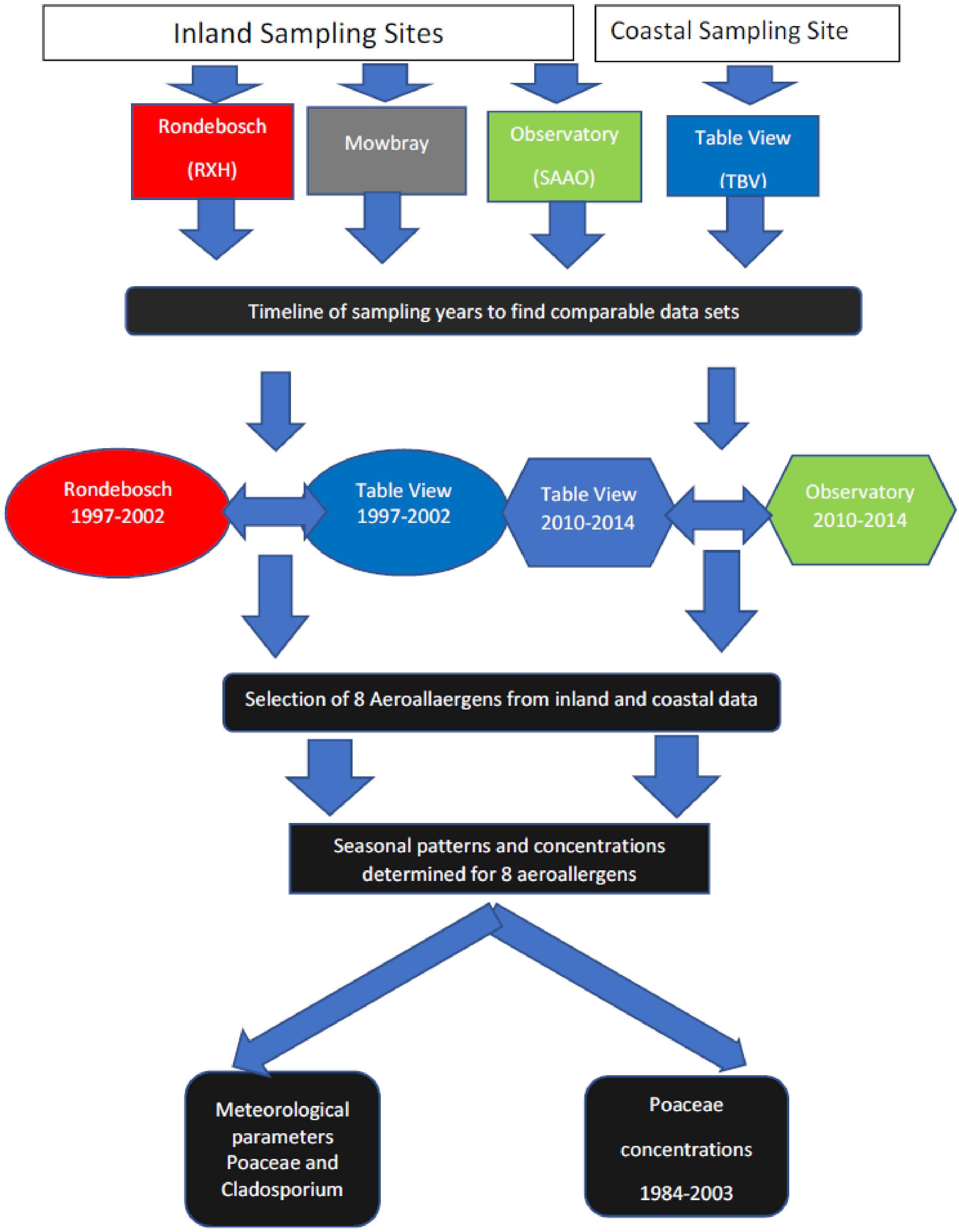



Figure 3.7 The flow chart shows the selection of comparable data sets from the timeline of sampling site data, the selection of eight aeroallergens for detailed analysis, the influence of meteorological parameters on Poaceae and Cladosporium and the change over time of Poaceae concentrations

### 3.8.2 Start, end, and duration of season

First, for a site and taxon, we obtained the average spore/pollen count per day of the year (including 29 February), averaged over all years for which data were available; day 1 = 1 January. We repeated this starting to count in July: day 1 = 1 July, because for many pollen and spore taxa in the Southern Hemisphere this allows us to better capture the season which may include the end of one calendar year and the beginning of the next.

Start / median / end of season was defined as the first day on which the cumulative count exceeded 5% / 50% / 95% of the total daily average count for the year. Duration was calculated as the difference between end and start (Myszkowski 2010).

*Bootstrapping*

To obtain standard errors and confidence intervals for start, median, and end of the season, we used bootstrapping:

1. For each of the 366 days (including 29 February) in a year, one count from the observed counts on that day (excluding missing values) was randomly sampled.

2. From these daily counts we calculated the cumulative count, start, end, median and duration.

3. Steps 1-2 above were repeated 200 times, resulting in 200 estimates for start, median, end and duration of season (see bootstrapdemo.pdf for illustration).

4. A 95% bootstrap percentile confidence interval was calculated from the 2.5th and 97.5 percentiles of the bootstrap distribution for each of these statistics.

4. The standard error for each statistic was estimated as the standard deviation of the corresponding bootstrapped values.

To compare start of season at two sites (or two periods) we estimated a 95% confidence interval for the difference assuming independent, normally distributed values, i.e. observed difference ± 2 x SE(difference), where the SE(difference) was calculated as the square root of the sum of the variances. 2-sided p-values for the difference, under a null hypothesis of no difference, were calculated under the same assumptions.

### 3.8.3 Seasonal profiles

To obtain a better picture of the seasonal profile per taxon and site, we smoothed the daily average counts using cyclic cubic penalized regression splines; cyclic to ensure that the estimated curve was continuous at the end and beginning of the year. These splines were fitted using the gam function from the R package mgcv (Wood 2006), in a generalized additive model framework, assuming a negative binomial distribution for the counts (original 2D counts taken), and a log link. To obtain the 95% confidence intervals, we exponentiated the estimated mean curve and its 95% confidence bands (± 2 SEs) obtained on the linear predictor scale (log-link), thus obtaining an estimated curve for the median count with 95% confidence bands. The degree of smoothness was determined using generalized cross validation (default in gam, Wood 2006).

### 3.8.4 Correspondence Analysis

Correspondence analysis is a method to explore relationships between rows and columns in tables of counts (Greenacre 2007). We used correspondence analysis to explore the taxa (columns) profiles at different sites over months and over years (rows), using R package ca (Nenadic and Greenacre 2007). The cell entries for a site-month for a taxon were calculated as the average daily counts for the taxon in the month for that site. Similarly, for site-years the entries were the average daily counts for the year and site for the taxon. By taking the average daily counts, we tried to avoid the problem of different numbers of observations/missing values per month or year. The plots presented are symmetric maps of the row and column principal coordinates.

*Principal Component*

The principal components analysis was performed on centred logratio-transformed counts (clr-transform). The axes on the biplots represent the first two principal components.

*Principle components analysis*

Principle components analysis is a useful way of summarising a multivariate data set in fewer dimensions. It was used to summarise the variations of eight species (eight dimensions) in two dimensions. For this, species contributions are mapped onto independent axes. In the two-dimensional biplot we use only the first-row axes, those that explain most of the observed variations in the eight-dimensional relative concentrations. For the biplot (5.9b), both the species (black arrows) and the data points (filled dots) are mapped onto these axes. This is an exploratory visual tool to study patterns (Aitchison and Greenacre, 2002).

*Selection of site-years*

To alleviate the problem of too many missing values, we selected only site-years with *Pleospora* and *Epicoccum* average counts > 0, and *Quercus* and *Platanus* average counts > 0.

### 3.8.5 Agreement of daily counts between sites

To evaluate how much counts for the same taxa at two different sites agreed on a day to day basis we used Spearman's rank correlation coefficient, and Cohen's Kappa statistic. We used only data from TBV and SAAO measured between 2010-2014, and only days on which counts were available for both sites. For Cohen's Kappa we divided the data into categories according to the 40%, 60% and 80% quantiles (for most taxa a large number of counts were either zero or very low). For *Epicoccum* and Poaceae these categories corresponded to no, low, moderate and high counts; for *Alternaria* and *Cladosporium* to low, moderate, high, and very high.

We chose this quantile method because the absolute count levels between sites differed considerably. We also calculated percent agreement between categories. Cohen's Kappa adjusts this for chance agreement, and the weighted form of Cohen's Kappa statistic takes into account the case where disagreement in nearby categories is closer than disagreement in categories further apart. We used the cohen.kappa function in R library psych (Revelle 2017).

### 3.8.6 Relationship with meteorological variables

To model the relationship between pollen counts (2D) and meteorological variables we used a generalized additive model (GAM) framework (Wood 2006). Because of the large variability in the counts we assumed a negative binomial distribution, with a log link. To model the yearly seasonal cycle we used a cyclic cubic regression spline (as above). We expected non-linear relationships between counts and temperature and wind speed. Therefore, we also used cubic regression splines to model the relationship with temperature (maximum and minimum) and wind speed. Because wind direction is a circular variable, we modelled this relationship with a cyclic cubic regression spline. The GAM models were fitted using the gam function from R's mgcv package (Wood 2006).

### 3.9 Summary

The concentrations and seasonal limits of the eight selected air spora were compared within the sites and between the sites, across the 30-year time series and for shorter time periods, using the statistical methods detailed above.

The database of data points for the study was vast, despite the selection of eight air spora and specific years: 1997-2001 and 2010/11-2014. Preliminary assessments only were made of the relationships with meteorological variables, but the steps in the flow chart (Figure 3.5) were completed.

Chapter 4

# The Sampling Sites

## 4.1 Introduction

Cape Town is situated at 34°S and 18°E has a population of 4 300 000 people who live in suburbs in the Cape Metropolitan Area (CMA). Cape Town suburbs are scattered across the Cape Peninsula, a narrow strip of land and they extend up the western and eastern coastlines. Table Mountain rises steeply to a height of 1086 metres from its position close to the port of Cape Town in Table Bay. The mountain is close to the city bowl, the area that houses the business district. It is flanked by residential homes, some built on the lower slopes of the mountain. The three academic hospitals in Cape Town are: Groote Schuur Hospital in Observatory, Red Cross Children's Hospital in Rondebosch and Tygerberg Hospital in Bellville. The demographics of the patients attending the academic hospitals have changed since pollen sampling began half a century ago and most of the outpatients who attend respiratory clinics at the academic hospitals now live in the area known as 'The Cape Flats'. The suburbs on the Flats include the large suburb of Mitchell's Plain and informal settlements like Khayelitsha and Gugulethu. Rhino-conjunctivitis was a reported symptom by 20.7 % of Cape Town children aged 13-14 years who answered questionnaires in the ISAAC Phase III in Africa study (Ait-Khaled et al. 2007).

Emeritus Professor Eugene Weinberg established air spora monitoring in 1968 using Durham spore traps, as an adjunct to the diagnosis and management of the patients, who attended the Allergy Clinic at Red Cross Children's Hospital, in Rondebosch, Cape Town. The Durham spore traps were replaced with Hirst type spore traps, using slides that were changed daily. This trap, in turn, gave way to the Burkard seven-day recording volumetric spore trap, which was operated at the hospital from 1980 until 2006. The pollen count and an explanatory pollen report were sent to two academic hospitals in the Greater CMA and to private Allergy clinics every week. The pollen and fungal spore concentrations were published in the media each week and broadcast by a local radio station, 567 Cape Talk. Pollen sampling in other areas of the Western Cape, were added and formed a small network of sampling sites. I established each one of these sampling sites and was solely responsible for the operation of the spore traps, the microscopy, the reports and the data storage. The sites are shown in Figure 4.1 below.

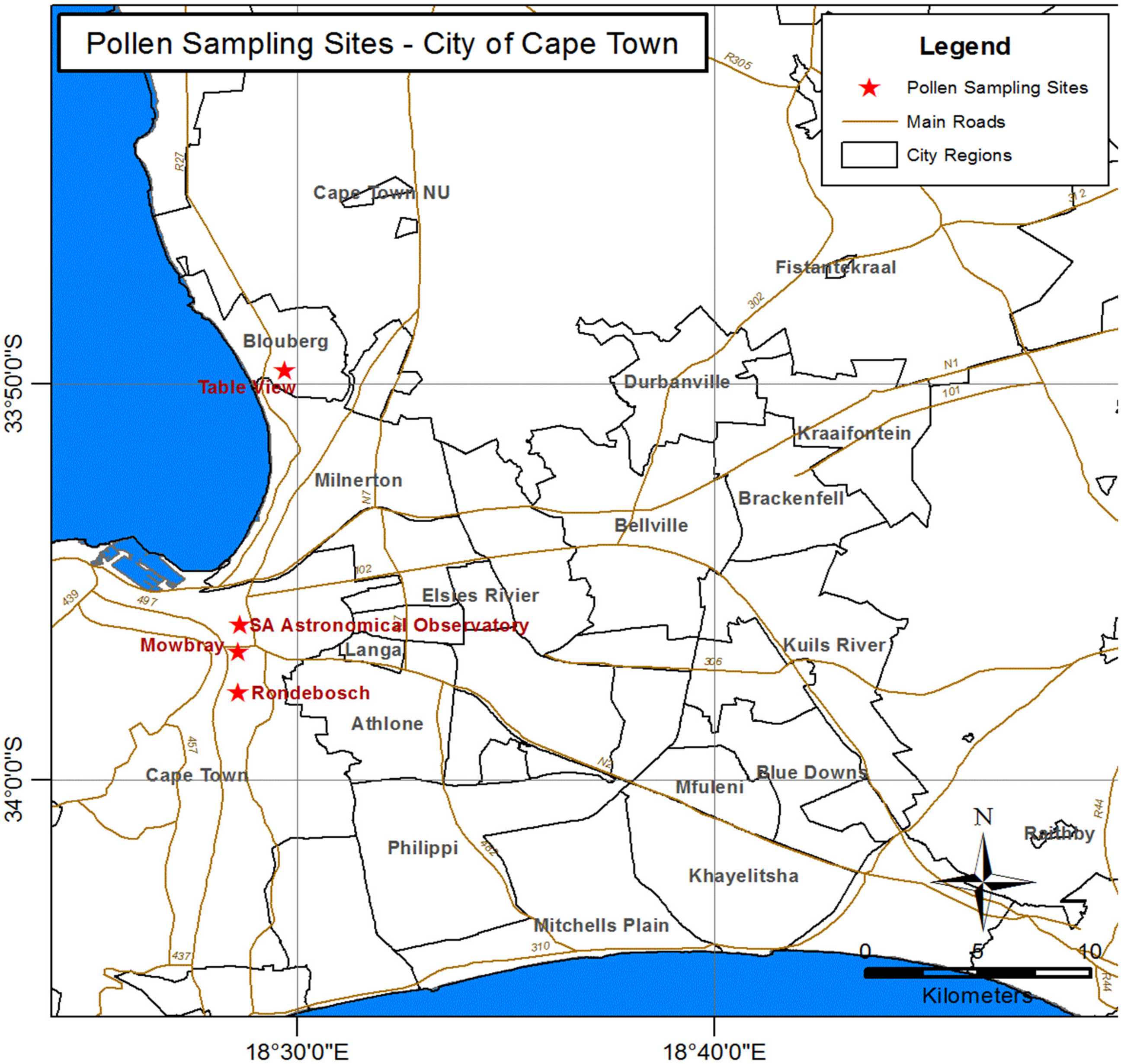



Figure 4.1 The Cape Town sampling sites in Rondebosch Mowbray Observatory and Table View. Custom designed by Michael Weston of E-science

## 4.2 The topography of the Cape Metropolitan area

Cape Town is situated at the south-western tip of South Africa on the Cape Peninsula, occupying 2460 $km^2$. (Rebelo et al. 2011) Spiny mountain ranges and two long coastlines characterize the area and the land frequently rises steeply from the shoreline. There are two major regions within this area; the mountainous areas and the 'Cape Flats' which are low lying and characterized by sandy dunes. The coastlines differ, as the Indian Ocean, with its warm Agulhas current flanks the east coast, while the Atlantic Ocean with its cold Benguela current, sweeps north along the West Coast. These unique features contribute towards the formation of minor climate zones, that exist within a relatively short distance from one another (Lutjeharms et al. 2001).

## 4.3 Climate and weather in the Western Cape

Strong winds blow throughout the year in the Cape, especially on the Cape Flats. A singular feature of the Cape Peninsula is the strong, summer, south easterly wind, colloquially known as 'The Cape Doctor' as it is this wind that blows pollutants away from the city and dispels smog. The south easterly wind sometimes blows mists and clouds over Table Mountain and this is referred to by Capetonians as the mountain's 'tablecloth'. During the winter months, the north westerly wind reaches gale force on occasion and has been known to uproot mature trees and wrench roofs from houses. Again, it is the suburbs of the Cape Flats that bear the brunt of the NW wind. Suburbs in the lee of Table Mountain are shadier than the suburbs of the Cape Flats, or the coastal areas and residents of these quiet, lush areas, set in tree lined streets experience quite different weather patterns to the inhabitants of suburbs on the Atlantic seaboard, or those who live on the windswept Cape Flats. During the change of season from summer to spring, or autumn to winter, these differences are more pronounced and it is not uncommon to experience 'four seasons in a day' when moving from Cape Point to Blouberg (blue mountain) on the West Coast, a mere 83 km distance.

## 4.4 Seasonal patterns of Western Cape air spora

Pollen and fungal bioaerosols are strongly associated with climatic conditions (Hjelmroos, 1993) and both pollen and fungal spore levels are directly affected by changing weather patterns (D'Amato et al. 2015). There is marked variation in the weather patterns within the same vegetation biome in the south-western Cape. It is therefore not surprising that there are differences in the pollen catch and in the fungal spore load from sites that are relatively close to each other, but are situated in regions with distinctive features, which support different vegetation. While the inland sites, i.e. the Rondebosch, Mowbray and Observatory sites are all close to Devil's Peak alongside Table Mountain, the Table View site is close to the sea and borders a distinctly different vegetation biome of sandy dune vegetation. This vegetation is *Strandveld* with elements of *Renosterveld (*Rebelo et al. 2011*).* Precipitation, temperature, relative humidity and especially wind speed and direction, are dissimilar to the extent that separate weather forecasts are assembled for these regions.

### 4.5 The Red Cross Hospital site in Rondebosch

A Burkard seven-day recording spore trap was fixed 20 $m^{-1}$ above ground on the roof of Red Cross Children's Hospital in Rondebosch, Cape Town. The hospital occupies a large corner plot, with busy double carriage roads on two sides, but the main buildings are set back from these roads. The buildings are adjacent to a quiet access road, which acts as a buffer between the spore trap and Milner Road, a major road on one boundary, while a helicopter landing pad lies between the spore trap and the equally busy Klipfontein Road. A large common of 40 hectares abuts Milner road and this public open space is home to many species of indigenous plants as well as untended grass, most of which have been introduced and naturalized. Hospital and research buildings line the other two sides of the sampling site. This inland site is a mixture of Cape Flats Sand Fynbos, and Peninsula Granite Fynbos (Rebelo et al. 2011).

*The Flora of the Cape Floristic Kingdom*

The mountain vegetation is protected and is known as Table Mountain National Park. The vegetation of the Cape Peninsula forms The Cape Floristic Kingdom. Measuring 90 000 $km^{-2}$ in area, it is the of the 6 floristic kingdoms, or phytogeographical areas found worldwide, but despite its small size, it is one of the richest, with the most abundant plant species (Meadows, 1993). The vegetation is xerophytic bush and limited evergreen forest. The shrubby plants are known as fynbos (fine bush) because the tough shrubs are fine needled and characterised by the dominant species: Ericas. Pollen from the indigenous fynbos plant species, so named for the thin, needle-like, or ericoid indigenous plant species, is seldom trapped from the ambient air. The Proteaceae, with their broader, sclerophyllous leaves are also well represented in the vegetation as are the Restionaceae and Iridaceae families (Manning & Goldblatt, 2012). The fynbo*s* plants are rarely wind pollinated; they are largely pollinated by insects, birds or small mammals and are therefore unlikely to be allergenic. The vegetation map of Cape Town shows the different vegetation zones and this may be seen in figure 4.2 below

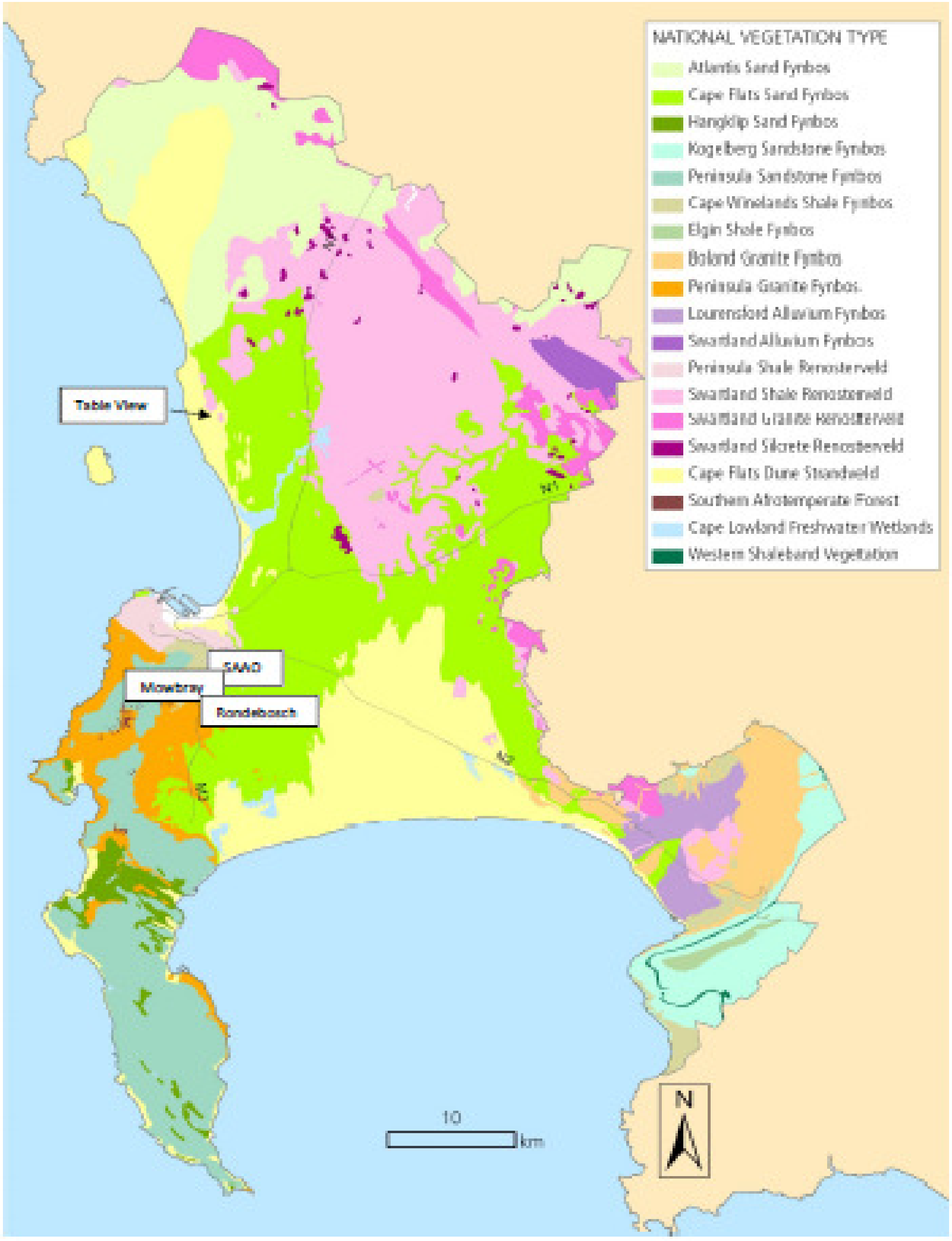



Figure 4.2 Cape Town Vegetation. South African National Biodiversity Institute with Cape Town sampling sites added

*Trees near the Hospital sampling site*

The trees in the area are mainly exotics; they are Northern Hemisphere trees that have been introduced. The predominant species are *Platanus (plane*, often London Plane*), Pinus,* often

*Pinus radiata*, but *P pinaster*, *P hepensis* and *P pinea* are also found) and *Quercus* sp., predominantly *Quercus robur*) species in the suburbs that surround the spore trap. Olive trees, including the indigenous *Olea capensis* (Ironwood) are seen as well as many species of *Rhus* (especially *Rhus angustifolia* and *Rhus lancea* or *Karee).* Yellowwood or *Geelhout trees (Podocarpus latifolius*) have decreased enormously since 1652, when the first settlers arrived at the Cape from Europe, but they are still seen in parks and gardens. *Acacia longifolia* is plentiful and together with *Casuarina spp,* was introduced from Australia to bind the soil of the sandy Cape Flats and coastal areas of the Cape. (Moran and Hoffman, 2012) Several Myrtaceae, or gum species, introduced from the Mediterranean, are found in abundance and were planted for shade. The trees are found on the lower slopes of Devil's Peak, which flanks Table Mountain, on Rondebosch Common and lining roads in the suburbs around the sampling site (Adamson &Salter, 1950). Many ornamental trees, such as Tamarisk, or *Cupressus macrocarpa* (Monterey cypress) now renamed *Hesperocyparis macrocarpa*, have been planted in residential gardens. A large number of grasses, most of which have been introduced and naturalised are found on Rondebosch Common and on roadsides.

*Grasses around the Hospital sampling area*

The commonly found grasses of the Cape Peninsula include the following: *Andropogon sp, Anthoxanthum sp, Avena barbata and A fatua, Briza maxima and minor, Bromus spp*, *Chloris gayana*, *Cymbopogon spp, Cynodon dactylon, Digitaria spp, Festuca spp*, *Holcus lanatus, Hordeum spp, Lagurus ovatus, Lolium perenne, Lolium temulentum, Poa annua, Paspalum spp, Panicum spp, Pennisetum clandestinum, Phalaris spp* and *Stenotaphrum secundatum* (Oudtshoorn 1999). *Cynodon dactylon*, or Bermuda grass, does not cross react with the other grass species (Prescott & Potter, 2000) and a separate skin prick or specific IgE should be used in the grass testing diagnostic panel to distinguish this allergenic grass.

*Climate and weather in Rondebosch*

The elevation of the Hospital sampling area, in the suburb of Rondebosch, is 16 metres above sea level (http://www.capetown.gov.za/waterservices). Cape Town enjoys a Mediterranean climate with a typical winter rainfall area and dry summer. The winter months (June-Aug) are cool in contrast to the dry, warm summer months (December-February). Strong north-westerly winds, frequently >2m/s blow in winter and south-easterly winds prevail in the summer. While the spring months (Sep-Nov) may be cold and windy with rain. Autumn (March-May) is generally calm and clear.

### 4.6 The Table View sampling site

The Table View site was established as part of an Air Quality study, in an area close to a petrol refinery. The height of Table View above sea level is 9 metres (http://www.citipedia.info). The spore trap was positioned 2 $m^{-1}$ above ground level at a secure caravan site in Circle Road, Table View. The spore trap was positioned at the same place as the pollutant monitors in the study and the height at which the spore trap was positioned, was dictated by the fencing that caged the site. The study (1994/95) was designed in response to complaints from the communities of the suburbs, that were close to the refinery and it was decided to measure pollen and fungal spore bio-aerosols together with pollutants, to determine the health risk to the residents. The external auditor for this study recommended that the aeroallergen measurements be continued after the study was concluded, as grass pollen levels showed a seasonal peak in October. The measurements have continued at the Table View site since that time, although the pollen and fungal spore measurements were not continuous and there are some gaps in the dataset for this site.

*Table View Climate and weather*

The coastal site has features of the Cape Flats Dune Strandveld, Atlantis Sand Fynbos and Cape Flats Pure Sandveld. It is close to the sea and is buffeted by offshore and onshore winds. The coastal town is not shaded by the mountain and enjoys longer sunshine hours.

*Coastal vegetation*

Although there are plant species that are common to the inland and the coastal pollen monitoring stations, there are also plants that are unique to each. The Table View site is on the border of the West Coast Strandveld, which is a strip of land, 50km wide, that runs along the extreme edge of the West Coast from Melkbos to Vredendal. (Warnich & Verster, 2004). The sandy soil is quite different from the rich, peaty and sometimes clay mountain soil of the inland sites and is home to a plethora of dune plant species, such as Asteraceae and Mesembryanthemums, colloquially known as *vygies.* This vegetation is known as the *Renosterveld.* Palynological analysis of boreholes dug in this area at Rietvlei, (Reed lake) to compare recent pollen spectra with the fossil pollen spectrum, have identified many pollen taxa found in the modern pollen rain such as: *Chenopodiaceae, Cyperaceae, Ericaceae,* *Gramineae, Myricaceae, Myrtaceae, Plantaginaceae, Podocorpaceae, Proteaceae, Restionaceae, Typhaceae and Urticaceae*. This indicates that the vegetation of the Table Bay area contains many of the plant species that have flourished in the area since the Holocene (Schalke, 1973).

**4.7 The Mowbray site: National Geo-Spatial Building in Mowbray**

The pollen sampling site in Mowbray (2007-2009) was set up by the Allergy Diagnostic and Clinical Research Unit for the benefit of allergists and pulmonologists at the University of Cape Town (UCT) Lung Institute. A new Burkard seven-day recording spore trap was positioned as close as possible to the Institute building. Asthmatic and allergic patients attend the clinic at The Allergy Immunology Unit (AIU) where allergen immunotherapy to grass pollen is practised. Clinicians involved in clinical drug trials for seasonal symptoms, such as rhinitis and conjunctivitis, need to know the daily atmospheric pollen and fungal spore levels for the duration of the clinical trial. The spore trap was set up on the roof of the National Geo-Spatial Information building in Rhodes Avenue, Mowbray, at a height of approximately 12 $m^{-1}$. Very low levels for pollen and fungal spores were obtained from the site and there was a concern that nearby buildings were creating wind funnels and adversely affecting the pollen catch. Consequently, the spore trap was moved to the South African Astronomical Observatory, in the nearby suburb of Observatory in August 2010.

The distance between this sampling site and the Red Cross Children' Hospital sampling site is slight (3 km) and it is unlikely to have been significantly different in terms of sunshine, relative humidity or rainfall but it was observed that even this small difference in distance affected the pollen catch. The Mowbray sampling site is close to the mountain, just below Devils' Peak, as well as a large four-lane motorway and is surrounded by buildings. In addition, the summer wind speed is thought to be stronger at this site than at the Observatory (SAAO) site, by the local population, although this could not be measured. There are many student residence buildings nearby and although playing fields can be seen from the Mowbray site, the buildings seem to have formed a pollen barrier, obstructing the trapping of air spora. The vegetation surrounding this site is similar to the vegetation at the Rondebosch sampling site.

### 4.8 The Observatory site: South African Astronomical Observatory

The Burkard 7-day recording spore trap was removed from the Geo-Spatial building in Mowbray and repositioned on a rooftop, 5 $m^{-1}$ above ground level at The South African Astronomical Observatory, in Observatory, in August 2010. It is still in operation. Fortuitously, there is a weather mast at this site, owned and operated by the South African Weather Service (SAWS) and so daily meteorological parameters are available for the duration of the sampling period. The area of the Observatory grounds is 9 hectares. It is set back from major roads and it is surrounded by open land at the confluence of the Black and Liesbeek rivers in the Two Rivers Urban Park (TRUP). The Raapenberg Bird Sanctuary, which occupies 10 hectares and Valkenberg Hospital abuts the Observatory grounds. The vegetation of the large grounds of the Observatory was originally composed of small indigenous shrubs, grasses, reeds and bulbs, some of which remain. In the grounds of the Astronomical Observatory and in the nearby park Eucalyptus, *Pinus*, *Ulmus*, *Betulus*, *Kiggelaria africana* (wild peach) *Rhus spp*, *Morus* spp and *Ficus spp* are planted (http://www2.astronomicalheritage.net/). Details of the four Cape Town sampling sites are shown in Table 4.1 below.

**Table 4.1. The location of the Cape Town sampling sites**

| Location of the Western Cape Sampling Sites | Rondebosch | Table View | Mowbray | Observatory |
|---|---|---|---|---|
| GPS Coordinates | 33.95°S, 18.48°E | 33.48°S, 18.29°E | 33.57°S, 18.28°E | 33.93°S, 18.47°E |
| Height of Sampler | 20M | 2M | 12M | 5M |
| Operational period | 1984-2014 | 1985-2014 | 2008-2009 | 2009-2014 |
| Aim | Patient care | Air Quality | Clinical Research | Patient Care |

*Sampling height*

The heights of the spore traps were not standardised and varied from two metres above ground level at the Table View site, to 20 metres above the ground at the Red Cross Hospital site. The spore trap at the Mowbray site was positioned at a height of twelve metres and the Observatory spore trap was set up 5 metres above the ground.

### 4.9 Summary

A key feature of Cape Town is the biodiversity of its plants species. The Cape Floristic Kingdom has a huge variation in plant species. There are 19 vegetation types in the city of Cape Town. The most widespread are the *Cape Flats Sand Fynbos, Swartland Shale Renosterveld, Cape Flats Dune Strandveld* and *Atlantis Sand Fynbos* (Rebelo et al. 2011). Few of the indigenous plants supported by these different vegetation zones produce anemophilous, or windblown pollen. However, the variation in vegetation types is indicative of a pronounced variation in climate and soil. These factors would affect alien plant species, resulting in a variation of wind pollinated species in the different vegetation zones. I have compared the pollen and fungal spore catches from the sampling sites described in this chapter and examined the differences in frequencies of the air spora in the differing vegetation zones of the inland and coastal sampling sites.

Chapter 5

# Mapping the air spora

## 5.1 Introduction

Fifty-one pollen taxa and twenty-four fungal spore genera were identified from the four Western Cape sites: Rondebosch, Mowbray, Observatory and Table View from 1984-2014 during different time periods. Sampling was not continuous at all the sites. Pollen and fungal spores from each site were ranked and the major pollen and fungal aeroallergens were identified and compared in the following way: The pollen taxa were divided into the categories of trees, grasses and weeds and these categories of pollen were compared at the sampling sites. Peak months for selected fungal spores and seasons for trees, grasses and weeds were identified and the pollen and fungal spore profiles for each site were described.

After the completion of these broad comparisons eight aeroallergens were selected for detailed analysis: The pollen taxa Poaceae, Cupressaceae, Platanaceae and *Quercus* and the fungal genera: *Alternaria, Cladosporium, Epicoccum* and *Pleospora* were chosen based on their prevalence and known allergenicity, which will be described in detail below. Cupressaceae (cypress tree pollen) and *Pleospora* have not been included in skin prick/specific IgE testing in Cape Town and were selected based on their high prevalence and unusual seasonal patterns. I reasoned that cypress and Pleospora might be important unrecognized aeroallergens in Cape Town.

The eight aeroallergens were compared from matching years at Rondebosch, Table View and Observatory to assess the similarities and differences between the sampling sites.

**Part I. Pollen**

## 5.2 Pollen seasonal patterns

Seasonal pollen peaks were seen at all the sites but the timing of the peaks differed. The total pollen index (TPI) were compared at the three sampling sites. The peak pollen peak occurred in September at the Observatory sampling site while the peak concentration for total pollen was seen in October at the Table View and the Rondebosch sampling sites. This difference reflects the different vegetation patterns at the three sites and can be attributed to the different compositions of tree, grass and weed pollen at the three sites. Tree pollen was dominant at the Observatory site, grass pollen was more prevalent at the Rondebosch and Table View sites. The seasonal peaks for total pollen concentrations at the sites are shown in Figure 5.1 below.

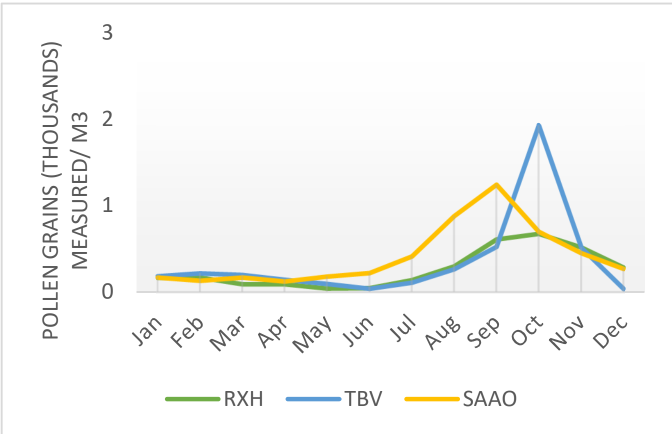



Figure 5.1 Seasonal pollen patterns at Rondebosch (RXH) 1984-2002 Table View (TBV) and Observatory (SAAO) 2010-2014

### 5.2.1 The Total Pollen Index (TPI)

The total pollen index was calculated by summing the pollen concentration at each site from 1 January to 31 December. The mean TPI was measured at each site by summing the total pollen catch for each year and finding the mean annual concentration. The total tree, grass and weed pollen indices were calculated in the same way. The mean values for total pollen, total tree, grasses and weeds were calculated from the 1984-2006 data sets at Rondebosch and Table View and from the 2010-2014 data sets at Observatory and Table View. The TPI for each site was similar in quantity but the proportion of pollen in the categories of trees, grasses and weeds varied. Each site was characterized by differences in the composition of its pollen spectrum and a unique pollen distribution of trees grasses and weeds for each site was identified. This is shown in Figure 5.2 below.

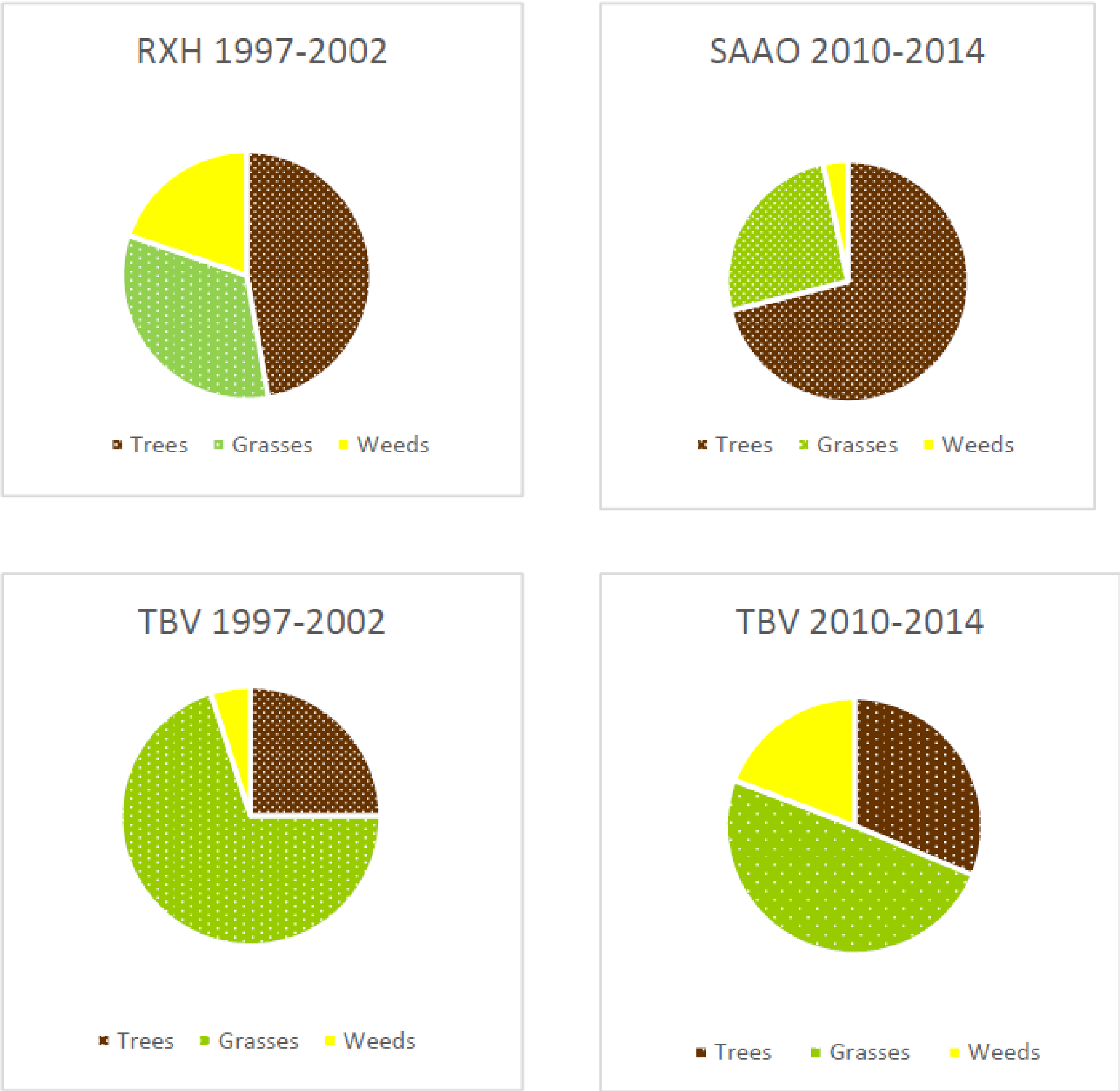



Figure 5.2 Proportions of tree grass and weed pollen (%) at Red Cross Hospital Rondebosch (RXH) Observatory (SAAO) and Table View (TBV)

### 5.2.2 Pollen categories at the sampling sites

*The Rondebosch sampling site*

A higher proportion of tree pollen, than grass or weed pollen was trapped at the Rondebosch sampling site. The TPI consisted of trees 47%, grasses 33% and weeds 20%.

*Table View sampling site*

Two data sets from Table View were assessed: 1997-2002 and 2010-2014. The findings from the two periods differed and were as follows:

*Table View 1997-2002*

Poaceae, or grasses, dominated the pollen catch for these sampling years. The TPI was divided into grasses 70%, tree pollen 25% and weed pollen 5%.

*Table View 2010-2014*

The grass component of the pollen load decreased for the later data set at this sampling site. Grasses were 49% tree pollen increased to 31% and weed pollen increased to 19%.

*Observatory 2010-2014*

Tree pollen taxa were the most prevalent of the categories at this site. The TPI was divided into 71% tree pollen, 26% grass pollen and 3% weed pollen.

### 5.2.3 Pollen frequencies at the sites

*The Mowbray sampling site*

The findings from the Mowbray site were added to discussions about unusual fungal spores and tree pollen only. The findings from the sampling site were excluded from the general analysis as the pollen record was less than the required 3 years of sampling data.

The tree, grass and weed species were identified from botanical sources (Palgrave, 1977, Adamson and Salter 1950). The percentage of the TPI was found for each pollen taxon at the four sampling sites in the categories: Trees, grasses and weeds and these percentages are compared at the sampling sites in Tables 5.1 a, b and c below.

