THE POLLEN AND SPORES OF METROPOLITAN CAPE TOWN AND THEIR RELATIONSHIP WITH METEOROLOGICAL CONDITIONS

IN COMPLETION OF THE DEGREE M.SC.

BY : PHILIP HAWKE APRIL 1989

### DEPARTMENT OF ENVIRONMENTAL AND GEOGRAPHICAL SCIENCE UNIVERSITY OF CAPE TOWN

The University of Cape Town has been given the right to reproduce this thesis in whole or In part. Copyright is held by the author. The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

#### ABSTRACT

Aeropalynology remains an under-researched discipline in South Africa. Cape Town has a wide variety of flora, a complex local climate and a history of allergy related complaints, thus justifying an aeropalynological survey. A literature review indicates that Poaceae is the dominant atmospheric pollen at most research locations, worldwide, while <u>Cladosporium</u>, in general, is the dominant atmospheric fungal spore. Fungal spores are, quantitatively, the dominant partner the in atmosphere. Meteorological factors such as wind speed and wind direction. precipitation, temperature, relative humidity, atmospheric atmospheric stability have been identified pressure and as affecting air spora concentrations and an attempt is made  $\mathbf{to}$ explain the relationships involved. A thorough review of particle behaviour and current sampling methods indicates that the Burkard volumetric sampler was best suited for airspora sampling in Cape Town. Results of the research confirm that Poaceae is the dominant pollen, but basidiospores are the dominant spores in the atmosphere. Spores, numerically, outnumber pollen 4:1 in the distinct atmosphere. Atmospheric pollen shows seasona] fluctuations, while seasonal fluctuations for spores are less distinct. In this investigation, the affect of meteorological variables (Wind speed and wind direction, temperature, relative humidity, preciptation, atmspheric pressure and atmospheric airspora concentrations, varies according stabilty) on to different airspora. Stepwize regression analysis (performed bγ the BMDP R2 statistcal package) provided models for predicting airspora concentrations. However, r and R<sup>e</sup> values are low be suggesting that an alternative for model should found building. The use of synoptic charts as an alternative to computer modelling, is explored.

#### ACKNOWLEDGEMENTS

Scherag (Pty) Ltd. generouosly provided the grant that made this research possible. Dr Mike Meadows is thanked for his advice, creative ideas and moral support during the research period. Dr Peter Moll of the Economics Department, U.C.T, provided useful advice on the use of the BMDP statistical package. Jean Sugden is warmly thanked for her patience in explaining the technicalities of laboratory work, and helping in identifing troublesome pollen. Andy Vinnicome kindly constructed the wind roses. Greg Sneddon is thanked for the use of the spore phototgraphs. The D.F. Malan meteorological office personnel are also to be thanked for compiling and supplying the meteorological data so willingly during this period. The amalgamated Beveraging & Canning Company, in particular the director, Mr Peters, are thanked for supporting the research and generously fitting and supplying the electrical for the Burkard volumetric sampler. The Cape Town lead Municipality willingly allowed us to use their Burkard volumetric sampler at Bothasig. To Debbie, my immense gratitude for the patience and good cheer you displayed, long after it was reasonable to do so!

## CONTENTS

CHAPTER 1 : INTRODUCTION	1
CHAPTER 2 : THEORETICAL FOUNDATION	9
CHAPTER 3 : REVIEW OF MATERIALS AND METHODS USED IN AEROPALYNOLOGY	54
CHAPTER 4 : RESEARCH METHODOLOGY	94
CHAPTER 5 : RESULTS	108
CHAPTER 6 : DISCUSSION	148
CHAPTER 7 : CONCLUSION	175
REFERENCES	181
AFFENDICES	•
APPENDIX 1 : LABORATORY AND COUNTING METHODS	203
APPENDIX 2 : FLATES SHOWING POLLEN AND SPORES	214
APPENDIX 3 : SYNOPTIC CHARTS	220

1

FAGE

### LIST OF FIGURES

### FIGURES

١

		1
1.	Day and night pollen profile	19
2.	Vertical distribution of uredospores	19
з.	Pollen release of <u>A. excelsa</u> and <u>A. spinosus</u> $^{\diamond}$	38
4.	Mean bi-hourly conidia total for <u>Pyrenophora teres</u>	44
5.	Vertical profiles of pollen and spores.	51
6.	Forces acting on particles during passive fallout	57
7.	Deposition of airspora on a culture plate	62
8.	Hyde's gravity sampler	64
9.	Hyde's aeroscope	64
10.	Theoretical impaction efficiencies for particle	
	encountering a culture plate	66
11.	Behaviour of particles at the intake of a suction device	69
12.	Andersen sieve impinger	74
13.	Hirst spore trap	77
14.	Map showing Epping and Bothasig sampling site	97
15.	Distribution of particles along the length of a slide	
	used in the Burkard volumetric sampler	102
16.	Epping and Bothasig airspora content	115
17.	Epping and Bothasig pollen spectrum	116
18.	Epping and Bothasig spore spectrum	117
19.	Epping monthly pollen counts	119
20.	Epping monthly spore counts	119
21.	Bothasig monthly pollen counts	120
22.	Bothasig monthly spore counts	120
23.	Epping monthly weed counts	121
24.	Epping monthly Fynbos counts	121
25.	Bothasig monthly weed counts	122
26.	Bothasig monthly Fynbos counts	122
27a.	Epping daily pollen concentrations versus atmospheric	
	variables (July 87-October 87)	138

PAGE

. .

27Ь.	Epping daily pollen concentrations versus atmospheric	
	variables (November 87-Febuary 88)	139
27c.	Epping daily pollen concentrations versus atmospheric	
	variables (March 88-June 88)	140
2 <b>8a.</b>	Epping daily spore concentrations versus atmospheric	
	variables (July 87-October 87)	141
2 <b>86.</b>	Epping daily spore concentrations versus atmospheric	
	variables (November 87-Febuary 88)	142
28c.	Epping daily spore concentrations versus atmospheric	
	variables (March 87-June 88)	143
29.	Wind rose showing wind direction during sampling period	145
30.	Wind rose showing pollen peaks and corresponding wind	
	direction	145
31.	Wind rose showing spore peaks and corresponding wind	*
	direction	146

III

### LIST OF TABLES

### TABLES

1.	The most common airborne pollen families	14
2.	The most common genera of airborne fungi	14
з.	Difference in mean spore concentrations over	
	distance	84
4.	Epping pollen spectrum in decending order of	
	significance	111
5.	Bothasig pollen spectrum in decending order of	
	significance	112
6.	Epping spore spectrum in decending order of	
	significance	113
7.	Bothasig spore spectrum in decending order of	
	significance	114
8.	Correlation coefficients for pollen and spores	
	versus meteorological variables (Introductory	
	investigation)	126
9.	Multiple regressional equations for pollen and	
	spores (Introductory investigation).	127
10.	Correlation coefficients for selected pollen and	
	spores versus meteorological variables	130
11.	R <sup>2</sup> and F Ratio values for forward and backward	
	stepwize regressional analysis	134
12.	Regression equations based on the results of the	
	stepwize regression analysis	135
13.	Wind direction during the sampling period	144

PAGE

-

## LIST OF PLATES

### PLATES

1.	The Burkard system (Epping)	88
2.	The Burkard system (Bothasig)	88
з.	The Burkard sampler	8%
4.	Sampling drum	90
5.	Sampling drum installed on timing device	90
6.	The timer	91
7.	Timer : Winding mechanism	91
8.	Vacuum pump (battery operated)	92
9.	Vacuum pump (mains operated)	<b>9</b> 2
10.	Components of box on Epping sampler stand	93
11.	The Epping sampling site showing distorting vegetation	107
12.	Tape removed from drum	210
13.	Tape placed on calibrated ruler	210
14.	Melinex tape divided into daily sections	210
15.	Vaseline dissolved off tapes	211
16.	Zeiss swing out head centrifuge	211
17.	Acetolysis process	212
18.	Hydrofluoric acid and plastic tubes	212
19.	Mettler scale.	213
20.	Nikon microscope	213
21.	Poaceae pollen grain	215
22.	Cyperaceae pollen grain	215
23.	Ericaceae pollen grain	216
24.	Fabaceae pollen grain	216
25.	Asteraceae pollen grain	217
26.	Proteaceae pollen grain	217
27.	<u>Alternaria</u> spore	218
28.	Pithomyces spore	218
2 <b>9.</b>	<u>Epiccocum</u> spore	219
30.	Cladosporium and Aspergillus spores	219

PAGE

V

.

1

## 1. INTRODUCTION

·

## 1. INTRODUCTION

ţ

1.1 Project rationale

4

1.2 Project aims

(

1.3 Project objectives

#### 1. INTRODUCTION

### 1.1 Project rationale

defined by Hyde (1969: Aeoropalynology as 579) is "...concerned with the composition of airspora and its variations with time and place both at or near ground level and up aloft".(1) During the course of this century aeropalynology has received considerable attention from scientists, particularly since the 2nd world war. (Hyde, 1969). The reason for this increase can be summarised in three steps. Firstly, there was a growing realisation in the scientific coummumity that many diseases were transmitted through the atmosphere and it was thus necessary to identify the organisms that were resident in the atmosphere. Secondly, as a the ongoing research into airspora and result of disease, many scientists believed that it was necessary to study meteorological factors such as wind direction temperature in conjunction with airspora and concentrations, in order to derive some understanding of the weather conditions that are associated with high airspora concentrations in the atmosphere. Thirdly, having made the connection between airspora concentrations and meteorological variables scientists then `attempted to build predictive models to help predict those days where particularly high concentrations of pathogenic airspora would probably occur. These steps in part explain the growing interest in the subject of aeropalynology, the bottom line of which is the improvement in the quality of life of humans. It should be noted that the steps outlined above indicate a trend rather than a fixed progression in research. What follows is a brief resume of the above steps and an attempt is made to demonstrate the nature of the research that has been conducted in each of the steps.

1

Hyde uses the word "airspora" to include both spores and pollen. For the purposes of this project "airspora" will refer to both spores and pollen in the atmosphere.

disease transmission of Firstly, the Ьу airspora dispersed in the atmosphere has obvious implications for humans. their domesticated animals and plants. This serves initially as an incentive for researchers to record the type of airspora present in the atmosphere. Nilsson & Palmberg-Gotthard (1982), for example, have established the pollen calendar for Huddinge (Sweden). Pateria & Sahu (1982) have analysed the atmospheric fungal spore population of Sagar (India). Secondly. microbiolgists and plant pathologists identify the fungal spores, study their life cycles and identify those atmospheric variables which tend to influence their distribution and concentration. Hilderbrand & Sutton (1982) studied the effect of temperature and reltive humidity on the fungus, Peronospora destructor, which severely diseases onion crops in Bradford Ontario. Fitt et al. (1985) studied the manner in which wind and rain affected the dispersal of Botrytis fabae conidia. This fungus profoundly influences the development of field beans. McHardy & Gadoury (1986) were able to demonstrate that the concentration and distribution of Venturia inaequalis, scab, is heavily the apple influenced by maximum temperature and the time of day that precipitation occurs. With respect to so-called "hayfever", Anderson (1985) drew up a pollen and spore callender for Anchorage, Alaska. Anderson found that the fungal spore, Basidiomycetes and the pollen grain, Betula papyrifera, were the most dominant spore and pollen grain respectively in the atmosphere in the 1982 and 1983 season. Further, it was considered that the threshold value for this birch which, incidentally is a well known aeroallergen, approximates 50 grains m<sup>-3</sup> of air. Anderson also argued that it is the previous season's temperatures and total rainfall which are instrumental in creating the conditions for high pollen concentrations the following year. These four examples demonstrate the fact that, although the identification of the fungal spore and pollen grain is central to the

theme of aerobiology, an understanding of meterological principles is also required.

Thirdly, the end result of much of the work in aerobiology (2) is the creation of predictive models for the most part, based that are. on multiple regressional analysis which tests the relationship between airspora concentrations and selected atmospheric variables. These models are helpful, for in example. predicting days when allergy sufferers can expect high concentrations of ambient pollen in the atmosphere. Spieksma (1980) has shown that considerable success has been achieved with hayfever forecasting in the Netherlands. Forecasts were correct 72%, 85%, and 88% of on 150 occasions analysed in 1977, the time 1978 and 1979 respectively. Savary (1986) studied the spore, Puccinia arachidis. This fungus is responsible for rust Ivory coast. on groundnuts on the Using multiple regression analysis he was able to show that wind relative humidity were the most important velocity and variables to consider when predicting high concentrations of this fungus. Thus the equation indicates that relatively dry and calm weather (hioh spore concentrations were negatively correlated with relative humidity and wind velocity) will produce high concentrations of this fungus in the atmosphere.

The importance of the above studies is found in the fact that they underiably provide humans with the knowledge to improve the quality of their existence on this planet. A knowledge of the origin and distribution of atmospheric air spores helps scientists to control plant diseases that are fungus related. This ultimately must provide for better crop production. From the point of view of "hayfever", a knowledge of the airspora that are responsible for allergies in humans and a knowledge of the type of weather conditions that are responsible for

Г. С. Г

<sup>2</sup> Aeropalynology is a subset of "aerobiology". "Aerobiology" refers to the study of all microorganisms in the atmosphere and in the context of this project the two words are used inter-changeably.

the atmospheric distribution of airspora, must be of help to medical personnel who deal with patients suffering from allergies. However, having stated the relevance of aerobiology to the human condition in general, it is now necessary to look at the South African situation and Cape Town in particular.

Aeropalynology is a discipline which is not much in evidence in the Republic. The earliest known study was that of Davidson (1941). Since that date Ordman has been the work responsible for most of completed in aerobiology. (Ordman, 1947,1955,1961,1963; Ordman 8 Etter, 1956) Most of Drdman's work was related to allergies, and a number of important facts emerge. Firstly South Africa is no exception with regard to seasonal respiratory allergy when compared to the rest of the world. (Ordman, 1955) Secondly, Ordmán (1956) suggested that the fungi play an important role in Thirdly, Ordman (1955)believed that allergies. respiratory allergies were a dominant feature of South and that the climate of African Eastern coastal areas the east coast appravated the situation. Fourthly, Ordman (1955) reported that in Cape Town respiratory allergies are a common feature. However, despite the aeropalynology remains work of Ordman, an under researched area in this country and very little effort has been made to identify those meteorological variables responsible for high airspora concentrations. For this reason and particularly in the case of Cape Town which according to Ordman(1955) has a history of respiratory allergy amoungst its population, it was felt that а study should be undertaken in order to establish the nature of the airspora spectrum in Cape Town, Besides this, Cape Town has a wide variety of flora (both garden exotics and the plant kingdom, "fynbos") and experiences seasonal extremes in weather patterns, for example a dry summer and wet winter, summer SE winds and winter NW winds. These factors alone would justify a study of aeropalynology in Cape Town.