**Table 5.1a Grasses Bulrushes Reeds and Rushes**

| Pollen Taxon | Common Name | Rondebosch % of Total Pollen | Mowbray % of Total Pollen | Observatory % of Total Pollen | Table View % of Total Pollen |
|---|---|---|---|---|---|
| Poaceae | Grass | 31 | 22,5 | 23 | 63 |
| Typhaceae | Bulrush | 0,2 | 0,9 | 0,3 | 1,7 |
| Restionaceae | Reed | 0,1 | 0,8 | 0,9 | 0,5 |
| *Juncus* | Rush | 0,0 | 0,2 | 0,03 | 0,1 |

The pollen compositions in this comparison were calculated from all available data from the years 1984-2005 at Rondebosch 2008-2009 at Mowbray and 2011-2014 at Table View and Observatory

**Table 5.1b Tree pollen comparisons at the sampling sites**

| Pollen Taxon | Common Name | Rondebosch % of Total Pollen | Mowbray % of Total Pollen | Observatory % of Total Pollen | Table View % of Total Pollen |
|---|---|---|---|---|---|
| *Acacia* | Includes Port Jackson willow | 1,4 | 0,1 | 0,1 | 0,5 |
| Betulaceae | Birch | 0,2 | 0,5 | 0,2 | 0,0 |
| **Cupressaceae** | **Cypress** | **2,2** | **7,1** | **24** | **2,9** |
| Hippocastanaceae | Horse chestnut | 0,0 | 0,0 | 0,05 | 0,1 |
| Moraceae | Includes fig, mulberry | 0,8 | 0,2 | 1,0 | 0,7 |
| Myrtaceae | Eucalyptus | 0,5 | 3,1 | 12,1 | 1,8 |
| Oleaceae | 0live | 1,7 | 2,8 | 3,0 | 2,2 |
| *Pinus/ Podocarpus* | Pine, yellowwood | 31 | 25 | 10,9 | 4,5 |
| ***Platanus*** | **Plane** | **10,5** | **15,9** | **5,0** | **1,4** |
| *Populus* | Poplar | 0,0 | 0,0 | 0,4 | 0,0 |
| *Prosopis* | Mesquite | 0.8 | 0,0 | 0,01 | 0,0 |
| *Rhus* reclassified *Seersia* | Includes kareeboom or White karee | 0,1 | 1,9 | 0,9 | 0,2 |
| ***Quercus*** | **Oak** | **0,6** | **4,0** | **4,7** | **0,4** |
| Salix | Willow | 0,1 | 0,0 | 0,07 | 0,0 |
| Tiliaceae | Lime/linden | 0,0 | 0,0 | 0,04 | 0,1 |
| | | | | | |
| Ulmaceae Incl Celtis africana | Incl. white stinkwood | 0,1 | 0,9 | 1,5 | 0,4 |
| *Casuarina* | Beefwood | 0,3 | 0,0 | 0,01 | 0,0 |

The tree pollen compositions in this comparison were calculated from all available data from the years 1984-2005 at Rondebosch 2008-2009 at Mowbray and 2011-2014 at Table View and Observatory The study trees: Cupressaceae, *Platanus* and *Quercus* are printed in bold typeface

**Table 5.1c. Herbaceous shrubs/weeds**

| Pollen Taxon | Common Name | Rondebosch % Total Pollen | Mowbray % Total Pollen | Observatory % Total Pollen | Table View % Total Pollen |
|---|---|---|---|---|---|
| Amaryllidaceae | Includes Nerine and Haemanthus | 0,1 | 0,2 | 0,9 | 0,4 |
| Asteraceae | Includes daisy | 3,5 | 2,6 | 0,5 | 3,8 |
| Boraginceae | Includes Echium, Lobostemon | 0,0 | 0,0 | 0.01 | 0,0 |
| Bruniaceae | Indigenous includes Berzelia | 0,0 | 0,0 | 0,01 | 0,1 |
| Caryophyllaceae | Includes Dianthus | 0,3 | 0,2 | 0,4 | O,5 |
| Chenopodiaceae | Goosefoot | 0,4 | 0,6 | 0,5 | 2,8 |
| Cyperaceae | Sedge grass | 8,2 | 1,7 | 0,9 | 1,7 |
| Ericaceae | Heath | 2,0 | 1,3 | 0,3 | 1,1 |
| Euphorbiaceae | May be succulent | 0,0 | 0,0 | 0,0 | 0,0 |
| Iridaceae | Includes Chasmanthe gladiolus and ixia | 0,0 | 0,0 | 0,06 | 0,0 |
| Liiaceae | Includes Agapanthus Kniphofia Lachenalia | 0,4 | 0,1 | 0,02 | 0,3 |
| Loranthaceae | Includes mistletoe | 0,0 | 0,1 | 0,0 | 0,0 |
| Malvaceae | Herbaceous shrub | 0,2 | 0,0 | 0,8 | 0,3 |
| *Myrica* | Waxberry | 1,4 | 1,6 | 0,3 | 4,9 |
| Oenotheraceae | Includes Evening Primrose | 0,0 | 0,6 | 0,0 | 0,1 |
| Oxalidaceae | Oxalis | 0,1 | 0,0 | 0,07 | 0 |
| Plantaginaceae | Plantain | 1,7 | 0,6 | 0.3 | 0,7 |
| Polygonaceae | Weed | 4,5 | 0,5 | 0,3 | 0,7 |
| Polypodiaceae | Fern | 0,4 | 0,7 | 0,2 | 0,3 |
| Proteaceae | Indigenous shrub | 0,1 | 0,2 | 0,03 | 0,0 |
| Ranunculaceae | Annual herb | 0,0 | 0,0 | 0,04 | 0,0 |
| Rosaceae | Includes Cliffortia | 0,4 | 0,0 | 0,04 | 0,2 |
| *Rumex* | Herb incudes. dock | 0,2 | 0,1 | 0,08 | 0,0 |
| Rutaceae | Includes Agathosma | 0,1 | 0,4 | 0,06 | 0,3 |
| Solanaceae | Includes Nicotiana | 0,0 | 0,1 | 0,02 | 0,0 |
| *Taraxacum* | Dandelion | 0,0 | 0,1 | 0,07 | 0,0 |
| Thymeaelaceae | Includes Passerina, Gnida | 0.0 | 0,1 | 0,04 | 0,0 |
| Umbelliferae | Herb includes Thunbergia | 0,0 | 0,1 | 0,08 | 0,0 |
| Urticaceae | Nettle | 1,1 | 2,0 | 0,9 | 0,3 |
| Parietaria | Pellitory of the wall | 0,0 | 0,0 | 2,8 | 0,0 |

The pollen compositions in this comparison were calculated from all available data from the years 1984-2005 at Rondebosch 2008-2009 at Mowbray and 2011-2014 at Table View and Observatory

*Ranking the pollen taxa*

The pollen taxa at each of the sites: Rondebosch (Red Cross Children's Hospital) Table View (TBV), Observatory (SAAO) and Mowbray were ranked and compared. In order to gain an idea of the prevalence of the different trees, grass and weed plant species that produce anemophilous pollen and to compare the pollen taxa at the four sites. These comparisons will be described in detail below, but it was immediately apparent that tree pollen dominated the catch at Rondebosch, Observatory and Mowbray, with a strong seasonal peak for grasses. In contrast, tree pollen was poorly represented at Table View, the coastal site, where grass pollen dominated the catch and the percentage of grass pollen was close to double the proportion at the sampling sites further inland. Pollen taxa from each site that exceeded 1% of the total pollen catch for that site as shown in Tables 5.3 a, b and c were ranked and are shown in Figures 5.3 a, b, c and d below.

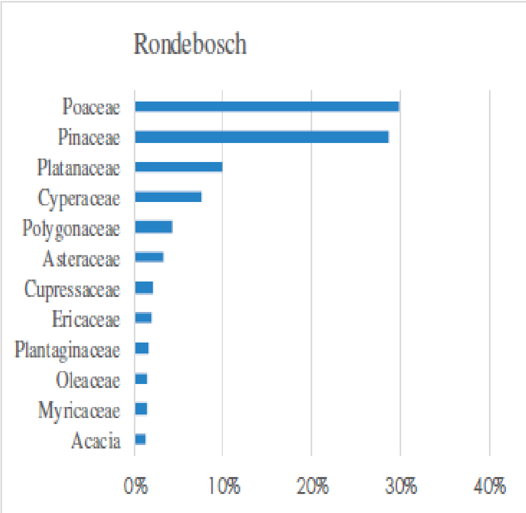



Figure 5.3a. Rondebosch 1984-2002

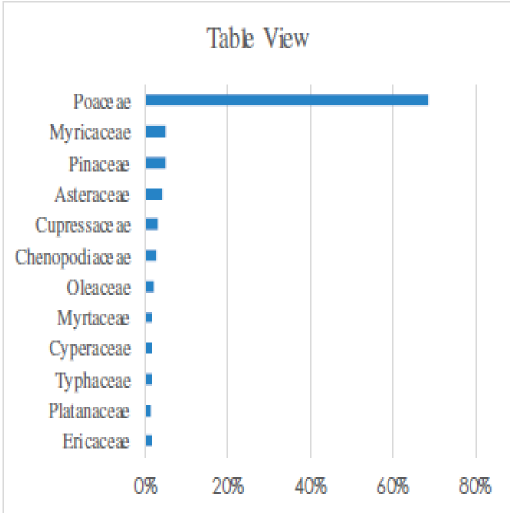



Figure 5.3b. Table View 1994-2014

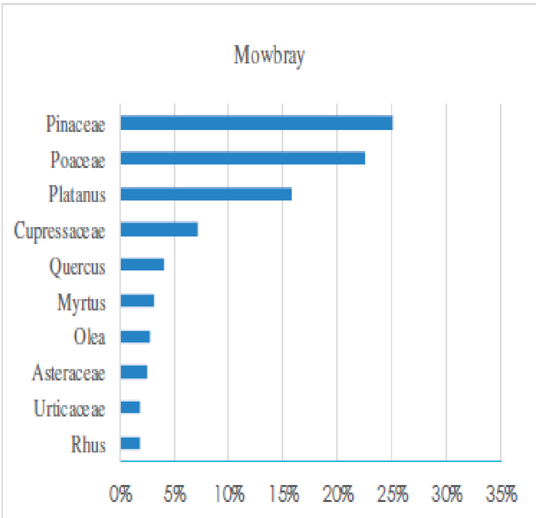



Figure 5.3c. Mowbray 2008-2010

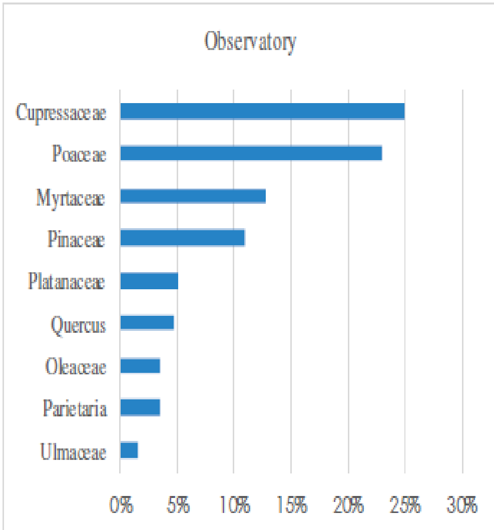



Figure 5.3d. Observatory 2010-2014

Figure 5.3 a, b, c and d. Ranked pollen taxa in order of magnitude at (a) Rondebosch 1984-2002 (b)Table View 1994-2014 (c) Mowbray 2008-2009 and (d) Observatory 2010-2014

### 5.3 Tree pollen concentrations

The annual cumulative pollen concentration was calculated for each year from 1 January to 31 December.

*Observatory*

Tree pollen concentrations were higher at the Observatory site than at the other sites. *Cupressus* concentrations ranked highest, exceeding Poaceae. Other trees that exceeded 1% of the annual pollen catch were: *Pinus spp*, (pine) *Myrtus spp*, (gum) *Platanus spp*, (plane) *Quercus spp.* (oak) *Olea* (olive) *spp*, and *Ulmus spp* (elm). Most of these trees are aliens that have been introduced to the Western Cape except for Oleaceae, which includes the indigenous *Olea europaea subsbspecies Africana* and *Ulmus* which includes *Celtis Africana*, the white stinkwood tree.

*Mowbray* 2008-2010

Tree pollen concentrations at this site were low but reflected the tree frequencies at the nearby Observatory sampling site. An unusual finding was the presence of *Rhus* at the Mowbray site, which until that time had not been identified at any other sampling site. Some *Rhus* species e.g. *Seersia* belonging to this family, are indigenous.

*Rondebosch Red Cross Children's Hospital 1997-2001*

Tree pollen dominated the pollen load in Rondebosch. Tree pollen concentrations at this site that exceeded 1% of the TPI included *Pinus spp*, (pine) *Platanus spp*, (plane) *Olea spp*, (olive)*, Cupressus spp*, (cypress) and Acacia.

*Table View 1997-2001 and 2010-2014*

Tree pollen concentrations were lowest at the Table View coastal site, in keeping with the *Cape Flats Dune Sandveld* vegetation, an open scrub vegetation type. Tree pollen that exceeded 1% of the TPI included Pine, *Cupressus spp, Olea spp, Myrtus spp and Platanus spp* but tree pollen amounted to only 26% of the TPI.

*Seasonal limits*

The seasonal limits were calculated using the 90% method whereby the start of the season was defined as the date on which 5% of the cumulative seasonal concentration was reached. The end of the season was defined as the date on which 95% of the cumulative seasonal concentration was reached. The median date was set as the date when 50% of the cumulative seasonal concentration was reached and the duration was the difference between the start and end dates.

### 5.3.1 Tree pollen seasonal patterns

The major allergenic trees identified from allergy clinic audits of skin prick and specific IgE testing in Cape Town were *Cupressus* (cypress), *Quercus* (oak) and *Platanus* (plane). The pollen release seasons for these tree taxa were similar at the three sites but differences in pollen concentration were seen at the sampling sites. Seasonal peaks for these trees were compared.

### 5.3.2 Cupressaceae *Quercus* and *Platanus* seasons

The daily average counts were smoothed using cyclic cubic penalized regression splines from the gam function of the R package mgcv (Wood 2006). Cyclic splines ensured that the curve was continuous at the start and end of the year. The method is explained in section 3.6.4. Smoothed splines are shown in Figure 5.4 below.

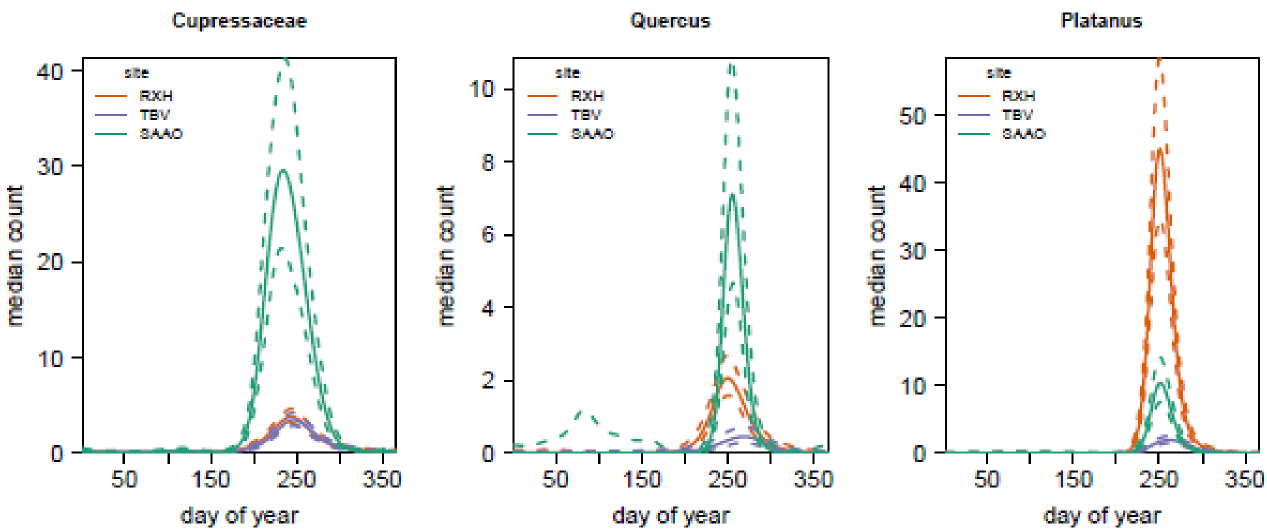



Figure 5.4 Tree pollen seasonal patterns at the three sampling sites 1997-2001at Rondebosch (RXH) and Table View (TBV) and at Table View and Observatory (SAAO) 2010-2014

### 5.3.3 *Cupressus* flowering patterns

*Cupressus* concentrations were compared at Rondebosch, Table View and Observatory. The mean daily *Cupressus* concentration value was calculated by finding the average *Cupressus* daily concentration for all the years for which data was available and log transforming the values because the counts were extremely skew and therefore difficult to show using absolute concentrations. All available data were used when histograms were drawn provided the data collection was continuous during the relevant flowering season. Years with gaps in the data were excluded. Spreadsheets containing all the sampling years can be found in Appendix A. The *Cupressus* concentrations from 1 January until 31 December at the three sites are shown in Figure 5.5 below.

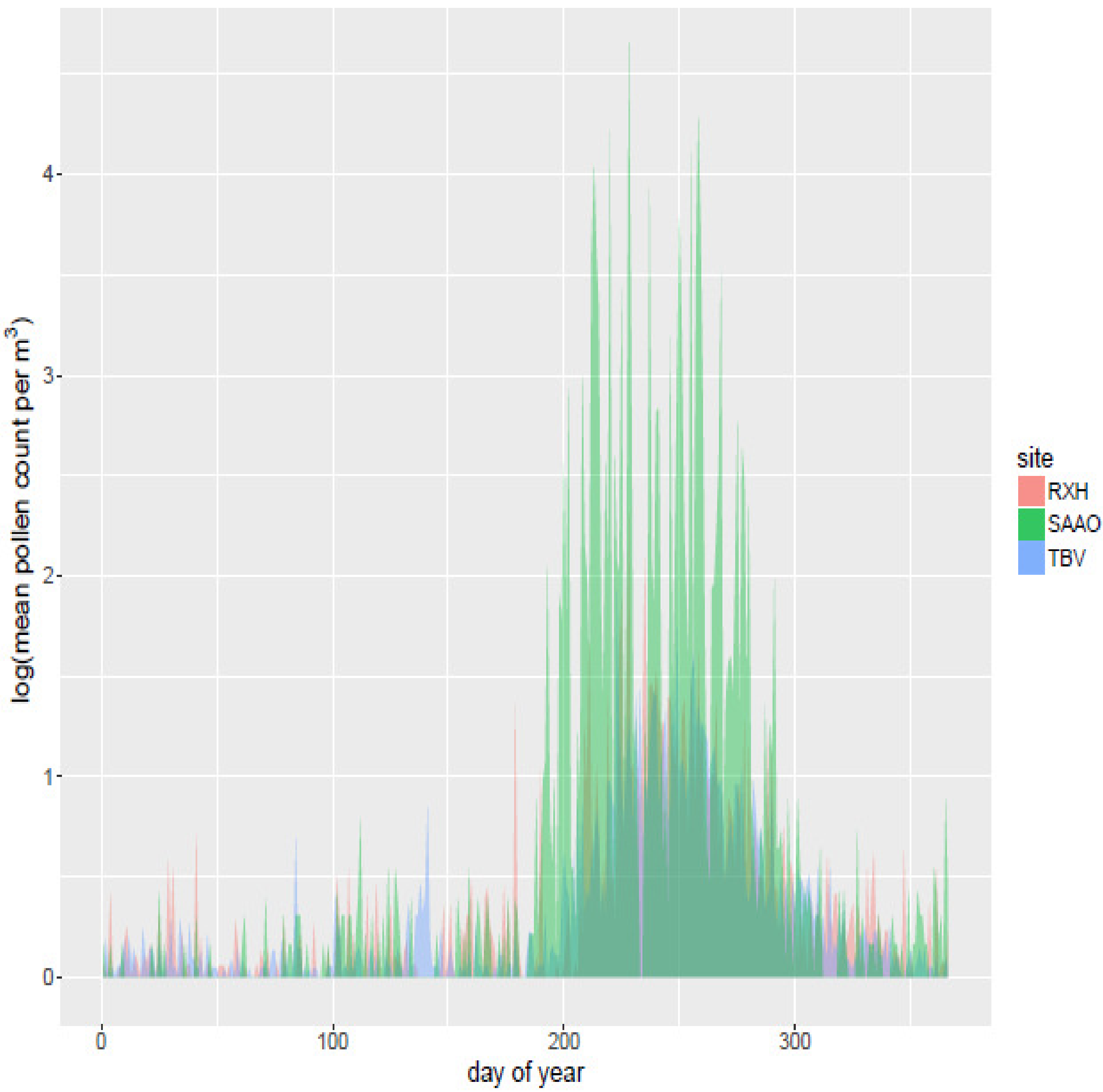



Figure 5.5 *Cupressus* daily averaged counts at Rondebosch (RXH) 1984-2001 Table View (TBV)1995-2005 and Observatory (SAAO): 2010-2014

*Cupressaceae seasonal variations*

The total pollen indices of Cupressaceae for the years 1997-2001 (Comparison I) at Rondebosch and Table View were all less than 250 grains. The concentrations at Table View were low for accurate analysis, but were compared.

*Comparison II. Cupressaceae at Table View and Observatory: 2010-2014*

The Pollen Index for *Cupressus* was consistently higher at the Observatory site. The average season started at the end of July and finished in October. Seasons were compared according to their annual index, or the total *Cupressus* annual sum start, median and end dates. The threshold value for *Cupressus* was 15 pollen grains/m$^{-3}$ according to the Burge Scale (see Methods 3.5.1). The highest score for the season was found and these measurements are shown in Table 5.2 below.

**Table 5.2. Comparison II. *Cupressaceae* seasonal variation at Table View and Observatory 2011-2014**

.

| Year | Sampling site | Start date | Median date | End date | Season duration (days) | Peak concentration grains/m$^{-3}$ | Annual *Cupressus* Index |
|---|---|---|---|---|---|---|---|
| 2011 | Table View | 20 July | 7 September | 6 October | 48 | 28 | 292 |
| | Observatory | 30 July | 12 August | 6 October | 39 | 200 | 828 |
| 2012 | Table View | 24 July | 1 September | 28 October | 66 | 17 | 332 |
| | Observatory | 26 July | 10 September | 1 October | 37 | 296 | 1713 |
| 2013 | Table View | 18 July | 3 September | 29 October | 73 | 30 | 297 |
| | Observatory | 17 July | 24 August | 5 October | 51 | 130 | 1267 |
| 2014 | Table View | 22 June | 18 September | 29 October | 68 | 16 | 181 |
| | Observatory | 29 July | 16 August | 8 September | 42 | 311 | 1443 |

*Cupressus seasonal variations*

Variations in the seasonal start, peak, end and duration dates for the 2011-2014 datasets at Observatory and Table View were found. The cumulative *Cupressus* sum (Annual Cupressus Index) for the years 2010- 2014 was summed. The cumulative *Cupressus* index at Table View was 1,102. This was only 20% of the mean annual index at Observatory which was 5,247. The season duration at Table View was longer than the season at Observatory. The start date was earlier than Observatory and the end date occurred later. Seasonal dates for this taxon are shown in Table 5.3 below.

**Table 5.3. Mean *Cupressus* seasonal dates and cumulative concentrations 2011-2014**

| Sampling Site | Mean start date | Mean Peak date | Mean end date | Mean season duration (days) | Mean *Cupressus* Index |
|---|---|---|---|---|---|
| Table View 2011-2014 | 14 July | 7 September | 23 October | 64 | 275 |
| Observatory 2011-2014 | 26 July | 24 August | 29 September | 42 | 1314 |

### 5.3.4 *Platanus* flowering patterns

*Platanus* (plane) pollen was trapped at Rondebosch and Observatory in significant concentrations but the Table View *Platanus* concentrations were too low for comparative analysis. The threshold value for *Platanus* was 15 pollen grains/m$^{-3}$ according to the Burge Scale (see Methods 3.5.1). The flowering season for this taxon was very short at all the Cape Town sites, spanning just six weeks. Significant concentrations were recorded within a three-week period which included the last week of August and the first two weeks of September. This pattern was shown by comparing the average daily count for all the sites where complete datasets were obtainable from the 1984-2014 data collection. The flowering patterns for *Platanus* at Rondebosch, Table View and Observatory may be seen in Figure 5.6 below.

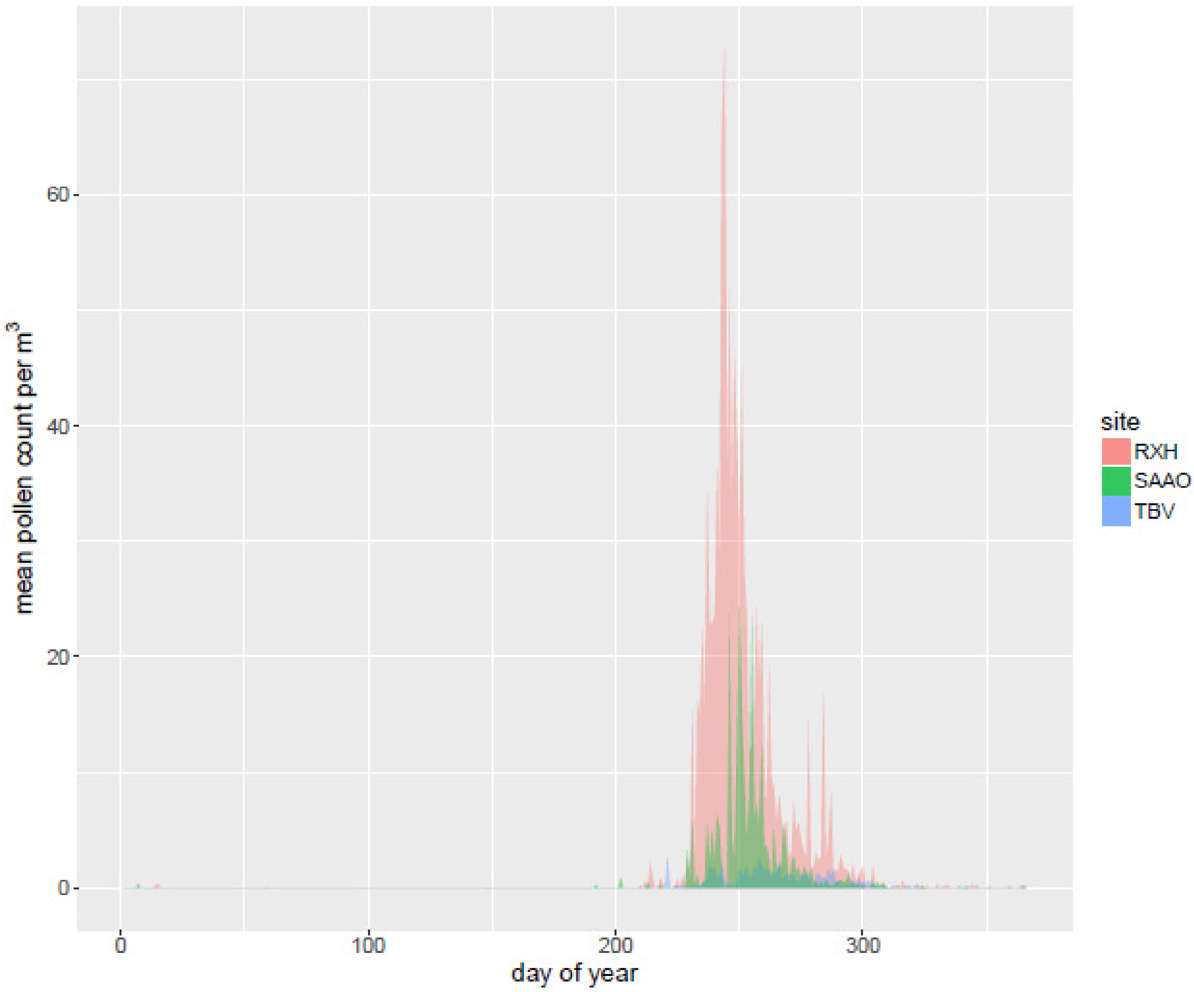


Figure 5.6 *Platanus* daily averaged counts at Rondebosch (RXH) 1984-2006 Table View (TBV) 1995-2005 and Observatory(SAAO): 2010-2014

Although plane trees began their pollen release cycle abruptly, the flowering cycle tapered to an end gradually and a weak pollen signal for this taxon persisted until the end of November or in some years, early December. The *Platanus* flowering season was defined by analysing the flowering patterns at Rondebosch from the sampling years 1997-2002 and at Observatory for the years 2010-2014. The start, median and end dates and the seasonal duration were assessed. The annual Pollen index for *Platanus* and the highest score were found. Annual concentrations and seasonal patterns for this sampling site are shown in Tables 5.4a and 5.4b below.

**Table 5.4a**. ***Platanus* seasonal variations in Rondebosch 1997-2001**

| Year | Start date | Median date | End date | Season duration (days) | Peak concentration grains/m$^{-3}$ | Annual *Platanus* Index |
|---|---|---|---|---|---|---|
| 1997 | 28 August | 10 September | 11 October | 41 | 72 | 644 |
| 1998 | ND* | ND | ND | ND | ND | ND |
| 1999 | 31 August | 5 September | 29 October | 59 | 25 | 251 |
| 2000 | 30 August | 8 September | 8 October | 39 | 41 | 339 |
| 2001 | 5 September | 12 September | 13 October | 33 | 17 | 90 |

* No data. Pollen counts from 25 August- 6 September 1998 were absent

**Table 5.4b. *Platanus* seasonal variations in Observatory 2010-2014**

| Sampling year | Start date | Median date | End date | Season duration (days) | Peak concentration grains/m$^{-3}$ | Annual *Platanus* Index |
|---|---|---|---|---|---|---|
| 2010 | 25 August | 2 September | 20 September | 26 | 95 | 279 |
| 2011 | 18 August | 6 September | 05 October | 48 | 27 | 264 |
| 2012 | 4 September | 11 September | 28 September | 24 | 66 | 254 |
| 2013 | 24 August | 8 September | 12 October | 48 | 37 | 209 |
| 2014 | 29 August | 8 September | 01 October | 33 | 32 | 182 |

*Platanus seasonal parameters*

The dates for the start, peak and end of the season were calculated. Variations in the season duration and the cumulative *Platanus* pollen sum for each period, 1997-2001 and 2010-2014 were calculated and these are shown in Table 5.5 below.

**Table 5.5. Mean *Platanus* seasonal dates and cumulative concentrations at Rondebosch 1997-2001 and Observatory 2010-2014**

| Sampling Site | Mean start date | Mean Peak date | Mean end date | Mean season duration (days) | Mean annual *Platanus* index |
|---|---|---|---|---|---|
| Rondebosch 1997-2001 | 31 August | 9 September | 15 October | 43 | 331 |
| Observatory 2010-2014 | 26 August | 7 September | I October | 36 | 238 |

*Platanus peak flowering dates*

Peak dates for the three sites were plotted regardless of their value. Peak dates fell within a narrow window that included the last week of August and the first two weeks of September at the Rondebosch and Observatory sampling sites. The low concentrations of *Platanus* pollen at Table View showed more scatter, first appearing from August 24 and maintaining a low presence in the atmosphere until the third week in October. Table View peaks did not exceed the significant threshold. A plot was drawn to show peak values and dates at the three sites, using filled dots to show the major peak dates at all the sites and unfilled dots to show smaller, isolated peaks. The peak flowering times at Observatory and Rondebosch are in keeping with the peak dates in Table 5.5. The peak flowering times at Table View are later but the peaks are low and significant counts more than 15 grains $m^{-3}$ were infrequently recorded. The peak flowering dates are shown in Figure 5.7 below.

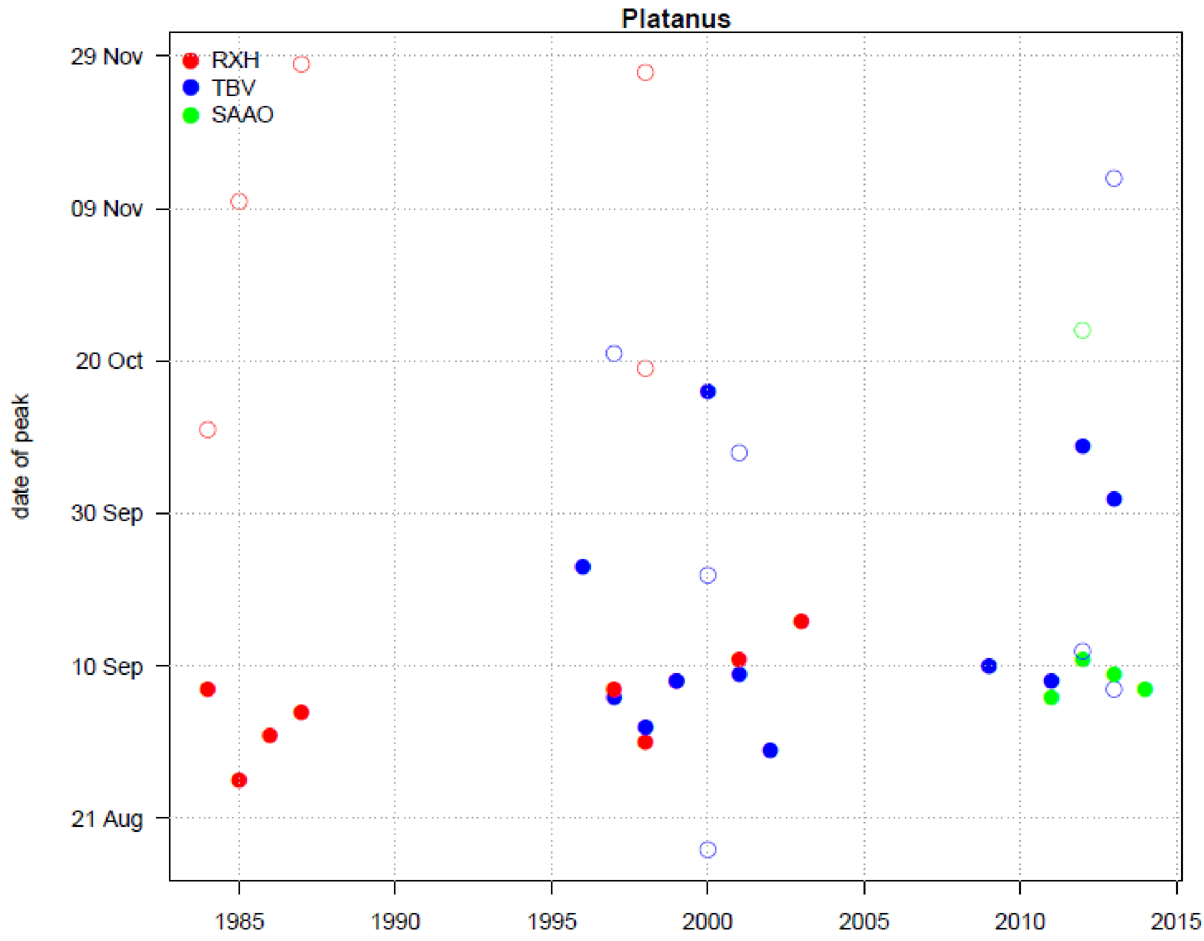



Figure 5.7 Platanus peak flowering dates at Rondebosch (RXH) Table View (TBV) and Observatory (SAAO) Filled dots show the main peak dates. Unfilled dots show isolated high counts outside the peak flowering season.

### 5.3.5 *Quercus* flowering patterns

*Quercus robur* is the oak species most commonly found in the suburbs where the pollen sampling sites were located and it is likely that *Quercus robur* is the pollen grain that is most often trapped. Insignificant concentrations of *Quercus* were seen at Table View. The threshold value for *Quercus* was 15 pollen grains/$m^{-3}$ according to the Burge Scale (see Methods 3.5.1) At the Rondebosch site, the findings were variable, but concentrations rarely rose above moderate levels. The highest concentrations were obtained from the Observatory site, where a narrow window of significant concentrations was seen in early spring. Flowering patterns for Quercus at the sites are shown in Figure 5.8 below.

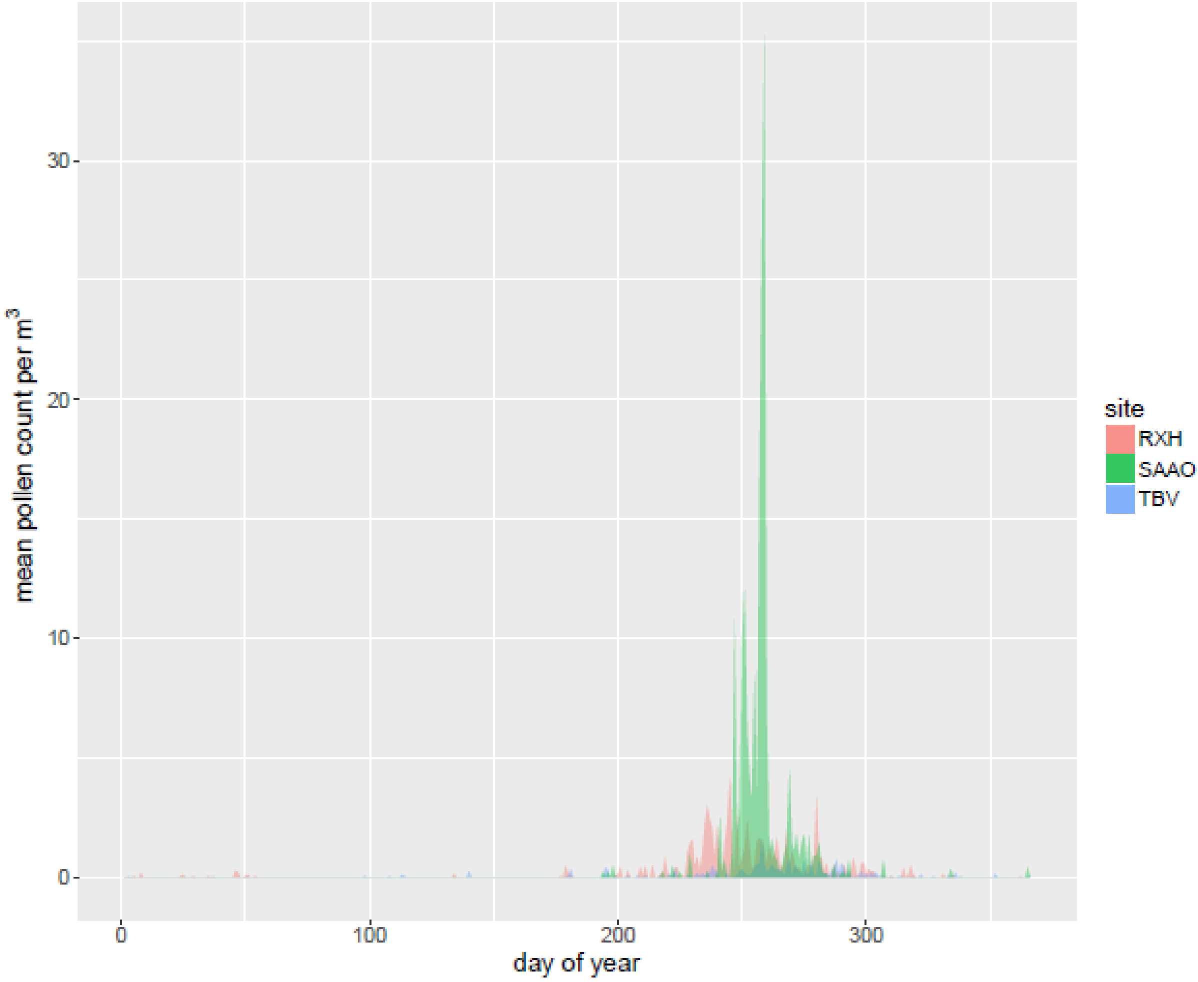


Figure 5.8 *Quercus* daily averaged counts at Rondebosch (RXH) 1984-2001 Table View (TBV)1995-2005 and Observatory (SAAO): 2010-2014

*Quercus seasonal parameters*

The annual *Quercus* pollen index and date of the highest concentration at the Observatory site were found. The start, median and end dates and the duration of the *Quercus* season were calculated and are shown in Table 5.6 below.

**Table 5.6. *Quercus* seasonal variations at Observatory**

| Sampling year | Start date | Median date | End date | Season duration (days) | Peak concentration grains/m³ | Annual *Quercus* Index |
|---|---|---|---|---|---|---|
| **2010** | 30 August | 7 September | 25 September | 26 | 42 | 197 |
| **2011** | 27 August | 19 September | 6 October | 40 | 12 | 90 |
| **2012** | 6 September | 15 September | 25 September | 19 | 140 | 401 |
| **2013** | 12 August | 10 September | 3 October | 51 | 9 | 46 |
| **2014** | 9 August | 8 September | 2 October | 55 | 28 | 137 |

*Quercus seasonal variations*

The dates for the start, peak and end of the season were calculated. The variations in season duration and the overall *Quercus* pollen index were calculated for the 2010-2014 data set and these measurements are shown in Table 5.7 below.

**Table 5.7 Mean *Quercus* seasonal dates at Observatory (SAAO).**

| Sampling Site | Mean start date | Mean Peak date | Mean end date | Mean season duration (days) | Mean Quercus annual index |
|---|---|---|---|---|---|
| Observatory 2010-2014 | 23 August | 12 September | 30 September | 39 | 174 |

### 5.3.6 Tree Pollen Summary

Tree pollen concentrations were higher at the inland sites at Rondebosch and Observatory than at the coastal site of Table View. The most prevalent tree pollen taxa at the coastal and inland sites were *Cupressus, Pinus* and *Platanus*. Pine pollen levels were not assessed due to the low incidence of responders to *Pinus* on Skin Prick Tests and specific IgE testing from audits of patients that attended the Red Cross Children's Hospital Allergy Clinic. (Potter et al. 1991). *Cupressus* concentrations were trapped in the highest concentrations at Observatory. There was a 5-fold difference between the annual *Cupressus* index at Observatory and the annual cumulative *Cupressus* index at Table View. *Quercus* pollen exceeded 1% of the TPI at the Observatory sampling site only. *Quercus* pollen concentrations did not ever exceed the threshold value of 15 pollen grains/$m^{-3}$ at Table View. The flowering season for all the major tree pollen taxa was short, spanning just six weeks for the taxa *Cupressus*, *Platanus* and *Quercus.* The exception was *Cupressus* at Table View, where the season was 14 weeks. Significant concentrations of *Platanus* pollen were measured at Rondebosch and Observatory only. No significant *Platanus* concentrations were measured at Table View.

*Tree pollen Plates 1-5*

The following plates with pollen photomicrographs illustrate trees and their pollen found in the south-western Cape. The photographs depicting the trees were all taken near the pollen sampling sites in Newlands and Rondebosch. Plate 4 depicting *Salix babyonica*, or weeping willow is added for interest as it is found in low quantities. The photomicrographs were taken by the author.

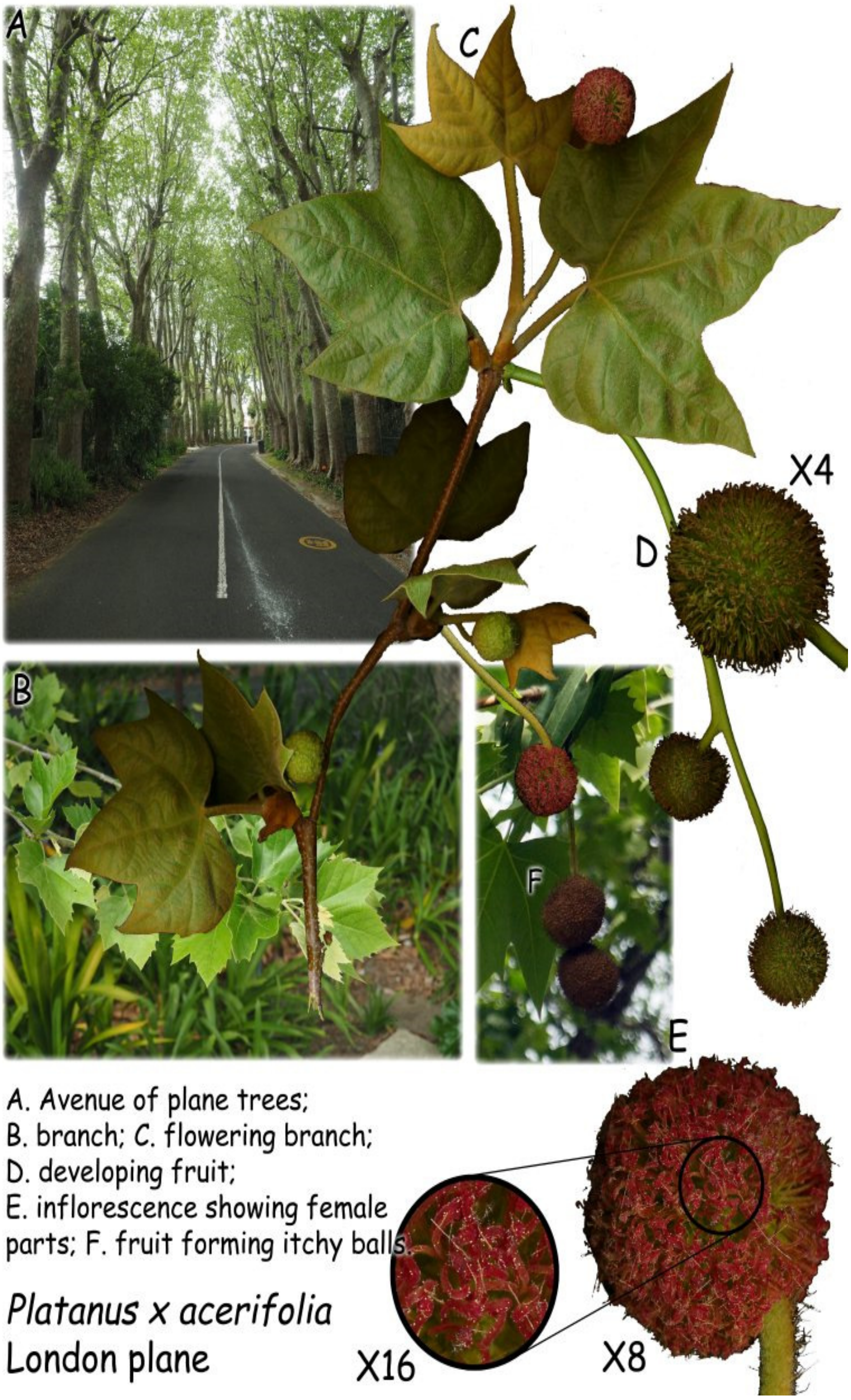
Plate 1. Cupressus sempervirens - cypress tree


*Cupressus intact pollen grain*

*Cupressus pollen grain showing ruptured exine or cell outer wall*

**X** = Magnification factor

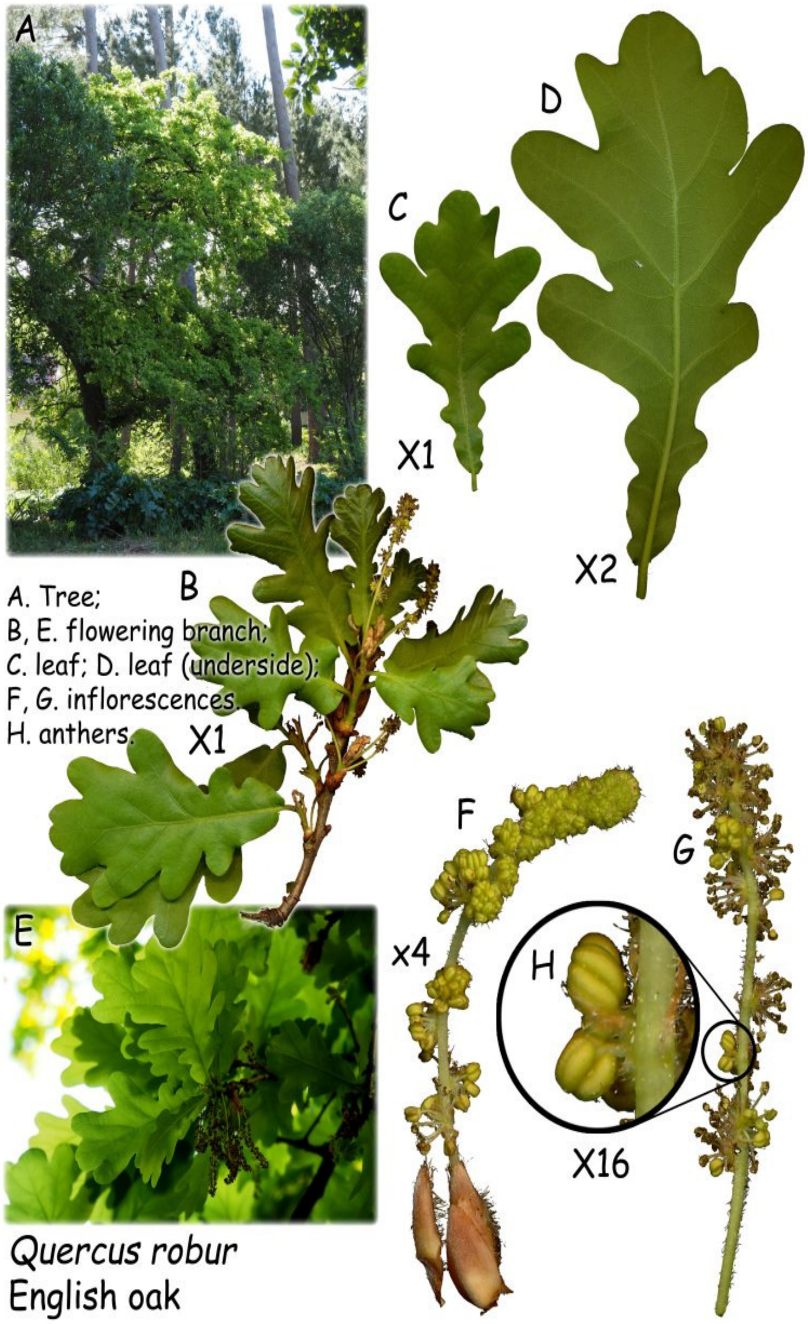
Plate 2. Platanus acerifolia - London plane tree

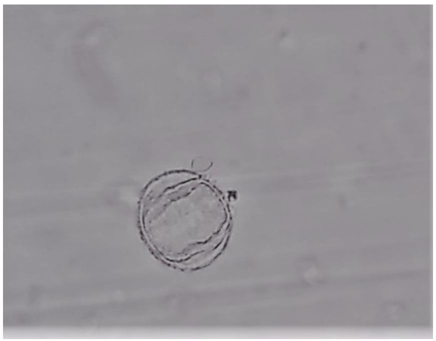



**Tricolpate *Platanus spp* pollen grain**

**X** = Magnification factor

# Plate 3. Quercus robur- English oak tree

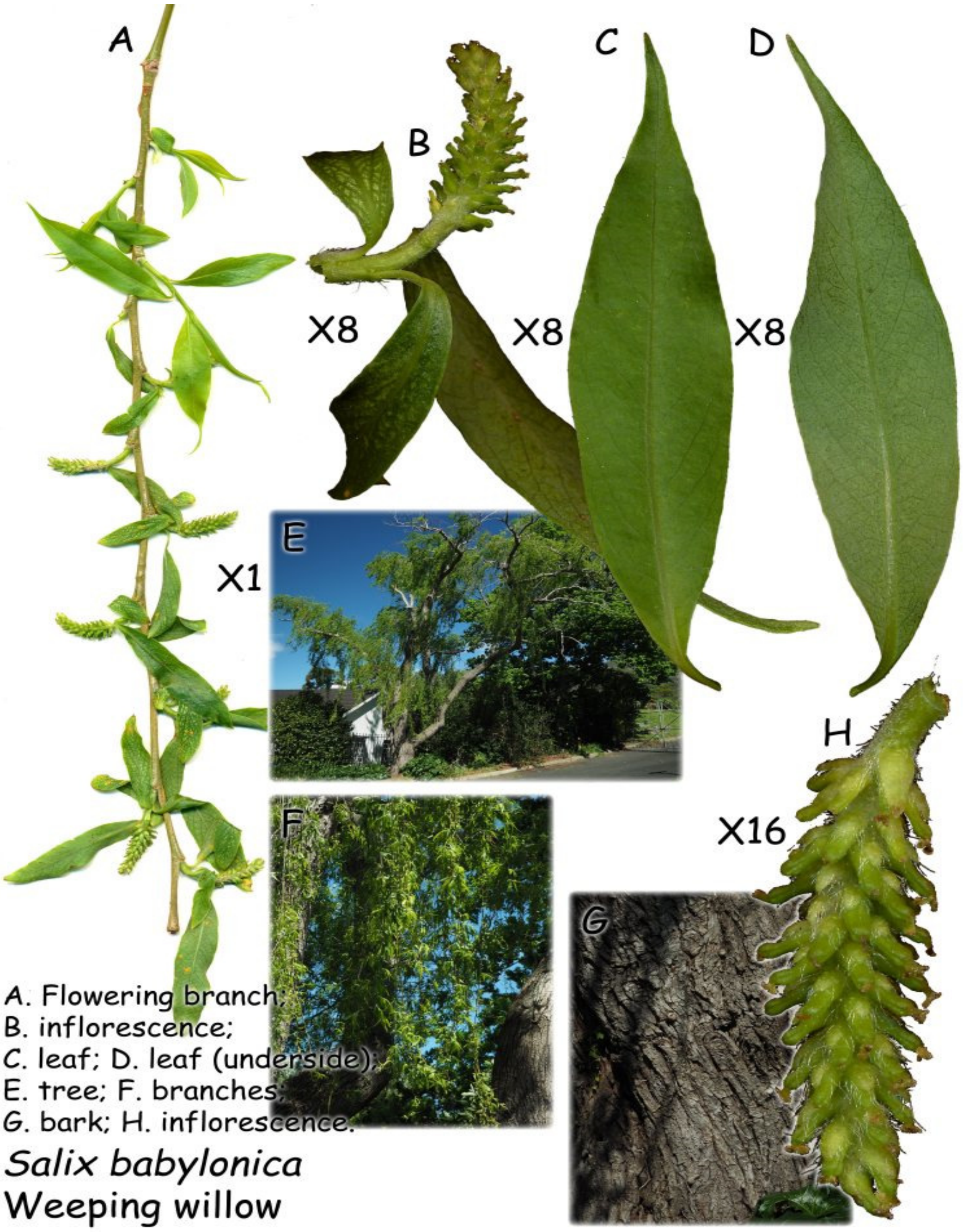


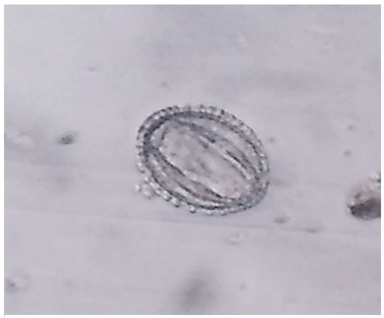



*Quercus sp pollen grain*

**X** = Magnification factor

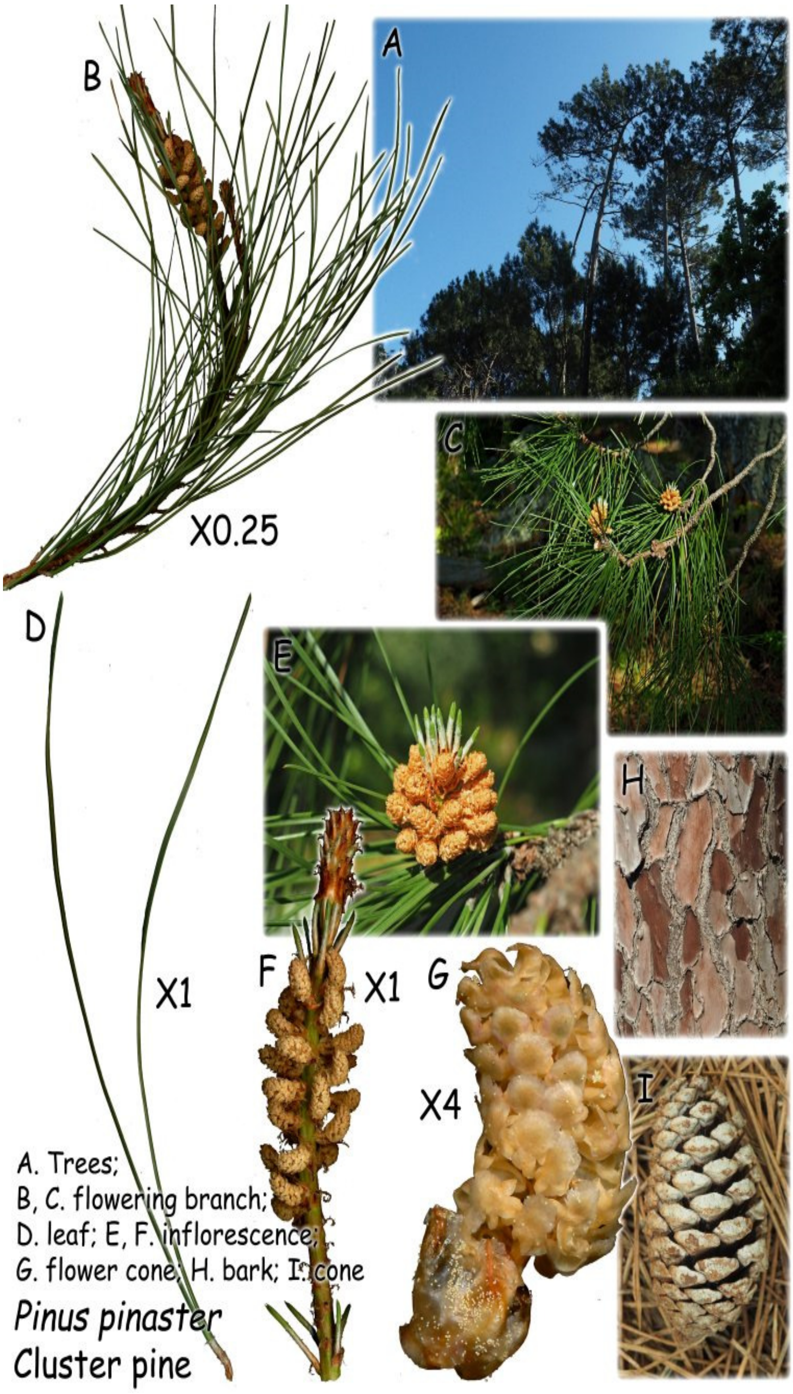
Plate 4. Salix babylonica - Weeping Willow tree



*Salix pollen grain*

# Plate 5. Pinus pinaster - pine tree




*Pinus pollen grains have air bladders*

## 5.4 Poaceae

Grasses belong to the family Poaceae. Allergenic grasses are found amongst the tribes of the sub families Pooideae, Panicoideae, Paniceae and Chlorodoideae. Insignificant concentrations of Typhaceae, or bulrushes, were identified from December to March. Restionaceae, or reeds flowered from October to March and were identified in low concentrations of less than 10 pollen gains/m $^{-3}$. Grasses begin their flowering cycle in August in the Cape and continue to pollinate until autumn but grass concentrations did not increase significantly until September at the Cape Town sampling sites. The lower Poaceae threshold was set at 5 pollen grains/m$^{-3}$ according to the Burge Scale (see Methods 3.5.1). Mean daily grass concentrations for all the years were calculated from the daily averaged concentrations from the datasets of the sampling sites. The grass seasons and peak flowering dates from these sampling sites were compared. The annual distribution of grass pollen, measured from 1 January -31 December may be seen in Figure 5.9 below.

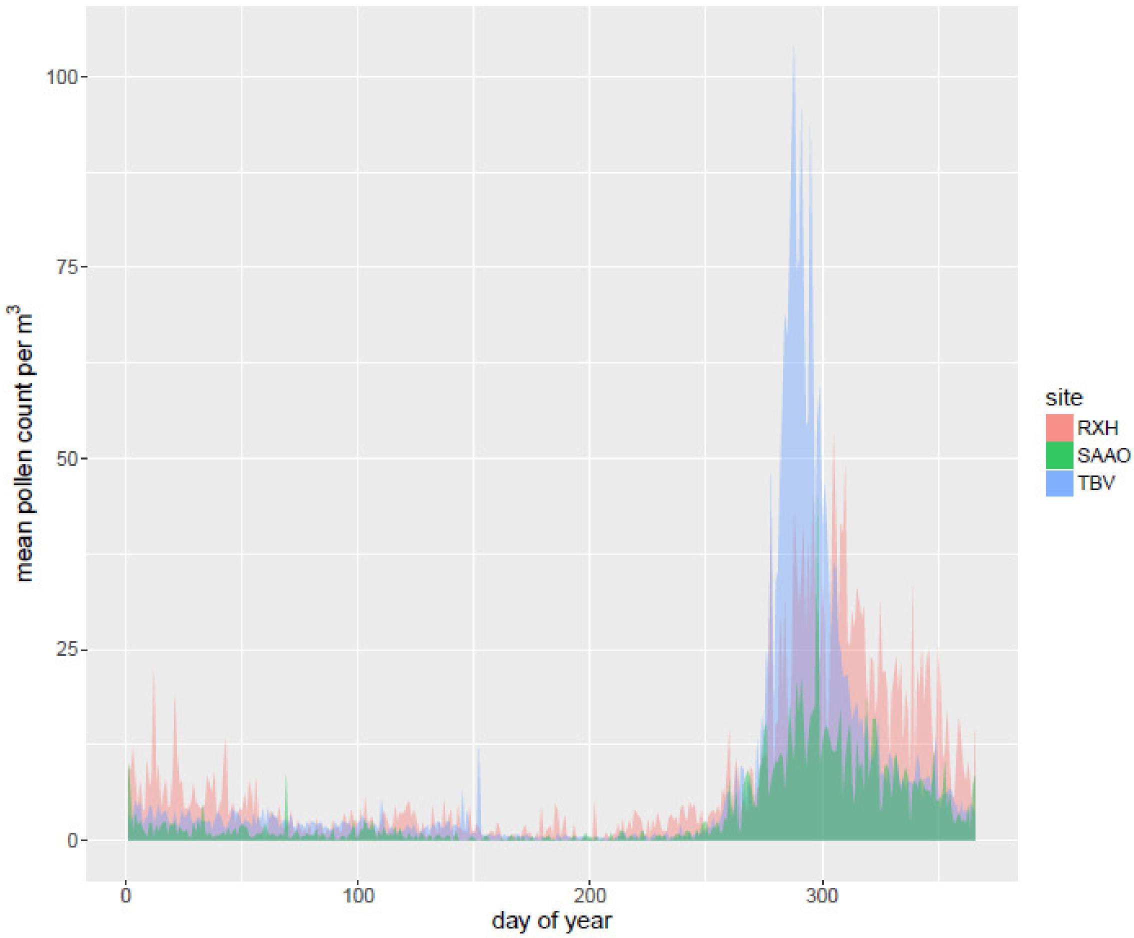



Figure 5.9 Poaceae daily averaged counts at Rondebosch (RXH) 1984-2001 Table View (TBV)1995-2005 and Observatory (SAAO): 2010-2014

### 5.4.1 Defining the grass pollen season

The start and end dates for the grass flowering seasons are used by allergists for selecting the correct months for clinical trials and grass immunotherapy. The start/median/end of season was defined as the first day on which the cumulative count exceeded 5%/50%/95% of the total daily average count for the season. For the start and end of the grass season we applied larger cut-offs, 5% rather than 2.5% to eliminate long tails at each end of the season when grass concentrations gradually decrease to less than 10 pollen grains. The duration of the season was calculated as the difference between the start and end dates.

*Annual Poaceae Index (API)*

The seasonal dates were calculated by summing the mean daily grass pollen counts throughout the grass flowering period. Grasses begin to flower in July and are a constant presence in the atmosphere in concentrations that are initially low i.e. less than 5 pollen grains/m$^{-3.}$ Grasses start to increase after the winter rain, peaking in spring and the flowering cycle continues after the end of the calendar year. For this reason, the grass season in Cape Town is not calculated according to the calendar year as it is for the trees. The grass concentration for each day is summed from 1 July to 30 June for each year, labelled according to the year containing the start of the season. This total is known as the Annual Poaceae Index (API). An average API was calculated from sampling years with sufficient data from the 1984-2014 datasets. The API for every sampling year could not be calculated as some years had missing data. These are listed in the Appendix. The grass distribution from 1 July-30 June at Table View and Observatory for the years 2010-2014 with a threshold line of 10 pollen grains/m$^{-1}$ was plotted. This threshold was chosen as it is a clear indication that grass pollen levels are increasing and is shown in Figure 5.10 below.

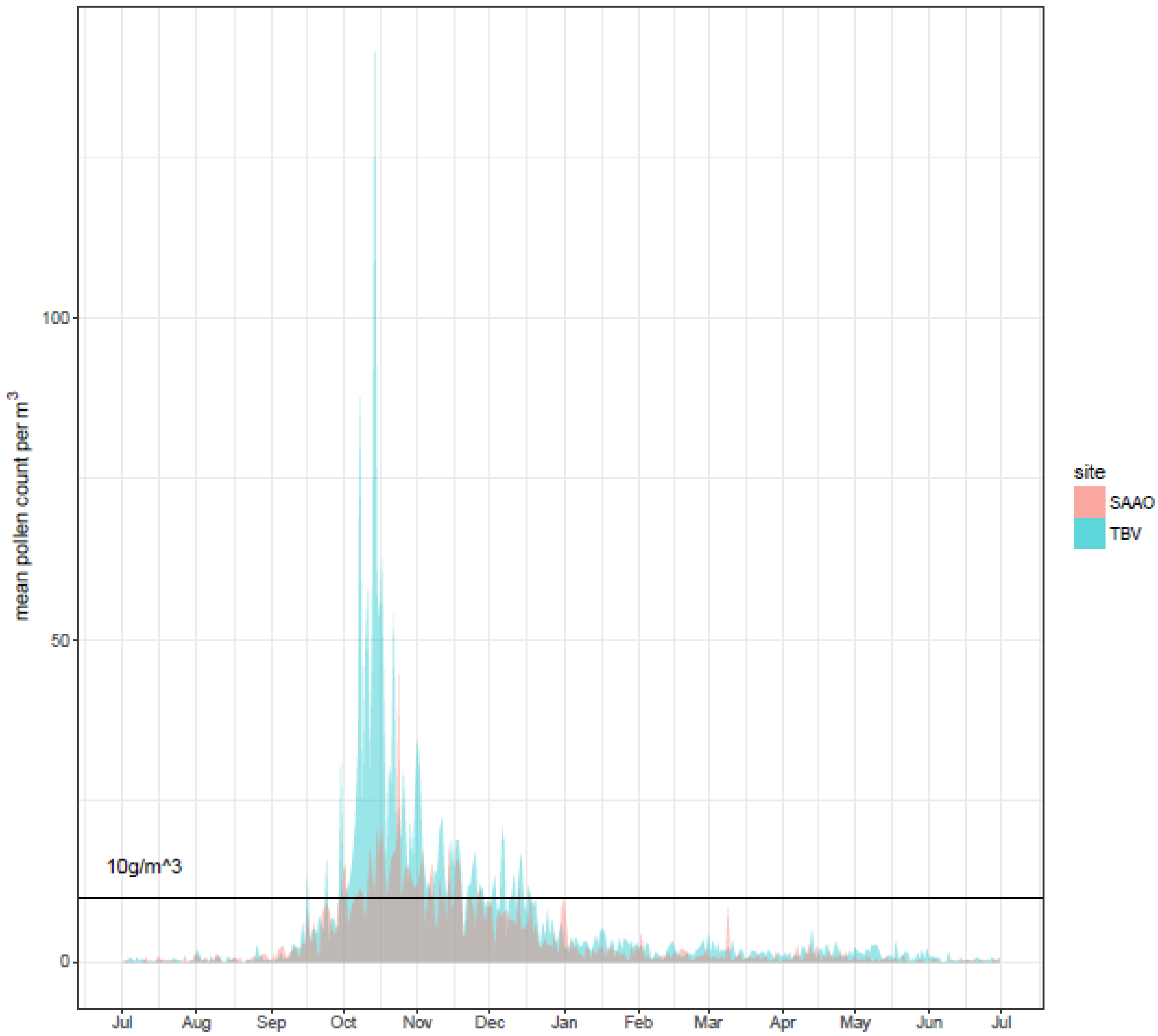



Figure 5.10 The grass season at Observatory (SAAO) and Table View (TBV) from 1 July-30 June 2010-2014 showing a 10grains/$m^3$ threshold line to identify the time of year (September-January) when grass counts exceed the grass threshold.

### 5.4.2 Comparison of grass seasons at the sites

The seasonal variation of grasses at the sites was compared at the Table View and Observatory sampling sites by analysing grass pollen concentrations collected from the years 2010-2014. To compare the start of the season at two sites (or two periods) we estimated a 95% confidence interval for the difference assuming independent, normally distributed values, i.e. observed difference ± 2 x SE(difference), where the SE(difference) was calculated as the square root of the sum of the variances. 2-sided p-values for the difference under a null hypothesis of no difference, were calculated under the same assumptions.

*Day of the year (DOY)*

We calculated the start, median and end days as the numerical day of year (DOY), 1=1 July for the years 2010-14 at Table View and SAAO from the mean daily counts (mean count from 4 years of data) for each site and found the mean of the four years for each site These values are shown in Table 5.8 below.

**Table 5.8. Start median and end of the grass season at Table View and Observatory 2011-14**

| | V1 | V2 | V3 | july.obs.quantiles | X2.5. | X50. | X97.5. | SE.july | avg.july |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Poaceae | TBV | start | 90 | 81.975 | 90 | 96 | 4.069237455 | 89.21 |
| 2 | Poaceae | TBV | median | 114 | 109 | 115 | 123 | 3.515847111 | 114.875 |
| 3 | Poaceae | TBV | end | 273 | 238.95 | 267 | 336 | 24.89838646 | 273.4 |
| 4 | Poaceae | TBV | duration | 183 | 145.975 | 179.5 | 246.025 | 25.57094575 | 184.19 |
| 5 | Poaceae | SAAO | start | 80 | 77 | 81 | 85 | 2.707072442 | 80.72 |
| 6 | Poaceae | SAAO | median | 126 | 120 | 126 | 134 | 3.643232178 | 126.865 |
| 7 | Poaceae | SAAO | end | 253 | 232.95 | 253 | 278.1 | 11.40761889 | 253.63 |
| 8 | Poaceae | SAAO | duration | 173 | 152.975 | 172 | 198.05 | 11.56158515 | 172.91 |

DOY: 1=1 July. V1 V2 V3= value 1 value 2 and value 3. Observed quantiles correspond to the observed start median, end and duration. 2.5%, 50% and quantile 97.5%. Quantile standard error and average were obtained from bootstrapped cumulative curves.

*Day of year as a calendar date*

The day of year for the start, median and end dates represents a calendar date. DOY was converted to calendar dates and these may be seen in Table 5.9 below.

**Table 5.9. Average grass seasonal dates 2011-2014**

| Sampling Site | Start Date | Median date | End date | Duration/Length of season (days) |
|---|---|---|---|---|
| Table View (TBV) | 27 September | 22 October | 30 March | 183 |
| Observatory (SAAO) | 17 September | 3 November | 10 March | 173 |

### 5.4.3 Table View and Observatory seasonal differences

Confidence intervals and P-values were calculated to examine the difference between the Table View and Observatory site datasets for the years 2010-2015 and these are shown in Table 5.10 below.

**Table 5.10**. P-values applied to mean start, median and end dates for the Poaceae season At Table View and Observatory 2010-2015. The difference is measured in days (diff).

| | class | stat | diff | CI.low | CI.upp | p.value |
|---|---|---|---|---|---|---|
| 1 | Poaceae | start | -10 | -19.77485236 | -0.2251476383 | 0.04074980787 |
| 2 | Poaceae | end | -20 | -74.77457137 | 34.77457137 | 0.4652276862 |
| 3 | Poaceae | median | 12 | 1.873930356 | 22.12606964 | 0.01778231362 |
| 4 | Poaceae | duration | -10 | -66.12641152 | 46.12641152 | 0.7215870975 |

We used confidence intervals to compare the start, median and end dates at the Table View and Observatory pollen sampling sites. We found some evidence that the grass season started earlier at Observatory. Range 0-20 days, Confidence Interval 95%. There was no evidence of a difference in the end or duration of the grass flowering season between the sites.

### 5.4.4 High concentration Poaceae days

The number of days during the season when grass exceeded 20 grains/$m^3$ was calculated from the grass pollen concentrations from each of the sampling sites. The annual cumulative score or Annual Poaceae Index (API) was calculated by summing Poaceae counts from 1 July to 30 June. The cumulative score was calculated for the year from 1 July to 30 June. The highest daily Poaceae score for each year was found and labelled the highest concentration date. The start date was the day on which 5% of the API was reached, the median date was the date on which 50% of the annual grass pollen sum was reached and the season end date was the date on which 95% of the API was reached. These comparisons are shown in Table 12 below. Poaceae, or grass concentrations first exceeded 10 grains/$m^{-3}$ during September at the sites and March was the final month before concentrations declined to less than 10 pollen grains/$m^{-3}$. Anomalous grass concentrations that exceeded 10 pollen grains were occasionally measured outside of the main season.

*Annual Pollen Index (API)*

The API at Table View was consistently higher than the API at the Rondebosch site. The median date occurred earlier at the coastal site and the highest concentration date occurred earlier for most of the years in the 1997-2001 comparison. The peak date was defined as the date on which the median grass concentration for the season was trapped and it usually occurred close to the date of the maximum concentration.  The maximum concentration showed the highest daily grass concentration. These values are shown in Table 5.11 below.

*Comparison I*

**Table 5.11. Poaceae seasonal variation at Rondebosch (RXH) and Table View (TBV) 1997-2001**

| Year | Sampling Site | Start date | Median date | End date | Season duration (days) | Maximum concentration date and value | No days ≥ 20 grains/m³ | Annual Poaceae Index (API) |
|---|---|---|---|---|---|---|---|---|
| 1997 | Rondebosch | 11 September | 1 November | 12 February | 154 | 16 October (41) | 11 | 1058 |
| | Table View | 21 September | 26 October | 7 February | 139 | 9 October (84) | 13 | 1474 |
| 1998 | Rondebosch | 30 September | 20 November | 12 February | 219 | 19 November (45) | 12 | 1007 |
| | Table View | Data missing | Data missing | Data missing | Data missing | Data missing | Data missing | Data missing |
| 1999 | Rondebosch | 27 September | 8 November | 4 May | 219 | 11 +25 October (23) | 6 | 937 |
| | Table View | 27 September | 20 October | 31 March | 184 | 16 October (389) | 31 | 3722 |
| 2000 | Rondebosch | 29 September | 31 October | 29 March | 182 | 20 October (66) | 6 | 1122 |
| | Table View | 10 October | 20 October | 17 February | 130 | 13 October (321) | 30 | 2813 |
| 2001 | Rondebosch* | 9 October | 12 November | 21 February | 135 | 12 November (30) | 6 | 831 |
| | Table View* | 2 October | 14 October | 26 April | 206 | 9 October (430) | 26 | **5350** |

* No data 20-26 August

*Seasonal Poaceae ranges 1997-2001 at Rondebosch and Table View*

The 2001-2002 grass season at Table view was flagged as a severe grass season. Very high concentrations >200 grains/$m^{-3}$ were recorded from 9-14 October. The grass concentration on 14 October was 544 grains/ $m^{-3}$. The seasonal ranges for the grasses for the years 1997-2001 were calculated for the two sites and are shown in Table 5.12 below.

**Table 5.12. Poaceae mean seasonal variations at Rondebosch and Table View 1997-2001**

| Sampling Site | Mean start date | Mean peak date | Mean end date | Season duration (range in days) | Mean maximum concentration grains/ $m^{-3}$ | Average no of days ≥ 20 grains/ $m^{-3}$ | Mean Poaceae Index |
|---|---|---|---|---|---|---|---|
| Rondebosch | 27 September | 9 November | 11 March | 182 | 41 | 8 | 991 |
| Table View | 3 October | 20 October | 14 March | 165 | 306 | 25 | 3339 |

*Poaceae seasonal parameters*

The 2011-2014 Poaceae API, median score =50% of the API, highest concentration dates, highest scores and number of days with grass concentrations more than 20 pollen grains/$m^{-3}$ at Table View and Observatory were found. 20 pollen grains/ $m^{-3}$ was the threshold between moderate and high concentrations according to the Burge scale (see Methods 3.5.1). At Table View the grass pollen concentrations were ≥20 pollen grains/ $m^{-3}$ on 98 days of this five-year cycle. At Observatory the grass concentrations were ≥20 pollen grains/ $m^{-3}$ on 25 days of this five-year cycle. The measurements are shown in Table 5.13 below.

*Comparison II*

**Table 5.13. Poaceae seasonal variations at Table View and Observatory 2011-2014**

| Year | Sampling Site | Start date | Median date | End date | Duration | Maximum concentration Grains/m$^{-3}$ | No days ≥ 20 grains/ m$^{-3}$ | Annual Poaceae Index (API) |
|---|---|---|---|---|---|---|---|---|
| 2011 | Table View | 18 September | 23 November | 23 April | 217 | 17 October (45) | 6 | 1049 |
| | Observatory | 14 September | 27 October | 14 April | 212 | 17 October (34) | 10 | 1246 |
| 2012 | Table View | 21 September | 17 October | 12 March | 173 | 8 October (260) | 25 | 2509 |
| | Observatory | 15 September | 28 October | 13 March | 180 | 21 October (37) | 8 | 1234 |
| 2013 | Table View | 30 September | 27 October | 1 February | 124 | 14 October (289) | 39 | 2599 |
| | Observatory | 19 September | 10 November | 14 March | 145 | 15 October (37) | 5 | 1018 |
| 2014 | Table View | 17 September | 20 October | 27 March | 191 | 14 October (128) | 18 | 1591 |
| | Observatory | 7 September | 23 October | 22 December | 104 | 14 October (30) | 2 | 610 |

*Seasonal Poaceae ranges 2011-2014 at Table View and Observatory*

The seasonal range for the start, peak, end and duration of the grass season at the Table View and Observatory sampling sites were calculated. The maximum grass concentration for a 24-hour period and the number of days when the grass concentration was ≥ 20 grains/m$^{-3}$ were found and these measurements are shown in Table 5.14 below.

**Table 5.14 Poaceae mean seasonal ranges at Table View and Observatory 2011-2014**

| Sampling Sites | Mean start date | Mean peak date | Mean end date | Season duration (range in days) | Mean maximum concentration grains/ $m^{-3}$ | Mean no of days >20 grains/ $m^{-3}$ | Poaceae cumulative sum |
|---|---|---|---|---|---|---|---|
| Table View | 21 September | 29 October | 17 March | 124-217 | 289 | 98 | 1937 |
| Observatory | 14 September | 29 October | 24 February | 104-212 | 37 | 25 | 1027 |

### 5.4.5 Peak Poaceae dates at the pollen sites

The grass concentrations peaked in mid-October at Table View and Observatory except for 2012 when the Table View peak occurred on 8 October. Grasses in Rondebosch peaked from the October 26- November 20. There is within-site variation as the grass season may peak on a range of dates, even at the same site during different years. Peak Poaceae dates were compared for the three sites and are shown in Figure 5.11 below.

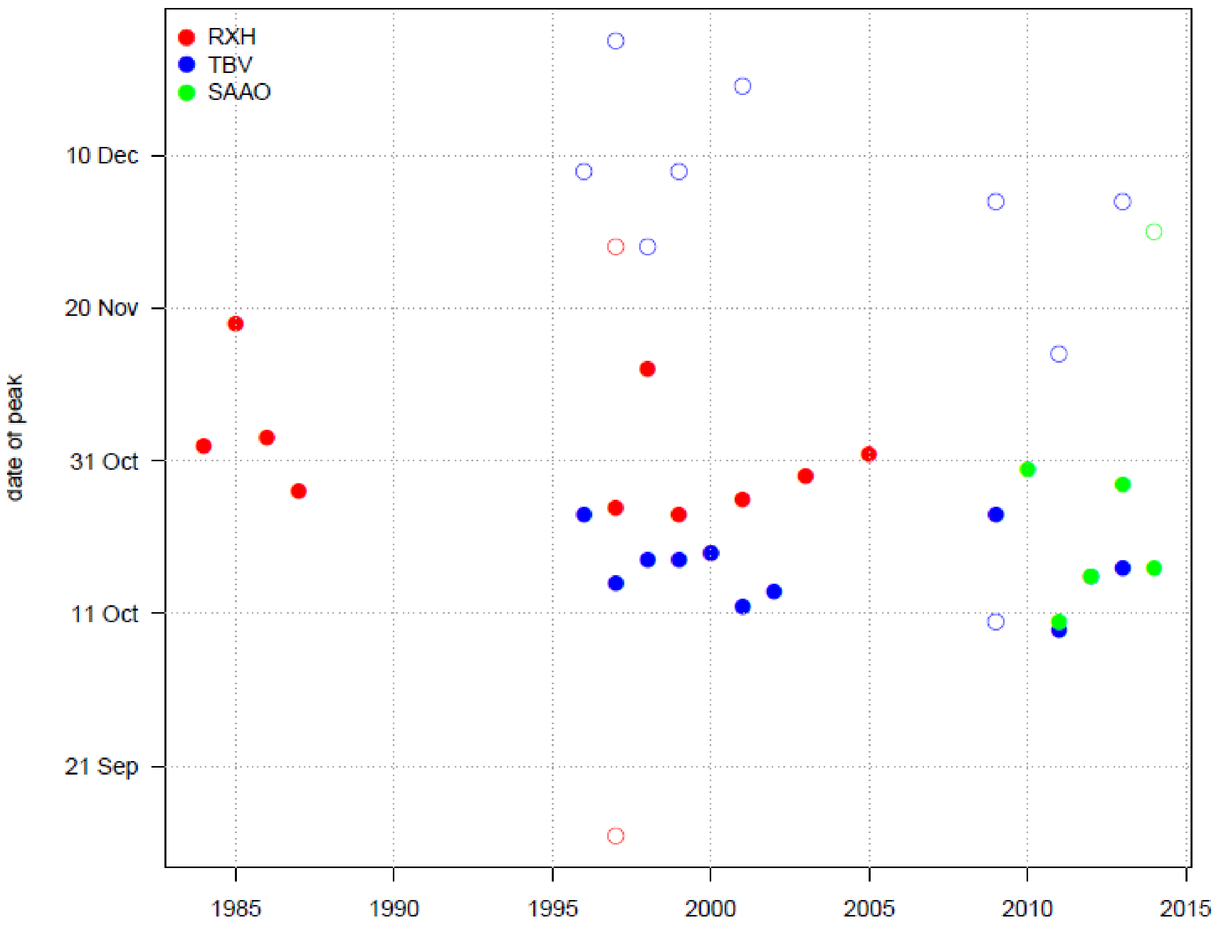



Figure 5.11 Peak pollen dates for Poaceae at Rondebosch Table View and Observatory 1985-2015 from data sets: Rondebosch 1984-2006, Table View1995-2004, 2010-2014 and Observatory 2010-2015. Filled dots show the main peaks of the grass flowering season, open dots show minor isolated peaks

### 5.4.6 Severity of the grass season

When the Annual Poaceae Index (API) exceeded 5 000 it was flagged and placed in the category: Severe Grass Season. Table View experienced a severe grass season during 2001. The mean API for the 1997-2001-time period at Table View was 3 266. When the API was measured for the series 2010-2014 grass concentrations were found to have declined sharply at Table View and no severe grass seasons were recorded for these years. At Table View the API was consistently higher than the API at Rondebosch. The mean API at Table View was also consistently higher than the API at SAAO for the later data set of years 2010-2014.

### 5.4.7 Changes in Poaceae concentrations over time

*Table View*

The mean API at Table View decreased from a 5483 for the years 1997-2003 to a mean API of

1937 for the years 2011-2014.

*Rondebosch*

The Poaceae data collection from the Red Cross Hospital site in Rondebosch was incomplete and some years could not be included for this reason. Grass and tree pollen data for years with complete data sets for the months September to December were averaged and compared and are shown in Figure 5.12 below.

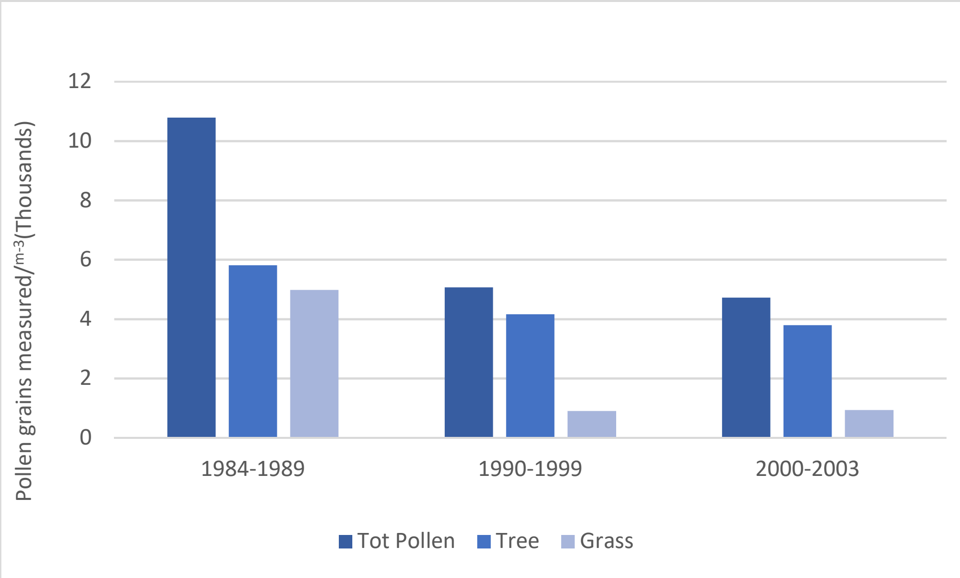



Figure 5.12 Averaged total pollen, total tree and total grass concentrations at Red Cross Hospital September-December 1984-2003

Total pollen, tree and grass concentrations declined sharply from 1989 to 1999 but the pollen concentrations for the years 1990-1999 were comparable with the years 2000-2003.

### 5.4.8 Poaceae Summary

Poaceae pollen frequencies exceeded 22% of the pollen load at all three inland sites. 68% of the pollen load at the coastal site of Table View consisted of grass pollen. Grass pollen ranked first of the pollen taxa at the coastal site of Table View and at the inland site at Rondebosch. Grass pollen ranked second at the Mowbray and Observatory sites. When the grass seasons for the years 2010-2014 at Table View and Observatory were compared, the Table View annual pollen indices were consistently greater than those of Observatory. The number of days with grass pollen scores ≥20 at Table View was 4-fold more than Observatory. The grass season started earlier in Observatory than at Table View but the duration and end dates of the grass season at the two sites were similar. Table View grass concentrations were higher than those of Observatory and sometimes exceeded 100 grains/$m^{-3}$/day during the peak grass pollen period. Daily grass concentrations at Rondebosch and Observatory rarely rose above 30 grains/$m^3$/day. The annual grass index decreased by 65% at Table View between 1997 and 2010. The grass count at Rondebosch decreased by 80% between 1989 and 1999 and these low grass concentrations continued for the years 2000-2003.