#### 1.2 Project Aims

Bearing in mind the foregoing discussion, the aims of this dissertaion are fourfold.

- a) To produce a pollen and spore calendar for greater metropolitan Cape Town based on data from two sampling stations.
- b) To quantify the annual variation in airspora based on the data from the two sampling stations.
- c) To establish which meteorological factors are responsible for high airspora concentrations in the atmosphere.
- d) To produce a predictive model which will aid in the prediction of airspora concentrations in the atmosphere

In order to achieve these aims the following objectives were established.

#### 1.3 Project objectives

 a) A review of available literature covering the early history of aerobiology and the period after the 2nd World War until the present. In this section an attempt will be made to establish :

i) The global nature of the airspora spectrum in a quantitative sense.

ii) The relationship between airspora and meteorological variables and the manner in which pollen and spores respond to different atmospheric stimuli.

In i) and ii) above, an attempt will be made to isolate the differences between pollen and spores.

 b) A thorough review of the techniques and methods used in aeropalynology, emphasizing the advantages and disadvantges of the various techniques and methods adopted by investigators globally and justifying the choice of the collector used in this project - the Burkard sampler.

- c) An explanation of the research methodology used in this dissertation covering such aspects as the choice of sampling sites, laboratory techniques and the use of the statistical test, forward and backward, multiple regression analysis.
- d) A presentation of the results of the research in this study emphasizing:

 i) The quantitative aspects of metropolitan Cape
 Town's airspora spectrum, based on data from two sampling sites.

ii) The annual variation amoungst different types of pollen and spores

iii) The relationship between atmospheric airspora concentrations and meteorological variables.

iv) The creation of predictive models based on the relationships developed from iii) above.

e) A discussion on the results of this research based on i, ii, iii and iv above, comparing the results to similar research done in South Africa and elsewhere in the world.

### 2. THEORETICAL FOUNDATION

### 2. THEORETICAL FOUNDATION

2.1 Developments in aeropalynolgy until 1945.

2.1.1 Shortcomings of early research

- 2.2 Post 1945 developments in aeropalynology
  - 2.2.1 Quantitative aspects of atmospheric pollen and spores
  - 2.2.2 Pollen : The dominants
  - 2.2.3 Spores : The dominants
  - 2.2.4 Differences between atmospheric pollen and spores
  - 2.2.5 Periodicity
  - 2.2.6 Airspora and their relationship to meteorological factors.
    - 2.2.6.1 Wind direction
    - 2.2.6.2 Wind velocity
      - a) Pollen
      - b) Spores
    - 2.2.6.3 Relative humidity, temperature,

precipitation and leaf wetness

- a) Pollen
- b) Spores
  - i) <u>Alternaria</u>
  - ii) Pyrenophora teres
  - iii) Eutypha armeniacea
- 2.2.6.4 Atmospheric stability
- 2.2.7 Concluding remarks on atmospheric pollen and spores

#### 2.1 Developments in aeropalynology until 1945

In the nineteenth century, while the causes of infectious diseases of man and animals were being of laboratories, unraveiled in a band researchers attempted to clarify whether diseases such as cholera, typhoid and malaria could be connected to fluctuations of microbes in the atmosphere. Besides being the early hygienists this group also, unwittingly, could lay claim to being the first aeropalynologists. Salisbury (1866), by exposing sheets of glass above marshy places in the Mississippi Valley at night, was able to show the existence of spores growing on the surface of prairie soil. These spores were liberated at night up to a vertical dictance of 30 metres and collected on the glass sheets, which Salisbury then investigated with the Cunningham (1873) was able aid of a microscope. to demonstrate that moist weather diminshed inorganic dusts but it increased the total number of funcal spores. According to Gregory (1973) the most intensive sustained analysis of bacteria and moulds in the atmosphere was made in Paris in the last guarter of the nineteenth century under the auspices of Pierre Miguel. He designed a water driven pump which sucked air at a rate of 20 litres per hour over a glycerine-covered glass slide. Miquel, using this volumetric method, was able to show that airspora varied greatly in the same place at different times and that this variation bore а relationship to season, weather, district and altitude. Specifically, Miquel demonstrated that mould spores averaged about 30 000 m<sup>-3</sup> of air in summer, increasing to 200 000 m<sup>-3</sup> of air in rainy weather. (Compared to the dissertation findings in this these values are extraordinarily high.) In prolonged dry weather the numbers were reduced, while in winter they were only 1000 m-∍. also observed that, He in the event of rainfall, the spore content dropped dramatically (termed "wash-out" by modern aeropalynologists) but recovered rapidly afterwards.

Miquel can also be credited with being the first to establish a regular diurnal periodicity in mould spores. He observed two maximum peaks at approximatley 07h00 and 19h00 and two minimum peaks at 02h00 and 14h00. He also noted that the maximum peaks were correlated with increased passage of horse-drawn traffic and street sweeping! Miquel apparently realised the rich potential of his early observations in aeropalynology and remarked that "... the micrographer who has the leisure could studies of the subject." (Gregory make some nice 1973:11). Perhaps most importantly, Miquel was able to prove conclusively that airspora were not responsible for the dreaded diseases which concerned the hygienists, viz. cholera.

However, there remained certain diseases which could not be explained by pathogenic or parasitic invaders. These could be loosely grouped as nutritional diseases, cancer allergies. The third group proved to be an and intriguing avenue of research, since patient reaction to various allergens is inconsistent and individualised. Further, "normal" individuals can tolerate substances which allergics cannot. It had long been hypothesized that hay fever was attributable to pollen, although it Blackley demonstrate this remained for (1873) to conclusively.

Blackley proved the allergenic properties of pollen by inhaling pollen himself. He also suspected that there vertical gradient ìn atmospheric pollen may be a content. He was able to examine this hypothesis by placing a sticky slide at breathing level. He then placed slides at various heights up to 430 metres using kites. He concluded that in windy conditions slides at higher altitudes caught up to 20 times more pollen than at breathing level (Gregory 1973:13), thus showing not only that a vertical gradient existed for pollen but also that this gradient bore a relationship to wind velocity.

At the turn of the century aeropalynological studies the continent, specifically to the moved back to Teutonic countries, Austria and Germany. Schmidt (1918), an Austrian meteorologist, was interested in quantifying the relationship between the rate of diffusion of a spore cloud and distance travelled by that cloud. From this research he was able to establish that pollen grains, if affected only by horizontal currents, would all fall to the ground at a distance easily computable from Stokes Law. However, if subjected to atmospheric turbulence, the pollen would be transported to much greater distances. albeit in rapidly diminishing numbers. Schmidt also examined the vertical profile of spore concentrations. He argued that "... the number of particles falling, under the influence of gravity, across any horizontal boundary is compensated for by the number of particles moved upwards by diffusion, and so the concentration of particles in the air should decrease exponentially with increasing height ..." (Gregory 1973:179). He quantified this hypothesis with the following equation :

> V\_z X = X<sub>e</sub> exp - ----A

 $X = X_{o} \exp X_{o} = concentration at height z = 0$  $V_{a} = terminal velocity of fall$ A = Schmidt's intermixing variable

It is worth noting that this thesis was based on the assumption that the atmosphere was stable and thus does not necessarily contradict Blackley's (1873) findings.