### 5.4.9 The flowering sequence of the Cape grasses

All the grasses were photographed on Rondebosch Common and on the University of Cape Town sports fields under my direction except *Stenotaphrum secundatum* (Buffalo grass) and *Pennisetum clandestinum* (*Kikuyu)*. The latter two photographs were taken from http://www.ispot.org.za

*Poaceae flowering sequence Plates 6-13*

The Plates which illustrate grasses found in abundance in the Greater Cape Town Metropolitan Area are shown in the same order as the timing of their appearance in spring in Cape Town. *Poa annua* appears first, as early as July. This species disappears from open sunny areas by October. *Avena barbata* and *Avena fatua* appear next, growing tall in stands of waving fronds along roadsides and on waste land. This grass is dried and appears to have dehisced by October in many Cape Town areas. *Lagurus ovatus*, the beautiful 'bunny tail' grass is abundant on Rondebosch Common and the Cape Flats and similar sandy areas, but seldom seen close to the mountain in shadier suburbs. It flowers from October and continues its flowering period until December. *Briza maxima* and *minor*, colloquially known as '*klokkies*' meaning 'little bells' in the Cape, is found in sunny and shady suburbs alike and flowers from October to January. It is joined in the pollen release cycle of grasses by *Lolium perenne*, *Lolium temulentum* and *Lolium multiflorum.* The *Lolium* species flower prolifically in October and continues its flowering cycle until January. The flowering period of *Cynodon dactylon* is December-March but this grass may flower at other times, especially in exposed, sunny regions. In areas with long sunshine hours *Cynodon dactylon* may continue to flower into autumn. *Stenotaphrum secundatum*, or buffalo grass, a hardy, indigenous coastal grass flowers from October to May. Buffalo grass was originally confined to coastal areas but is now a popular lawn grass and it is commonly found in the Cape Metropolitan Area. (Adamson & Salter 1950, Oudtshoorn 1999) and personal observation from trail walking.

Plate 6. *Poa annua*.

Common name: Winter grass

Plate 7. *Avena barbata*.

Common name: Wild oats

Plate 8. *Lagurus ovatus*.

Common name: *Bunny tail*

Plate 9. *Briza maxima*. Common name:

*Great quaking grass*

Plate 10. *Lolium temulentum*.

Common name: *Ryegrass*

Plate 11. *Cynodon dactylon*.

Common name: *Bermuda grass*

Plate 12. *Stenotaphrum secundatum.*

Common name: *Buffalo grass*

Plate 13. *Pennisetum clandestinum*

Common Name: *Kikuyu*

### 5.5 Herbaceous shrubs

Herbaceous shrubs are commonly known as weeds. Weed concentrations were low i.e. more than 10 grains/$m^{-3}$ at all the sites and comprised more than 5% of the annual catch at Red Cross Hospital in Rondebosch, and at the sampling sites at Table View and Observatory.

#### 5.5.1 Weed pollen variation at the sites

There was greater variation in the weed species at the Rondebosch and Table View sites than at the Observatory site where only *Parietaria* was trapped in quantities that exceeded 1% of the annual pollen catch. In Mowbray Urticaceae and Asteraceae exceeded 1% of the annual pollen catch. The poorer, sandier soil at the coastal site of Table View, which has *Cape Flats Dune Sandveld* vegetation and the Rondebosch site that is situated in Rondebosch *Cape Flats Sand Fynbos* vegetation was reflected in the weed pollen concentrations. The presence of *Erica spp* at the sites with sandier soil and the low concentrations, and the absence of *Erica* pollen from Observatory which has a heavier clay soil, emphasises this distinction.

The threshold value for weed pollen is 10 pollen grains/ $^{-3}$ according to the Burge Scale (see Methods 3.5.1). Myricaceae includes many species and may be a shrub or a tree. *Myrica spp* ranked second on the frequency list at Table View and eleventh at Rondebosch although the mean annual concentration was lss than 5% of the TPI at both sites. *Chenopod spp* ranked sixth at the Table View site. *Chenopod* was a constant low presence in the atmosphere at Table View from the midsummer months of November to February but weed concentrations remained below the threshold of 15 pollen grains/$m^3$/24-hour period.

*Anemophilous vs insect pollinated weed pollen*

Many of the identified pollen taxa are not anemophilous or wind pollinated. They are pollinated by insects, birds or mammals. This possibly accounts for the low levels found in aerobiological studies. Weeds identified at the sites are listed in Table 5.15 below.

**Table 5.15. Weeds identified from Cape Town sampling sites 1984-2014**

| | | |
|---|---|---|
| Asteraceae = Compositae | Loranthaceae | Proteaceae |
| Boraginaceae | Malvaceae | Ranunculaceae |
| Bruniaceae | Myricaceae | Rosaceae |
| Caryophyllaceae | Oenotheraceae | Rutaceae |
| Chenopodiaceae | Oxalidaceae | Solanaceae |
| Ericaceae | *Parietaria/Urticaceae* | *Taraxacum* |
| Iridaceae | *Plantago* | Thymelaeaceae |
| *Juncus* | Polygonaceae | Tiliaceae |
| Liliaceae | Polypodiaceae | Umbilliferae |

### 5.5.2 Weed pollen 2011-2014

Low daily weed concentrations less than 10 grains/ $m^{-3}$ were measured at Table View and Observatory for the years 2011-2014. The monthly weed scores, mean monthly concentrations and annual weed summed scores are shown in Table 5.16 below.

**Table 5.16. Weed pollen concentrations at Observatory (SAAO) and Table View (TBV) 2011-2014**

| SAAO | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Cumulative annual score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2011 | 27 | 167 | 70 | 25 | 30 | 13 | 12 | 2 | 63 | 18 | 19 | 15 | 461 |
| 2012 | 44 | 11 | 51 | 50 | 510 | 135 | 17 | 15 | 17 | 46 | 22 | 14 | 932 |
| 2013 | 7 | 17 | 65 | 46 | 30 | 15 | 15 | 2 | 28 | 61 | 23 | 25 | 334 |
| 2014 | 20 | 22 | 43 | 28 | 16 | 16 | 9 | 7 | 13 | 24 | 9 | 11 | 218 |
| **Total** | **98** | **217** | **229** | **149** | **756** | **179** | **53** | **26** | **121** | **149** | **73** | **65** | **2115** |
| Mean | 25 | 54 | 57 | 37 | 189 | 45 | 13 | 7 | 30 | 37 | 18 | 16 | 528 |
| | | | | | | | | | | | | | |
| TBV | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Cumulative annual score |
| 2011 | 36 | 60 | 120 | 0 | 27 | 32 | 42 | 35 | 81 | 20 | 12 | 25 | 490 |
| 2012 | 58 | 52 | 105 | 41 | 28 | 11 | 22 | 76 | 175 | 208 | 39 | 34 | 849 |
| 2013 | 23 | 21 | 102 | 12 | 43 | 1 | 4 | 13 | 20 | 28 | 94 | 36 | 397 |
| 2014 | 22 | 60 | 31 | 66 | 28 | 11 | 8 | 16 | 35 | 29 | 27 | 27 | 360 |
| **Total** | **139** | **193** | **358** | **119** | **126** | **55** | **76** | **140** | **311** | **285** | **172** | **122** | **2096** |
| Mean | 35 | 48 | 90 | 40 | 32 | 14 | 19 | 35 | 78 | 71 | 43 | 31 | 524 |

*Weed pollen concentrations 2011-2014*

Weed pollen concentrations were generally low throughout the year. The highest concentrations occurred in the autumn months of February to March and spring months September to October at Observatory and at Table View There was one exception to this pattern. An anomalous peak was seen for *Parietaria* from 6 May to 30 June 2012 at the Observatory site. The total score of 510 in May 2012 at Observatory has been highlighted in yellow. *Parietaria* concentrations exceeded the significant threshold of 10 grains/m$^{-3}$ on 13 days of this five-week period during 2012. The *Parietaria* pollen release pattern may be seen in Table 5.17 below.

**Table 5.17.** ***Parietaria*** **concentrations May-June 2012 at the Observatory (SAAO) site**

| May 2012 (days 1-31) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Parietaria*/m³ | 0 | 0 | 0 | 0 | 0 | 39 | 3 | 0 | 0 | 20 | 4 | 4 | 41 | 13 | 45 | 93 | 1 | 1 | 0 | 0 | 13 | 0 | 208 | 10 | 0 | 0 | 0 | 0 | 1 | 0 | 12 |
| June 2012 days 1-30) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
| *Parietaria*/m³ | 18 | 31 | 40 | 1 | 0 | 0 | 0 | 0 | 0 | 31 | 1 | 0 | 0 | 8 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

Significant concentrations for the May-June 2012 *Parietaria* concentrations were counted and this may be seen in Figure 5.13 below.

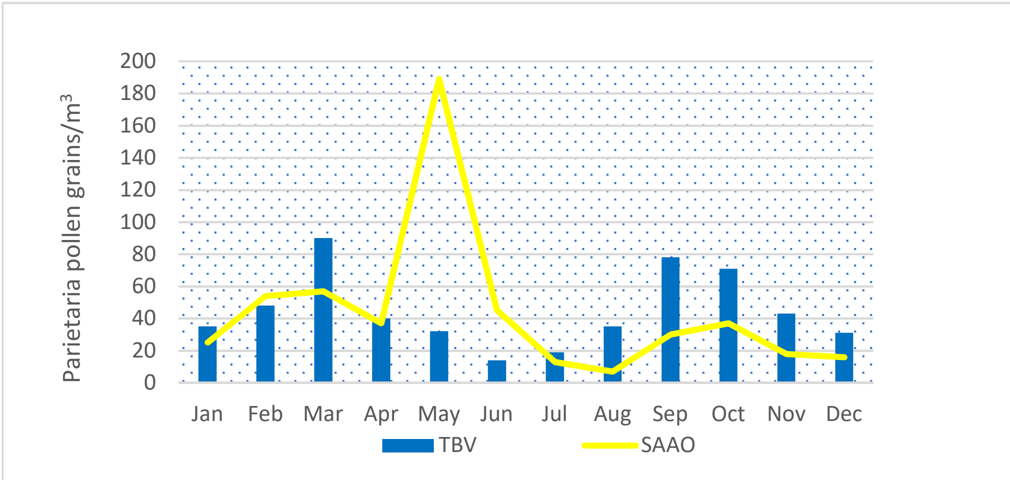



Figure 5.13 Mean weed concentrations 2011-2012 at Table View (TBV) and Observatory (SAAO)

### 5.5.3 Weed Summary

The weed pollen concentrations were insignificant at all the sampling sites throughout the 1984-2014 sampling years except for one sampling year at Observatory, when *Parietaria* concentrations exceeded the significant threshold in May and June 2012. *Erica spp* were rarely seen at Observatory, but were regularly trapped at Rondebosch and Table View, in low concentrations below the threshold of 10 pollen grains/$m^3$. *Myrica*, or sweet gale appeared in low concentrations in the atmosphere at Table View and Rondebosch i.e. less than 10 pollen grains/$m^3$. Weed pollen concentrations usually increase during the autumn months but this pattern was not apparent at the Cape Town sites. A similar low incidence was observed for Plantaginaceae, or English plantain at all the sites.

*Fynbos pollen*

The beautiful indigenous proteas, ericas and reeds of the fynbos= thin leaved vegetation that characterises the floral kingdom in the south-western Cape formed less than 5% of the annual pollen load.

*Weed distribution patterns*

Distribution profiles were not compiled for weeds, except for *Parietaria* at one site only, because their atmospheric concentrations were insignificant.

*Weed Pollen Plates 14-20*

The following plates illustrate some of the introduced and indigenous herbaceous shrubs of the south-western Cape. The photographs were taken by myself and by others (see Acknowledgements).

Plate 14. *Plantago lanceolata* - English Plantain

Introduced

Plate 15. *Taraxacum officinale*- dandelion

Introduced

Plate 16. *Dimorphotheca pluvialis* - rain daisy.

Family Asteraceae. Indigenous

Plate 17. *Arctotheca populifolia* West coast sand pumpkin

Family Asteraceae. Indigenous

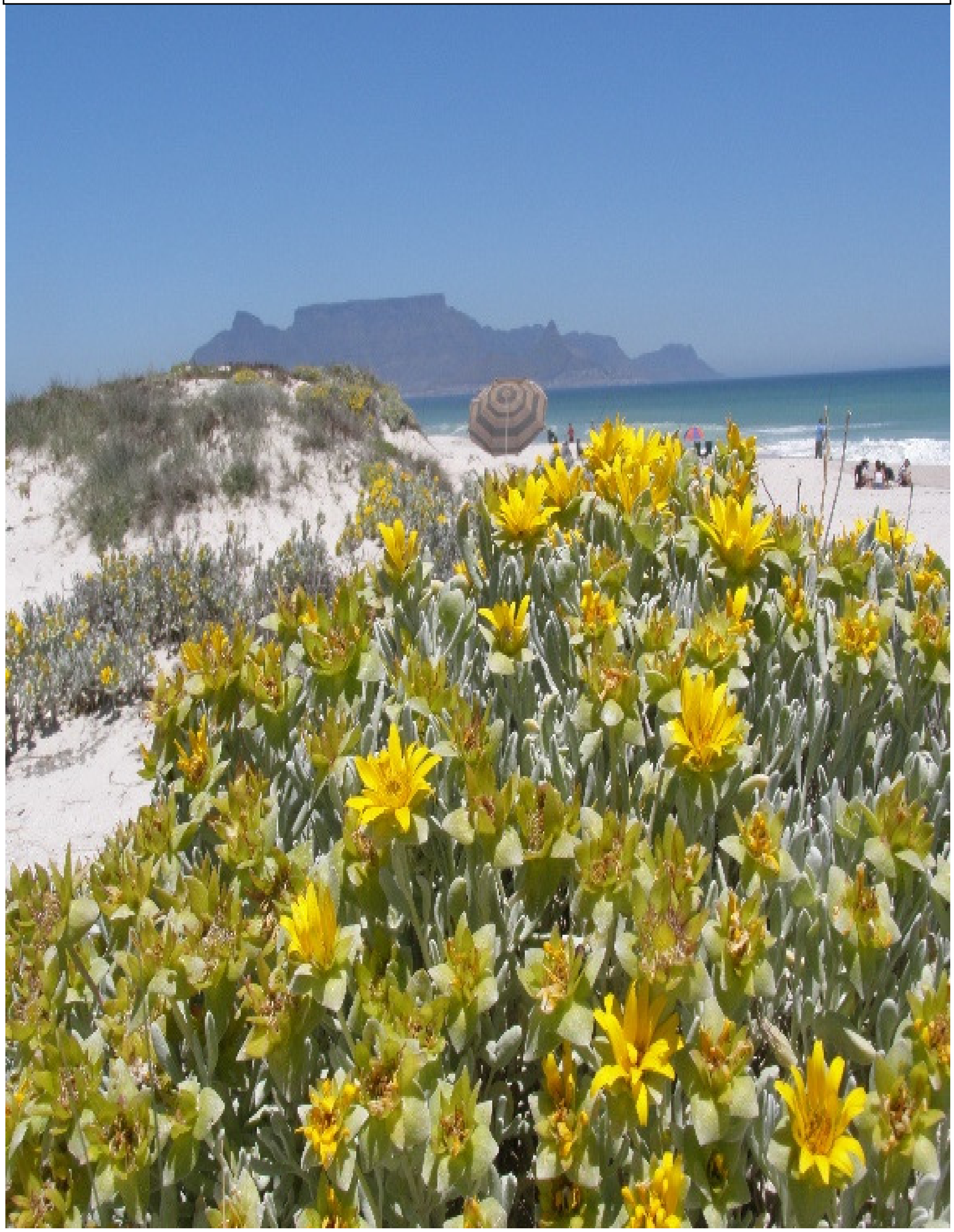


*Plate 18. Protea stokoei.* Fynbos plant. Indigenous.

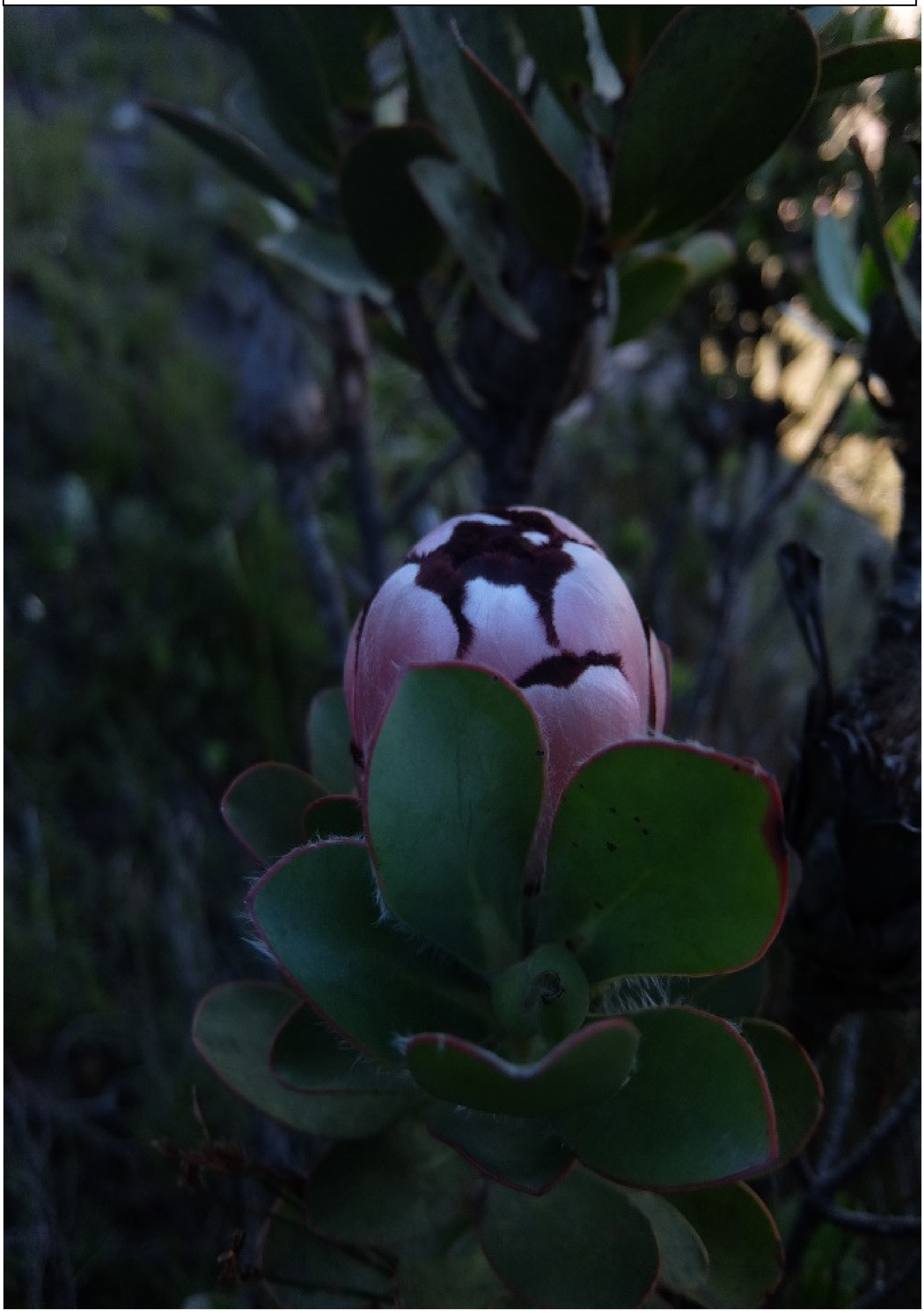


Plate 19. *Erica perspicua.* Typical fynbos plant. Indigenous

Plate 20. Restionaceae. Reed. Typical fynbos plant.

Plate 21. Aizoaceae. *Vygie*. Indigenous

### 5.5.4 Pollen season summary

*Pollen proportions*

Tree pollen was more prevalent at the inland sites of Rondebosch and Observatory than at Table View, where a paucity of tree pollen was found. Grass pollen dominated the catch at Table View. Weeds contributed insignificant amounts of to the assemblages of atmospheric pollen at all the sites except Observatory.

*Tree pollen seasons*

Trees began their annual flowering cycle in July with *Cupressus*, (July-October) and were followed by *Quercus* (August to September) and *Platanus* (August-October).

*The grass pollen season*

Grasses began flowering earliest in Observatory, starting in mid-September, followed by Table View where grasses began their flowering cycle at the end of September. Grass peaks were higher i.e. more than 10 pollen grains/m $^{-3}$/day in Table View and more days with high grass counts were identified at that site, but there was no significant difference between the duration and the end of the grass season at the two sampling sites. Grasses continued to flower at the sites until March.

*Weed pollen seasons*

Weed concentrations were low and diffuse at all the sites with no clear pattern for taxa or season except for *Parietaria*. Consistently high concentrations more than 10 pollen grains/m$^{-3}$ were identified in the autumn months of May-June in Observatory in 2012.

*Changes in pollen concentrations over time*

The two long time series at Red Cross Hospital, Rondebosch and Table View both showed decreases in pollen concentrations. At the Rondebosch site the average annual grass count decreased from 4,890 for the decade 1980-1989 to 902 during the years 1990 to 1999 and remained low for the subsequent years: 2000-2003.

## Part II

### 5.6 Fungal spores identified

24 fungal spore genera were identified from pollen sampling studies in Cape Town. Ascospores and basidiospores were undifferentiated. *Aspergillus* and *Penicillium* are indistinguishable when using light microscopy and so they were grouped together as *Aspergillus/Penicillium*. The list of identified fungal spores is shown in Table 5.18 below.

**Table 5.18. Fungal spores identified from Cape Town sampling sites**

| | | |
|---|---|---|
| Ascospores | *Epicoccum* | *Puccinia* |
| *Aspergillus/Penicillium* | *Fusarium* | Rusts |
| *Alternaria* | *Drechslera* | Smuts |
| Basidiospores-coloured | *Leptosphaeria* | *Spegazzinia* |
| *Bispora* | Mildew | *Stemphyllium* |
| *Chaetomium* | *Periconia* | *Tetraploa* |
| *Cladosporium* | *Pithomyces* | *Torula* |
| *Curvularia* | *Pleospora* | *Ulocladium* |

### 5.6.1 Selection of fungal allergens for analysis

The annual fungal spore load was summed and daily fungal spore concentrations were reported as spores/m$^{-3}$. There is a wide range of threshold values for the different fungal aeroallergens. *Alternaria* sensitisation has been set at 50 spores/m$^{-3}$ (Sadyś, Adams-Groom and Kennedy, 2016) while the threshold for *Cladosporium* is much higher at 3,000/m$^{-3}$ (Burge and Rogers, 2000). The differences in threshold values for fungal spores is unlike the pollen sensitization thresholds where the threshold ranges are lower and the range is far smaller. Threshold values for pollen may range from 5 grains/m$^{-3}$ for grasses to 15 grains/ m$^{-3}$ for tree pollen (Burge 1992).

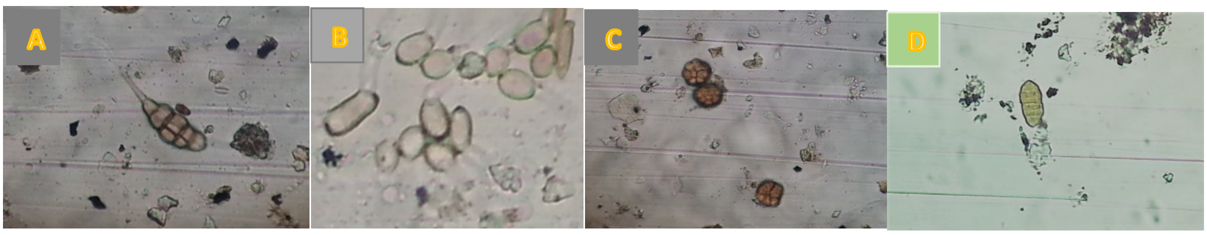



Figure 5.14 A: *Alternaria* B: *Cladosporium* C: *Epicoccum* D: *Pleospora* The Photomicrographs (x 400 magnification) photographer: D Berman

Fungal spores selected for comparison were chosen that were known to sensitize patients e.g. *Alternaria, Cladosporium and Epicoccum. Pleospora* was added to the study after observing consistent increases in concentrations at Table View during July. The selected fungal spores were compared at the sites by measuring their relative abundance in the atmosphere in frequency charts. These charts listed all the fungal spores trapped in amounts that were more than 1% of the annual fungal index. In Cape Town the seven most prevalent fungal groups or spores were: ascospores, basidiospores, *Alternaria, Aspergillus/Penicillium, Cladosporium, Epicoccum* and *Pleospora*. The group labelled 'Other' contained: *Bispora, Chaetomium, Curvularia, Fusarium, Drechslera (Helminthosporium), Leptosphaeria, Periconia, Pithomyces, Puccinia, Spegazzinia, Stemphylium, Tetraploa, Torula and Ulocladium.* The frequency charts may be seen in Figure 5.15 a, b, c and d below

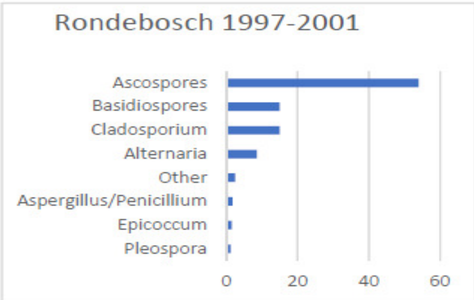



**Figure 5.14a Rondebosch fungal spore frequencies**

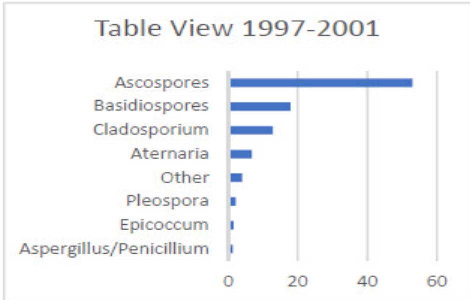



**Figure 5.14b Table View fungal spore frequencies**

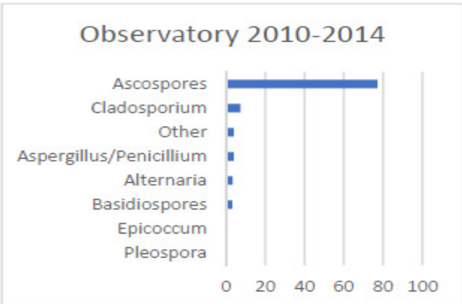



**Figure 5.14c Observatory fungal spore frequencies**

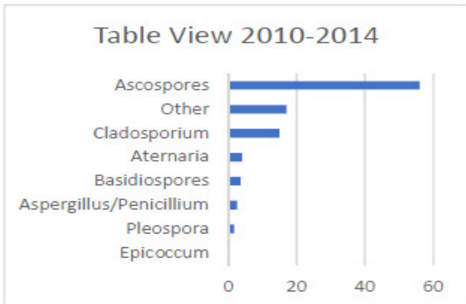



**Figure 5.14d Table View fungal spores frequencies**

Figure 5.15 a, b, c and d. Fungal spore Frequencies at the Cape Town Sites. The category 'other' includes a range of fungal spores that were less than 1% of the total fungal load

### 5.6.2 Method of comparison

Histograms and bi-plots were composed from all the data available for all the years from the Rondebosch (RXH) Table View (TBV) and Observatory (SAAO) sites. Distribution patterns and seasons of the selected fungal genera: *Alternaria, Cladosporium, Epicoccum* and *Pleospora* were found. For each individual spore, the data sets from 1997-2001 at Rondebosch and Table View and the data sets from 2010-2014 at Table View and Observatory were selected for comparison. The annual scores, peak dates and seasons were found and compared. There was marked variation in the seasonal distribution of the four fungal genera. A unique seasonal pattern was identified for each genus and this is demonstrated by splines which showed the seasonal variation of the four selected fungal genera compared from daily measurements over the years 1985-2003 at Rondebosch, 1995-2003 at Table View and from the 2010-2014 data sets when the Table View and Observatory sampling sites operated concurrently. The seasonal patterns are shown in Figure 5.16 below. (see 3.6.4. for method).

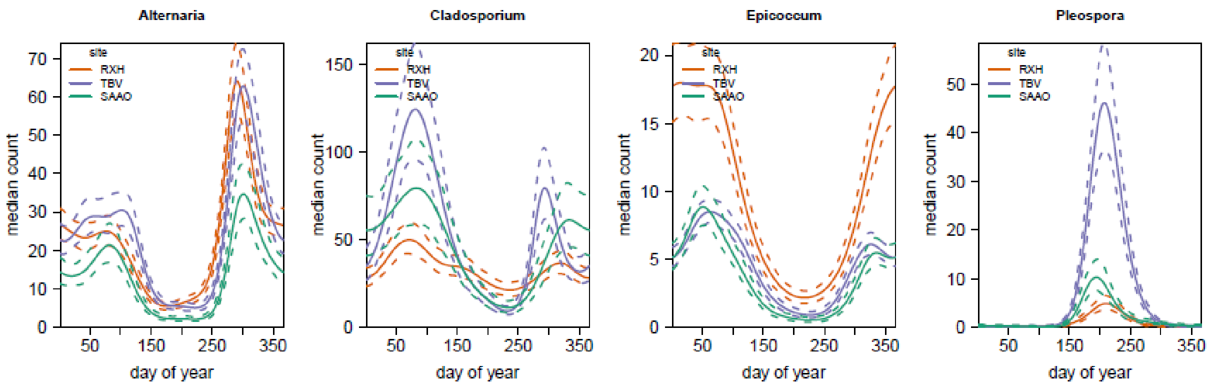



Figure 5.16 Seasonal patterns for Alternaria, Cladosporium, Epicoccum and Pleospora at the sampling sites at Rondebosch (RXH) for years 1985-2003, Table View (TBV) years 1995-2003 and also 2010-2014 and at Observatory (SAAO) 2010-2014

### 5.7 *Alternaria*

*Alternaria* concentrations were compared by means of a histogram at the three sites. Not all years had complete records, or recorded Alternaria each day so years with more than 300 data points were included in the analysis for all the sampling years. 300 data points was chosen as representing a year during which sufficient daily analyses had been captured. The *Alternaria* histogram is shown in Figure 5.17 below.

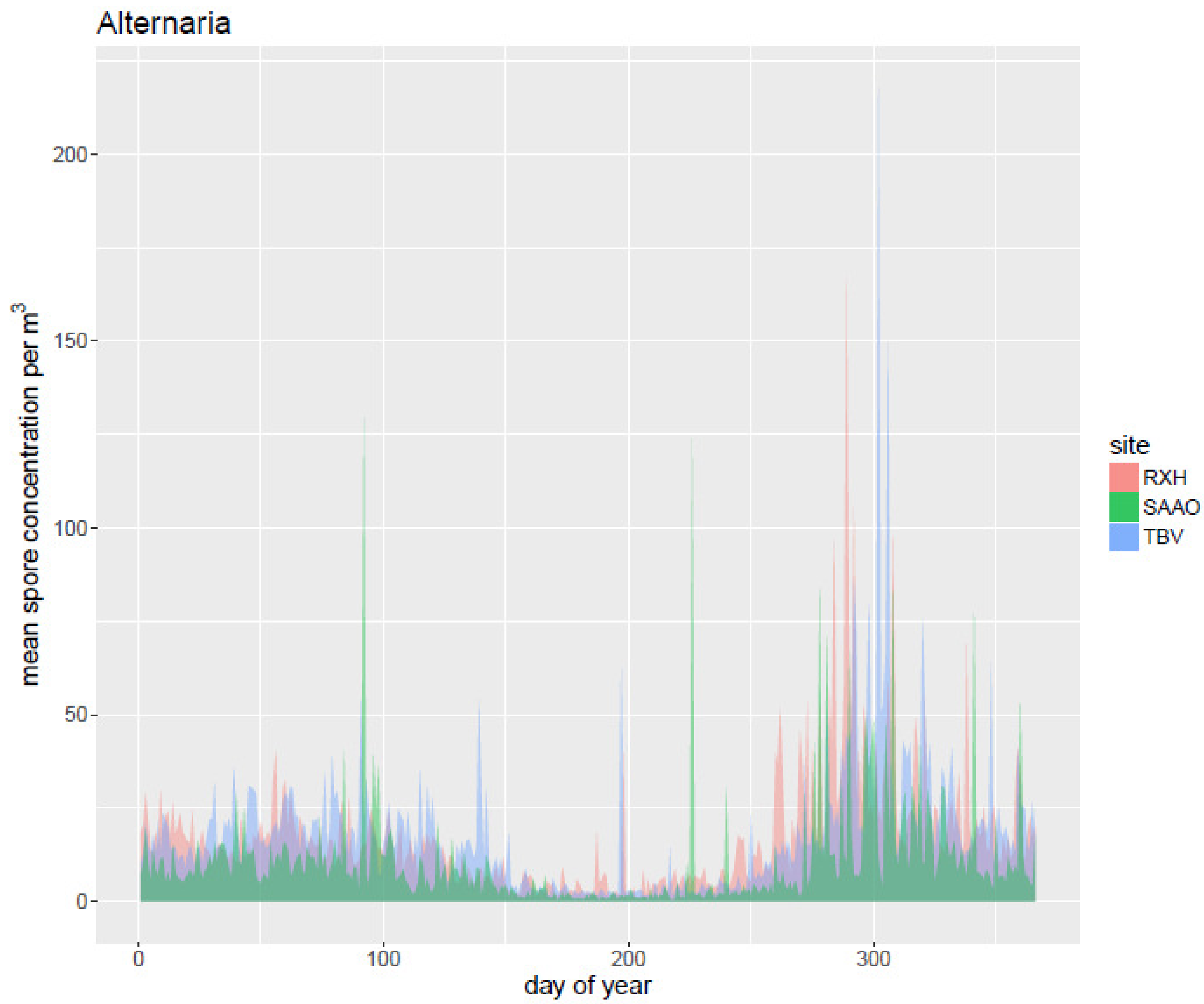



Figure 5.17 Alternaria mean annual distribution at Rondebosch (RXH) 1984-2003, Table View (TBV) 1995-2003 and also 2010-2014 and Observatory (SAAO) 2010-2014

### 5.7.1 The *Alternaria* season

The Bootstrapping method was used to identify the standard errors and confidence intervals for the start/median/mean/end of the season by randomly sampling one day from each of the 366 days of the year, inclusive of February 29. Missing values were excluded. From these daily counts the cumulative count, the start/end/median dates and the duration were calculated. The steps were repeated 200 times.

A 95% interval was calculated from the 2.5 and 97.5 percentiles of the bootstrap distribution for each of these statistics. The standard error for each statistic was estimated as the standard deviation of the corresponding bootstrapped values. A demonstration of this method is given in Figure 5.18 below.

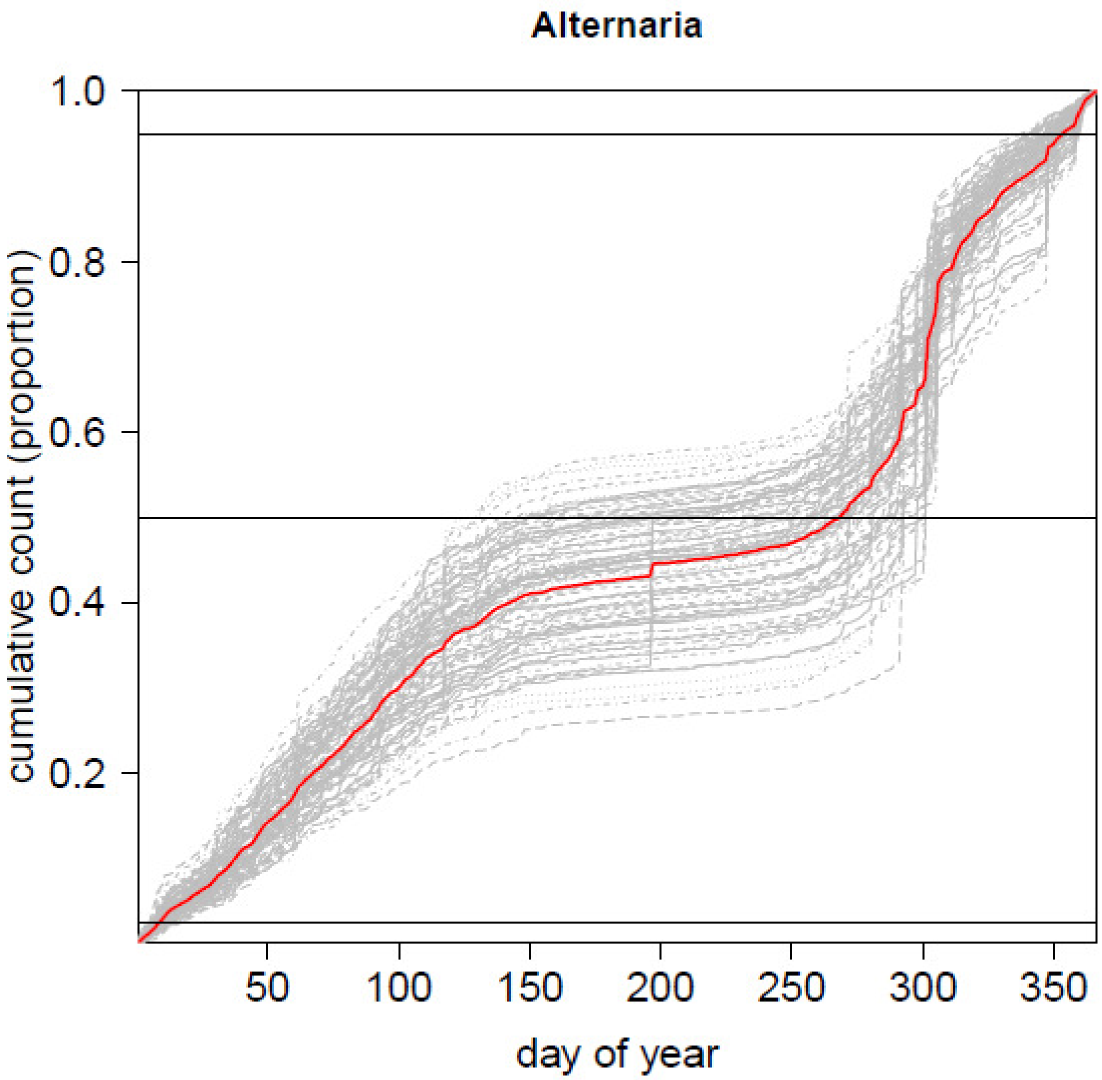



Figure 5.18 The annual accumulation Alternaria sum using bootstrapped values.

The *Alternaria* histogram shows the peak dates as the day of year. The histogram shows that occasional concentrations that exceeded 50 spores/m$^{-2}$ were seen in the autumn months of March-April and in mid-winter in the month of August. The main sporing season for *Alternaria* occurred during the spring to summer months of October and November, close to day 300 (DOY 300= 27 October) at all the sites. To show this more clearly a scatter plot was drawn, comparing the peak sporing patterns at all the sites. The days and months when the peaks occurred are shown in Figure 5.19 below

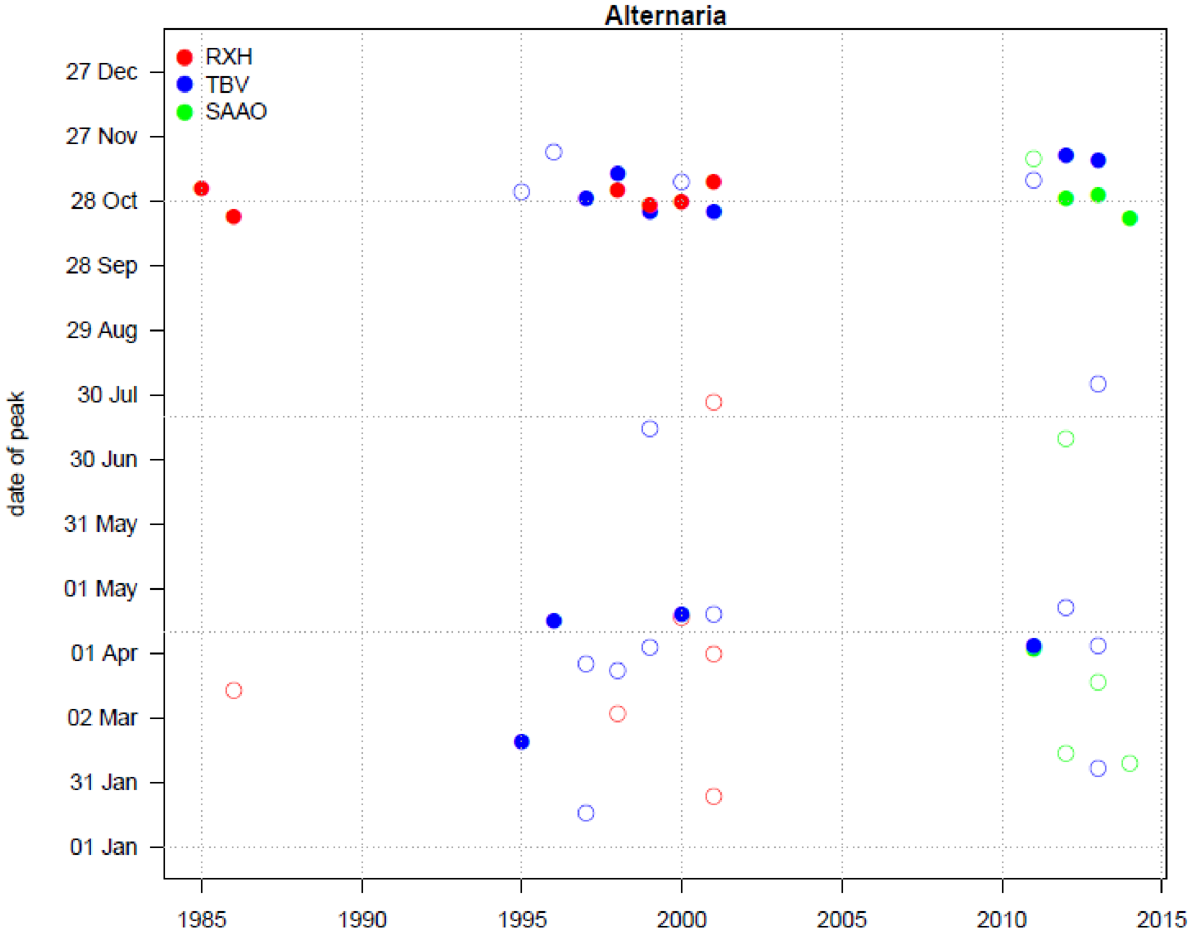



Figure 5.19 Peak dates for Alternaria at the Rondebosch (RXH) Table View (TBV) and Observatory (SAAO) sites for years 2010-2014. Filled dots represent the maximum concentrations. Unfilled dots show peaks outside the main season

### 5.7.2 Comparing *Alternaria* concentrations at the sampling sites

The annual *Alternaria* load was measured and summed for each year from the data sets at the three sites: Rondebosch (RXH), Table View (TBV) and Observatory (SAAO). The cumulative *Alternaria* concentrations for the compared years and the date and value of the maximum, or peak *Alternaria* concentration for each year were found. The Annual Fungal Index (AFI) was measured. These values are shown in Table 5.19 below.

**Table 5.19. The *Alternaria* season at the Cape Town sites**

| Year | Site | Peak value | Peak date | *Alternaria* annual score | Annual Fungal Index (AFI) (all spores) |
|---|---|---|---|---|---|
| **1997** | Rondebosch | 441 | 17.09 | 7 607 | 39 156 |
| | Table View | 221 | 2.04 | 6 043 | 121 212 |
| **1998** | Rondebosch | 134 | 10.10 | 4 251 | 60 156 |
| | Table View | 2 010 | 1.11 | 11 882 | 209 255 |
| **1999** | Rondebosch | 86 | 20.10 | 2 637 | 78 022 |
| | Table View | 1013 | 15.7 | 7 985 | 190 821 |
| **2000** | Rondebosch | 238 | 21.11 | 5 485 | 118 822 |
| | Table View | 199 | 23.11 | 8 035 | 156 450 |
| **2001** | Rondebosch | 222 | 16.11 | 4 514 | 121 856 |
| | Table View | 3 554 | 28.10 | 16 479 | 224 172 |
| **2010** | Observatory* | 417 | 4.10 | 3 640 | 125 684 |
| | Table View | 744 | 18.05 | 8 910 | 129 703 |
| **2011** | Observatory | 702 | 1.04 | 6 518 | 47 171 |
| | Table View+ | 614 | 31.03 | 9 289 | 70 370 |
| **2012** | Observatory | 245 | 16 & 31.10 | 4 741 | 94 470 |
| | Table View | 725 | 15.11 | 8 808 | 136 066 |
| **2013** | Observatory | 360 | 7.10 | 3 318 | 66 515 |
| | Table View | 361 | 18.11 | 6 559 | 95 506 |
| **2014** | Observatory | 550 | 3.11 | 4 411 | 25 373 |
| | Table View | 390 | 4.10 | 8 235 | 108 823 |

- Preseason data missing Jan-July

\+ October data missing

### 5.7.3 Mean *Alternaria* values

The mean values were found for *Alternaria* at the three sites. The mean *Alternaria* peak dates sometimes occurred outside the main spring/ summer season, during the late summer- early autumn months March-April. 5.8-6.2 % of the AFI was composed of *Alternaria* at all the sites except Table View, where it was 7.7%. The mean values for the data sets may be seen in Table 5.20 below.

**Table 5.20. The *Alternaria* mean values at the Cape Town sites**

| Years | Sampling Site | Mean peak *Alternaria* value | Mean peak date | Mean annual *Alternaria* load | %age of AFI | Mean annual fungal load (AFI) | Peak months |
|---|---|---|---|---|---|---|---|
| **1997-2001** | Rondebosch | 224 | 23.10 | 4 898 | 5.8% | 83 602 | Sept-Nov |
| | Table View | 1 399 | 19.09 | 10 085 | 5.5% | 180 383 | April Oct-Nov |
| **2010-2014** | Table View | 567 | 30.08 | 8 360 | 7.7% | 108 093 | March, May, Oct-Nov |
| | Observatory | 455 | 14.09 | 4 526 | 6.2% | 7 1842 | April, Oct Nov |

### 5.7.4 Seasonal shifts for *Alternaria* over time

The peak dates were compared over this 20-year time span. The 1997-2001 and 2010-2014 Alternaria seasons from the Table View data set (1997-2001 and 2010-2014) showed similar peak dates. The proportion of the annual fungal load increased from 5.5% in 1997-2001 to 7.7% of the AFI during 2010-2014 at Table View. This is a 40% increase.

## 5.8 *Cladosporium*

The seasonal distribution of *Cladosporium* at the three sampling sites at Rondebosch, Table View and Observatory was assessed by comparing all the years in the sampling collections at the three sites that had more than 300 data points for an annual year. The histogram drawn from these data is shown in Figure 5.20 below.

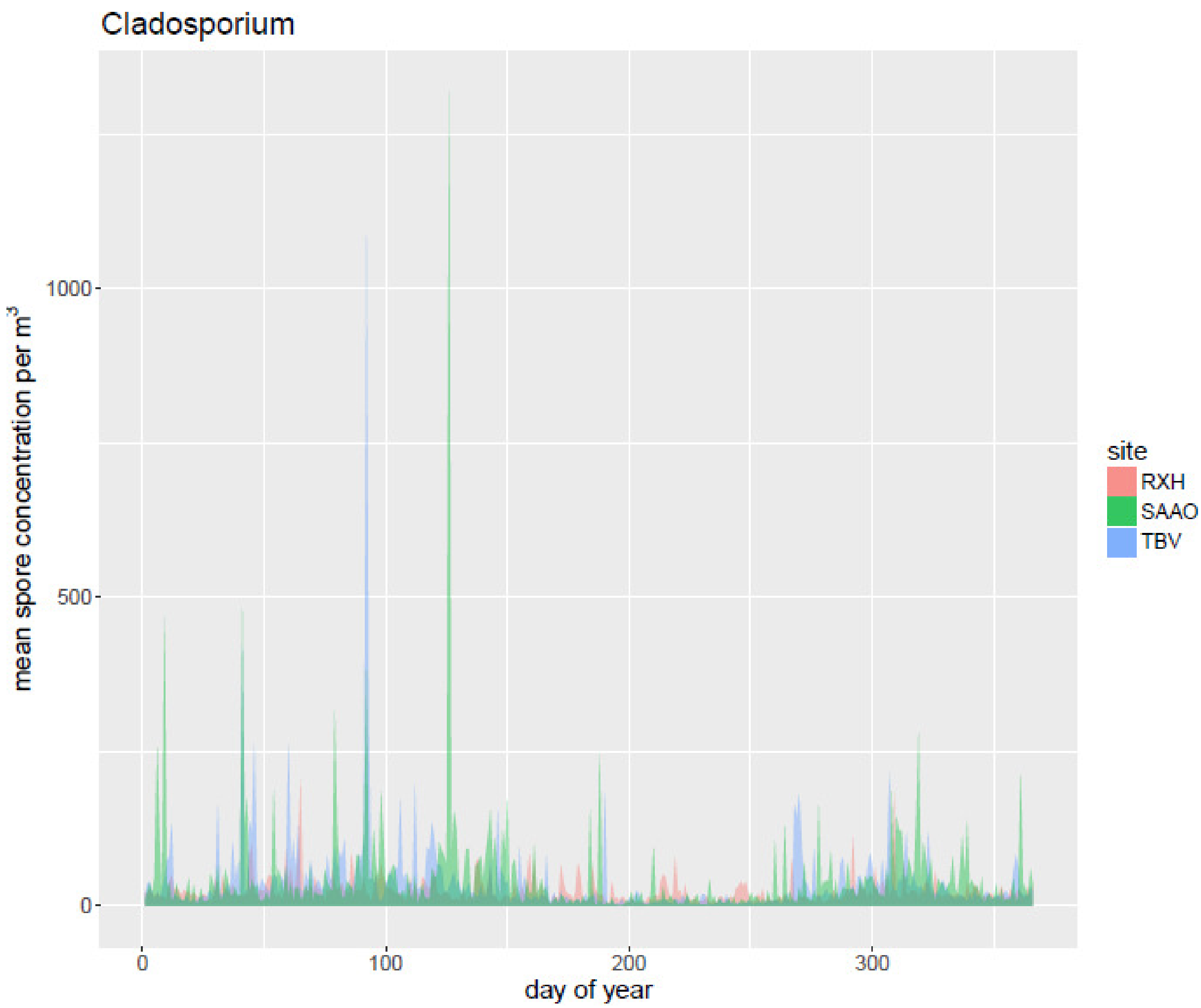



Figure 5.20 Annual *Cladosporium* distribution at Rondebosch for years (RXH) 1984-2003 Table View (TBV) 1995-2003 and also 2010-2014 and Observatory (SAAO) 2010-2014

### 5.8.1 *Cladosporium* seasonal peaks

*Cladosporium* concentrations were surprisingly low at all the sites for all the years. Few counts above the threshold value of 3,000 spores/m$^{-3}$ were recorded so that the mean scores were uniformly low. This was an unexpected finding considering that Cape Town has a temperate climate. Temperate climates are favourable for *Cladosporium* (Solomon 1978). The distribution shows a decline during the winter months of May-August for days 150-240 but small spikes can be seen during this time. The data were log transformed so that the individual *Cladosporium* concentrations at the sites can be seen more easily in Figure 5.6 below.

### 5.8.2 Comparing *Cladosporium* concentrations

The annual *Cladosporium* load was measured and summed for each year from the data sets at the three sites. The cumulative *Cladosporium* concentrations for the compared years and the date and value of the maximum, or peak *Cladosporium* concentration for each year were found. The Annual Fungal Index (AFI) was measured. These values are shown in Table 5.21 below.

**Table 5.21. *Cladosporium* annual concentrations at the Cape Town sites**

| Year | Site | Peak *value* | Peak Date | *Cladosporium* Annual Score | Proportion of AFI | Annual Fungal Index (AFI) |
|---|---|---|---|---|---|---|
| **1997** | Rondebosch | 259 | 30.10 | 3 456 | 8% | 39 156 |
| | Table View | 893 | 06.02 | 6 900 | 3.2% | 121 212 |
| **1998** | Rondebosch | 391 | 21.02 | 7 798 | 12.9% | 60 156 |
| | Table View | 416 | 20.04 | 13 404 | 6.4% | 209 255 |
| **1999** | Rondebosch | 287 | 22.12 | 4 800 | 6.1% | 78 022 |
| | Table View | 438 | 9.01 | 12 126 | 6.3% | 190 821 |
| **2000** | Rondebosch | 2 562 | 03.03 | 27 676 | 2.3% | 118 822 |
| | Table View | 2 105 | 15.04 | 26 234 | 16.8% | 156 450 |
| **2001** | Rondebosch | 431 | 17.11 | 15 381 | 12.7% | 121 856 |
| | Table View | 1 689 | 04.03 | 11 280 | 5% | 224 172 |
| **2010** | Observatory* | 316 | 16.10 | 4 315 | 3.4% | 12 5684 |
| | Table View+ | 1 584 | 28.04 | 18 638 | 14.3% | 129 703 |
| **2011** | Observatory | 1 974 | 01.04 | 27 171 | 5.8% | 47 171 |
| | Table View | 15 584 | 01.04 | 61 474 | 44% | 140 992 |
| **2012** | Observatory | 1 030 | 23.02 | 10 078 | 10.7% | 94 470 |
| | Table View | 1 072 | 14.06 | 21 023 | 15.5% | 136 066 |
| **2013** | Observatory | 2 597 | 01.02 | 14 253 | 21.4% | 66 515 |
| | Table View | 8 048 | 10.02 | 30 757 | 32.2% | 95 506 |
| **2014** | Observatory | 3 203 | 09.01 | 7 020 | 7% | 100 935 |
| | Table View | 2 250 | 13.02 | 11 062 | 10.2% | 108 823 |

- Jan-Aug no data

\+ 31 July-4 Oct no data

### 5.8.3 Mean *Cladosporium* values

The mean values were found for *Cladosporium* at the three sites. The mean peak dates were not included as the peak date months were extremely variable. The peak concentration values were very low when compared to Northern European aerobiological surveys (Calderón et al. 1997, Fernández-Rodríguez et al. 2014; Sadyś et al. 2015) where the peak concentrations sometimes exceeded the annual cumulative score for *Cladosporium* in Cape Town reaching concentrations of 20,000 to 47,000 (Sadyś, Adams-Groom and Kennedy, 2016). One elevated concentration for Cladosporium, measuring 15,584 spores/ $m^{-3}$ occurred on 1 April 2011 and a concentration of 1,421 spores/ $m^{-3}$ was recorded at Observatory on the same day. The meteorological parameters surrounding these two events and *Cladosporium* concentrations from the Cape Town sampling sites will be discussed in Chapter 6. The range of months when Cladosporium peak concentrations were found and the mean values for the data sets may be seen in Table 5.22 below.

**Table 5.22 *Cladosporium* mean values at the Cape Town sites**

| Years | Sampling Site | Mean peak *Cladosporium* value | Mean annual *Cladosporium* Index | Mean AFI | Proportion of AFI | Months of peak concentrations |
|---|---|---|---|---|---|---|
| **1997-2001** | Rondebosch | 786 | 11 822 | 83 602 | 14% | October-March |
| | Table View | 1 425 | 24 027 | 180 382 | 13% | November-April |
| **2010-2014** | Table View | 6 920 | 28 590 | 108 093 | 26% | October-April |
| | Observatory | 1 824 | 12 567 | 86 955 | 14% | November-May |

*Comparison of Cladosporium concentrations at the sampling sites*

The *Cladosporium* prevalence was similar at the Rondebosch and Observatory sites, measuring 14% of the annual fungal index, or AFI at both the sites. The annual *Cladosporium* sum at Table View from the years 2010-2014 was 26%. This was twice the *Cladosporium* proportion of 13% from the years 1997-2001. This increased proportion was due in part to the high Cladosporium on 1 April 2011, which alone was 25% of the annual Cladosporium load.

*Cladosporium seasons at the sites*

The peak *Cladosporium* months were similar over the years at Table View and occurred from October/November to April. A similar prevalence pattern was seen at Rondebosch, where the highest concentrations were found from October to March. The *Cladosporium* season was slightly longer in Observatory where the highest concentrations were found from November to May.

### 5.9 *Epicoccum*

A histogram compiled from sampling years with more than 300 data points as described for *Alternaria* and *Cladosporium* was plotted to show the annual distribution and is shown in Figure 5.21 below.

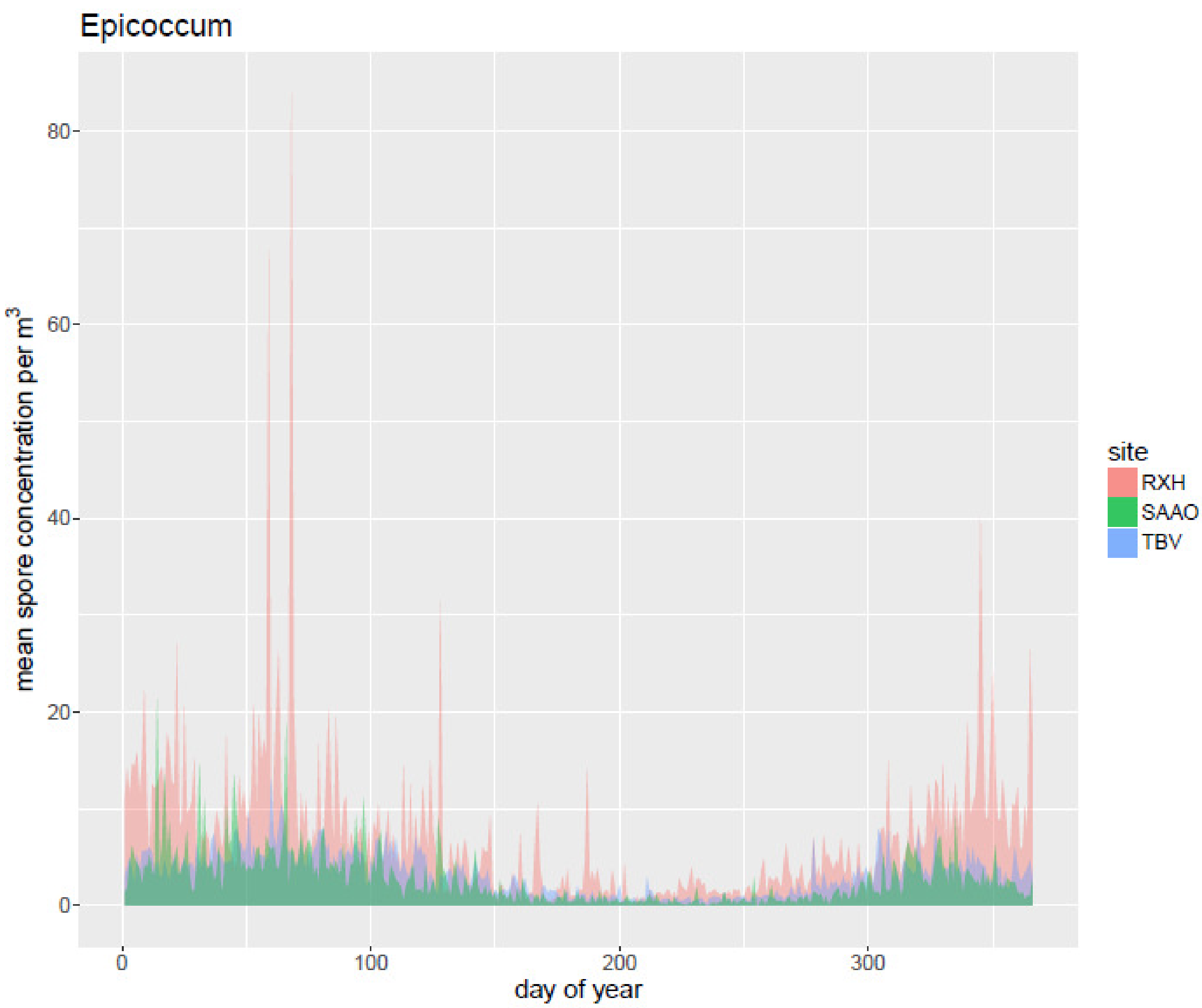



Figure 5.21 Annual *Epicoccum* distribution at Rondebosch (RXH) for years 1984-2003, Table View (TBV) 1995-2003 and also 2010-2014 and Observatory (SAAO) 2010-2014

#### 5.9.1 *Epicoccum* seasonal concentrations

The annual *Epicoccum* load was measured and summed for each year from the data sets at the three sites. The cumulative *Epicoccum* concentrations for the compared years and the date and value of the maximum, or peak *Epicoccum* concentration for each year were found. The Annual Fungal Index (AFI) was measured. These values are shown in Table 5.23 below.

**Table 5.23. *Epicoccum* annual concentrations and the annual fungal index**

| Year | Site | Annual *Epicoccum* Concentration | Proportion of AFI | AFI |
|---|---|---|---|---|
| **1997** | Rondebosch | 736 | 1.8% | 39 156 |
| | Table View | 2 088 | 1.7% | 121 212 |
| **1998** | Rondebosch | 1 060 | 1.8% | 60 156 |
| | Table View | 2 460 | 1.1% | 209 255 |
| **1999** | Rondebosch | 642 | 0.8% | 78 022 |
| | Table View | 1 361 | 0.7% | 190 821 |
| **2000** | Rondebosch | 1 034 | 0.9% | 118 822 |
| | Table View | 2006 | 1.2% | 156 450 |
| **2001** | Rondebosch | 1 122 | 0.9% | 121 856 |
| | Table View | 1 476 | 0.6% | 224 172 |
| **2010** | Observatory* | 676 | 0.5% | 125 684 |
| | Table View+ | 1 226 | 0.9% | 129 703 |
| **2011** | Observatory | 1 827 | 3.8% | 47 171 |
| | Table View | 1 096 | 1.6% | 70 370 |
| **2012** | Observatory | 1 197 | 1.3% | 94 470 |
| | Table View | 775 | 0.6% | 136 606 |
| **2013** | Observatory | 874 | 1.3% | 66 515 |
| | Table View | 1 012 | 1.0% | 95 506 |
| **2014** | Observatory | 597 | 0.6% | 100 935 |
| | Table View | 593 | 0.5% | 108 823 |

- Jan-Aug no data

\+ 31 July-4 Oct no data

Consistently low concentrations of less than 60 spores/$m^{-3}$ were seen in spring, summer and autumn at all the sites throughout the years with just two exceptions for Rondebosch when the mean concentrations exceeded 60 spores/$m^{-3}$.

### 5.9.2 *Epicoccum* mean concentrations

The annual *Epicoccum* index at the Rondebosch site from the 1997-2001 and the *Epicoccum* cumulative load at Observatory and Table View were similar and constituted 0.8% - 1.2% of the AFI. The Table View cumulative *Epicoccum* load was 2.9% of the AFI. The significant threshold was not exceeded at any of the sites during these time periods. Significant concentrations of *Epicoccum* were regularly counted at Rondebosch for 1985-1989. High concentrations were not seen after these years. A possible source of the high *Epicoccum* concentrations during the earlier sampling years was the municipal garden refuse dump that operated for those years opposite the Rondebosch pollen sampling site. The mean annual concentration from the listed data sets is shown in Table 5.24 below.

**Table 5.24. Mean annual *Epicoccum* cumulative scores and proportions**

| Years | Sampling Site | Mean annual *Epicoccum* load | AFI | Proportion of AFI | Months of peak concentrations |
|---|---|---|---|---|---|
| **1997-2001** | Rondebosch | 919 | 83602 | 1.1% | None |
| | Table View | 1583 | 180382 | 2.9% | None |
| **2010-2014** | Table View | 948 | 108093 | 0.8% | None |
| | Observatory | 1034 | 86955 | 1.2% | None |

*Epicoccum* distribution for the years 1997-2001 and 2010-2014 matched the histogram distribution. The histogram shows occasional concentrations >60 spores/$m^{-3}$ at Rondebosch and consistently higher values throughout the years 1985-1995. These high concentrations decreased after those years.

### 5.10 *Pleospora*

*Pleospora* is a wet weather spore. Some form-species of *Stemphyllium* are the asexual stage of *Pleospora* (Burge 1983) and *Pleospora/ Stemphyllium* is closely related to *Alternaria* (Weber 2015). A strong seasonal peak was demonstrated for this fungal spore in the mid-winter months of June and July at Table View and Observatory. The signal was weaker at the Rondebosch site, but *Pleospora* concentrations increased in mid-winter in that suburb. A histogram showing the distribution of *Pleospora* may be seen in Figure 5.22 below.

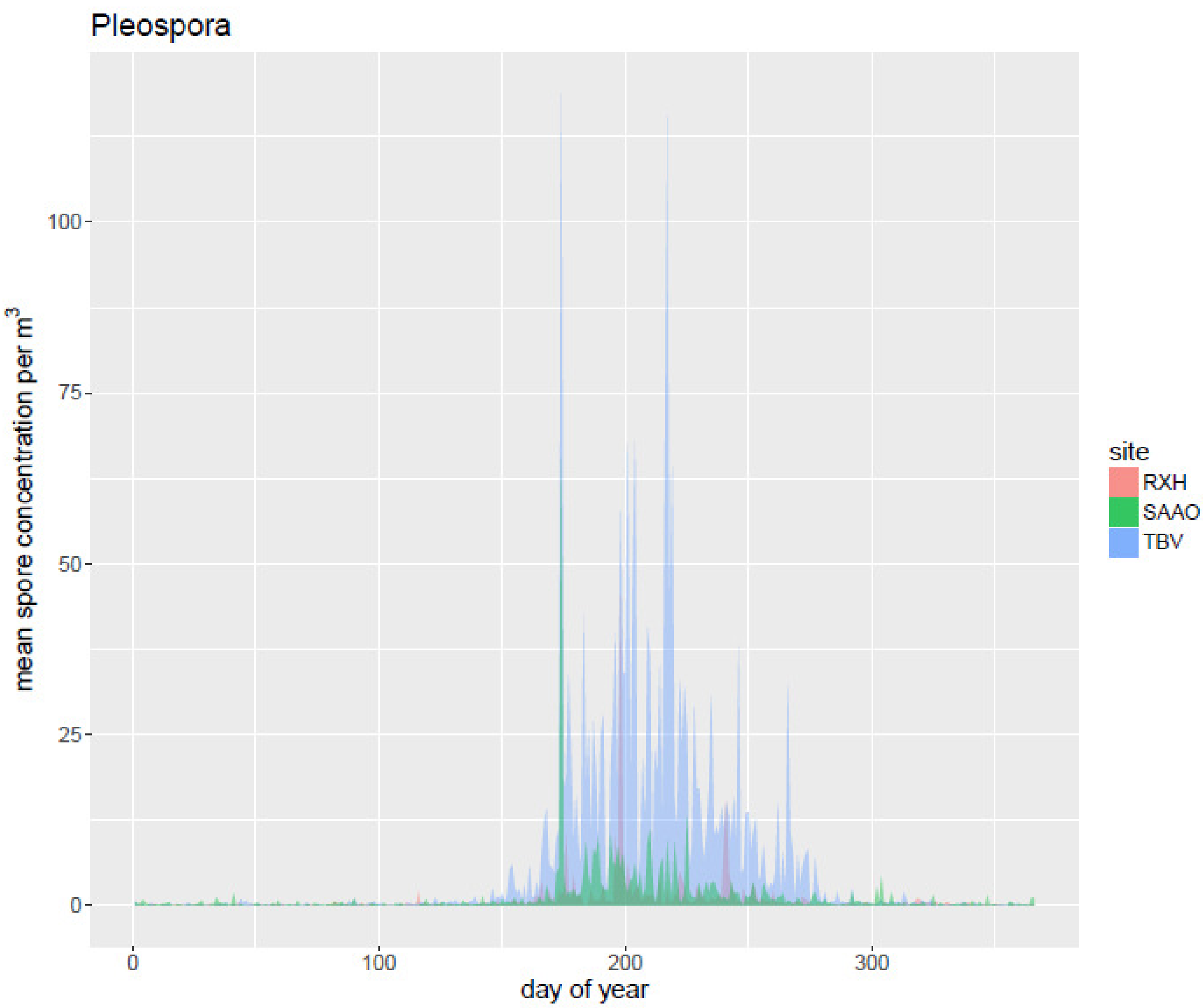



Figure 5.22 Annual *Pleospora* distribution at Rondebosch (RXH) for years 1984-2003, Table View (TBV) 1995-2003 and also 2010-2014 and Observatory (SAAO) 2010-2014

### 5.10.1 Comparing *Pleospora* seasonal values

The annual *Pleospora* load was measured and summed for each year from the data sets at the three sites. The cumulative *Pleospora* concentrations for the compared years and the date and value of the maximum, or peak *Pleospora* concentration for each year were found. The Annual Fungal Index (AFI) was measured. These values are shown in Table 5.25 below.

**Table 5.25. The *Pleospora* season at the Cape Town sites**

| Year | Site | Peak *Plesopora* Concentratio n | Peak *Pleospora* date | *Pleospora* annual score | Proportion of AFI | Annual Fungal Index (AFI) |
|---|---|---|---|---|---|---|
| **1997** | Rondebosch | 27 | 29.08 | 193 | 0.5% | 39156 |
| | Table View | 246 | 01.08 | 1635 | 1.3% | 121 212 |
| **1998** | Rondebosch | 111 | 14.07 | 377 | 0.6% | 60156 |
| | Table View | Data lost | Data lost | 3652 | 1.7% | 209 255 |
| **1999** | Rondebosch | 506 | 16.07 | 1159 | 1.5% | 78 022 |
| | Table View | 601 | 19.7 | 4178 | 2.1% | 190 821 |
| **2000** | Rondebosch | 62 | 1.08 | 367 | 0.3% | 118 822 |
| | Table View | 382 | 09.07 | 2533 | 1.6% | 156 450 |
| **2001** | Rondebosch | 129 | 24.06 | 755 | 0.6% | 121 856 |
| | Table View | 610 | 14.07 | 3315 | 1.5% | 224 172 |
| **2010** | Observatory* | Missing data | Missing data | Missing data | Missing data | Missing data |
| | Table View+ | 248 | 21.07 | 990 | 0.8% | 129 703 |
| **2011** | Observatory | 64 | 28.07 | 805 | 1.7% | 47 171 |
| | Table View | 247 | 27.07 | 1947 | 2.8% | 70 370 |
| **2012** | Observatory | 446 | 22.06 | 931 | 1% | 94 470 |
| | Table View | 1770 | 22.06 | 5309 | 3.9% | 136 066 |
| **2013** | Observatory | 61 | 07.08 | 555 | 0.8% | 66 515 |
| | Table View | 506 | 06.08 | 2866 | 0.4% | 95 506 |
| **2014** | Observatory | 45 | 02.07 | 1046 | 1% | 100935 |
| | Table View | 5515 | 04.08 | 6556 | 6% | 108 823 |

- Jan-Aug no sampling

\+ 31 July-4 Oct no data

### 5.10.2 Mean *Pleospora* values

Mean values were found for *Pleospora* at the three sites. Low levels of *Pleospora* were present in the atmosphere outside the peak season. *Pleospora* concentrations increased sharply from the end of June and declined by the end of August. The highest concentration was recorded at Table View when a concentration of 5,515 spores/m$^{-3}$ was counted on 4

August 2014. There was marked variation between sites and in different years but at each sampling site, an annual *Pleospora* concentration of more than 450 spores was recorded during the studied years. The mean peak dates and their concentrations may be seen in Table 5.26 below.

**Table 5.26. *Pleospora* mean values at the Cape Town sites**

| Years | Sampling Site | Mean annual *Pleospora* load | AFI | Proportion of AFI | Months of peak concentrations |
|---|---|---|---|---|---|
| **1997-2001** | Rondebosch | 570 | 68013 | 0.8% | June-August |
| | Table View | 1386 | 180382 | 0.8% | June-August |
| **2010-2014** | Table View | 3039 | 108093 | 2.8% | June-August |
| | Observatory | 836 | 86955 | 1.1% | June-August |

### 5.10.3 Fungal spore summary

The three dry air spores, *Alternaria, Cladosporium* and *Epicoccum* all increased in concentration during the spring and autumn months. They were least prevalent in the winter months of May-July. Strong seasonal peaks were seen for each year and at each site for *Alternaria.* Peak months were October to November at all the sampling sites when concentrations more than 50 $m^{-3}$ were regularly found at all the sampling sites. An unexpected finding was the low concentrations of less than the 3,000 spores/$m^{-3}$ for *Cladosporium*. *Epicoccum* concentrations were consistently low at all the sampling sites after 1997. Significant concentrations were seen prior to that year from Rondebosch. *Pleospora*, a wet air spore, peaked in June and July. The highest concentrations were found at the coastal site of Table View where a concentration more than 5,000$m^{-3}$ was seen in one 24-hour period.

*Pleospora changes over time*

The *Pleospora* cumulative score at Table View for series 2010-2014 increased by a factor of 3.5 when compared with the *Pleospora* cumulative score site from 1997-2001.

## 5.11 Monthly relative proportions of eight selected air spora

All the data points from all the years were combined to graphically represent the eight pollen taxa and fungal genera selected for analysis in the study.  Compositions, which were colour coded to show the monthly proportions, were drawn. This was done by summing all available daily concentrations (1984-2015) for the months January to December and the sites (Rondebosch, Table View and Observatory). The relative proportion of each taxon was calculated as the proportion of the total concentration of all eight taxa for that site and month.

Figure 5.23 below does not show absolute concentrations. It shows only relative proportions so it is not informative about the total monthly concentrations of the selected pollen and fungal spores. It does illustrate how the relative proportions change over the months. The dominant pollen or fungal spore may be clearly seen for each month and it also shows how these proportions change throughout the months of the year.

It shows the relative proportions of the eight selected pollen and fungal spores by month (January-December) and site (Rondebosch, Table View and Observatory). For each month, site and taxon, all available daily concentrations were summed (1985-2015). The relative proportion for each taxon was calculated as the proportion of the total concentration of all eight taxa for that month and site. The relative proportions can be understood as the average relative contribution of these eight taxa to the total concentration over time. The bars show the monthly distribution patterns at the sampling sites and these may be seen in Figure 5.23 below.

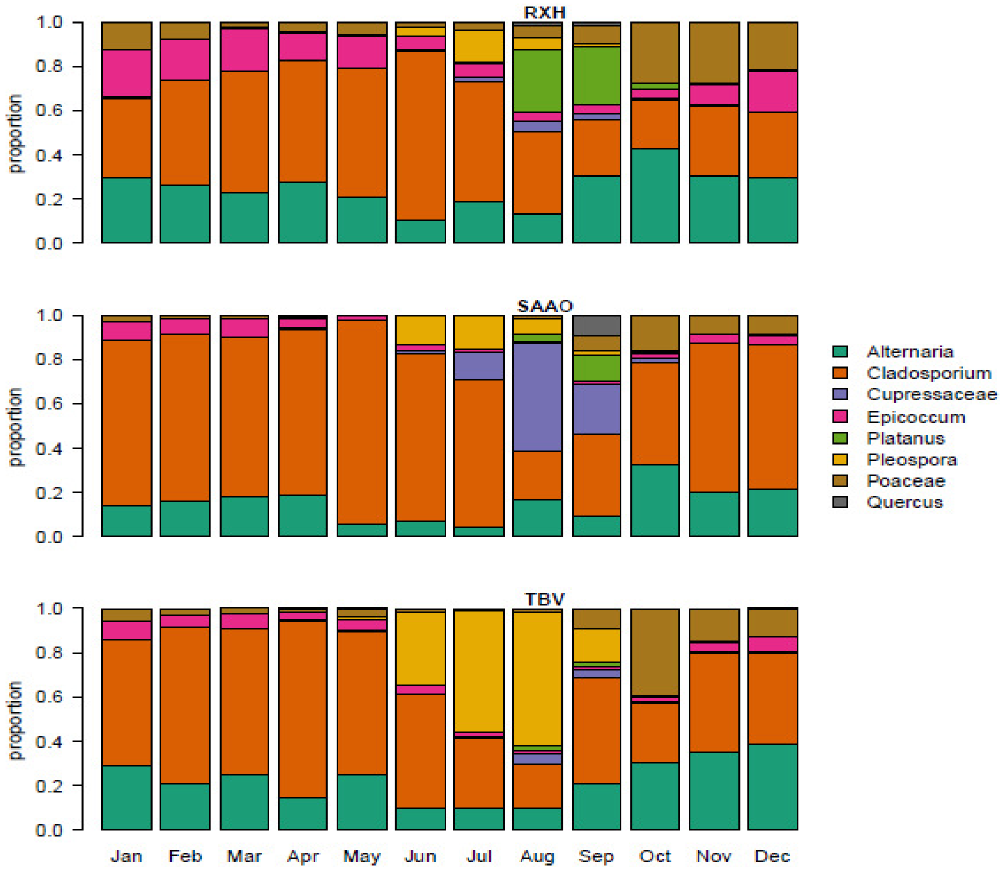



Figure 5.23 Monthly relative pollen and spore concentrations expressed as proprtions of the summed concentrations of these eight selected taxa/genera. For each month and site we summed concentrations for all days available between 1984 and 2015 before calculating the proportions

*Pollen and fungal spore proportions*

The pollen patterns and sporing fungi change month by month and this can be seen from the chart as the proportions of the pollen air spora vary with the changing seasons.

*Fungal spore proportions at the sites*

The fungal genera: *Alternaria, Cladosporium and Epicoccum* are seen throughout the year in varying proportions at all three sites. *Cladosporium* was the dominant air spore at Observatory (SAAO) and Table View (TBV) from January to May and for an even longer period, from January to June at Rondebosch RXH). *Epicoccum* was a constant low presence in the ambient air at all the sites. *Pleospora*, the wet weather spore was visible on the chart from June-September at all the sites and outnumbered *Cladosporium* at Table View in July and August.

*Pollen proportions at the sites*

Tree pollen was strongly represented in spring by *Cupressus* (cypress). *Platanus* (plane) and *Quercus* (oak) pollen. The contribution of *Cupressus* to the pollen load was much greater at Observatory (SAAO) than at the other sites and *Platanus* (plane) showed clearly in the August-September months at Rondebosch (RXH) and to a lesser extent at Observatory (SAAO) but was seen in very small quantities at Table View (TBV). *Quercus* pollen made a noticeable contribution at the Observatory site in September, a far smaller contribution at Rondebosch and was not seen at Table View. Poaceae pollen was present throughout the year but dwindled to small amounts in the winter months of May-July then reappeared in August, peaking in October.

### 5.12 Compositional changes in the 8 aeroallergen levels over time

Compositional covariance biplots were drawn to illustrate changes in the composition of the eight selected aeroallergens over time. All available data was entered into the database to find patterns in the relationship between the eight taxa. The components in this group of aeroallergens: *Alternaria*, Poaceae, *Pleospora*, Cupressaceae, *Platanus, Quercus*, *Cladosporium* and *Epicoccum* are connected by black arrows.

The way each taxon is positioned in relation to the other taxa, indicates the changing proportions. The closer two taxa are in the 2-dimensional plot, the more alike they are in proportion to each other. If the arrows for two taxa point in opposite directions, this indicates that their proportions are inversely related; as one increases, the other decreases. When the arrows are close together as may be seen for *Alternaria, Epicoccum* and *Cladosporium* it is an indication that that the proportions increase or decrease together. The months or years that lie along this axis have relatively higher spore concentrations relative to other taxa or years or months. Two biplots were drawn to show the relative composition of the air spora at the sites over time. Plots were drawn firstly to show the proportions at the sites over years and then across months. These biplots are 2-dimensional simplified pictures of the composition of the taxa selected for this study.

*Yearly variations of the eight aeroallergens*

The first plot shows compositions over the proportions of the years at the three sites. The group with black lines is shown on the right and indicates the positions of the eight selected taxa in relation to the two axes. The dot indicating the position of a particular year on the plot shows how the year has taken up that position because of the weight of the heaviest taxon or taxa in the group. The highest concentrations for trees were seen at the Observatory site and the yellow coded years for Observatory are positioned furthest to the right, indicating higher concentrations for tree pollen at that site than at Rondebosch or Table View site. The Table View years show a shift to the left, that suggests higher proportions of Poaceae and *Pleospora,* with no substantial weight of tree pollen to oppose the migration. The outliers for 2014 seen at the top of the plot reflect the lower Poaceae, *Quercus* and *Platanus* pollen concentrations that were counted at the Table View and Observatory sites in that year. These variations in the pollen and fungal spore proportions are shown in Figure 5.24a below.

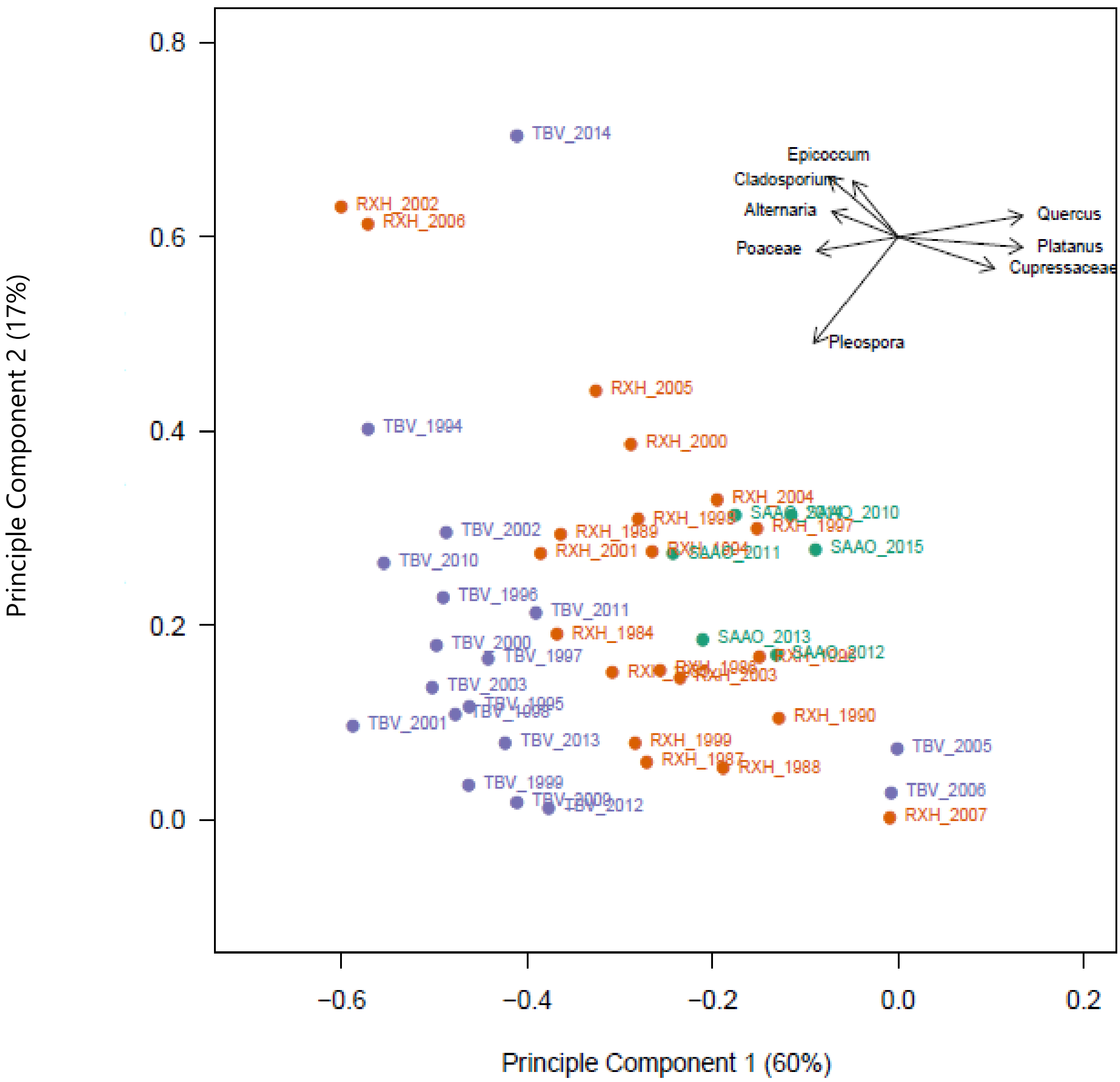



Figure 5.24a Biplots with the first two principle components summarising variations in the yearly study of the eight studied taxa at Rondebosch (red) Table View (blue) and Observatory (orange) 1984-2014. The black arrows plot the individual species in relation to the two principle component axes.

*Principle components analysis*

Principle components analysis is a useful way of summarising a multivariate data set in fewer dimensions. It was used here to summarise the variations of eight species (eight dimensions) in two dimensions. For this, species contributions are mapped onto independent axes. In the two-dimensional biplot we use only the first-row axes, those that explain most of the observed variations in the eight-dimensional relative concentrations. For the biplot (5.9b), both the species (black arrows) and the data points (filled dots) are mapped onto these axes. This is an exploratory visual tool to study patterns.

*Monthly variations of the eight aeroallergens*

The monthly variations in the proportion of the selected pollen and fungal taxa were similarly displayed. The most congested area on the plot, in the upper left quadrant, holds the month of October= month 10 when grasses and weeds flower and *Alternaria* levels peak. The Observatory site is much lower on the Poaceae axis, due to the strong opposing pull of Cupressaceae which is found in high concentrations at that site. May, June and July are the months when the lowest pollen counts are recorded in Cape Town and fungal spore concentrations are often below the threshold level of 900 spores/$m^{-3}$ especially if the temperature is below 15°C. The months of August= month 08 in Rondebosch and August and September= month 09 in Rondebosch and Observatory are situated closest to the arrow trajectory for *Platanus*, in keeping with the high plane tree pollen concentrations at those sites in August-September. These combinations may be seen in Figure 5.24b below.