With the advent of aircraft, researchers were able to investigate upper air conditions. However, earlier researchers such as Harz (1904) had used balloons to sample the upper air, demonstrating conclusively that airspora existed up to heights of 4000 metres. Stakman et al. (1923) were the first to use an arcraft to sample Table 1 The most common airborne pollen families

Aceraceae Acanthaceae \*Amaranthaceae \*Asteraceae Betulaceae Bignoniaceae \*Chenopodiaceae \*Cupressaceae \*Cyperaceae \*Ericaceae Euphorbiaceae Fabaceae Juglandaceae Moraceae Myricaceae Myrtaceae Oleaceae

\*Pinaceae \*Plantaginaceae Platanaceae Poaceae Polygonaceae Rosaceae Ranunculaceae Rutaceae Salicaceae Tilaceae Thymelaeaceae Umaceae Unticaceae

Table 2 The most common genera of airborne fungi.

Alternaria Aspergillus Chaetomium Cladosporium Cordana Curvularia Drechslera Epicoccum Fusarium Helminthosporium Leptosphaeria <u>Mucor</u> <u>Penicillium</u> <u>Periconia</u> <u>Pithomyces chartarum</u> <u>Nigrospora</u> <u>Stemphylium</u> <u>Streptomyces</u> <u>Trichoderma</u>

the upper air. Using vaseline-coated slides over the Mississippi Valley, they were able to demonstrate the existence of rust spores up to a height of 5400 metres. Peturson (1931) and Hubert (1932) both demonstrated that altitude atmospheric with increasing spore content decreased exponentially. These findings would appear to support an ideal altitudinal profile. However, they were contradicted by later researchers who identified particular meteorological conditions that contributed to contradicting the ideal altitudinal profile. Rempe (1937), making a series of aeroplane flights over German forests by day and night, was able to demonstrate that temperature inversions occurring at night caused the maximum number of grains to occur above the 200 metre temperature inversion level. He also showed that with moderately windy conditions and with cumulus clouds up to 2000 metres the maximum number of grains might occur as high as 200-500 metres. By implication, one can assume that the strong convection currents associated with cumulus cloud caused the spores to diffuse to far greater heights than normal. Conversely, in stable conditions with a stratified cloud layer and high wind velocities a marked decrease of pollen with height was found. In general, Rempe demonstrated that at night the numbers trapped usually decreased with increasing height much more than by day and that fewer numbers were trapped at all heights at night than by day. This is clearly illustrated in Figure 1. Craigie's (1945)research added a futher dimension to Rempe's earlier findings. Figure 2 serves to illustrate the point. Craigie sampled rust spores - uredospores - over the Canadian prairies. curves A and D tend to approximate the ideal altitudinal profile. Curve B was interpreted by Craigie as indicating a transfer of spores into an area that was not producing uredospores. this area thus acts as a sink, removing spores to the lower atmosphere while the greater concentration remains at 14000 feet. Profile С interpreted could be indicating as convectional activity to a height of 5000 feet (cumulus

cloud formation) after which the uredospore concentration would fall off.

#### 2.1.1 Shortcomings of early research

Early researchers, at least those prior to 1945, did not have access to the battery of sophisticated inferential statistical tests that we have today or for that matter, use of advanced computers. Thus they were not able to do more observe what appeared to strong than be asssociations between different sets of data. Specifically, because computer-aided analyses were not available at the time these observed associations between sets of data could only be hinted at. The strength of the relationship could effectively guantified. never be Therefore, questions such as " to what extent does wind velocity affect spore concentration in a vertical air profile? " and " how important is temperature affecting as a variable in the diurnal periodicity of airspora? ", would be left to researchers who would benefit from the tremendous advances made in technology as a result of the Second World War.

#### 2.2 Post 1945 developments in aeropalynology

1

Since the research under review in this paper deals with airspora and the relationship of airspora to selected meteorological parameters, the following section deals with a survey of literature, concentrating firstly on the quantitative aspects of the airspora spectrum .and secondly on the relationship between appropriate meteorological factors and airspora concentrations. Moreover, since the period prior to 1945 has been dealt with in a general sense , this section will concentrate on the period 1945 to the present. It is worth noting that only the literature immediately pertinent to each section is reviewed. Literature covering aspects such as the advantages and disadvantages of various samplers will be dealt with in the next chapter.

## 2.2.1 <u>Duantitative aspects of atmospheric pollen</u> and spores.

Researchers generally accept that spores are the dominant partner amoung the airspora (Street ደ Hamburger, 1976; Chaubal & Kotmire, 1982; Sapute et al., 1983; Anderson, 1985; Kumar, 1985; Royes, 1987). The balance between these two factors in the atmosphere must indeed vary from place to place, but the following two examples give an idea of their proportions in the atmosphere. (The following figures represent the total number of spores and pollen in a year.) Satpute et al., (1983) report from Shillong, India, that 22% of all airspora are pollen while spora represent 72% of the total spectrum. The remaining 6% constitute other biological matter. Royes (1987) report that in Kingston, Jamaica, spores are extremely dominant making up 97,73% of the spectrum whilst pollen comprises only 0.40% nf the total material observed. Without labouring the point, spores do dominate but the ratio between spores and pollen probably fluctuates considerably at any time depending on the time of year, season and daily meteorological conditions.

#### 2.2.2 Pollen : The dominants

Pollen from an aerobiological point of view can be divided into two major categories. The first category are the anemophilous (wind pollinated) They taxa. constitute the chief Source of inhalant pollen allergens and include grasses. Chenopodiaceae, Amaranthaceae and some trees such as oak, birch and elm (Singh, 1987). The second category are the entomophilous (pollination through vector mediation) taxa. Because they are

not wind pollinated they do not occur in the same concentrations in the atmosphere compared to the anemophilous taxa. However, Singh (1987) warns that he has found that some entomophilous taxa are found in abundant concentrations in the atmosphere and it would thus be scientifically hazardous to ignore them simply because they are not wind pollinated.

It is difficult to find trends in the global pollen spectrum because, as mentioned earlier, plants are not nearly as adaptable as fungi and thus in most instances taxa are specifically located on the earth's surface. However there are some taxa at the family level that are reasonably their distribution. ubiquitous in These are primarily the orasses (Poaceae) and weeds (Chenopodiaceae, Amaranthaceae and Plantaginaceae). Moreover these taxa are prodigious producers of pollen and are wind It pollinated. is thus not surprising that scientists the world over have found that these taxa are often the common (and in many cases the most dominant) contributers to the pollen spectrum (Street & Hamburger, 1976; Anderson etal., 1978; Chen & Huang, 1980; Singh & Babu, 1981; Chaubal & Kotmire, 1982; Frenguelli et al, 1983; Halwaqy & Halwaqy, 1984; Anderson, 1985; Kumar, 1985; Dhorranintra et al., 1987; Hurtado & Riegler-Goihman, 1987: Longo & Cristofolini. 1987). However depending on the vegetation in the region where collection is taking place, tree or shrub pollen may be the most dominant member of the pollen spectrum eg Pinus in Sweden (Nilsson & Palmberg-Gotthard, 1982), Casuarinaceae in Egypt (El-Ghazaly & Fawzy, 1987) and Cupressaceae in Italy (Longo & Christofolini, 1987). It is also of interest to note some of the percentage contributions of various families to the pollen spectrum. Frenguelli et al. (1983), researching