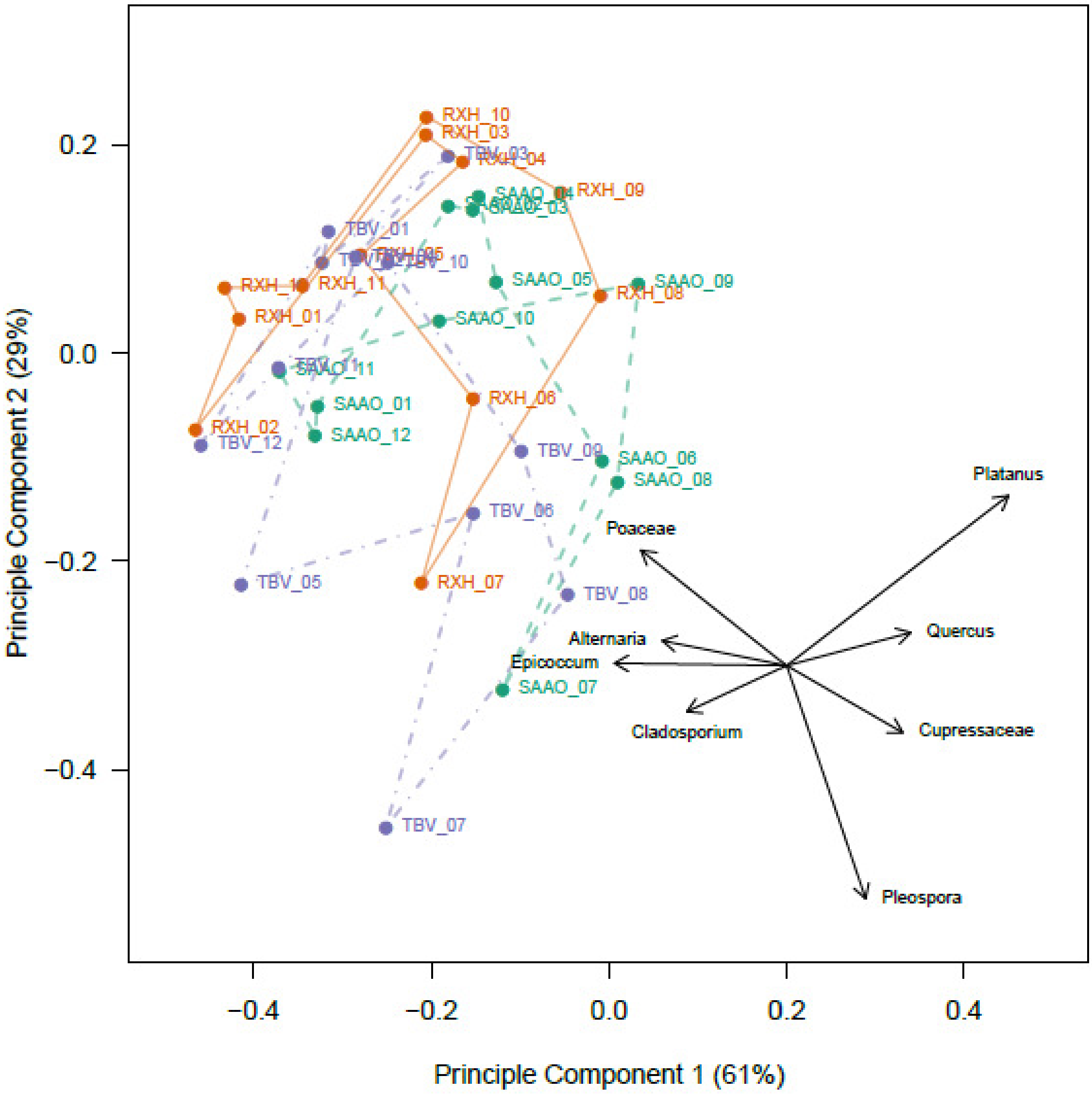



Figure 5.24b. The first two principle components summarising monthly variations in the composition of the eight studied taxa at Rondebosch (red) Table View (blue) and Observatory(orange) Years:1984-2014. The black arrows plot the individual species in relation to the two principal component axes.

### 5.13 Aerobiological Zones

There is some overlap between the sites but biplot 5.9a shows clear separation between the sites of the eight selected pollen and fungal taxa for most of the sampling years.

What we see here are distinct aerobiological zones. The Inland Aerobiological Zone, or the Inland Aerobiome includes the Rondebosch, Mowbray and Observatory sampling sites. We have named this zone the Valkenberg Aerobiome after the area where the sampling sites are situated. The coastal Aerobiological Zone, or the West Coast Aerobiome contains the Table View sampling site, on the western coastline of the Western Cape, South Africa.

**5.14 Overall Summary**

The eight selected taxa were compared directly with each other from the three sites using the data sets 1997-2001 (Rondebosch and Table View) and 2010-2014/5 (Observatory and Table View). To compare the eight selected pollen taxa and fungal spore genera the compositional plots and bi-plots were drawn. The seasonal patterns and flowering times mirrored the findings of the direct analysis, showing the dominance of *Quercus* and Cupressaceae at Observatory, and *Platanus* in Rondebosch. The long Poaceae season was seen at all the sites, especially in Table View. The wet air spore, Pleospora can clearly be seen at Table View in the mid-winter months of July and August. *Cladosporium* was the most abundant of the selected fungal spores and seen throughout the year, followed by *Alternaria* and showed a distinct decrease during the mid-winter months of June, July and August. *Epicoccum* was a constant low contributor to the fungal spore spectrum at all the sites.

Relative proportions were not constant for all the years and fluctuated at the three sites over time when months and years were compared. There were some differences in the start dates for these pollen taxa between the sites but the start date for the seasons at the same site was consistent.

Chapter 6

# The influence of weather on *Cladosporium* and Poaceae

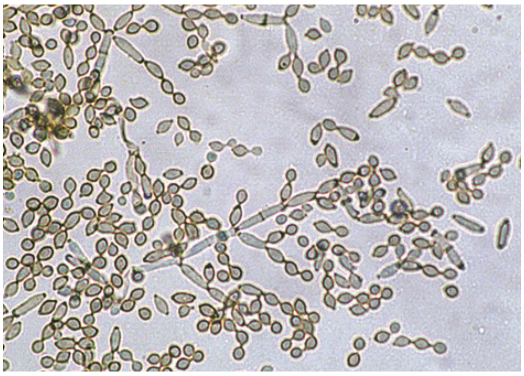


Figure 6.1a *Cladosporium cladosporioides* culture. *University of Adelaide by Ellis D*

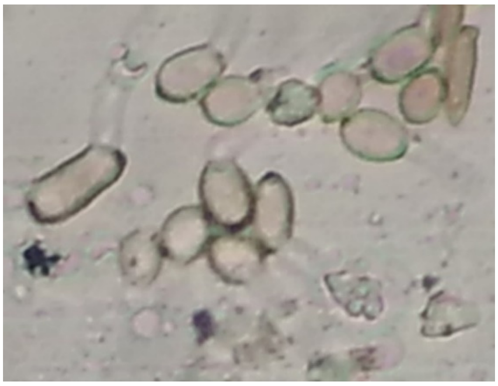


Figure 6.1b *Cladosporium* x400 *Photomicrograph by author.*

### 6.1 Introduction

We chose to investigate the presence of *Cladosporium* in the ambient air of Cape Town because the concentrations were unexpectedly low in this temperate region. We compared the components of the weather that are known to influence the occurrence of high i.e. more than 3,000 spores/m$^{-3}$ concentrations using our sampling records and weather data from the South African Weather Services (SAWS). It has been shown that the meteorological parameters that have been correlated with high atmospheric *Cladosporium* concentrations are: Minimum and maximum temperature, precipitation, humidity and wind speed and direction (Kurkela 1997). We compared one very high *Cladosporium* concentration with the suggested ranges of these parameters and then applied the table of meteorological parameter ranges to concentrations that were greater than 1,000 spores/m$^{-3}$.

### 6.2 *Cladosporium* and the weather

The meteorological parameters listed above that influence the presence of *Cladosporium* in the atmosphere are discussed separately below.

### 6.2.1 Precipitation

*Cladosporium* concentrations were compared with rainfall at Table View and Observatory and the washing out effect of rain on the hygroscopic *Cladosporium* conidia as described above was clear (see 2.7.6). This may be seen in Figures 6.2 a and b below.

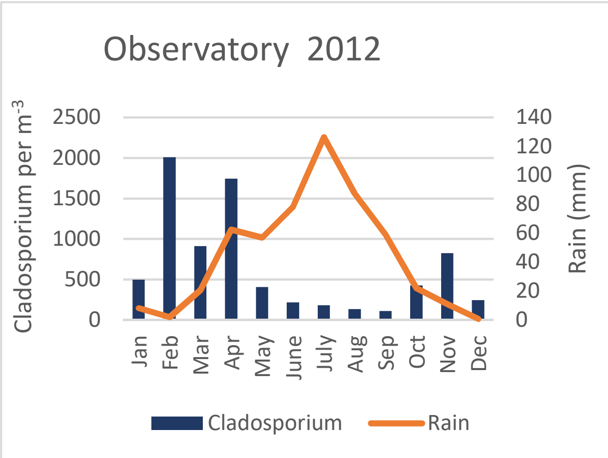



Figure 6.2 a. *Cladosporium* and rain measurements in Observatory in 2012

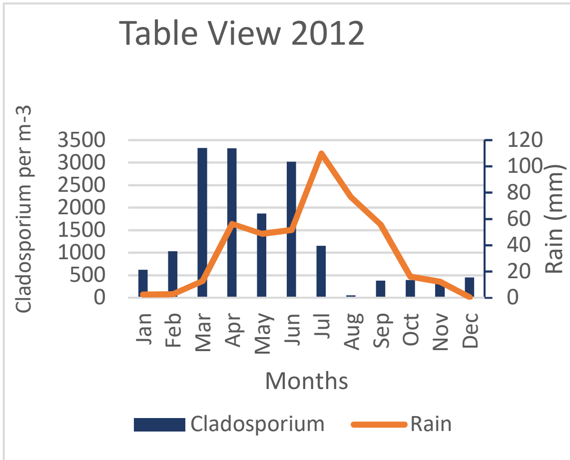



Figure 6.2b. *Cladosporium* and rain measurements in Table View in 2012

### 6.2.2 Temperature

When investigators correlated empirical data from long term studies of *Cladosporium* levels with meteorological parameters, a significant positive association with maximum temperatures was found, which varied according to whether *Cladosporium cladosporioides* or *Cladosporium herbarum was* more prevalent in the sampling area. *Cladosporium cladosporioides* levels greater than 200 spores/m$^3$ were found to occur within the temperature range 13-21°C and Cladosporium *herbarum* levels greater than 60 spores/m$^3$ were seen within the temperature range 18-25°C.

*Cladosporium concentrations and temperature in Cape Town*

Cladosporium concentrations in Observatory during 2013 were compared with $T_{\text{max}}$, $T_{\text{mean}}$, and $T_{\text{min}}$. The trend was towards higher concentrations of Cladosporium during the spring/summer/autumn months of October-March but *Cladosporium* concentrations were as low in January as they were in June. This comparison with temperature may be seen in Figure 6.3 below.

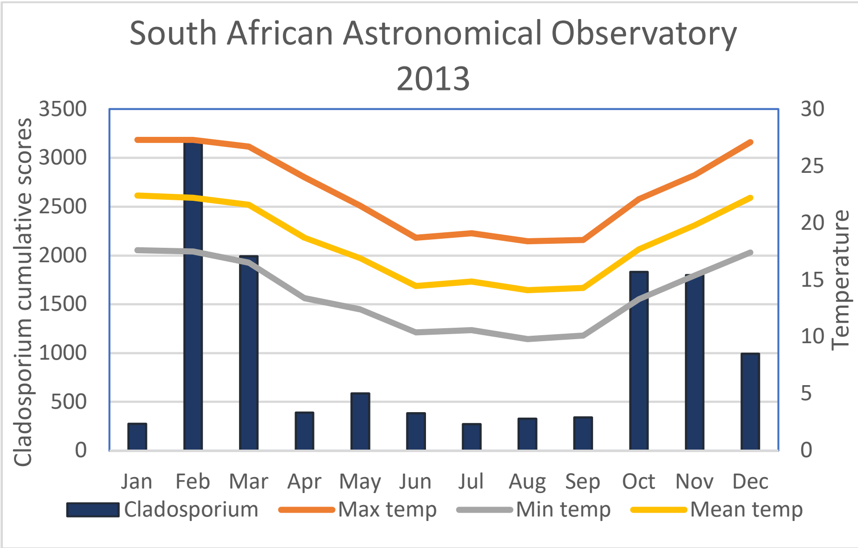



Figure 6.3 *Cladosporium* and temperature in Observatory 2013

### 6.2.3 Relative humidity

Conflicting results have been found when the correlation between relative humidity and high *Cladosporium* levels have been examined. Several authors found a significant negative correlation with relative humidity (Mitakakis et al. 1997, Calderón, Lacey & McCartney 1997) but Katial found a positive correlation with relative humidity in Denver, Colorado in the United States (Katial et al. 1997).

*Relative humidity and spore release*

In vitro experiments conducted to study the dispersal of *Cladosporium carygenum* conidia demonstrated the association between relative humidity and spore release. Gottwald (1983) notes that spore release increased when the relative humidity was > 40%. Controlled studies have measured the hygroscopic property of *Cladosporium. Cladosporium cladosporioide*s was shown to be the most hygroscopic of the fungal spores, which would also affect its aerodynamic properties, increasing its settling rate under conditions of high relative humidity. (Reponen et al.1996).

*Cladosporium concentrations and relative humidity in Cape Town*

No correlation could be seen between relative humidity levels and Cladosporium concentrations in Table View in 2012 and this may be seen in Figure 6.4 below.

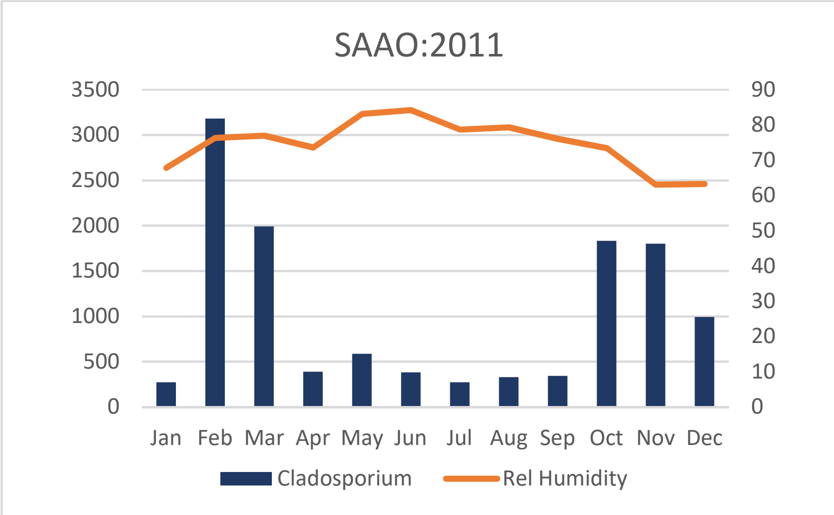



Figure 6.4 Cladosporium and relative humidity levels Observatory 2011

**6.2.4 Wind speed and direction**

The size of the spore carried on air streams is relevant when measuring wind speed. *Cladosporium* conidia are variable in size and shape but nonetheless they are in the lowest range for size of the fungal aerospora, together with other small diameter spores like *Aspergillus*, *Penicillium* and *Botrytis*. Wind lifts spores into the atmosphere, especially when the wind speed is > $0.5m^{-s}$ (Kurkela, 1997) but when the velocity exceeds 2 m/s for a prolonged period, *Cladosporium* spore levels decreased, as was demonstrated above a Kansas wheat field. (Eversmeyer & Kramer 1975) and observed in a Western Cape study (Hawke & Meadows 1989).

Gregory (1973) showed that increased wind speed has been shown to 'significantly decrease' the incidence of certain spores, including basidiospores, *Cladosporium* and *Botrytis.* Aerobiological studies from suburbs of Cape Town reported low *Cladosporium* levels (Potter et al. 1991: 82; Cadman & Dames, 1996: p 83). Conversely, high *Cladosporium* levels, or concentrations that exceeded 3000 spores/$m^{-3}$ were reported in inland areas of South Africa such as Johannesburg (Cadman, 1997: 178) and Mpumalanga where a concentration of 30 000 spores/$m^{-3}$ was counted on a single day (Berman, 2013:200) and from a study in the coastal city of Durban (Dames, 1994: p347). The direction of the wind is important for coastal areas, as on shore winds that blow from the direction of the sea, would be much less likely to carry fungal spores, than off shore winds that pass over vegetation, according to Brown & Jackson (1978) and Jones & Harrison (2004).

*Prevailing wind at Table View*

Strong prevailing winds blow along the coastal areas where Table View is situated. It is a favourite kite surfing area and wind reports are published daily for this coastline. Strong southerly and south easterly winds may reach gale force in summer and the north-westerly wind may reach similar speeds during winter months. Wind roses chart the average daily wind speed and direction for the month. Wind roses are graphical charts that characterize the speed and direction of wind at a specific location. Presented in a circular format, the length of each "spoke" around the circle indicates the amount of time that the wind blows from a particular direction. Colours along the spokes indicate categories of wind speed. (https://www.climate.gov). Wind roses for the months of the year were drawn from wind soundings at Chevron, an oil refinery 7 km from the sampling site. The wind roses are shown in Figure 6.5 below.

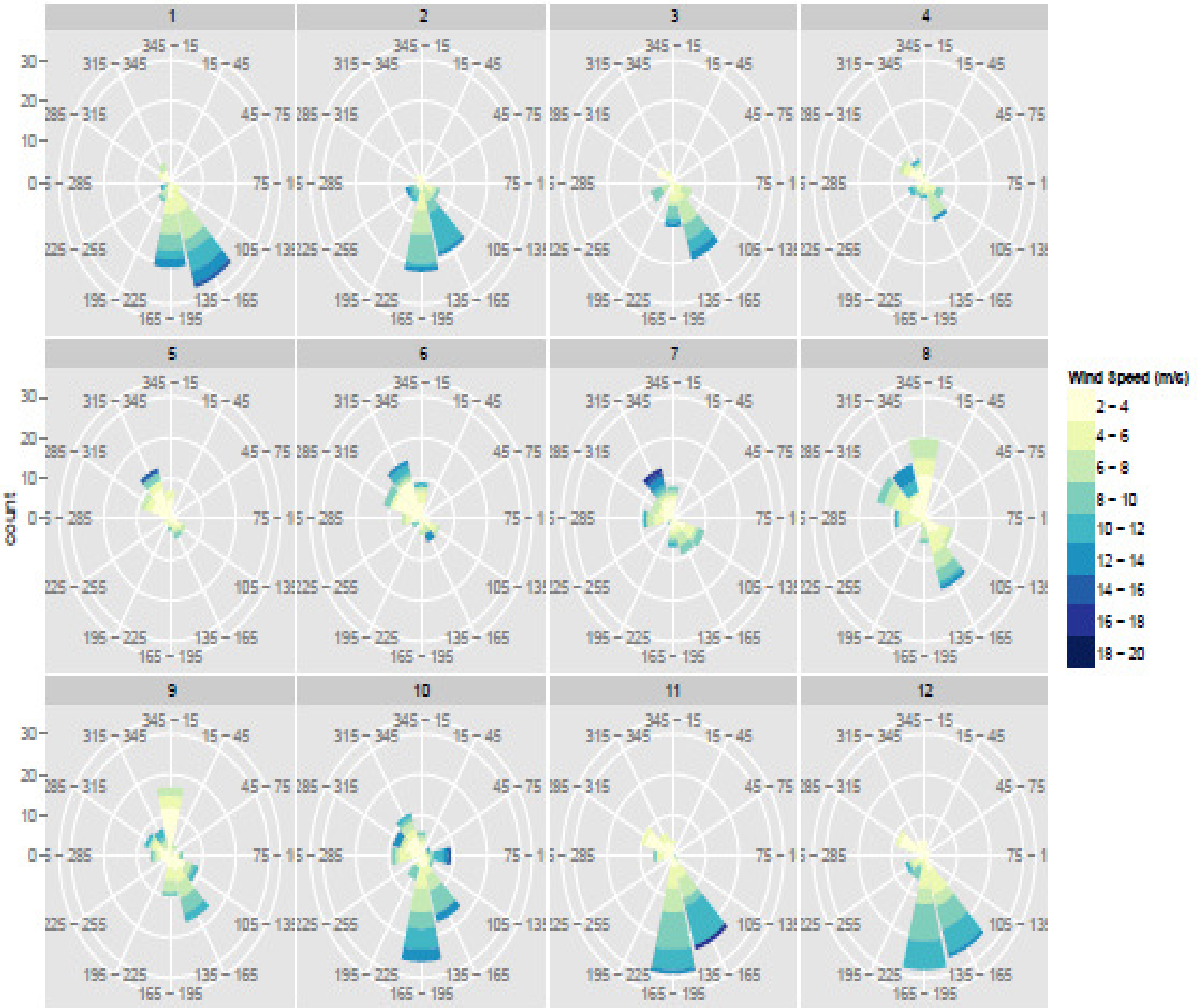
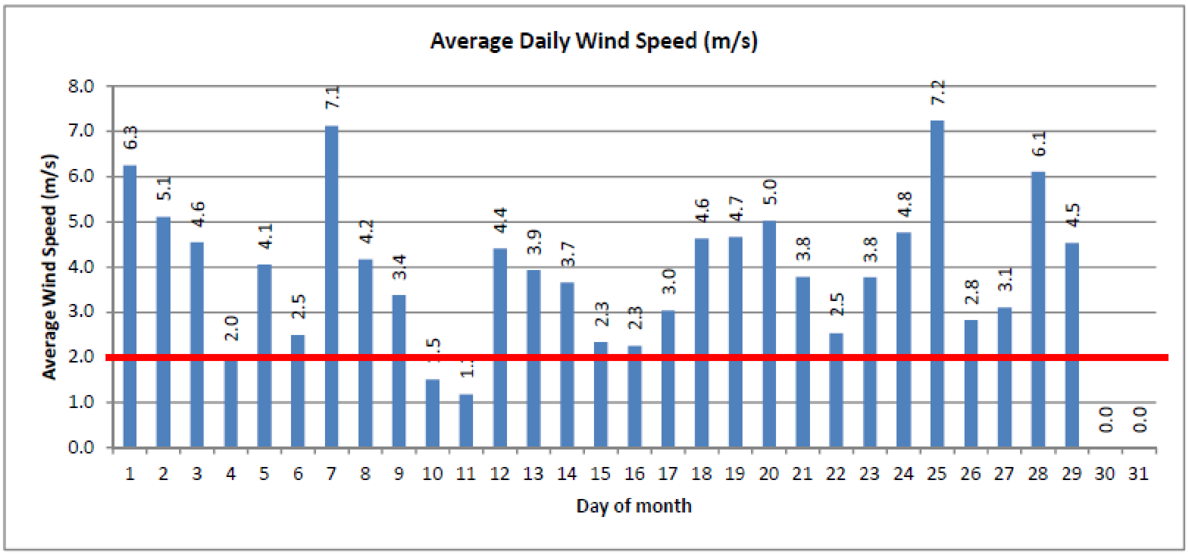



Figure 6.5 Wind roses at Table View 2012 for calendar months. 1=January-12= December

High wind speeds that approach gale force may be seen in the months of January, July and November, when wind speeds exceeded 17 metres per second (m s) or 33 knots (https://www.windfinder.com).Wind speeds reach 12-14 m s during the months of February, March, August, October and December. Optimum wind speed months for *Cladosporium* are April, May and September when average speeds are low.

Detailed analysis showed wind speed and direction for the months when the highest *Cladosporium* concentrations were obtained for the year 2012. A red line divided the wind speeds above and below 2 metres per second and average daily values and these measurements are shown in Figure 6.6 below.



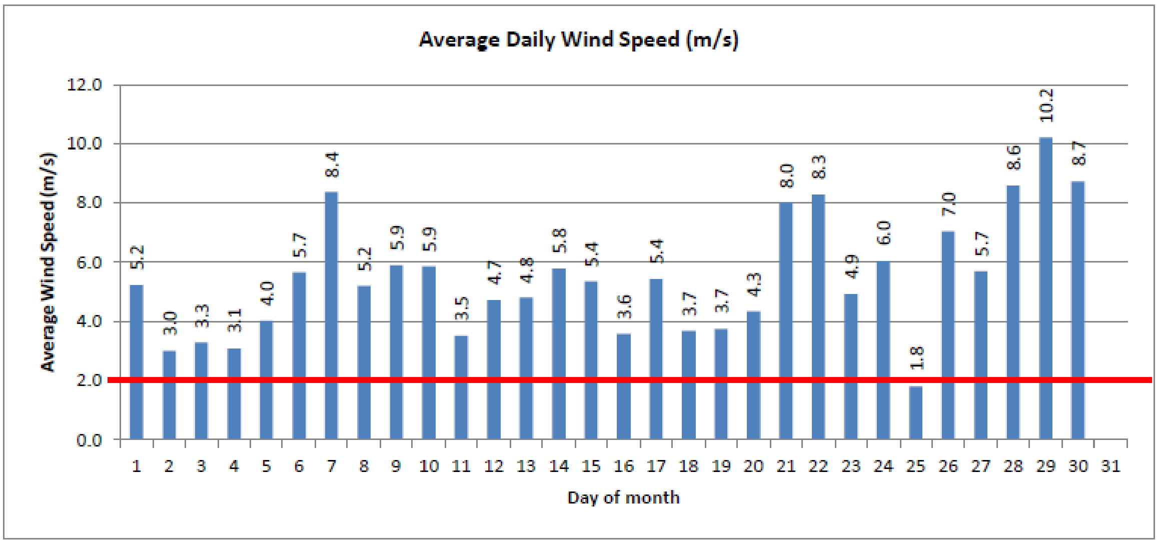

| Average Daily Wind Direction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Degrees:** | 169 | 165 | 168 | 185 | 136 | 218 | 200 | 236 | 157 | 100 | 139 | 134 | 157 | 153 | 147 | 186 | 95 | 156 | 154 | 160 | 148 | 137 | 138 | 158 | 200 | 145 | 189 | 144 | 198 | | |
| **Direction:** | SSE | SSE | SSE | S | SE | SSW | S | SW | SE | E | SE | ESE | SE | SE | SE | S | E | SE | SE | SSE | SE | SE | SE | SSE | S | SE | S | SE | S | | |
| **Day:** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |

| | | |
|---|---|---|
| **Montly Avarage Wind Speed:** | 3.95 | m/s |
| **Monthly Average Wind Direction:** | 161 | Degrees |
| | SSE | Direction |

Figure 6.6 Daily wind speed and direction for June 2012 in Table View. The exact wind speed for each day is shown on the bar chart. Wind speeds that were below 2m s are highlighted in red.

*High wind speeds*

The average wind speed was 3.95 m/s in June but wind speeds increased during the summer months. The average wind speed in November 2012 increased to 5.46 metres per second and winds gusted up to speeds > 10 metres per second. The daily wind speed and direction are shown in Figure 6.7 below.




**Average Daily Wind Direction**

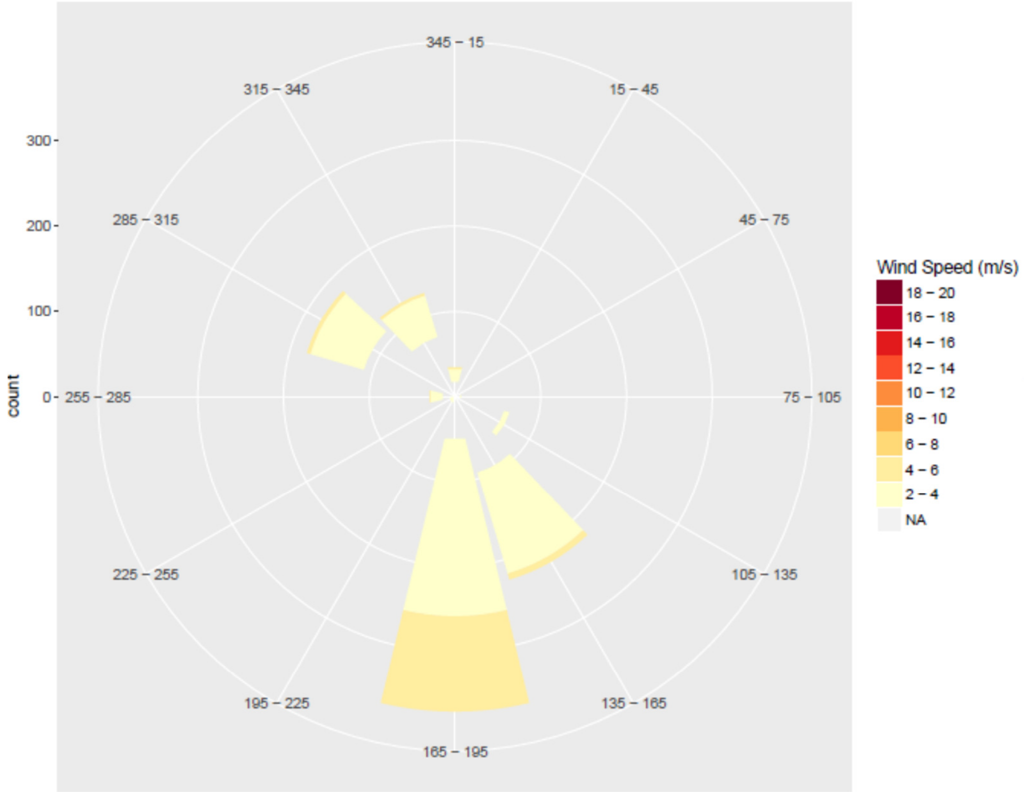
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Degrees: | 154 | 245 | 289 | 280 | 195 | 172 | 172 | 165 | 168 | 174 | 215 | 174 | 185 | 197 | 184 | 241 | 194 | 205 | 238 | 199 | 169 | 173 | 217 | 277 | 72 | 169 | 163 | 165 | 168 | 146 | |
| Direction: | SE | SW | W | W | S | SSE | SSE | SSE | SSE | SSE | SSW | SSE | S | S | S | SW | S | SSW | SW | S | SSE | SSE | SSW | W | ENE | SSE | SSE | SSE | SSE | SE | |
| Day: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |

| | | |
|---|---|---|
| Montly Avarage Wind Speed: | 5.46 | m/s |
| Monthly Average Wind Direction: | 192 | Degrees |
| | S | Direction |

Figure 6.7 Daily wind speed and direction during November 2012 in Table View. The exact wind speed for each day is shown on the bar chart. Wind speeds below 2 m s are highlighted in red.

*Wind speed and direction at Observatory and Table View*

Wind speed and direction is extremely variable in the South-Western Cape and may change within a comparatively short distance. The wind speeds of the two sampling sites at Observatory and Table View were compared and are shown in the wind roses 6.8 a and b below.



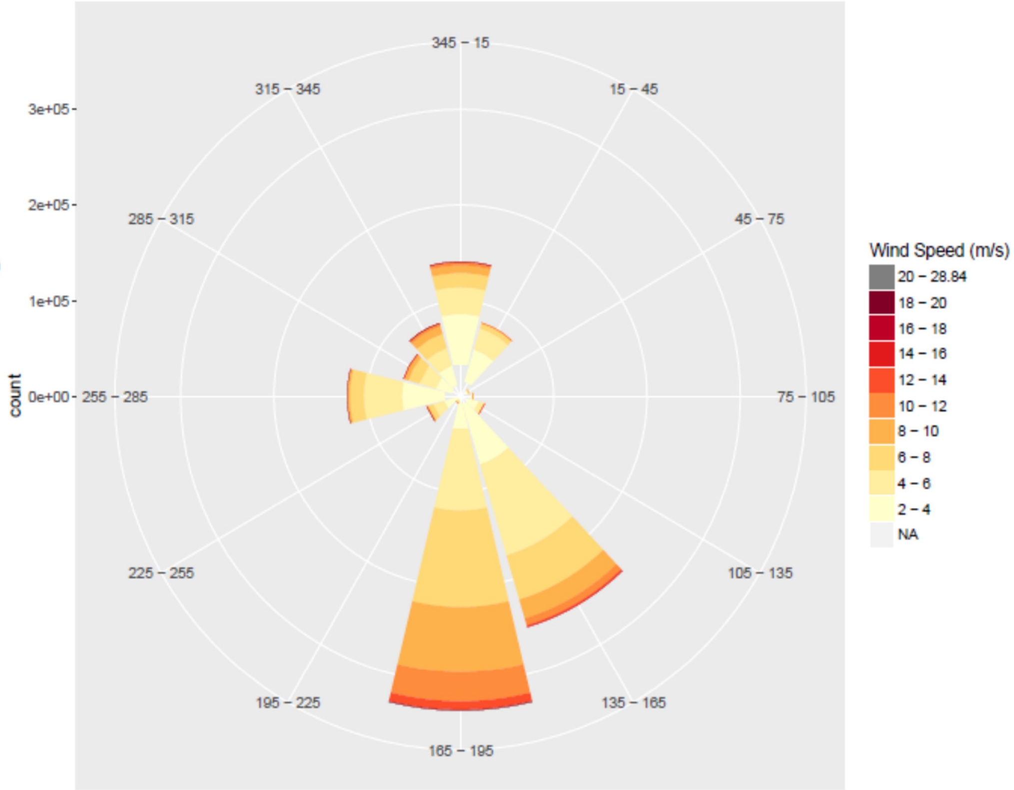




Figure 6 8a Wind rose shows the average wind speed and direction direction for Observatory 2012.

Figure 6 8b Wind rose shows the average wind speed and for Table View 2012.

*Observatory wind speeds*

The average wind speed at the Observatory site was low and did not exceed 8m s. By contrast Table View average wind speeds reached speeds of 14-16 metres per second during the summer and winter months. Gale force winds greater than 16 m s were recorded. This not only shows the difference between wind speed at the two sampling sites, but explains how strong south-easterly or north-westerly winds in Table View could carry spores from farming areas to the north or vegetation to the south of the sampling site.

### 6.3 Meteorological ranges for *Cladosporium*

To summarize the findings of investigators who studied the effect of selected meteorological parameters on *Cladosporium* concentrations, *Cladosporium* is a dry air spore and in addition it is hygroscopic. The spore will not remain airborne in conditions of high relative humidity yet it requires moisture to produce conidia. The rain or dew that is necessary for sporulation for this spore must be followed by dry conditions for the liberation of spores. The optimum temperature range for this fungus explains why it is more commonly found in temperate climates. Higher concentrations tend to be seen in calm weather, or at wind speeds below the suction speed of the volumetric spore trap. The prerequisites for high concentrations are shown in Table 6.1 below.

**Table 6.1. Meteorological parameters required for high *Cladosporium* concentrations**

| | |
|---|---|
| Relative Humidity | >40% |
| Temperature | 15-28°C |
| Wind speed | Low < 2m/s |
| Wind direction | Offshore over vegetation |
| Rain | 1-2 days prior to *Cladosporium* spike |

When these factors are applied, it becomes obvious that the timing of the release of the conidia is incidental, whether it be morning, afternoon, evening or night. Of much greater importance is the coincidence of the factors listed above. Seasonality of the sporing of the fungus is also subject to this list of specifications. This was shown in the unusual peak

*Cladosporium* concentrations in Kuwait, which occurred during the cooler winter months as the high summer temperatures exceed the optimum temperature range for *Cladosporium*. The occurrence of winter peaks for *Cladosporium* are in contradiction to the peaks commonly seen during spring and autumn months in temperate regions around the world

### 6.4 *Cladosporium* concentrations at the Cape Town sampling sites

We found low levels of *Cladosporium* (less than 3,000 spores/m$^3$) from pollen sampling studies from 1985-2014 in the Western Cape. This was a consistent finding at all four of the sampled sites and it contrasted with the findings from pollen sampling sites in most temperate areas. It has been noted that *Cladosporium* levels are generally lower in coastal cities, like Cape Town. Cadman, Hawke and Meadows reported low *Cladosporium* levels from their Cape Town sampling sites. Researchers found high levels of *Cladosporium* from pollen sites in other sampling sites of South Africa, e.g. Secunda, Mpumalanga (Berman, 2013) Johannesburg, Gauteng (Cadman, 199) and Durban, KwaZulu Natal. (Dames & Cadman, 1994). A review of the Northern Hemisphere literature indicated that *Cladosporium* obeys well defined ranges of meteorological parameters and these are; temperature, rainfall, relative humidity and wind speed and direction. Seasonality for this fungal spore has been frequently reported but differs according to climate. Researchers have reported positive and negative associations with the weather parameters.

#### 6.4.1 Optimum weather parameters

It is necessary for clearly defined ranges for selected weather parameters to be met, for high *Cladosporium* concentrations to occur at any given geographic locations. Table 6.1 lists the ranges for the meteorological parameters that should be present in concert for *Cladosporium* spikes greater than 3,000 spores/m$^{-3}$ to occur.

#### 6.4.2 *Cladosporium* concentrations >1000 spores/m$^{-3}$ and weather

Cladosporium concentrations from 2011-2014 at Table View and Observatory that exceeded 1000 spores/m$^{-3}$ were compared with the temperature, relative humidity and wind speed and direction recorded on that day. 1000 spores/m$^{-3}$ was chosen because there were insufficient days with more than 3,000 spores/m$^{-3}$. A lag effect was shown after rainfall, so precipitation values for two days prior to the *Cladosporium* spike were included. Values that agreed with the range of weather parameters in Table 6.1 were highlighted in red. The comparisons are shown in Tables 6.2 and 6.3 below.

**Table 6.2 *Cladosporium* concentrations and weather parameters at Table View 2011-14**

| Date | *Cladosporium* >1000/m³ | Temperature Min-max 15-28°C | Relative Humidity (should be >40%) | Rainfall prior to the *Cladosporium* spike (1-2 days prior to spike) | Wind speed and direction (speed should be <2m/s) |
|---|---|---|---|---|---|
| 01.04.2011 | 15584 | 16.3-20.9°C | 86% | 30.03 6.4mm 31.03.0.2mm | NNW: 330 1.3 m/s |
| 02.04.2011 | 4038 | 15.7°C- 22.6°C | 86% | 1.04.2011 0.4mm | 0° Calm |
| 09.11.2011 | 1275 | 12.7°C-19.5°C | 71% | ND | NNW 300 3.3m/s |
| 08.07.2011 | 2163 | ND | ND | ND | ND |
| 10.02.2013 | 5795 | 15.7-28.6 | 91% | 9.2: 13.4 mm 10.2:14.6mm | W 1.7m/s |
| 15.02.2013 | 2336 | 15.5-31.5 | 55% | 13.2: 3.2 | SE 8.1m/s |
| 02.11.2013 | 2963 | 16.1-26.8 | 51% | 31/10.13: 19.1mm 1/11/13: 0.2mm | SSE 9.0m/s |
| 31.01.2013 | 1265 | 19.6-32.6 | 56% | ND | SSE 10.3 m/s |
| 13.01.2014 | 1620 | 17.9-26.8 | 55% | Nil | WSW 8.6m/s |
| 14.02.2014 | 1146 | 16.3-36.8°C | 52% | Nil | 0° Calm |

Code: The red highlight shows that the suggested met parameter range was met

**Table 6.3 *Cladosporium* concentrations and weather parameters at Observatory 2011-14**

| Date | *Cladosporium* >1000/m³ | Temperature Min-max 15-28°C | Relative Humidity (should be >40%) | Rainfall prior to the *Cladosporium* spike (1-2 days prior to spike) | Wind speed and direction (speed should be <2m/s) |
|---|---|---|---|---|---|
| 1.04.2011 | 1421 | 15.7-21.7°C | 87% | 30/4: 0.2 31/4: 1.8 | SSE 1.4m/s |
| 06.07.2011 | 1154 | 12.3-17.3 | 60% | 3/7: 0.2 | SSE 2.4m/s |
| 14.11.2011 | 1068 | 10.3-23.4°C | 74% | 12/11:16mm 13/11: 2mm | W 1.4m/s |
| 10.2.2013 | 1870 | 16.3-21.7 | 91% | 9/2: 13.4mm 10/2: 14.6mm | 0°C Calm |
| 13.2.2013 | 1642 | 16.8-30.5°C | 98% | 11/2: 0.2mm 13/2: 3.2mm | N 1.5m/s |
| 19.3.2013 | 1182 | 15.0-26.3°C | 85% | 18/3: 1.4mm | 0° Calm |
| 09.01.2014 | 2306 | 16.9-23.6°C | 82% | 7/1:7.6mm 8/1: 0.2mm 9/1: 2.8mm | N 1.5m/s |

Code: The red highlight indicates that the suggested met parameter range was met

## 6.5 Results

*Temperature*

The temperature range for all the elevated Cladosporium concentrations at both sites fell within the suggested temperature range of 15-28°C as shown in Table 6.1.

*Humidity*

Humidity was more than 40% on all the days examined at both sites. This agreed with the suggested range. On shore winds were recorded at Table View on days with the highest and second highest *Cladosporium* concentrations. This is contrary to the suggestion that on shore wind would be less likely to carry *Cladosporium* conidia.

*Wind direction*

Wind direction was variable at both sampling sites.

*Wind speed*

Wind speed was less than 2m s on 86% of the high concentration days at Observatory but only on 40% of the high concentration days at Table View.

*Rain*

Rain preceded high Cladosporium days on 71% of the days tested at Table View and on 100% of the high *Cladosporium* days at Observatory as suggested in Table 6.1.

*One Cladosporium concentration >15000 spores/m-$^{3}$*

An unusually high *Cladosporium* concentration of 15,584 spores/m-$^{3}$ was measured in Table View on 1 April 2011. The Cladosporium concentration in Observatory on the same day was 1,420 spores/m-$^{3}$. On the following day, 2 April, the Cladosporium concentration had decreased to 4,038 in Table View and to 102 spores/m-$^{3}$ in Observatory. The meteorological parameters from the nearest weather mast at the Royal Cape Yacht Club were examined and the following readings for 1 April 2011 are shown in Table 6.4 below.

**Table 6.4 Meteorological parameters at Table View and Observatory 1.04.2011**

| Site | Date | *Cladosporium* spores/m-3 | Temperature | Humidity | Rain prior to *Cladosporium* spike | Wind speed and direction |
|---|---|---|---|---|---|---|
| Table View | 1.4.2011 | 15524 | 16.3-20.9°C | 86% | 30.03 6.4mm 31.03.0.2mm | NNW: 330 1.3 m/s |
| Observatory | 1.4.2011 | 1421 | 15.7-21.7°C | 87% | 30/4: 0.2 31/4: 1.8 | SSE 1.4m/s |

Code: The red highlight indicates the suggested met parameter range was met

*Weather on 1 April 2011 at Table View and Observatory*

All the criteria listed in Table 6.1 were met on 1 April 2011. The meteorological parameters at The Royal Cape Yacht Club for Table View and Observatory from the weather mast at the sampling site were similar except for rain. 6.4mm was measured at Table View on 30.3 and 1.8mm on 31.03.2011 At Observatory the rainfall figures were lower; 0.2mm on 30.03 and 1.8mm on 31.04.2011

The meteorological parameters listed in Table 6.1 were compared at Table View on 1 April by plotting the individual measurements and comparing them and this comparison may be seen in Figure 6.9 below.