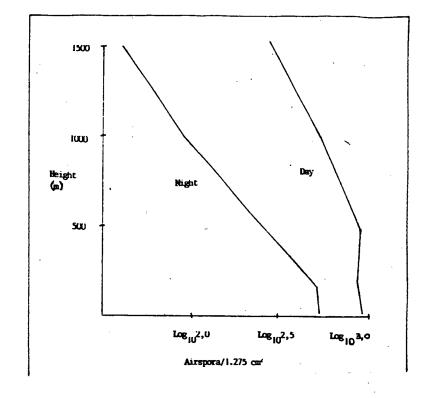
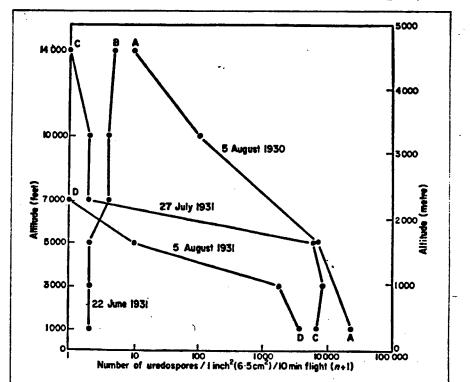


Figure 1 Day and night Pollen Profile (Rempe, 1937).

Figure 2 Vertical distribution of <u>Puccinia graminis</u> uredospores. Numbers of uredospores (plotted as log<sub>10</sub> n+1) trapped per square centimetre of sticky slide trap per 10 minutes of flight at various altitudes over Southern Manitoba during rust epidemics (Craigie, 1945).



in Italy, note that Poaceae contributes 17.47% , Fagaceae 19.30%. Oleaceae 17.48%, Cheno-Amaranthaceae 2.19% and Pinaceae 2.16% to the total pollen spectrum. Chen & Huang (1980) note that , corporately, trees contribute 56%, weeds 9.1% and grasses 22.7% to the pollen spectrum in the Taipei Basin, Taiwan. In their study, the 12.2% was made ferns remaining up of and unidentidied objects. Al-Doory et al. (1980) were able to show that in Washington, D.C., Poaceae formed only 5,4% of the pollen spectrum while oak trees constituted 35.1%. The balance was made up of a collection of decidious trees. These three case studies show that the balance between grass, shrub and tree pollen weed. does vary considerably over space. Moreover, the balance between the different pollen types varies over time as well. Kumar (1985) demonstrates that pollen proportions vary from one year to the next in the same place. For example in the 1980 season Poaceae contributed 33.28 % to the pollen spectrum in Bareilly, India. However, this figure changed to 18% the following year - a decline of nearly 50%. Table 1 indicates a list of some of the more common airborne pollen families. The families marked with an asterisk are the more prodigious producers of airborne pollen (Anderson et al. 1978).

20

### 2.2.3 Spores : The dominants.

Before looking at the dominants in this section something must be said about the classification of fungi. Recently there has been a tendency to separate the fungi into a kingdom of their own (Myceteae)(Mims, 1984). This has led to considerable disagreement amoung mycologists as to the most effective method of classification. It seems that many mycologists simply embrace whatever scheme is most suitable to them. (Alexopoulos & Mims, 1979). Bearing this in mind the major division that interests aerobiologists is the Amastigomycota division which is by far the largest division. Within this division there are four subdivisions, Zygomycotina, Ascomycotina, Basidiomycotina and Deutermycotina (Mims, 1984).

The subdivision Zygomycotina contain the bread moulds and by and large are of little interest to the aerobiologist. The class Ascomycetes, is the dominant class in the Ascomycotina . Within this class are the yeasts, mildews, and cup fungi. Most of the ascomycetes produce conidia( an asexual state), with the exception of the yeasts. In the atmosphere, these organisms are more likely to be encounted in the conidial form rather than in the sexual or ascus-producing state. This means that a researcher must identify the fungus from its asexual state, which is hazardous because the conidia of many Ascomycetes resemble those of the Deuteromycetes (Mims, The class Basidiomycetes in the 1984). subdivision Basidiomycotina, includes the fungi such as mushrooms, puffballs, jelly fungi and the rusts and smuts. plant-pathogenic These Basidiomycetes produce sexually and asexually. Basidiomycetes produce specialised structures, basidia, from which the basidiospores are derived sexually. These are often prominent in the atmosphere. Conidia are also produced asexually but are not a dominant feature, as with the Zygomycetes. Lastly the "fungi imperfecti", the Deuteromycetes, from the subdivision Deuteromycotina, are a class which are known to reproduce by asexual means only, ie. through the use of conidia. They are of considerable importance to those concerned with mould allergies. It is worth emphasizing again that the

conidial stages of the Deuteromycetes are similar to those of the Ascomycetes and thus confusion about the classification of organisms in this class easily occurs.

In looking for global trends it is apparrent that Deuteromycetes provide the dominant conidia in the atmosphere. Lacey (1981) demonstrated after surveying about 200 studies world wide, that the genera Cladosporium, Alternaria, Penicillium and Aspergillus, listed in decreasino order of significance, are most consistently associated with the highest mean percentages of total spore catches. Exceptions occur in areas conducive to the growth of basidiomycetous fungi ie. New Orleans (Salvaggio, 1970) and Auckland (Hasnain et al., 1984). In these cases, basidiospores are the most dominant form in the atmosphere. A1-Doory (1984) made a similar survey and came to the same conclusion. However, he included Fusarium as the fifth most important contributor to the spore spectrum. Notwithstanding the above trends, it is important to note that findings of these two scientists (Lacey, 1981; Al-Doory, 1984) are based on the averages worked out from their surveys. The order of significance does not hold firm over space. For example Sandhu et al. (1964)listed Alternaria (30%) as the most dominant form in Delhi, India, while Cladosporium (27%) and Fusarium (13%) followed in order of significance. Hotchkiss. (1963)et al. demonstrated that <u>Cladosporium</u> (28%) followed by Alternaria (24%) were the most dominant forms in Indianapolis, Indiana. Table 2 lists the most common genera of the fungi.

### 2.2.4 Differences between atmospheric pollen and spores

Having dicussed the quantitative aspects of atmospheric pollen and spores it is now necessary

to clarify the major differences between spores and pollen.

- a) Pollen on the whole is seasonal, being released in late spring and summer. Not all fungal spores are seasonal, occuring throughout the year, eg.
   <u>Aspergillus</u> and <u>Penicillium</u> (Sneller, 1984).
- b) Many spores are universal, occuring world wide Cladosporium, Alternaria, Fusarium eq. and These spores are thus highly Helminthosporium. adaptative and can survive an exraordinary diversity of climates. There are few if any plants which are as ubiquitous as this.
- c) The life cycle of plants and moulds bear little comparison. The production of pollen can begin up to 14 months before it is released (Fairley & Batchelder, 1986) while some spores can divide and be released within 24 hours.
- d) Spores, generally, are smaller than pollen having terminal velocities and lower thus probably remain bouyant for longer in the atmosphere. Further. in terms of shape they are less symmetrical than pollen which would, further account for their longer levitation in the atmosphere.

### 2.2.5 Periodicity

Periodicity refers to the regular peaks and troughs in pollen concentrations over a selected time period. In a 24 hr period this periodicity referred to as diurnal is or circadian periodicity. Diurnal periodicity is a well known phenomenon of pollen and spores (Reddi et al., 1980; Gottwald & Bertrand, 1982; Mallaiah & Rao, 1982; Shenoi & Ramalingam, 1983; Spieksma, 1983a; Steel, 1983; Chen, 1984; Hasnain et al., 1984: Martin & Clough, 1984; Spieksma et al., 1985; Burge, 1986; Okhuoya, 1986; Savary, 1986; A1-Eisawi & Dajani, 1987; O'Roùrke & Buchmann, 1987;

Royes, 1987). What is not nearly as clear is the variation. Some reason for this diurnal investigators a heavy emphasis on the place genetic aspect of the plant which provides for a pattern which is little basic and reqular influenced by meteorological factors (Davidson, Liem & Groot, 1973). 1941: Other scientists suppose that the fluctuations are actually caused by meteorological factors. (Singh & Babu, 1980; Spieksma, 1980; Leuschner & Boehm, 1981; Steel, 1983) Although this mystery has not been laid to rest, the truth may be found in the combination meteorological of genetic programming and factors. The relationship is at the very least complex and requires further research to the response of plants to climatic elucidate factors.

Most plants release pollen at a certain time of the year and thus have a seasonal periodicity as (Pennycook, 1980; Bai & Reddi, 1981: well Mallaiah & Rao, 1982; Verma & Kamal, 1982; Shenoi Ramalingam, 1983; Anderson, 1985; Okhuoya, & 1986; Royes, 1987). However moulds and fungi are capable of producing more than one peak per producing plants tend to annum. Pollen be seasonal with a well defined peak in the heart of the pollen season. Spores on the other hand can show as many as 3 peaks during the course of 12 1980; & Reddi, 1981; months. (Pennycook, Bai Okhuoya, 1986; Royes, 1987). Scientists as yet have not provided an adequate explanation for this difference between pollen and spore producers but Sneller(1984) has hinted that it is likely to be the greater adaptability of fungi to varying climatic conditions on a global scale. This means that spore producers can produce and over release spores а greater range of temperature, relative humidity and precipitation regimes. Should this be the case, then it is

possible that on more than one occasion during the year the various meteorological requirements of the fungi are met and they are able to produce and release spores. It is also possible that because of the advanced evolutionary adaptions that plants have had to make in order to compete and survive that they respond to only very specific stimuli while fungi/moulds being less advanced in an evolutionary sense are able to respond to a broader range of stimuli eg. temperature etc.

# 2.2.6 <u>Airspora and their relationship to meteorological</u> <u>factors</u>

The literature on aeropalynolgy is now vast, particularly in the area of the relationship of to airspora concentrations selected meteorological factors. Thus, although the review is considered comprehensive, only those papers that attempt to throw light some onto the immediate relationship between airspora and meteorological varaibles, will be reviewed. Further to this it should be noted from the outset that the relationship between weather and airspora is highly complex (Charpin et al., 1973; Davies & Smith, 1974; Makinen, 1977; Mullins et al., 1977; Stix, 1977; Anderson, 1980; Singh & Babu, 1980; McDonald, 1980; Spieksma, 1980 & 1983b). Initially each meteorological factor is studied separately in order to gain some insight into the operation of that factor as it relates to airspora. At the end of the section, however, an attempt is made to draw the various factors together to create a broad picture showing the relationship betweeen airspora and weather as a whole.

Before dealing with wind direction and wind velocity, one must bear in mind that different plants may respond differently to the same weather conditions. (Kapyla, 1984). Thus we must also assume that spores, compared to pollen, will also respond differently to weather. Within the group, the spores will also show variations amongst themselves in response to weather conditions. One may then query the wisdom of the relationship of airspora looking at to weather factors on such a broad scale. Will any meaningful information become apparent? Where researchers have attempted to 'paint with a broad brush', (Reddi et al., 1980; Steel, 1983 and Mandrioli, 1987), in most cases it is done so on the basis of past research into a variety of organisms. There are contadictions to the generalisations and hypotheseis they propose as will become apparent later on in this section, but it should be born in mind that progress in science requires broad propositions which need to be tested in detail in order to reach a model which is acceptable. Particularly in this sphere of scientific endeavour, it is apparent that we have some way to go before any such generally acceptable model is forthcoming. With the exception of wind direction and atmospheric stability, spores and pollen will be dealt with seperately under each sub-heading. The reason for this will be made apparent at the beginning of each sub-section.

#### 2.2.6.1 Wind direction

Pollen and spores are studied together in this section because wind is involved in the distribution of airspora and therefore its effect is mechanical on plants and fungal growths. It thus seemed appropriate to look at spores and

pollen together in this case. Street and Hamburger (1976), Mercuri et al. (1982) and Keynan et al. (1986) researching in San Diego, Siena and Tel Aviv respectively were not able to find any correlation between wind direction and pollen concentrations. On the other hand, Hyde (1950) and McDonald (1980) were able to show very strong correlations between wind direction and Researchers generally are loathe to pollen. explain these seemingly contradictory results and moreover most of the literature reviewed does not consider wind direction a factor worth studying. Nonetheless both the Hyde and McDonald papers argue strongly for the consideration of wind direction as an important factor. According to (1950:405), "...steady offshore Hyde winds produced a high catch; the highest catches of the year were made on days when such winds were recorded". The following explanation may provide a pointer in understanding the relevance of this factor, although neither Hyde or McDonald attempts such an explanation. McDonald showed that at Galway, Ireland, there were significantly higher airspora concentrations in 1977 than in 1978. He attributed this to a change in the summer seasonal wind direction. In the 1977 season, wind direction was predominantly from the W through N and E which was mainly from overland. In the 1978 season the wind varied from W to S which was from over the sea. Simply put, offshore winds were associated with high pollen concentrations while onshore winds were associated with low concentrations. Hydes findings seem to corroborate this assessment. Onshore winds, originating from the ocean surfaces obviously do not have the opportunity to 'pick up' pollen, thus they are relatively pollen free when they reach coastal areas. On the other the offshore winds originate from hand the hinterland and thus are able to pick up pollen

and hold it in suspension. On arriving at the coast these winds, one may hypothesize, are relatively rich in pollen content. Coastal areas should thus experience higher atmospheric pollen concentrations during offshore winds if the above line of reasoning is correct. Spieksma and Tonkelaar (1986) sampling Leiden at in the Netherlands able to similar were reach conclusions. They noted that air advected a long distance from a source area overland, provided that area was dry, was rich in pollen compared to winds which blew onshore from N through a W to S-W direction (basically from the North sea). In summary, wind direction is a significant variable if, as Hyde points out, the wind blows over or originates from a pollen source area. As far as spores are concerned this conclusion seems to be confirmed by Beaumont et al. (1985) who made an aerobiological survey of the NE Netherlands. They found that a typical "local" correlation was found between the occurrence of <u>Cladosporium</u> and easterly-winds. Apparently the area to the east of the sampler was largely grassland which serves as a host for this spore. When the W wind blew onshore from the N sea, very few spores were Bagni et al. (1977) also found that in apparent. Leiden (Netherlands) Cladosporium counts were higher when the wind was from the E (offshore). These findings confirm those of Spieksma Tonkelaar (1986) who studied pollen in the Leiden district.