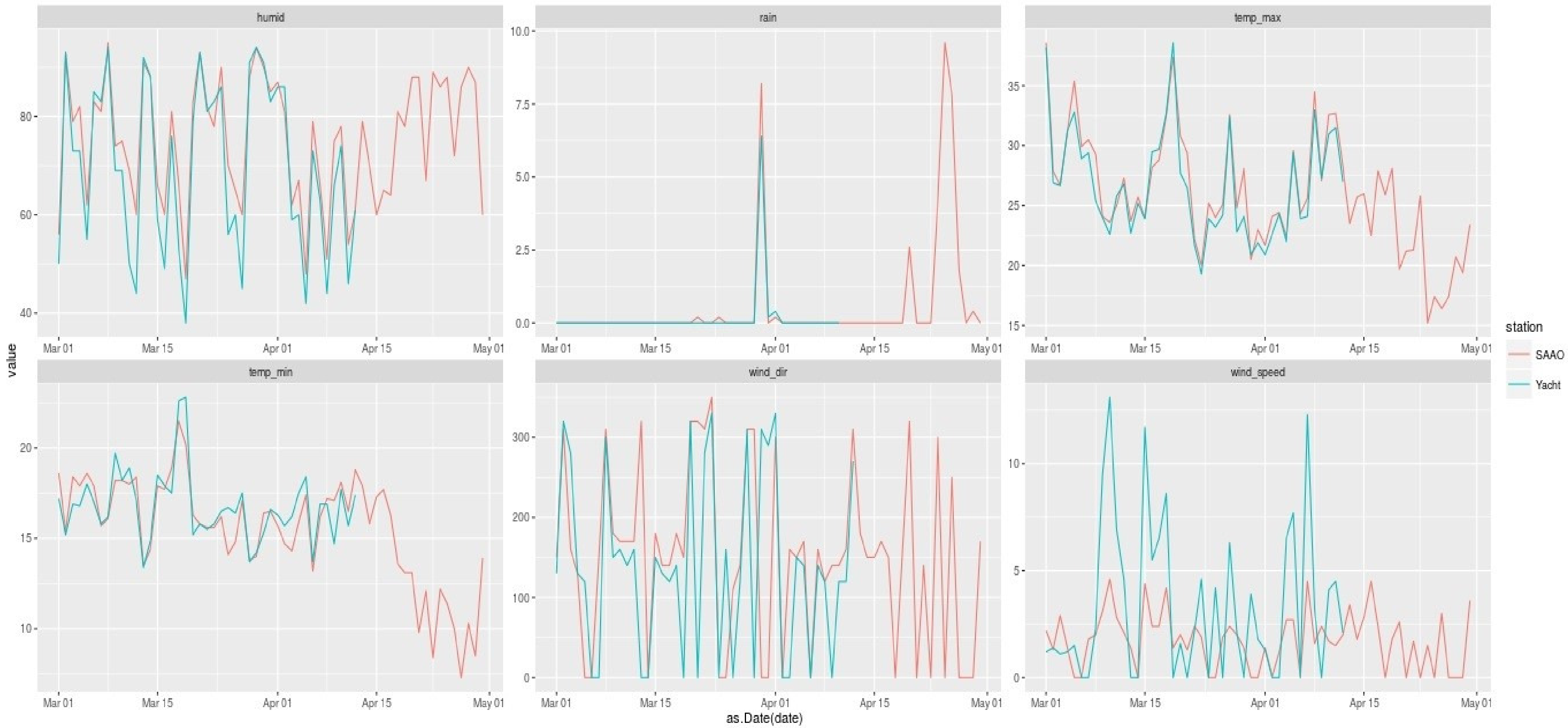



Figure 6.9 Meteorological parameters at Table View (TBV), at the Yachtclub weather mast (blue line) and Observatory (SAAO) at the on-site weather mast (red line), were compared on 1 April 2011 the date when high *Cladosporium* concentrations were recorded. Left-right top row: Humidity, rain and maximum temperature. Left to right bottom row: Minimum temperature, wind direction and wind speed.

*The Cladosporium spike and Table View weather*

To show the weather comparison with the *Cladosporium* spike the meteorological parameters were combined and plotted with the *Cladosporium* concentration. The *Cladosporium* concentration was converted to square root to fit the y-axis. It can be clearly seen that the sudden increase in *Cladosporium* concentration follows rain (blue broken line) on March 30 and 31 In Figure 6.10 below.

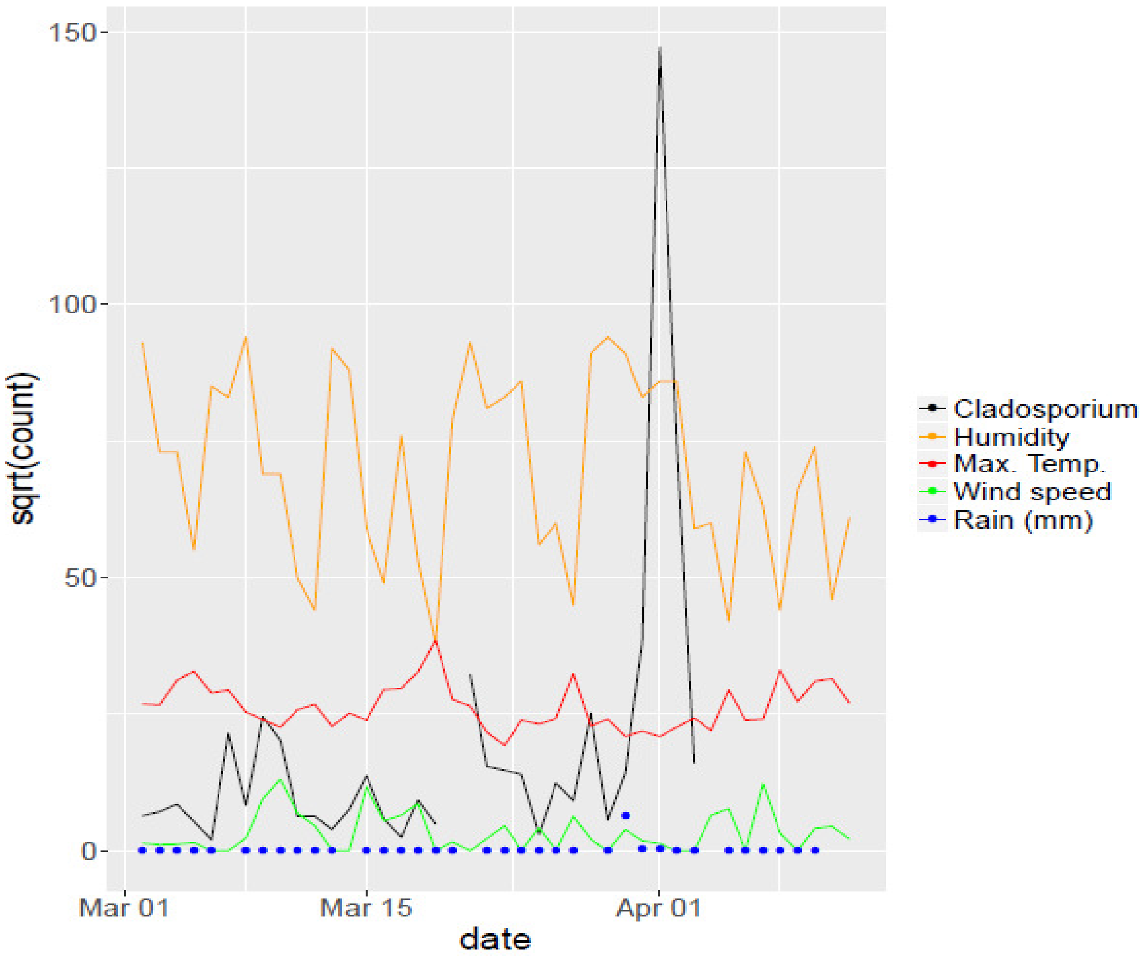



Figure 6.10 Cladosporium concentrations are compared with weather parameters at Table View showing the high concentration on 1 April 2011 in relation to humidity maximum temperature, rain and wind speed and direction.

## 6.6 Summary

*Cladosporium* concentrations rarely exceeded the suggested threshold concentration of 3,000 spores/m-$^{3}$ at all the sites. This was an unusual finding for a temperate area like Cape Town as *Cladosporium* has been identified as the most prevalent and ubiquitous of the fungal aeroallergens in many temperate areas as described above, where there are regular episodes of high concentrations.

A table of meteorological requirements for *Cladosporium* concentrations greater than 3,000 spores/m$^{-3}$ was designed, based on the quoted literature. The table included optimum ranges for temperature, humidity, wind speed and direction and rain. *Cladosporium* concentrations greater than 1,000 spores/m$^{-3}$ from the 2011-14 Table View and Observatory sites were extracted and the meteorological parameters from dates that matched the high concentration days were tabled for comparison.

*Results summary*

When the Cladosporium concentrations that exceeded 1,000 spores/m$^{-3}$ were compared with the meteorological parameters listed in Table 6.2 the results varied. Temperature and humidity were always within the suggested range. Wind speeds varied and wind direction was extremely variable. Wind speed was less than 2m s on 86% of the high concentration days at Observatory but only on 40% of the high concentration days at Table View. There was little consistency between wind direction and high concentration days at Table View or Observatory.

*Weather on 1 April 2011*

Rain, temperature, relative humidity and wind speed all fell within the suggested ranges on 1 April 2011, the day of the high *Cladosporium* concentration at Table View. Wind direction was off shore, or over vegetation, possibly coming from the wheat growing areas of the *Swartland* which is north-east of the sampling site.

## 6.7 Poaceae and temperature

Bousquet studied a group of 591 patients to better understand the classification of rhinitis. The former classification of allergic rhinitis (AR) was seasonal or perennial. The current Allergic Rhinitis Working Group (ARIA) classification is now intermittent or persistent rhinitis. Bousquet found that many patients with persistent rhinitis were sensitized to pollen only and although most patients with intermittent rhinitis were pollen sensitive, this was also true for patients who had persistent rhinitis. He concluded that seasonal and perennial rhinitis were not synonymous with intermittent and persistent rhinitis (Bousquet et al. 2007).

The grass pollen season is influenced by the weather. The meteorological parameters: Temperature, precipitation, humidity, length of day, or sunshine hours and wind speed and direction have all been studied in relation to grass pollen seasons. In the Northern hemisphere, models used to predict the start and severity of the grass season have included maximum temperature and rainfall in the months preceding the grass pollen season (Davies 1973, Clot 2003). The comparison of grass pollen in the cities of Bologna, Brussels, Munich and Strasbourg produced the first study where aerobiologists in the European Economic Community collaborated to reach a common goal (Bagni et al. 1976).

*Cape Town grass seasons*

Grasses were the most abundant of the pollen taxa at the Rondebosch, Mowbray and Table View sites and were second in abundance only to Cupressaceae at the Observatory site. The seasonal limits and severity of the grass season in Cape Town have been discussed above (see 5.4.1), but the influence of meteorological parameters on air spora has been analysed only once, during a five-week winter period in Cape Town (Hawke and Meadows 1989). A correlation between drought years and low grass pollen counts has been found in studies of grass seasons in Johannesburg (Cadman 1991). Weather affecting Poaceae has never been studied in Cape Town. The influence of selected meteorological parameters on the pollen release patterns of grasses at Observatory and Table View is considered below.

*Grass seasons at the sites*

We observed that there was a temporal pattern to the Cape Town grass seasons associated with spring season temperature, as seen in Northern Hemisphere studies discussed above. The seasonal curves were similar in timing but not in shape. The 1997-2001 grass seasons at the coastal Table View site occurred suddenly and increased rapidly, while the inland Rondebosch and Observatory grass seasons were preceded by a series of low concentrations. This pattern was less obvious in the 2011-2014 grass seasons at Observatory and Table View, than with the earlier Rondebosch and Table View time series, but the grass season at Table View again started abruptly during the later time series.

The shape and timing of the grass curves at the sampling sites in Rondebosch, Table View and Observatory differed. The grass distribution throughout the calendar year at the 3 sites is shown in the splines (see Methods 3.6.4) in Figure 6.11 below.

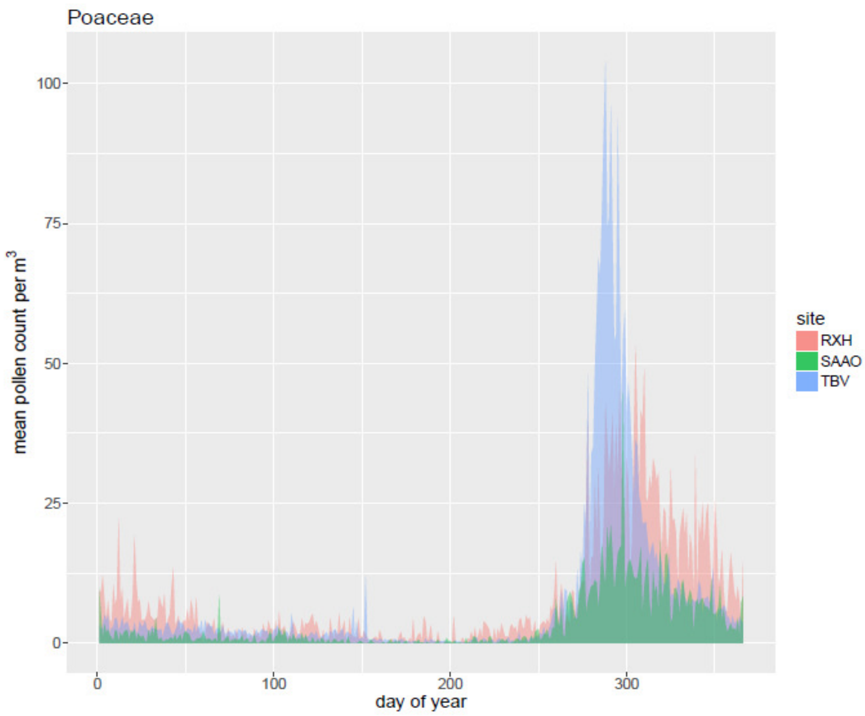



Figure 6.11. Poaceae daily averaged counts at Rondebosch (RXH) for the years 1984-2001 Table View (TBV) 1995-2005 and also 2011-2014 and Observatory (SAAO) 2011-2014

The differences in the start and shape of the grass splines in Figure 6.11 is apparent. The grass season at Observatory began with low but steadily increasing grass counts. The Table View grass season began suddenly and grass concentrations greater than 100 grains/m$^{-1}$ were seen during the first week after the grass concentrations exceeds 10 grains/m$^{-1}$. In Rondebosch the grass season began later than at the other two sites and grasses also peaked later, in November rather than October.

### 6.8 Variability in the grass seasons in Cape Town

In Cape Town the grass seasons at the different sites showed considerable variation between the sites during the same year and within the site, for different years. The variability may be the result of different rainfall patterns or fewer daylight hours and different temperature ranges. This variability in the shape of the seasonal grass curves is shown in a comparison of the 1997-2001 grass seasons at the Rondebosch and Table View sites and then for the 2011-2014 grass seasons at Table View and Observatory. These grass curves are shown in Figures 6.12 a and b below.

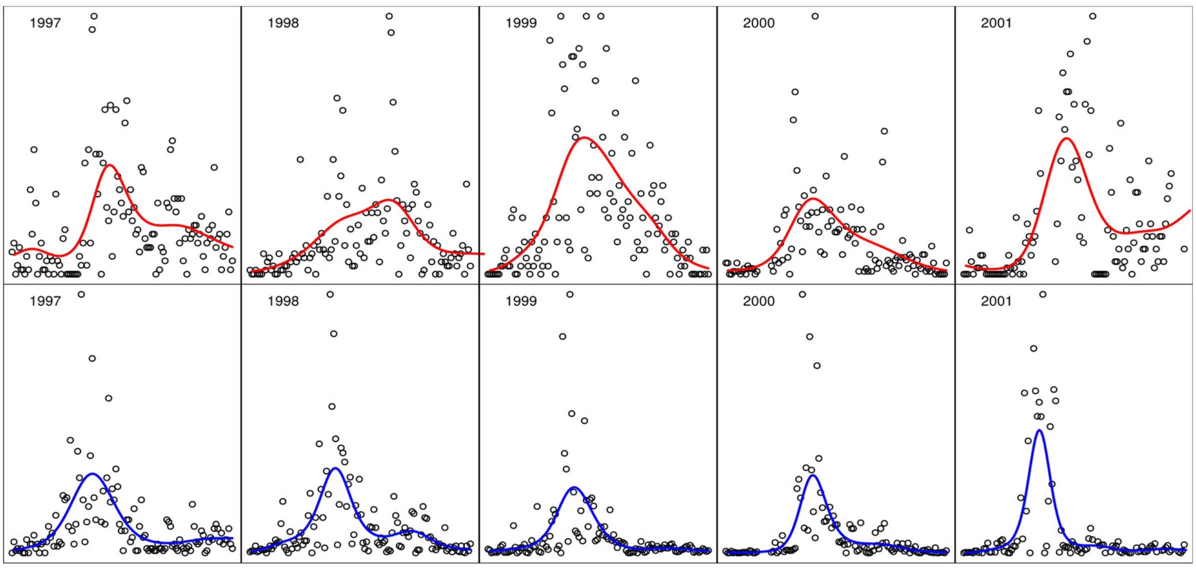



Figure 6.12 a. Cape Town grass pollen distribution 1 September to 31 December 2011-2014 at Rondebosch (red smoothing line) and Table View (blue smoothing line). The smoothed lines are splines fitted in a negative binomial generalized additive model and represent median pollen counts.

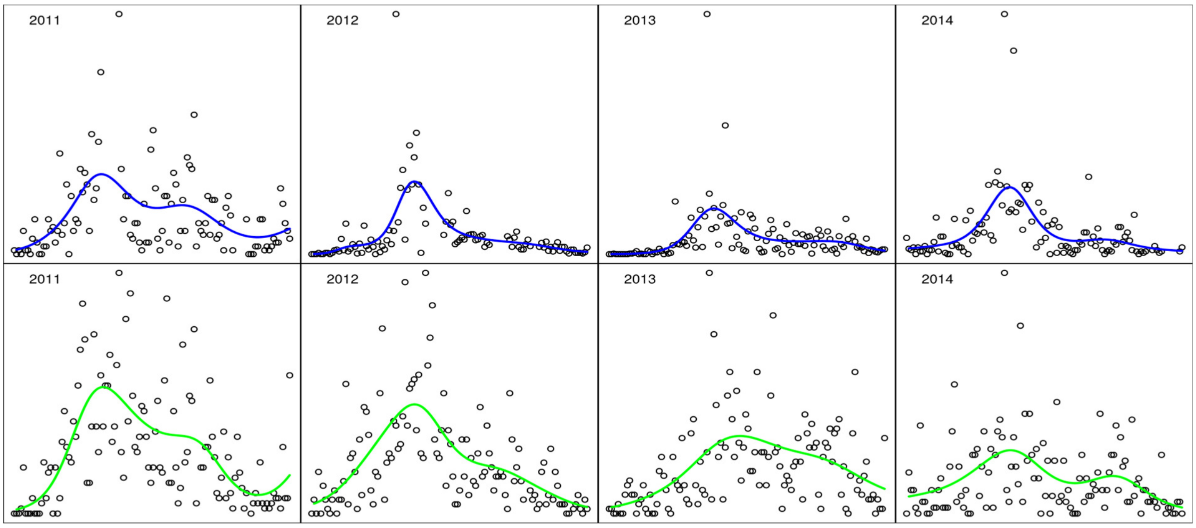



Figure 6.12 b. Cape Town grass pollen distribution 1 September to 31 December 2011-2014 at Table View (blue smoothing line) and Observatory (green smoothing line). The smoothed lines are splines fitted in a negative binomial generalized additive model and represent median pollen counts.

### 6.9 Monthly grass concentrations at the sites

*Coastal Table View grass concentrations*

The Annual Poaceae Index (API) at Table View was greater than the API at Rondebosch the years 1997-2001. The Table View API was greater than the Observatory API in 2012-2014 but not 2011. The total Poaceae concentration in the month of October was consistently higher in Table View than Rondebosch or Observatory throughout the time series. At the coastal site of Table View the highest Poaceae load was recorded in October for every sampling year.

*Inland Rondebosch and Observatory grass concentrations*

In Rondebosch the highest monthly summed concentration for grasses was recorded in November in 1997 and again in 1999. In Observatory, October was the peak grass flowering month each year from 2011-2014.

*Summed grass counts September-December at the sites*

The grass concentrations were compared for the months September-December at Rondebosch (RXH) and Table View (TBV) 1997-2001 and at Observatory and Table View 2011-2014 and are shown in Figure 6.13 below.


Figure 6.13. Summed monthly grass concentrations September-December at Red Cross (RXH) and Table View (TBV) 1997-2001 and at Table View and Observatory (2011-2014)

*Grass pollen and temperature*

Seasonal variations of grass counts are difficult to model when attempting to predict the start and severity of the grass season, because of the complexity of the variables. The variables include weather parameters and vegetation patterns as well as the variation in the flowering times of different grass species, which have different temperature requirements. Grasses were compared with the maximum temperature ($T_{max}$) from 1 July -30 June at Observatory (2010-2014). Here, the upward trend in spring temperatures across this 4-year time series is mirrored by the increasing grass counts during spring. The grass concentrations and maximum morning temperatures are plotted from 1 June-31 July for each of the calendar years. Temperature and grass concentrations are compared in Figure 6.14 below.

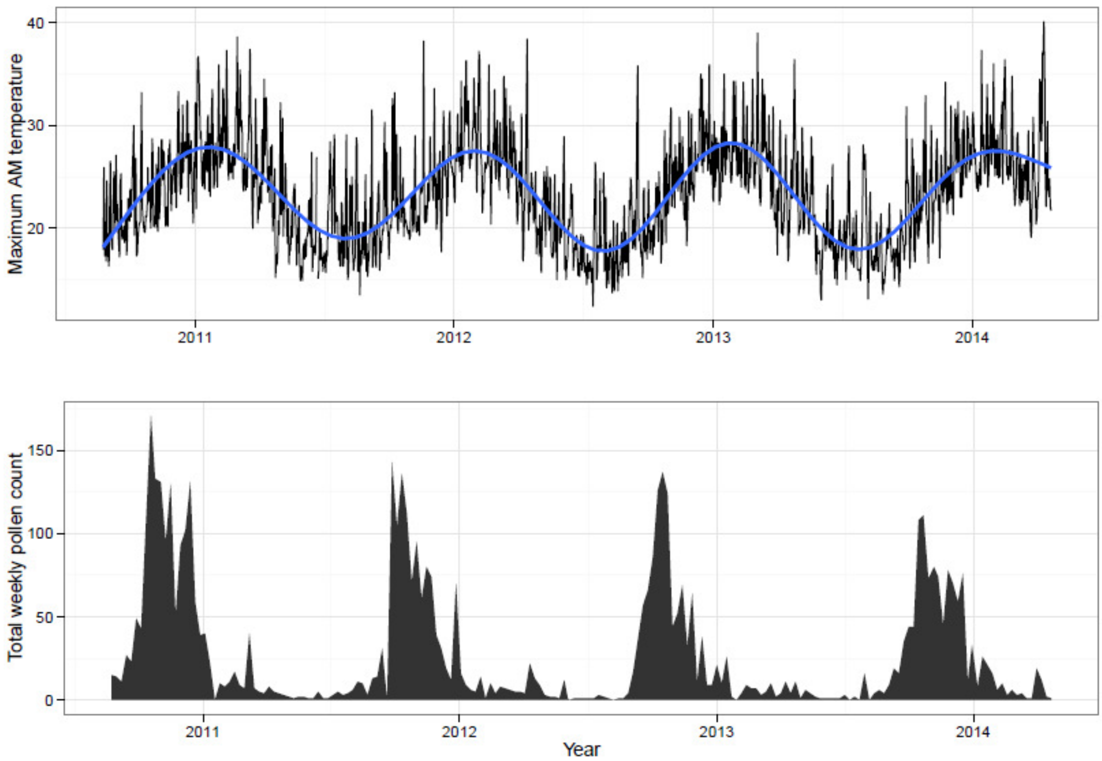



Figure 6.14 Comparison of the maximum morning temperature and total weekly grass pollen in Observatory: 1 July 2011-30 June 2014.

*Seasonal variations of grass seasons*

We observed seasonal variations in the grass concentration curves at all the sites in Figures 6.10a and b. The total grass concentration for September-December varied as did the distribution of the grass concentration. Grass was counted in varying amounts between the sites for the same year and at the same site for different years in the time series. This variation illustrates the point that grass seasons are difficult to model, when attempting to predict the start and severity of the grass season. The independent variables that influence the grass count are multifactorial. In addition, the inter-relationship between the variables and the length of time (lag) effect of variables such as rain, on the flowering of grasses is complex. The relationship with meteorological variables is not clearly understood and may differ in different climate zones and at different latitudes (Beggs 2015). This indicates that different models may need to be applied in different climate zones and this is seen in studies that predict grass season start dates (Leuschner 2000, Clot 2003).

*Grass seasons and temperature*

Grass pollen concentrations increased when the cumulative maximum temperature minus the cumulative minimum temperature measured daily from 1 July, reached a value of 660. This was tested using the 2012 and 2013 grass seasons at the West Coast and inland aerobiomes. Mean monthly temperatures at the two sites were not the same, relative to each other for 2012 and 2013. The mean monthly temperatures in Table View were consistently higher than the monthly average in Observatory in 2012 but in 2013 temperatures at the Observatory were higher than at Table View in June-September and this is shown in Figures 6.15 a and b below.

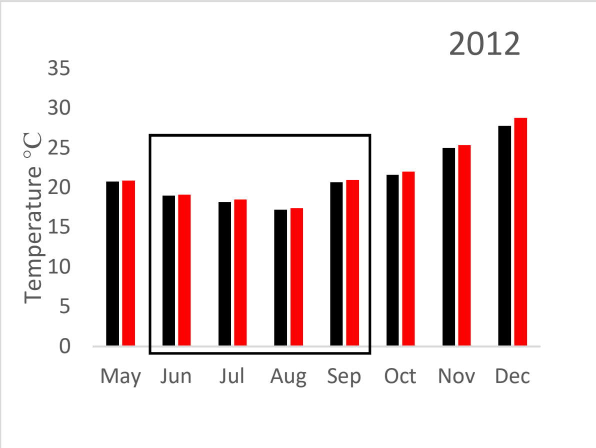



Figure 6.15a Average monthly temperatures at Observatory (black bar) and Table View (red bar) May-December 2012, The bracketed months show preseason temperatures.

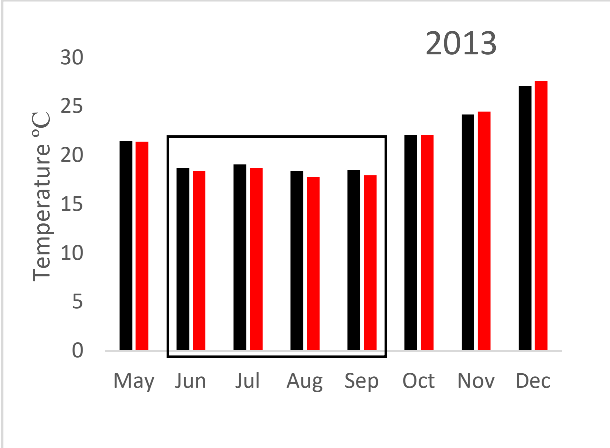



Figure 6.15b Average monthly temperatures at Observatory (black bar) and Table View (red bar) May-December 2013 The bracketed months show preseason temperatures.

**6.10 Mean ambient temperature and the grass season start date**

Clot devised a simple formula to predict the start date of the grass season in Basle and Zurich, Switzerland, using regression analysis. The cumulative maximum daily temperature was measured from 1 March. The start of the grass season was identified as the second rain free day after a total score of 500 was reached, excluding days with daily temperatures of less than 6°C. The forecast using these parameters was successful in two out of three cases (Clot, 1998).

Clot's method, using only the cumulative daily maximum temperature cannot be applied to South African grass forecasting which has different weather variables. There were no, or few days with temperatures below 6°C from the 2011-2014 weather variables at Observatory or Table View, for instance and it must be noted that the Cape Town minimum temperatures are higher than the minimum temperatures in the high altitude Northern Hemisphere cities.

*Adapting Clot's method to predict the start of the Cape Town grass season*

The cumulative maximum temperature minus the cumulative minimum temperature was measured from 1 July, the beginning of the Southern Hemisphere pollen season at Table View and Observatory and the cumulative values reached on the start date for the grass seasons at the sites were compared for 2012 and 2013. Temperature measurements, obtained from the South African Weather Services, for the years 2011 and 2014 were incomplete. This is shown Table 6.5 below.

**Table 6.5 Cumulative mean temperature and grass season start dates at Observatory (SAAO) and Table View (TBV) 2012 and 2013.**

| Site | Year | Start date | Summed $T_{max}$ (1July-Start date) | Summed $T_{min}$ (1 July-Start date) | Summed $T_{max}$ -Summed $T_{min}$ | Rain >10mm prior to start date |
|---|---|---|---|---|---|---|
| SAAO | 2012 | 15.09 | 1395.9 | 724.7 | 671.3 | 13mm (31.08) |
| TBV | 2012 | 21.09 | 1568.7 | 913.4 | 655.3 | 15.6 mm (20.09) |
| SAAO | 2013 | 19.09 | 1486.2 | 824.7 | 661.5 | 14 mm (13.09) |
| TBV | 2013 | 30.09 | 1671.7 | 1030.3 | 641.4 | 29.6 mm (10.09) |

$T_{max}$: Maximum temperature summed from 1 July to observed start date

$T_{min:}$ Minimum daily temperature summed from 1 July to observed start date

### 6.11 Predicted and observed grass season start dates

*Observed start dates*

The observed start dates were calculated by summing the grass concentrations for the year (12-month period) and calculating the start date by the 90% method. The sum of annual grass load or annual Poaceae Index (API) was 100%. The start date was the date reached when 5% of the grass index had been attained (see Methods 3.8.2).

*Temperature value found for grass seasonal start date*

The average difference between the sum of the daily maximum temperature minus the sum of the daily minimum temperature from 1 July to the start date for the 2012-2013 sampling years at Observatory and Table View was 657.3. This number was rounded to 660 and this value was taken as the predicting value for the start of the grass pollen season. When the summed maximum temperature minus the summed minimum temperature equalled 660, the start date for the grass pollen season would be reached, provided a rain day of 10mm or more occurred within two weeks prior to the grass start date. After applying the formula to the data, the predicted dates and observed grass season start dates were compared.

*Formula to predict grass season start dates*

The application of the formula:

X= Cumulative maximum daily temperature-cumulative minimum daily temperature

from 1 July until a value of 660 is reached

= Start date of grass season

was applied to the 2012 and 2013 grass pollen concentrations. Temperatures were obtained from SAWS. The weather mast for Observatory was operating on site and the nearest weather mast for Table View was at The Royal Cape Yacht Club in Cape Town harbour, 15km distant. The results of the comparison of real time and predicted grass season start dates are shown in Table 6.6 below.

**Table 6.6. Observed and predicted grass season start dates at Observatory and Table View 2012 and 2013**

| Site | Year | Observed grass start date | Predicted grass start date | Difference between Observed and predicted dates (days) |
|---|---|---|---|---|
| SAAO | 2012 | 15.09 | 14.09 | 1 |
| TBV | 2013 | 21.09 | 20.09 | 1 |
| SAAO | 2012 | 19.09 | 19.09 | 0 |
| TBV | 2013 | 30.09 | 01.10 | 1 |

### 6.12 Summary

Poaceae or grasses are a major trigger for allergic disease in Cape Town and were ranked first or second at all the sampling sites included in the study. Since 1973 Aerobiologists have attempted to predict the start and severity or peak concentration of the grass season (Davies, 1973). Correlations with meteorological parameters have been sought to identify the weather variables that influence the timing and shape of the grass season. We formulated a simple measuring tool to predict the start of the grass season, based on the method by Clot. This formula needs to be tested on a larger sample but shows some interesting consistencies

Chapter 7

# Discussion, Conclusions and Future Research

## 7.1 Introduction

The main aim of this study was to choose the most appropriate sampling site for the greater Cape Town Metropolitan Area by comparing the pollen and fungal spore taxa from the coastal and inland sampling sites. A pre-2000 matching dataset for the inland and coastal sampling site existed from the large body of sampling data. A prospective pollen sampling programme undertaken for the study created a contemporary post-2000 data set from the inland and coastal pollen sampling sites for comparison. This extended the dataset to 30 years.

The pollen and fungal taxa at the sites have been defined. The aims and objectives pertaining to the main aim set out in Chapter One, have been met. The project adds to the understanding of the aeroallergens in the atmosphere of Cape Town by broadly tracking eight selected pollen taxa and fungi, at all the sites across the 30-year time span, as well as directly, by comparing them at two sites for the same time periods.

Aerobiomes have been created to differentiate between the distinctly different air spora assemblages of the coastal and inland regions and these have been named the Valkenberg Aerobiome and the West Coast Aerobiome. Table View is on the Cape West Coast and Valkenberg is named after the historic complex between the Observatory and Rondebosch sites. The complex is a Cape Heritage site. The influence of meteorological parameters on one pollen taxon and one fungal spore were examined and a formula has been adapted to predict start dates for the grass season. Long term data is compared across decades at two sampling sites, to weigh the possible influence of global warming on decreasing grass pollen concentrations. The key findings and comparisons from the Valkenberg Aerobiome, in the Cape Peninsula, which contains the Rondebosch and Observatory sampling sites and at the West Coast Aerobiome where the Table View sampler is situated, are discussed. The project details the seasonal limitations and concentrations of pollen and fungal aeroallergens in the atmosphere of Cape Town.

A caveat must be added that the demographics of the population attending the academic hospitals has changed since pollen sampling began in Cape Town and large influxes of immigrants and migration of different ethnic groups has resulted in new settlements like Mitchells Plain and Khayaletsha, in the southeast Peninsula where the air spora had not previously been sampled. This has recently been addressed by other UCT researchers who are studying pollutants and air spora in relation to respiratory disease in Bellville South, close to Khayelitsha, Table View, close to Marconi Beam informal settlement and Noordhoek, close to Ocean View informal settlement.

### 7.2 Pollen assemblages in the Inland and West Coast Aerobiomes

The 2011-2014 pollen assemblages are compared at the Observatory and Table View sampling sites as these sites operated concurrently. The harvested pollen taxa reflect the vegetation surrounding the different sampling sites. Table View is in the West Coast Aerobiome on the west coastline of southern Africa. It is outside the Cape Peninsula and north of the inland sampling sites, just south of the West Coast region of the Western Cape. The Rondebosch and Observatory sampling areas are within the Valkenberg Aerobiome, close to the Table Mountain. Range. The vegetation and climate of both these aerobiomes is described in Chapter 4. They have been named 'aerobiome' to describe their unique composition of aeroallergens.

### 7.3 Tree pollen

The short tree season, averaging six weeks for *Platanus* and *Quercus* (August-September) and slightly longer for the Cupressaceae season (July-October) was striking in the Valkenberg Aerobiome, where tree pollen dominated the pollen catch in the spring months of September to November. Pollen concentrations increased sharply in September in the Valkenberg Aerobiome when maximum tree concentrations were recorded. The high concentrations were sustained throughout October as grasses began their flowering season. The September pollen peak was not seen at the West Coast Aerobiome, as tree pollen concentrations were low in that region. In the West Coast Aerobiome total pollen concentrations did not increase until October, when grasses began their annual flowering cycle.

The large contribution of tree pollen in the Valkenberg Aerobiome in September, caused the difference in the seasonal peak between the two sampling sites. This indicates that exposure to tree pollen is likely to be greater for residents who live inland than for the inhabitants of the coastal area. It also has implications for clinical drug trials with anti-histamines and immunotherapy regimes. Therapeutic interventions need to be conducted outside the pollen season. Preseason immunotherapy has been shown to be effective after six years in children (Eng, Reinhold and Gnehm, 2002, Durham et al. 2010) and we have therefore defined the seasonal limitations for specific pollen taxa like Poaceae and selected tree pollen taxa.

Pollen from *Pinus spp* was not differentiated from the indigenous *Podocarpus spp,* common name: Yellowwood tree. This pollen taxon was abundant at all the sampling sites. *Pinus spp* was the most abundant pollen in Mowbray, the second in frequency in Rondebosch and ranked fourth in Observatory. Concentrations were greater than 10% at each of the inland sampling sites. In Table View on the West Coast, *Pinus* ranked third and contributed 4,5% to the TPI. *Pinus* was not analysed in this study, as it was thought to be low in allergenicity by Cape Town clinicians and a more likely trigger for conjunctivitis than hay fever (Marcos et al. 2004). Above the Mowbray sampling site are remnants of the afrotemporate, or forest and fynbos (Galley and Linder 2006)) forest that extends from Newlands forest towards the area above the sampling site. The dominant alien pine is *Pinus pinaster* (Rouget et al. 2001).

The allergenicity of *Pinus spp* is contentious. Some researchers who have compared the relative abundance of pine pollen with sensitization rates recommend that pine pollen should be added to allergen testing panels (Gioulekas et al. 2004, Gastaminza 2009) while other researchers support the case for the low allergenicity of pine pollen (Bousquet et al, 1984, Subiza et al. 1995). Subiza points out that abundant pollen, may often be linked to sensitization but it does not always indicate that the pollen taxon is allergenic, noting the high concentrations of Pinus pollen and a low sensitization rate (4%) in contrast with the low relative abundance of *Plantago*, which had a high sensitization rate (32%). Benzoic acid leaches from pine pollen and it may be that it is this substance rather than pine pollen protein that is responsible for the effect on respiratory function (Fountain, Cornford and Shaw, 1995). Sensitization rates to *Pinus spp* in Cape Town are unknown, as this taxon has not been included in testing panels in allergy clinics.

The dominant allergenic trees in the Valkenberg pollen assemblages are the Northern Hemisphere alien taxa: *Cupressaceae*, *Platanus* and *Quercus*. In Cape Town these trees are favoured for the many parks, gardens and schools in the Valkenberg Aerobiome, situated in the mid -Cape Peninsula and are planted along roadsides in Rondebosch and the surrounding suburbs. Cupressaceae was the most abundant pollen in Observatory and *Platanus* (plane tree) was the dominant tree pollen taxon of the allergenic trees found in Rondebosch. During the sampling years 2010-2014 Cupressaceae averaged 24% of the Total Pollen Index (TPI) in the Valkenberg Aerobiome but only 2.9% of the TPI in the West Coast Aerobiome. Long distance transport of tree pollen has been reported. Taxa include cypress (Van de Water et al. 2003) and birch across borders, from Poland and Germany to Denmark (Skjøth et al. 2007) and within a country, from southern England to north London (Skjøth et al 2009) but this was not seen in our study, possibly due to the unusual topography and wind direction of our sampled areas.

The high inland Cupressaceae concentrations are consistent with findings in the Northern Hemisphere (Nicoleta 2009, Pérez-Badia et al. 2010) and the Southern Hemisphere (Katelaris and Burke 2003, Green et al. 2004) and this is not surprising as the cypress tree is a prolific pollinator. The sensitization rate to Cupressaceae in Europe is variable, at 2.4-9.6% in the general population, but in allergic patients this may increase to 30% (D'Amato et al, 2010).

Cupressaceae sensitization has implications for allergy sufferers in Cape Town, who live and work within the Valkenberg Aerobiome. These residents may present with rhinoconjunctivitis from July-August. D'Amato observes that the sensitization rate to this taxon is likely to increase, as allergologists increasingly identify Cupressaceae pollen allergy in late winter. Symptoms attributed to Cupressaceae in late winter could have been missed, due to the appearance of the allergen in late winter rather than spring (D'Amato et al. 2007).

In Canberra Australia, in the Southern Hemisphere, Medek (2012) correlated patients' symptoms with pollen counts and meteorological factors, using an internet-based hay fever diary. Cupressaceae and grass were the most abundant pollen allergens, as we found in our Cape Town study. Pollen counts that exceeded 20 grains/$m^{-3}$ invoked symptoms in rhinitis patients and symptoms persisted once this value was reached. Various pollen values have been suggested as the minimum amount necessary for the maximum symptoms to be triggered and a threshold of 50 grains/ $m^{-3}$ is still the concentration quoted in many studies (Davies and Smith 1973, Viander and Kovikko 1978, Negrini et al. 1992). 20 grains/$m^{-3}$ as a general threshold is similar to the Burge Scale (see Methods 3.5.1) that we used in the Cape Town study.

Cupressaceae was identified as an important late winter trigger for conjunctivitis and hay fever by Ordman in Johannesburg, South Africa and confirmed by skin prick tests. (Ordman 1970). Cadman reported that Cupressaceae comprised 24% of the catch in her two-year study in Kirstenbosch, Cape Town (Cadman and Dames 1996). Several Cupressaceae species exist in Cape Town but *Cupressus* pollen was not identified to species level from our Cape Town aerobiological studies.

There are several cypress tree species in Cape Town but testing with one species of Cupressaceae should not affect the sensitization rate as the different species share epitopes and there are high levels of cross-reactivity between the species. A close relationship has been shown between the Taxoideaceae (Cupressaceae) and Podocarpaceae (di Felice et al. 2001). This is relevant to Cape Town as Podocarpus (yellowwood tree) and Cupressaceae (cypress) trees co-exist in the region. *Cupressus* has not routinely been part of allergen testing panels in Cape Town. The high concentrations reported in this study indicate that this tree allergen could trigger seasonal symptoms and should be included in aeroallergen testing panels for patients with late winter to early spring symptoms. Diaz de la Guardia found that 30% of atopic patients in

Granada, Spain were sensitized to Cupressaceae and sensitization was highest in the age group: 21-40 (Diaz de la Guardia et al. 2006).

*Quercus* concentrations were less than 1% of the TPI in the West Coast Aerobiome. A seasonal *Quercus* peak was found in Observatory in August to September, but this was not seen in nearby Rondebosch. This is surprising as the suburb of Rondebosch is planted with numerous oak trees. Oteros used concentric rings around pollen sources, in this case oak trees, to map the correlation between emission and deposition. *Quercus* (oak) pollen was found at lower concentrations at sampling sites close to the emission source than at distances from 2 to 14 km from the flowering *Quercus* tree. (Oteros et al. 2017). This may explain why low concentrations of oak pollen were found in Rondebosch, but a strong seasonal peak (August-September) was seen in Observatory, 2.5 km distant. In Observatory *Quercus* concentrations were 4,7% of the TPI. *Quercus* was 29% of the pollen catch at the Kirstenbosch Botanical Gardens (Cadman 1996) but there are numerous oak trees at the site and in the adjacent suburb of Newlands, so this finding is not surprising.

*Betulus*, or birch pollen is not found in high concentrations in Cape Town but pollen is found each spring. The silver birch is increasingly used as an ornamental tree and silver birch glades have been planted in Cape Town. Corden found comparable concentrations of Betula in suburbs where the tree was largely ornamental, in a study comparing Betula pollen loads in suburbs of England and Ireland (Corden et al. 2000). There are few *Betula* trees in the Valkenberg Aerobiome but *Betula pendula*, or silver birch has become a popular garden tree and *Betul*a pollen was recorded in our study. It is advantageous that *Quercus* cross reacts with *Betula* and testing with *Quercus* antigen should cover *Betula* (Weber 2007).

*Platanus* was 1% of the TPI at the Coastal Aerobiome but 10% of the TPI in Rondebosch. 22% of Olympic athletes were found to be sensitized to *Platanus* when tested in Cape Town (Hawarden et al. 2002) and when skin prick test frequencies were compared, *Platanus* sensitization was ranked first of the tree allergens. In the West Coast Aerobiome, *Platanus* was less than 1% of the TPI during 2010-2014. In the Valkenberg Aerobiome, *Platanus* was 5.6% of the TPI. Considering that the plane tree pollen release period is relatively short, a sensitization frequency of 22% is remarkable and suggests that plane tree pollen sensitization occurs within a short time period.

The Observatory sampling site is a mere 2.5 km from the Rondebosch site, but it is closer to Table Mountain and the Afromontane forest and it is the only sampling site where Ulmaceae pollen exceeded 1% of the TPI. *Ulmus*, of the elm family formed 1% of the annual catch at Observatory in the Valkenberg Aerobiome. This family includes the indigenous white stinkwood tree, *Celtis Africana*, as well as introduced species, and the flowering times of the various species range from August to February (Palgrave 1977: p96-98). *Celtis spp* was 2.3% of the catch from a sampling site near Kirstenbosch in Cape Town (Cadman and Dames 1996). The sensitization rate to *Celtis africana* has not been studied in South Africa, but according to Cadman (1996) the tree releases large quantities of pollen.