## 2.2.6.2 Wind velocity

#### a) Pollen

There is little concensus amoungst researchers as to the exact effect of wind velocity on pollen, although much research has been done in this area. Hyde (1949:403), in the earliest known

study of this nature in Britain states, "...we have failed to confirm from our tables that wind velocity has any marked effect on the pollen catch ...". Street & Hamburger (1976) and Mercuri et al. (1982) seem to support this assertion. However, Anderson et al. (1978), Al-Doory et al. (1980), McDonald (1980), Reddi et al. (1980), Satpute et al.(1983), Steel (1983), Kumar (1985), Reddi & Reddi (1985), Fischbach (1986), Keynan et al. (1986), and Mandrioli (1987) have all found a definite relationship between wind velocity and pollen concentration. Looking carefully at the evidence, however, there may be no contradiction at all in these results.

"Pollen not shed under calm or stable is conditions. The emission of pollen into the atmosphere is thus a mechanical process, governed by wind speed and turbulence.". (Steel, 1983:131) In trying to explain the affect of wind on pollen concentrations in the atmosphere Steel points us to the fact that the EMISSION of pollen is a mechanical process. Others argue that there are other factors involved in the process but this will be dealt with later. In order to understand the significance of Steels point, the word 'emission' in the context that it is used must be clearly understood. Anthesis occurs when the anthers of the plant begin to protrude from the plant, laden with pollen. Indeed this process governed by several physiological, itself is ecological and biometeorlogical factors (Liem & Groot, 1973). When the tip of each anther lobe gradually diverges and the anther splits open exposing minute yellow pollen grains (Hubbard, 1968), a process known as dehiscence, the stage is now set for the dispersal of the pollen into the atmosphere. Reddi et al. (1980), Steel (1983), and Reddi & Reddi (1985) are in agreement that this final process of pollen liberation is

the result of wind and turbulence, basically a mechanical process. Reddi et al. (1980 : 176), however, points out that dehiscence is not the same as pollen release:

"When the anthers dehisce one would naturally expect that the pollen being dry and non-adherent are poured out. But this does not happen... the syngenesious nature and lateral position of the line of dehiscence facilitates the abutting pollen sacs to hold loosely the pollen until liberated... Even a slight breeze would suffice to dislodge part of the pollen load of the dehisced pollen sacs."

Thus, the dehisced pollen will remain on the anther until wind, turbulence or convectional currents cause the pollen to be dislodged.

What now of the authors who find that wind speed does not affect pollen concentrations? There are two possible explanations. In the first instance, the concept of terminal velocity of a particle should be understood. Stokes' formula, used to compute this velocity and the significance of this concept itself are explained in the section with methodology (Chapter dealing 3). Verv briefly, every pollen has a different shape and volume. This affects the way in which it responds to gravity, by either falling rapidly to the earth's surface (a high terminal velocity) or falling slowly toward the earth's surface (a low terminal velocity) For instance Xanthium strumarium, studied by Reddi et al. (1980), has a terminal velocity of 1.06 cm s<sup>-1</sup>. Although a wind with a speed of less than this figure may dislodge the pollen from the pollen sacs allowing it to fall to the ground, it would require a wind of at least the same strength as the pollen terminal velocity to keep it afloat in the

atmosphere. Following this line of argument any wind above this figure will liberate the pollen and keep it afloat. Higher wind speeds will not concentrations. cause higher Likewise, wind speeds below the terminal velocity of the pollen, while perhaps causing liberation of the pollen, would not cause an increase in atmospheric pollen concentrations. Thus no correlation between wind speed and pollen concentration will be found if the wind is consistently below the terminal velocity of the pollen being studied. Wind speeds consistently and markedly higher than the terminal velocity of the pollen being studied wi11 also not correlate with pollen concentrations because any variation in wind speed above the terminal velocity will still the pollen to liberate and float cause in aproximately the same numbers. Keeping the above it interesting to observe in mind is that Fischbach (1986) noted that a wind of 16 kmh was critical in floating pollen. Kumar (1985) put this critical figure at somewhere between 5-10kmh. Variation in these figures depends on the wind speed required to dislodge the pollen and the terminal velocity of the pollen, above which the wind must blow to keep the pollen afloat.

In the second instance. unless anthesis and dehiscence has taken place, no amount of wind will dislodge the pollen. Other factors such as season, relative humidity and temperature are responsible for these processes. If the requirements for anthesis and dehiscence have not been met then pollen formation and dispersal will not take place. In this context it is а relatively simple task to explain why pollen concentrations do not correspond well with wind speeds.

#### b) <u>Spores</u>

As there regards spores, is significant a difference in the dispersal mechanism. Whereas in pollen, dehiscence does not imply dispersal, in spores dispersal takes place at various stages in the life cycle of the fungus. Further. the rupturing of the sporangium (usually as the result of critical values in temperature and relative humidity) directly releases the spores into the atmosphere without the aid of wind. There are, however, a number of researchers who find that wind speed does not correlate with atmospheric spore concentrations (Aylor & Lukens, 1974; Hilderbrand & Sutton, 1982; Mallaiah & Rao, 1982; Lyon et al. 1984b; Martin & Clough, 1984; Beaumont et al. 1985: Fitt et al.. 1985 and Savary, 1986). Martin and Clough state that spores were trapped at a wind speed of 2.5 m sec-1, 5,5 m sec<sup>-1</sup> and 6,5 m sec<sup>-1</sup>. Although no correlation was found between wind speed and spore concentrations they argued that the lowest of these wind speeds was sufficient to keep spores in suspension in the atmosphere, thus higher wind speeds had no affect on spore concentrations. This corroborates the point made in the previous paragraph with respect to pollen. As with pollen, the critical factor seems to be the terminal velocity of the spore. Spores generally have smaller volumes and one would expect that their terminal velocities are less than pollen. It therefore follows that a gentle suspension wind would keep them in in the atmosphere. This seems to be the case when one notes that Aylor & Lukens (1974), Mallaiah & Rao (1982) and Lyon et al (1984b) found that the critical wind speed for spore suspension in the atmosphere was  $1,0 \text{ ms}^{-1}$ ,  $1,0 \text{ ms}^{-1}$  to  $2,5\text{ms}^{-1}$  and  $1,9 \text{ ms}^{-1}$  respectively. On average this seems to be half of what is required to keep pollen afloat.

Concluding this section on wind speed speed, it is important to make the point that wind cannot be looked at in isolation. When it acts iΠ concert with another factor such as relative humidity or temperature or both, then а significant correlation may be found. Should this be the case, then it is worth recognising that a simple correlation test is not adequate. Rather, multiple regression analysis would be more appropriate for identifying the significant variables.

## 2.2.6.3 <u>Relative humidity, temperature, precipitation</u> and leaf wetness.

#### a) Pollen

clear that, as a result of inter-It is relationships between the variables of relative temperature, preciptation and leaf humidity, wetness, their effects on pollen concentrations would be complex. Moreover, the variables should be examined in combination, since it is unlikely the effects of one of these factors alone would be significant in adjusting pollen levels (Davies . & Smith 1974, Raynor et al., 1976; McDonald 1980; Spieksma, 1980, 1983b; Leuschner & Boehm 1981). It is as well to heed a warning by Spieksma (1985:106):

" We are dealing with an extremely complex system of aerobiological and meteorological factors, in which not only local temperature (daily maxima and minima) and rainfall (quantity, duration, time of the day) but also inversion, longdistance transport of air masses, etc., may play a decisive role."