Myrtaceae, or eucalyptus pollen, contributed less than 5% to the annual pollen catch in the West Coast Aerobiome, but measured 12% in the Valkenberg Aerobiome. The eucalypts are alien trees introduced from Australia. This tree pollen has not been included in allergen testing panels in Cape Town but the sensitization rate to Myrtaceae in a group of South African Olympic athletes was 4%. This was the smallest number of responders to skin prick tests in the group of trees tested (Hawarden et al. et al. 2002).

*Acacia* concentrations comprised less than 1% in the Coastal Zone and 1,4 % in the Valkenberg Aerobiome (Rondebosch). This is of interest as patients often consider The Port Jackson willow, or *Acacia longifolia* to be the trigger for their seasonal symptoms, because of its obvious bright yellow pollen, when it begins its pollen release cycle. The pollen is heavy and sticky and is seldom found on Cape Town sampling strips, but *Acacia* sensitization rates have not been tested in Cape Town.

Athletes who are sensitized to trees, especially cypress, plane and oak should avoid training in the inland areas during the main tree flowering season, from September to November.
The tree pollen comparisons may be found in Table 5b.

## 7.4 Poaceae pollen in the Valkenberg and West Coast Aerobiomes

Grasses ranked highest of the pollen aeroallergens when sensitization frequencies were examined in the Western Cape. Grass was second only to house dust mite in the overall aeroallergen rankings (Motala unpublished audit of allergy clinic patients). The grass season duration is long, from September to March, but high concentrations occur from late September until November. Except for *Cynodon dactylon* or Bermuda grass, there are high levels of cross-reactivity amongst the grasses but their allergenic content varies, according to species and according to weather conditions (Buters 2015).

The grass season started earlier at Observatory, in the Valkenberg Aerobiome than at the West Coast Aerobiome (CI 95%) and grass counts increased slowly. In Table View in the West Coast Aerobiome, the season started abruptly and sudden, high grass concentrations were recorded in September. In the earlier sampling years: 1997-2001, grass concentrations were higher in the West Coast Aerobiome, where annual loads were placed into the category of ‘severe grass season’ when the annual grass index was more than 5,000 grains. A severe grass season occurred in 2001 in the West Coast Aerobiome.

Myszkowska (2014) found a strong correlation between preseason temperature increments and the start of the Poaceae season and this has been confirmed in other studies (Matyasovszky et al. 2011) but temperature increases alone cannot explain the later start to the grass season in the West Coast Aerobiome, where temperature differences in the two areas were inconsistent year on year. Different grass species, with later flowering times, could explain the later seasonal start for grasses in Table View (West Coast) when compared with Observatory(Valkenberg), which was a consistent finding in the time series, but temperature clearly plays an important role and is closely linked to daylight hours (Hyde 1952, Smart, Tuddenham and Knox 1979, Stach 2008).

According to Norris-Hill (1997) the highest grass concentrations occur when the temperature range is 20.1°C-25°C and temperatures above 25°C are associated with low grass pollen concentrations. This may partly explain why grass concentrations decrease rapidly in the West Coast Aerobiome, where the monthly average for the maximum temperature exceeds 25°C in

December, ahead of the Valkenberg Aerobiome. Grass concentrations increase earlier inland than at the coast, possibly due to urban heat islands, which artificially increase temperature (Khwarahm, 2014).

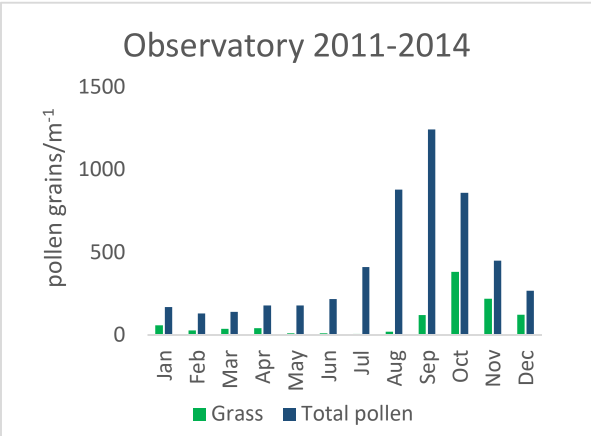



Figure 7.1a Averaged Grass and total pollen at Observatory 2011-2014

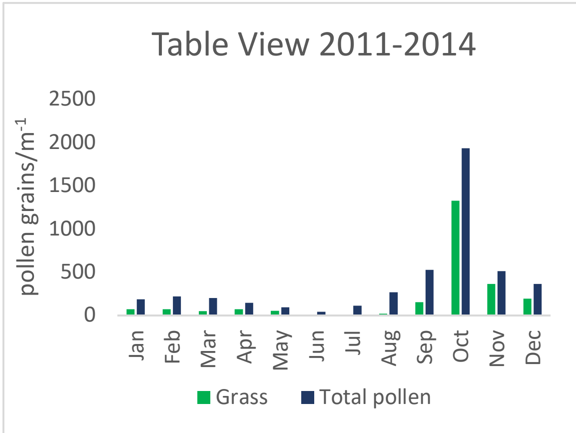



Figure 7.1b Averaged Grass and total Pollen at Table View 2011-2014

The variation in the pollen distribution patterns at the sampling site, Observatory in the Valkenberg Aerobiome and Table View, in the West Coast Aerobiome are shown in Figures 7.2 and b above. During August and September, the large contribution of tree pollen may be seen at the inland site, but there are no comparable tree pollen concentration spikes at the coastal site. The contribution of grass to the total pollen harvest for the months October-December was far greater at the West Coast site of Table View, where grasses were more than 50% of the pollen catch and were close to 80% of the catch in October and November.

### 7.5 Temporal and spatial variations in the Poaceae season

Grass seasons were compared for between-site and within-site variations. Grass seasons with more than one peak occurred in the Valkenberg and West Coast sampling sites, indicating that different grass species flower in response to different meteorological parameters, especially sunshine hours and daylight length (Fjellheim et al, 2014). Some aeropalynology studies in Northern Hemisphere countries showed an earlier start date for the Poaceae season when grass seasons were compared over decades, from long term series (Myskowska 2011) or where there was a pronounced variation in the start date (Emberlin et al. 1994, Jato et al. 2009). The grass seasons in the two Cape Town Aerobiomes, situated at 34°S agree with Medek's finding that grass seasons are longer below the latitude 37°S, with a tendency to more than one peak and lower Annual Grass Indices (Medek et al. 2015).

Poaceae, Typhaceae and Juncaeae are found in greater abundance in the West Coast Aerobiome. The West Coast sampling site is within 3 km of a body of water named Rietvlei (reed lake), where bulrushes are found in abundance. Restionaceae concentrations are highest in Observatory, possibly from the Black River, which is within 1 km of that site and has an abundance of reeds. The proportion of the total pollen for the groups Poaceae, Juncaceae, Restionaceae and Typhaceae may be found in Table 5.1.

### 7.6 Poaceae and land use

The annual grass concentrations declined at Table View, in the West Coast Aerobiome by 60% between 1997 and 2015. This large drop in grass pollen could be attributed to land-use. Rapid expansion has taken place in this area of the Cape Metropole and six new suburbs, shopping malls, hospitals and a bus transport system, now occupy land that was covered by grasses and shrubs in 1994, when sampling began in the West Coast Aerobiome.

The decline in the Annual Pollen Index in Rondebosch in the Valkenberg Aerobiome between 1984 and 2005 was 80%. Buildings have been erected close to the Rondebosch sampling site. Red Cross Children's Hospital, where the spore trap was positioned. The hospital has large new building extensions and parking areas and on surrounding land, commercial buildings have replaced open grassland. A municipal composting site has been removed from a site directly opposite the hospital. Urban expansion and densification cannot account for the even greater decrease in the Annual Poaceae Index in the Valkenberg Aerobiome. Rondebosch Common, an area of 40 hectares of common land and home to a variety of grasses and indigenous weeds (see 4.4) in front of the hospital is unchanged, as is the grassed emergency helicopter landing site at the hospital. The surrounding suburbs are older, established suburbs, where less densification would have occurred. Overall, there appears to have been less building in the area surrounding the Rondebosch site than at the sampling site at Table View in the West Coast Aerobiome over the study years.

The overall decrease in grasses at the long-term sampling sites is consistent with findings in regions with a Mediterranean climate (Ariano et al. 2010), where warmer temperatures in summer may have prematurely ended the season. A recent trend has shown decreasing grass concentrations at lower latitudes, contrasting with increased ragweed seasonal length associated with warming (Ziska et al. 2011). Global warming clearly affects different taxa in dissimilar ways as was shown when precipitation and temperature were correlated with Poaceae, *Populus* and Ambrosia (Makra et al, 2012). No significant difference in the duration of the grass season between the West Coast and Valkenberg Aerobiomes was seen in Cape Town.

The average start dates of the 2011-2014 grass seasons are 12 days earlier than the start dates for the 1997-2001 grass seasons in the West Coast and Valkenberg Aerobiomes. There was no statistical evidence that the start dates of the season had changed when compared to the 1997-2014 sampling years in Cape Town. This is different from the reports from some Northern hemisphere studies, where the start of the grass season has advanced and earlier start dates for grasses have been reported (Emberlin et al. 2000, Mesa et al. 2003). Zhang (2015) points out that the advancing start dates are very likely due to the greater increases in weather parameters, such as temperature and rain, at Northern Hemisphere latitudes and this point has long been noted in other studies (Frei 1998, Beggs 2006).

Beggs warns that the impact of Climate Change on aeroallergens such as ragweed and grass may be severe as changes in temperature and rainfall and increased $CO_2$ concentrations are not yet fully appreciated. These changes may increase aeroallergen concentrations thereby impacting negatively on allergic disease in affected communities (Beggs 2004). Furthermore, he postulates that the increased asthma prevalence within cities may be associated with elevated pollen, like ragweed and grass (Beggs 2006). He discusses the change in urban-rural differences in childhood asthma prevalence reported in the Western and Eastern Cape regions of South Africa (Weinberg, 2000). Beggs argues that the narrowing of the urban-rural gap in childhood asthma prevalence could be the result of increased $CO_2$ concentration and temperature, which is associated with increased aeroallergen concentrations.

The south-western Cape has experienced strong warming trends particularly during the summer months when grasses flower. In South Africa, the western half of the country has experienced the greatest increases in absolute maximum temperatures. The mean annual temperature in the south-western Cape has increased only slightly, by 0.018° C per decade, to a total increase of 0.05° C across the 30-year sampling span. However, it is important to note that the number of days with low minimum temperatures has decreased and days with high minimum temperatures has increased. The annual absolute minimum temperature showed a strong positive trend in Cape Town. (Kruger & Nxumalo, 2017). In Perugia, Italy, the mean annual temperature declined by 0.05 over 33 years, resulting in an earlier start date for the grass season and decreasing annual grass concentrations (Ghitarrini 2017).

Poaceae are treated as one taxon, because the grains of the many grass species are indistinguishable by light microscopy. However, the many and varied species of the temperate grasses have different growth requirements, so variations in temperature, precipitation and day length will have different effects on the different species 'The study of the seasonal timing of biological phenomena is known as phenology' and each grass species will adapt its growth and flowering time to suit climatic conditions. (Fjellheim, Boden and Trevaskis 2014). The grass season contains many grass species, each responding to its own ranges of temperature, day length, humidity, precipitation and wind speed and direction.

When all the factors that contribute to decreasing grass concentrations are weighed, increased land-use would undoubtedly have contributed to the decline in grass concentrations in the West Coast Aerobiome. However, the effects of global warming on grasses in the Valkenberg Aerobiome, where there has been less land use, cannot be excluded. Decreasing grass pollen counts have been shown in drought years and the effects of Climate Change with its concomitant higher temperatures are likely to be similar (Cadman 1991). Dry seasons have been experienced in Northern Hemisphere countries when pollen levels have declined (Gehrig 2006). The effects of higher temperatures have been shown on *Artemisia spp* in Spain (Cariňanos et al. 2013) when flowering seasons ended earlier, and it is likely that increased land use and Global Warming have together brought about the decrease in grass pollen concentrations in Cape Town.

### 7.7 Weed/ herbaceous shrub pollen

The Rondebosch sampling site has a large tract of open land, the Rondebosch Common, on one boundary and this species-rich area is reflected in the ranked pollen of the *Cyperus*, sedge grass, *Erica,* or heather and the large family of Asteraceae which includes the indigenous daisies, such as the rain daisy: *Dimorphotheca pluvialis*. These pollen taxa were trapped in small concentrations. Ericacaceae is the only ranked taxon that contains indigenous species only, as opposed to a mixture of alien and indigenous species.

Fynbos pollen is rarely anemophilous, so it is not surprising that it is found in low concentrations in pollen assemblages of anemophilous pollen. Cadman (1996) reported an incidence of 19.29% for fynbos pollen of the total pollen catch in a 2-year study. Considering that the sampling site for the study was in the Kirstenbosch National Botanic Gardens, home to a large collection of fynbos plants and situated on the slopes of Table Mountain, where fynbos plants grow in their natural habitat, this is not a surprising finding. It was not possible to quantify the percentage of fynbos pollen in our Total Pollen Indices as many of the taxa, i.e. Asteraceae, Oleaceae, and Ulmaceae contain species that are both indigenous and species that are alien.

Pollen concentrations below the threshold value of 10 grains/$m^{-1}$ of the three distinguishing taxa in the fynbos biome, Ericaceae, Restionaceae and Proteaceae were recorded in both aerobiomes, confirming the findings of earlier studies that showed low concentrations of fynbos pollen from Cape Town (Cadman 1996, Berman 2013). Other fynbos species were Olea and *Myrica*. *Olea* pollen was trapped in low concentrations in the Valkenberg and West Coast Aerobiomes and this taxon included the indigenous *Olea europaea* subspecies *Africana.*

At the Table View sampling site, ranked pollen taxa included Erica and *Myrica. Myrica* has been identified from a palynological analysis of Rietvlei, an area close to Table View and has been recovered from sediments dating to 36 500-33 000 BCE (Schalke 1973). Schalke observed that *Myrica cordifolia* was present in the dune vegetation of the study area. *Myrica spp* identified from pollen sampling studies in the West Coast Aerobiome is probably *Myrica cordifolia*, that has grown in the area for millennia. Myricaceae or sweet gale, a shrub or small tree was 4.9% of the TP at Table View and less than 2% at the inland sites, but *Myrica* concentrations were consistently less than 20 grains/m$^{-3}$ and seldom exceeded the threshold value of 10 grains/m$^{-3}$. Cyperaceae (sedge grass) exceeded 1% of the Total Pollen Index at Rondebosch in the Inland Aerobiome and at Table View in the West Coast Aerobiome. *Cyperus* concentrations that exceeded 30 grains were recorded in Rondebosch in the Valkenberg Aerobiome, from 1997-2001 during August but no equivalent elevated counts were seen in the West Coast Aerobiome. *Cyperus rotunda* was a major contributor to the pollen calendar in Saudi Arabia, when pollen calendars were constructed for comparison with patients' seasonal symptoms (Hasnain 2005).

The weed pollen category was smaller than the tree and grass categories in both the aerobiomes and at all the sites with a single exception. *Parietaria* at the inland site of Observatory, reached high concentrations during the late autumn-early winter months of May-June in 2012, when a maximum concentration day of 208 grains/m$^{-1}$ was counted, but the weed was seen in concentrations below the threshold concentration during the years 2011 and 2013-14. The erratic appearance of *Parietaria* may be due to rain patterns as it releases pollen in May, when the rainy season in Cape Town begins.

In the Northern hemisphere, the allergenicity of *Parietaria* has been well studied and is established (D'Amato et al. 1998). Negrini et al (1992) correlated symptom scores for rhinoconjuncticitis with pollen counts and found that patients experienced mild symptoms when *Parietaria* concentrations exceeded 10-15 grains/m$^{-3}$. Severe symptoms occurred when the concentrations reached 80 grains/m$^{-3}$. Concentrations greater than 80 grains/m$^{-3}$ have been recorded at the Observatory sampling site. *Parietaria* is likely to be a trigger for rhinoconjunctivitis in the Valkenberg Aerobiome. Concentrations above the threshold limit have been reported in sampling years beyond the years of the study, but sensitization rates to

Parietaria have not been studied in atopic subjects in the Cape Town area. The weed pollen taxa comparisons may be found in Table 5.1c.

Many of the weed pollen taxa are not anemophilous. It is testimony to the high wind speeds at the Rondebosch sampling site, that pollen from plants that are not wind pollinated such as Asteraceae, Ericaceae, Bruniaceae, *Oenothera* and *Oxalis* for example, should have been trapped by a spore trap set 20 metres above the ground.

**7.8 Fungal Spores**

The fungal spore loads from the sites at Rondebosch, Mowbray, Observatory in the Valkenberg Aerobiome and Table View in the West Coast Biome were compared as described above and the distribution of airborne fungal genera at the sites was defined. Ascospores were the most abundant spores at all the sites. When we ranked the fungi that exceeded 1% of the fungal spore load at all the sampling sites, basidiospores ranked second at the Valkenberg and West Coast sites for the years 1997-2001 but dropped in ranking and quantity at the West Coast site for the years 2010-2014. Basidiospores were found in low concentrations in Observatory for the same time span.

The Mowbray sampling site in the Valkenberg Aerobiome was not sampled for a sufficient length of time to be used in the comparison of sampling sites in the study, but it was the only site where *Curvularia* showed a strong seasonal peak in the autumn months of February and March, 2008-9. *Curvularia* was 12.1 % of the monthly fungal index. At all other sampling sites in the Valkenberg Aerobiome *Curvularia* was less than 2% of the fungal index for all months.

Hasnain investigated the allergenicity of basidiospores in the Auckland area by preparing skin prick test extracts from various basidiomycetes and found positive responses in 22% of allergic patients (Hasnain 1985). A study of 701 patients in the US and Western Europe using questionnaires and skin prick test extracts from eight basidiomycetes found a significant relationship between atopy and asthma and also between asthma and rhinitis and concluded that the basidiomycetes give rise to important aeroallergens (Lehrer et al. 1986, Lehrer et al. 1994). Rivera-Mariani et al showed in vitro reactivity in 29 out of 33 allergic subjects to basidiospores.

(Rivera-Mariani 2011) and basidiospores were significantly correlated with asthma and also with asthma reductions on holidays or weekends, in schoolchildren in a study of fungi in classrooms (Chen et al.2014). Considering that basidiospores were amongst the most prevalent spores from our study Cape Town allergists could consider adding basidiomycetes extracts to their skin prick testing panels.

### 7.8.1 Alternaria

*Alternaria* was rarely absent from the ambient air in daily pollen counts and a strong seasonal peak was shown in the early spring-early summer months of October- December in the Valkenberg and West Coast Aerobiomes, but the peak dates were variable at the sites. In countries at lower latitudes of Europe with a Mediterranean climate like Spain, a double *Alternaria* peak has been reported (Skjøth 2016) which is not seen in northern temperate countries with well-defined temperature ranges. Cape Town has a Mediterranean climate which would account for the occasional mid-winter peaks and, on occasion a double peak for *Alternaria* in the West Coast Aerobiome.

*Alternaria* is a dry air spore and it is sensitive to small temperature increments, so it begins to disappear from the air when temperatures exceed 25ºC. It may even appear after a succession of warm, dry winter days (Hatzipapas et al. 2002). A smaller *Alternaria* spike was seen in the autumn months of March-May at the West Coast site of Table View, but not at the Valkenberg sites and an unseasonal spike was seen at Table View in July. Geographic area as well as temperature should be considered when assessing factors that influence high concentrations as associations between high *Alternaria* concentrations and crop harvesting, were shown in European cities at different latitudes, the premise being that *Alternaria* that had colonised crops, and it might be carried on wind currents and collected by the spore sampler (Skjøth 2012).

Cape Town *Alternaria* proportions were similar at the Valkenberg and West Coast sites with a range of 5.5-7,7% of the Annual Fungal Index (AFI). Studies in central and southern Europe have found *Alternaria* relative abundance to be less than 10% of the Seasonal Fungal Index (SFI) (Sabariego et al. 2000, Kasprzyk, Rzepowska & Wasylów, 2004). The relative abundance of *Alternaria* at Table View in the West Coast Aerobiome increased by 40% from 1997 to 2014.

The increase could be due to changed agricultural practices from farm areas, altered vegetation or warmer temperatures.

Positive skin prick tests on 209 children in the Greater Cape Metropolitan area, with known allergic disease showed 18,6% positive responses to *Alternaria*, the highest number of responders to a fungal spore (Potter et al. 1991). In Universitas Hospital in Bloemfontein, in the Free State in central South Africa, 100 patients with allergic rhinitis confirmed by skin prick testing had the following response: *Alternaria* (25%). Sensitization rates showed *Alternaria* to be the first ranked fungal allergen and fourth in frequency, after Bermuda grass, maize and rye grass from a panel of 12 skin prick test allergens (Joubert 2006). In a multi-centre study, centres with a high prevalence of *Alternaria* sensitisation included centres in Australia and the US but substantial geographic variation in sensitization rates was shown across the group (Bousquet et al. 2007).

### 7.8.2 *Cladosporium*

*Cladosporium* concentrations were unexpectedly low in Cape Town from sampling studies in the Valkenberg and West Coast aerobiomes, when compared with studies in many temperate climates in the Northern and Southern Hemispheres (Sadyś 2016, Oliveira et al. 2010, Recio et al.2012). Similarly, low *Cladosporium* concentrations were seen in an aerobiological survey at Kirstenbosch (Cadman and Dames 1996) but were 42% of the AFI in a study in Durban on the east coast of South Africa. It was interesting to note that *Cladosporium* rarely exceeded the 3,000 spores/m in that coastal city. *Cladosporium* concentrations peaked during the winter months in Durban, a sub-tropical summer rainfall area, when temperatures were lowest. (Dames and Cadman 1994).

Although *Cladosporium* appeared in the atmosphere for most days of the year, no clear seasonal pattern could be seen at any of the Cape Town sampling sites. Cape Town *Cladosporium* concentrations did not often exceed the suggested 3,000 spores/$m^{-3}$ threshold (Bagni et al, 1977) nor even the more recent suggested threshold of 2,800 spores/$m^{-3}$. This second suggested threshold was found when patients with allergic rhinitis recorded their symptom on score cards and the severity of their symptoms was correlated with the atmospheric *Cladosporium* concentration. Symptoms of allergic rhinitis were positively correlated with *Cladosporium* concentrations of 2,800 spores/m$^{-3}$ (Rapiejko et al. 2007).

We formulated a table, with specific ranges of temperature, rainfall, humidity and wind speed and direction, assembled from studies of *Cladosporium* concentrations and their associations with meteorological parameters (Davies 1969, Katial, Jones and Dyer 1997, Hjelmroos 1993, Infante et al. 1999). *Cladosporium* peaks were reported in spring and autumn but in hot countries, peaks appeared in winter (Davies 1969, Dames and Cadman 1994). This indicated that the temperature range is critical, like the temperature requirements for *Alternaria* and that *Cladosporium* might be sensitive to temperatures greater than 28°C, when it might disappear from the air.

A 3-year study of the release of *Cladosporium* conidia in forest environments detailed the complex mechanisms of the release of the *Cladosporium* spores from their substrate, which was usually decaying vegetation and the meteorological parameters associated with *Cladosporium* concentrations (Kurkela, 1997). In a 5-week study of air spora and meteorological parameters, Meadows and Hawke observed that wind speeds greater than 2m s$^{-1}$ negatively affected the trapping of spores (Meadows and Hawke, 1989).

We examined the meteorological parameters on days when Cape Town *Cladosporium* concentrations were greater than 3,000 spores/m$^{-3}$. One high concentration of 15,584 spores/m$^{-3}$ in the coastal aerobiome on 01 April 2011 was selected and the temperature range, relative humidity, wind speed and direction and rainfall prior to this date were found as set out in our table. Rainfall is crucial for elevated *Cladosporium* concentrations. *Cladosporium* is a 'dry air' spore and it is hygroscopic, but as some moisture is required for sporulation, precipitation prior to the release of *Cladosporium* spores is essential (Lacey 1981). Kurkela disagrees with this theory and suggests that rain is a necessary mechanism for spore release by the splashing of raindrops, rather than to provide moisture for sporulation (Kurkela 1997).

Table 6.1 listed the meteorological parameters and their specific ranges. These were all met on the day of the elevated *Cladosporium* concentration. We compared *Cladosporium* concentrations at the sites that were greater than 1,000 spores $m^{-3}$ with the meteorological parameters and their ranges in our table and found that the weather conditions listed in Table 6.1 were usually met. We chose 1,000 spores $m^{-3}$ because our *Cladosporium* concentrations were lower than the 3,000 spores/$m^{-3}$ threshold. On 12 of the selected days, the full conditions were met. In the other five cases, rainfall or wind conditions were outside the suggested parameters and in most cases the parameter that did not fit was wind speed. The low *Cladosporium* concentrations are likely to be the result of meteorological parameters and lack of moisture. It has been seen that the concentrations of this spore increase rapidly during bouts of rare summer rain (personal observation). Cape Town has dry summers and the combination of heat and strong winds possibly inhibits the sporulation of *Cladosporium*.

Further study is needed to explore this theory of *Cladosporium* deposition in Cape Town. The West Coast Aerobiome is closer than the Valkenberg Aerobiome to wheat growing farmlands in the *Swartland* area, which are north-east of the sampling site and it is probable that *Cladosporium* conidia are transported to the West Coast spore trap from the farming area. The *Cladosporium* concentration on 1 April 2011 was 1,421 spores $m^{-3}$ at Observatory in the Valkenberg Aerobiome and 15,584 spores $m^{-3}$ at Table View, in the West Coast Aerobiome.

There have been few studies that have examined skin prick test responses to aeroallergens in Cape Town clinics. 8.6% of the 292 allergic children tested in a study of children with known allergic disease from Red Cross Children's Hospital Allergy Clinic had positive responses to *Cladosporium* (Potter et al. 1991). 14% of a cohort of 802 allergic children who were outpatients at the same hospital allergy clinic had positive responses to *Cladosporium* (Motala unpublished audit 2002). The ranking in the audit of aeroallergen skin prick test sensitization may be seen in Figure 7.2 below.

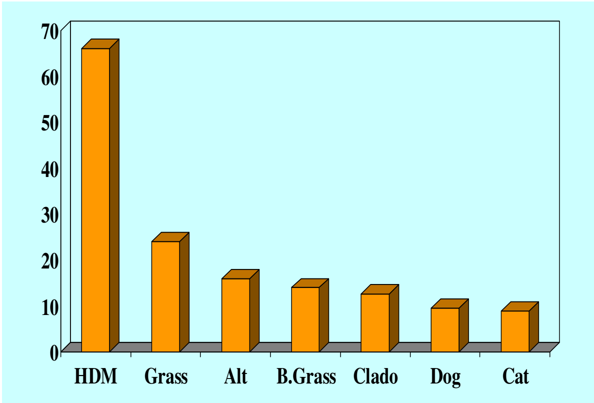



Figure 7.2 Audit of skin prick test sensitisation in children attending the Red Cross Children's Hospital Allergy Clinic: Motala 2002

*Cladosporium* was ranked second of the fungal spores in both studies which raises the question of exposure. Low *Cladosporium* concentrations were seen at both coastal and inland sites and at different sampling heights. The inland spore traps were set at different heights but the threshold level of 3,000 $^{-3}$ spore/m$^{-3}$ was exceeded on 10 days of the sampling period at the spore trap mounted at 2 m in the Table View, West Coast site and on 7 days at the Observatory sampling site in the Valkenberg Aerobiome, where the height of the spore trap was 5 metres so that sampling height did not appear to significantly affect the *Cladosporium* concentrations.

A study in Sardinia, Italy found that the correlation between *Cladosporium* concentrations and skin prick test frequencies was poor, despite the high concentrations found in that region. This contrasted with the lower concentrations of *Alternaria* and higher sensitisation rate (Cosentino, Faddo and Palmas 1995).

Despite the low *Cladosporium* concentrations in Cape Town, sensitization is on a par with sensitization in temperate countries like England where *Cladosporium* concentrations exceed the threshold as many as 47 times in one year (Sadyś, 2017). Rapiejko observes that symptoms are manifested in patients sensitised to *Cladosporium* when the threshold of 2,800 is exceeded. (Rapiejko et al. 2007) and yet sensitization to *Cladosporium* is occurring in Cape Town. Sensitization occurs despite far lower concentrations measured at three different sampling heights (2-20 metres) over 30 years in our study as well as in previous Cape Town studies (Hawke and Meadows 1989, Cadman 1996).

Katabatic winds sweep the lower reaches of the mountain in the inland sampling areas. The coastal area of Table View in the West Coast Aerobiome experiences on-shore and off-shore wind. This directly affects the impaction of the airborne bioaerosols in the Valkenberg and West Coast regions. These different wind transport systems that operate in the two areas, are delimited by topographical features, like the mountain range and the coastline.

In a study that examined *Cladosporium* concentrations greater than or equal to 6,000 spore/m$^{-3}$ a weak correlation was found between high *Cladosporium* concentrations and local wind. The highest concentrations were found on days with wind speeds less than 2m s (Sadyś, 2017).
It is possible that fewer days with wind speeds less than 2 m s occur in Cape Town and that it is a trapping problem, rather than a low incidence of *Cladosporium* that has resulted in the low concentrations (see Table 6.1).

### 7.8.3 *Epicoccum*

*Epicoccum nigrum* is one of the few fungal spores that can be identified to species level from air sampling (Burge and Rogers 2000). The conidia are verrucate or warty, and multi-celled with dark brown pigment and have an attachment scar. (Ellis 2007) They resemble small tortoise shells. *Epicoccum nigrum* is now known as *Epicoccum purparescens* (Bischt et al. 2004).

*Epicoccum* concentrations in Cape Town did not exceed the threshold concentration of 60 spores/ m$^{-3}$ (Burge and Rogers 2000) at the Valkenberg or West Coast Aerobiomes for the years 2011-2014 nor was the threshold exceeded for the 1997-2001-time series at either aerobiome. During the years 1985-1989, counts above the threshold were seen, attributable to different land use at that site. In these earlier years of the Rondebosch time series, *Epicoccum* concentrations peaked in autumn, during the autumn month of March (see Figure 5.7).

An unpublished study conducted at Red Cross Children's Hospital found a heavy growth of *Epicoccum* on potato dextrose agar settle plates that were exposed near kelp seaweed in coastal areas. It was assumed that *Epicoccum* colonises kelp. This may be so, but the Table View sampler is sited at the coast and it is within 2 kilometres of kelp beds and no high concentrations were seen for *Epicoccum* from that sampling area.

In a study conducted at the Red Cross Children's Allergy Clinic sensitization to *Epicoccum pupurascens* was 15% (Potter et al. 1991). The relative abundance of *Epicoccum* in Cape Town was found to be 1.2% in the Valkenberg Aerobiome and 0,8% in the West Coast Aerobiome of the Annual Fungal Index. These frequencies are comparable with the relative abundancy of 0.5% in the Southern Hemisphere city of Santiago, Chile (Henríquez, Villegas and Nolla, 2001) and 0,9% in Madrid, Spain (Herrero et al. 2006). Rizzi-Longo found lower concentrations for *Epicoccum* in Trieste, Italy than for *Alternaria* with a high correlation between the two fungi, likely due to similar responses to meteorological conditions (Rizzi-longo, Pizzulin-Sauli & Ganis 2009).

### 7.8.4 *Pleospora*

*Pleospora,* of the order Pleosporales, is a large group of Ascomycetes, containing 469 genera (Oliveira et al. 2010). We chose this fungus for detailed analysis as there was a consistent June-August seasonal peak in *Pleospora* concentrations at the West Coast Aerobiome. For the time series 2011 -2014 the *Pleospora* annual sum in the coastal aerobiome was 16,678. This was five times the annual *Pleospora* sum in the Valkenberg Aerobiome, which was 3,337. For the years 1997-2001, the *Pleospora* annual load was 11,661 in the West Coast Aerobiome and 2,474 in the Valkenberg Aerobiome. The coastal *Pleospora* annual sum was 4,7 times that of the inland annual sum.

*Pleospora* has been shown to be positively associated with rainfall (Oliveira 2010) We showed this in our study when peak concentrations for this fungus appeared during the months with the greatest rainfall. Rainfall figures for the years 2011 and 2014 were excluded as they were incomplete. Rainfall figures for 2012-3 are shown in Table 7.1 below.

**Table 7.1 Precipitation June-August at the inland and coastal sampling sites**

| Area | 2012 Rain *(mm)* | 2013 Rain *(mm)* |
|---|---|---|
| Valkenberg Aerobiome | 272.2 | 330 |
| West Coast Aerobiome | 242.2 | 272.8 |

Rainfall figures by permission of South African Weather Services

Awad observes that an important source of fungi is from crop harvesting, when crop pathogens become airborne (Awad, 2005). It is probable that *Pleospora* spores originate from crops grown in the farming areas north of the sampling site and that these fungi become airborne during harvesting activity, and are transported to the West Coast Aerobiome, where they colonize vegetation.

In a review to examine the association between outdoor fungi and the exacerbation of asthma in children, Tham observed that testing for sensitization is usually confined to *Alternaria, Aspergillus, Cladosporium* and *Epicoccum* (Tham et al. 2014). Allergenicity to *Pleospora* has not been extensively studied and the significance of this fungus as an aeroallergen is not yet fully understood but *Pleospora* and its anamorphs may induce allergic disease (Oliveira et al. 2010).

### 7.9 Summary

Comparisons of the pollen taxa and fungi at the sampling sites show wide variation, in relative abundance and species of the pollen taxa. Variation was also found in the distribution of the fungi in the Valkenberg and West Coast Aerobiomes. There were fewer differences in the seasonal limitations of the selected air spora when these were compared at the sites. The allergenic tree and weed pollen taxa are similar to the allergenic taxa found in the countries with Mediterranean climates like the Western Cape (Heinzerling et al. 2005, Pereira et al.2006) where Cupressaceae, *Platanus*, *Quercus* and *Parietaria* are major aeroallergens. Some similarities were seen when the Cape Town pollen and fungal spore spectra were compared with Southern Hemisphere countries situated at the same latitude, with similar seasons (Katelaris et al. 2004, Haberle et al. 2014). There have been few sensitization studies from allergy clinics in Cape Town that compare the air spora found from pollen sampling with the sensitization rates of atopic patients and this situation should be redressed.

### 7.10 Main Findings

1. Tree pollen was dominant in the Valkenberg Aerobiome, where it formed a strong peak in September. No corresponding tree peak in September was found in the West Coast Aerobiome.

2. Cupressaceae was the dominant pollen taxon of the three known allergenic trees analysed at the Valkenberg Aerobiome, followed by *Platanus* and *Quercus.*

3. Grass pollen was the dominant pollen taxon in the Coastal Aerobiome.

4. A formula to predict the start of the grass season using maximum and minimum cumulative temperature was adapted from a method designed by Clot.

5. There was a significant difference in the start of the grass seasons (0-20 days) between the Valkenberg and West Coast sampling sites (95% Confidence Interval).

6. There was a 60% decline in the Annual Grass Index in the West Coast Aerobiome between 1997 and 2014 possibly due to increased land use.

7. The decline in Poaceae in the Valkenberg Aerobiome was 80%. Declining Poaceae counts coincided with increasing temperatures at the Valkenberg Aerobiome and may be linked to Global Warming.

8. *Parietaria* pollen reached high concentrations, of up to 208 grains/$m^{-3}$ /day in the Observatory sampling site in late autumn (May-June).

9. *Myrica spp* exceeded 1% of the TPI in the West Coast Aerobiome but the allergenic status of this weed taxon has not yet been determined.

10. The pollen assemblages at the two sites have been defined and are different in composition. *Fynbos* pollen was a small component of the total pollen catch at both sites.

11. *Alternaria* was present at all the sites throughout the year, peaking in early summer and autumn.

12. *Cladosporium* concentrations were unexpectedly low for a city with a temperate climate This phenomenon was explored and associations with weather parameters were shown.

13. *Epicoccum* concentrations were insignificant at all the sites for all the years although this fungal spore maintained a constant, low presence for more than 300 days/annum in the air at the Valkenberg and West Coast sites.

14. *Pleospora* concentrations increased sharply in July-August in the West Coast Aerobiome.

**7.11 Conclusions**

Exposure to pollen and fungal spores is markedly different in the Inland and West Coast Aerobiomes in Cape Town. One coastal and one inland sampling site should be operated to obtain a pollen count for the city, where exposure to the major pollen and fungal spore aeroallergens varies in composition, abundance and seasonal limits. The major tree pollen concentrations form a strong September peak in the Valkenberg Aerobiome but not at the West Coast. *Parietaria* concentrations exceed the threshold inland but this has not been seen in the West Coast Aerobiome, Fungal spore peaks are greater and more frequent in the West Coast Aerobiome and high concentrations have been seen in this coastal area for *Pleospora* but they are absent from the Valkenberg Aerobiome.

*Limitations of the thesis*

- There were gaps in the data sets of the air spora and the meteorological data which made modelling difficult.
- The heights of the spore samplers were not uniform.
  It was not possible to accurately measure the effect of increased land use for urban housing on vegetation patterns but the Table View population increased from 38 543 to 78 528 (2001-2015) which indicates substantial loss of waste land, where many grasses grow'.

- The Table View site used wind soundings from the oil refinery 3km distant but other meteorological parameters were taken from The Royal Cape Yacht Club 15km distant.
- The effects of Climate Change on Poaceae could not be adequately assessed because of insurmountable gaps in the Rondebosch data between 1980 and 1989.

*Strengths of the thesis*

- The comparison of data collected specifically for the study identified the need for more than one sampling site in Cape Town.
- Long term data collected over 30 years, although incomplete and discontinuous has been analysed.
- New potential aeroallergens have been identified. These are the fungal spore, *Pleospora* in the Coastal Aerobiome and the weed, *Parietaria* at the Inland Aerobiome.
- The seasonal limits and relative abundance of eight major pollen and fungal spores were defined at Rondebosch, Table View and Observatory.
- Biplots were used to illustrate monthly variations in the relative abundance of eight selected air spora, at the inland and coastal sites.
- A relationship was demonstrated between a table of meteorological ranges and increased *Cladosporium* concentrations at the inland and West Coast aerobiomes.
- The Observatory site had a weather mast erected on site which was an ideal situation when air spora were examined in relationship to meteorological parameters at that site.
- The same methodology equipment was used and the same aerobiologist measured the air spora from 1984- to 2014.
- A possible predictive model for grasses has been developed: this requires testing prospectively.

## 7.12 Future Research

*Aerobiology projects*

1. Pollen monitoring should continue at both the Valkenberg and West Coast sites.

2. Pollen sampling should be conducted in the Southern Cape Peninsula region of the Greater Metropolitan Area, which has high urban density, due to expansion and population migration, to establish the possibility of extrapolating the pollen and fungal spore data from either the Valkenberg or West Coast Aerobiomes to this South Peninsula region.

3 Air quality monitoring should include pollen and fungal spore monitoring in Cape Town, especially in areas where pollutant levels, like sulphur dioxide and particulate matter are elevated.

4. The reliability of the formula to predict the start of the grass season should be tested ahead of the season in future pollen sampling.

5. The Table (6.1) I designed using meteorological parameter ranges to predict *Cladosporium* concentrations greater than 3,000 spores/m should be applied to future data to test the reliability of this set of criteria.

*Clinical studies*

5. Prevalence patterns of pollen and fungal spores should be correlated with patients' sensitization frequencies in Cape Town clinics, where allergic disease is managed.

6. Sensitization studies should be undertaken to a broader range of aeroallergens identified from this study such as Cupressaceae, *Parietaria*, *Pleospora*, *Myric*a, *Pinus* and Asteraceae to assess the significance of these atmospheric allergens, in atopic patients in the Cape Town area.

*Palynological research*

7. Fossil pollen assemblages from core samples in Cape Town could be compared with the modern pollen taxa found in Table View as in the comparison of modern and fossil pollen in the Drakensberg in the province of KwaZulu Natal (Hill 1996). This study would investigate possible changes over time between modern and fossil pollen at the same site, using existing fossil pollen assemblages from the study by Schalke in 1973.

## 7.13 Recommendations

The findings of the most prevalent pollen allergens raise questions regarding the appropriate selection of skin testing panels for patient diagnosis. *Cupressus*, *Parietaria* and basidiospores could be considered for inclusion in further patient testing. Skin prick test frequencies from Allergy Clinics in Cape Town academic hospitals should be audited and compared with pollen sampling frequencies.

Pollen sampling should be carried out in other major South African cities. Air spora calendars would guide allergologists and clinicians regarding the peaks in the aeroallergens in their city for diagnosis, immunotherapy regimes and clinical trials. These calendars would use pollen sampling sampling data wherever possible to produce year-round guides for aeroallergens like the example for Cape Town is shown in 7.3 below

### 7.14 Cape Town Air Spora Calendar

Calendars have been designed and circulated that show the flowering times of allergenic plants in the Cape Metropolitan Area but there is little information on the main sporulation seasons for allergenic fungal spores. Germany has a Pollen Information Service (PID) and pollen forecasts are linked to weather services (Melgar et al. 2012). The design and circulation of pollen calendars that show the seasonal parameters of the peak pollen release weeks, instead of the flowering times of allergenic plants have long been advised for Europe (D'Amato 1995). The seasonal aspects of the known allergenic fungi could be added to such a calendar for the benefit of allergists as allergy to fungal spores may be clinically significant (D'Amato et al. 1995). A calendar was compiled from the results of this study that show the months of peak pollen and fungal concentrations for the major aeroallergens we have identified in the Cape Town area and this is shown in Table 7.2 below.

**Table 7.2 Pollen seasons and fungal spore peaks in Cape Town**

| Aerobiome | Jan | Feb | Mar | April | May | June | July | August | September | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Valkenberg | Grass | Grass | Grass Alter naria | Cladosporium | Parietaria | Parietaria Alternaria | Cypress | Cypress Oak Plane | Grass Cypress Oak Plane | Grass Plane Alternaria | Grass Alternaria | Grass |
| West Coastal | Grass | Grass | Grass | Alternaria Cladosporium Alternaria | | Pleospora | Cypress Pleospora Alternaria | Cypress Pleospora | Grass Cypress | Grass Cypress Plane Alternaria | Grass Alternaria | Grass |

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# Appendix A: Mountants

**Carlberla's Solution for mounting slides for air spora identification**

25ml 40 per cent alcohol

5ml glycerine

2 or 3 drops saturated aqueous solution of basic fuchsin

Ogden, E. C., & Raynor, G. S. (1967). A new sampler for airborne pollen: the rotoslide. *Journal of Allergy*, *40*(1), 1-11.

**Glycerine Jelly for mounting slides for air spora identification**

20 g gelatin
70 ml water
60 ml glycerin (also known as glycerol)
2.4 g phenol (phenol is toxic and should be handled carefully using gloves)
Boil water. Measure 70 ml and add to gelatin. Boil again and mix. Add glycerin and phenol and mix. Add a small amount of stain (see section v. below) and mix again.

Sampler, B.P.V.A., Aeroallergen Monitoring Standard for The Asia Pacific Region.

## Appendix B: Spreadsheets

Total Summary Sheet: Total per category: tree/grass /weed

Weekly Classification: Total weekly concentration for each pollen/spore

Sheet 2D: Raw scores

Sheet 3D: Coverted to concentration/$m^3$

South African Weather Services Meteorological Data

Chevron wind soundings

SAAO spreadsheets

Table View spreadsheets

Red Cross Hospital spreadsheets

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