Further to the above point, when dealing with relative humidity, temperature, precipitation and must leaf wetness also consider the one possibility that these factors have a seasonal influence as well daily influence as a (Mandrioli, 1987), Therfore it is necessary to appraise this aspect before moving to the impact of daily fluctuations of these variables.

The timing of pollen release and the quantity of pollen produced are principally results of genetically controlled adaptations, but both can' be influenced by the climatic characteristics of the period before flowering. Mandrioli (1987:39) states that:

"...the quantity of pollen available for spring flowering is determined by weather conditions of the previous summer, because at that moment the cells designated to become pollen are already present, in fact, when in the previous summer abnormally high temperature and low precipitation occur, it is frequent to notice abundant pollen yield."

Reiss & Kostic (1976:609) note similarly, for North America that "...account must be taken of antecedent meteorological conditions which, to a large extent, predetermine the nature of a pollen season by affecting the growth of the plants." There are two important points to be made here. Firstly, regardless of the type of weather prior to anthesis, the plant is programmed genetically, so to speak, to produce pollen at a certain time of the year. How much it produces' and exactly when it will start producing pollen is influenced by meteorological factors. In Europe, Mandrioli (1987) has already hinted that high temperatures

the previous summer with low precipitation will cause abundant pollen production.

- Reiss & Kostic (1976) found that ragweed pollen season severity in North america is correlated positively with the mean minimum temperature of the four months before the pollen season began. They interpreted this to mean that ragweed growth is inhibited whenever temperatures fall below a threshold value which their regression formulae suggested was about 60°F. A similar positive found for the daily average correlation was temperature of the previous 4 months prior to the pollen season, another indication that a warm growing-season promotes ragweed growth. Lastly, they found that pollen season severity is with positively correlated mid-springtime precipitation. Al-Doory et al. (1980) claim that rainfall is the single most important factor influencing ragweed pollen season severity. Like Reiss and Kostic, they found that rainfall in late spring and early summer was a prerequisite to a severe ragweed pollen season. Fairley & Batchelder (1986) found that pollen release showed a positive correlation with the total rainfall a full year before pollen release but rainy season immediately before not with the pollen release. This is an unusual relationship and can perhaps be explained as follows. In non-Mediterranean climates, warm summers with low
- precipitation stimulate pollen production. this study took However, because place in California, region with Mediterranean a a climate, rain does not occur in summer. It would follow that some water-dependent physiological activities such as pollen production, become winter-precipitation dependent. This may explain why the study demonstrates that heavy oak-pollen release occurs 12-14 months after a winter with heavy rainfall (Fairley & Batchelder, 1986) and

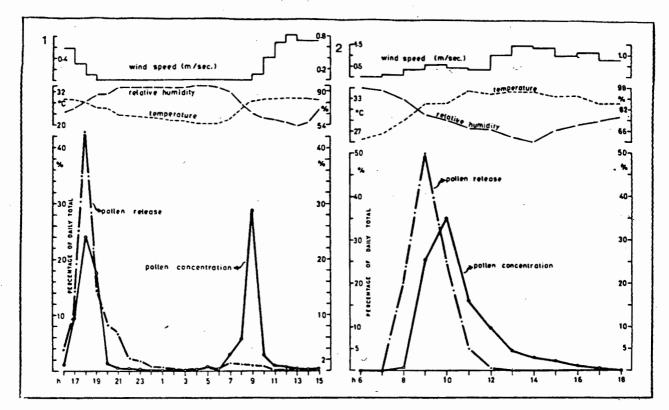
is not dependent on rains in the late spring and early summer period. It is of interest to note that it is only recently that researchers have given any emphasis to the early growing season and previous season's meteorological activity. Our knowledge in this area as far as pollen is concerned is still relatively thin and it is difficult to make any testable hypothesis. (1987)However, if we are to take Mandrioli seriously. then the previous summer seasons temperatures(high) precipitation(low) and determines at an early stage the quantity of pollen grains to be produced because the cells designated to become pollen grains are already present. In the growing season (late spring and ealy summer) high temperatures and preciptation encourage plant growth leading to high pollen production, although this will clearly vary from species. Finally if the species to region experiences a seasonal drought, such as in a Mediterranean region, then plants adapt to the summer drought by allowing the previous rainy season to determine the extent of pollen cell Very little has been said of division. the previous season's relative humidity values, but this is probably autocorrelated with temperature and precipitation. From this point on the impact of daily meteorological factors in relation to pollen concentrations is reviewed.

Before embarking on this discussion it is necessary to clarify the processes leading to pollen release (dehiscence). It has already been that pollen dispersal is а mechanical shown process reliant on wind velocity. However, the up to pollen release are stages leading а combination meteorological, genetic of and physiological factors that to difficult are unravel. Initially, when flowering begins the androecia of the mature florets in a capitulum

protrude as a result of the elongation of the filaments - anthesis (Hubbard, 1968). This protrusion, the result of rapidly dividing cells in the filament, (Reddi et al., 1980) is dependent on varíous enviromental factors. Firstly, moisture or dew is necessary because, in order for the filament cells to divide, water is required. Thus rain during the day and/or dew at night plus a high relative humidity would hasten this process. However anther protrusion still occurs when there is no rain and Reddi et al. (1980) maintain that this could be induced by increased water uptake from the soil. Reddi et (1980), were able to show experimentally on al. the weed Xanthium strumarium, that a sudden increase in temperature also caused rapid protrusion of filaments. These observations seem to be partly verified by Liem & Groot (1973) who studied the grasses of F.rubra and H.lanatus on the Dutch Fresian Islands. Initially they were able to demonstrate that the emergence of anthers started when air temperatures dropped after noon. anther protrusion rapidly However increased towards a maximum when the relative air humidity was at its lowest and the air temperature `at its highest, usually about midday. Again one must bear in mind that not every plant responds in the same way to meteorological factors but as far as anther protrusion is concerned it appears that some form of water is necessary for initial filament cell division, while this process is accelerated by increasing temperatures and decreasing relative humidity during the day.

Dehiscence of the anthers (release of pollen) as opposed to anthesis, according to Reddi et. al. (1980), will only occur when dry conditions are apparent. They argue that for <u>X. strumarium</u> the critical values of  $40^{\circ}$ C and a relative humidity of below 95% must be met before dehiscence can

Figure 3 Pollen release and pollen concentration profile for <u>A. excelsa</u> and <u>A. spinosus</u> (Reddi & Reddi, 1985:110).



38

1.

take place. Presumably this must take place during the day. Wet conditions caused by dew or This rain will delay and prolong dehiscence. seems to be the case in Liem & Groot's (1973) study where they argued that decreasing relative humidity was clearly associated with increased pollen release. From the evidence available, dehiscence or release of pollen is dependent on the desiccation (exhaustion of moisture) of the pollen sac walls (Reddi et al. 1980). Kapyla (1984:175) sums up this cause and effect relationship " the opening of anthers is probably caused by drying. So it is understandable that in high relative humidities pollen concentrations were clearly lower." Work by Ljungkvist et al. (1977) and Al-Doory et al. (1980) tends to support these findings. Thus, dehiscence occurs when temperatures increase at the beginning of the day leading to a drying of the atmosphere and thus a reduction in relative humidity. As a result the pollen sac walls become desiccated which leads to pollen liberation. This relationship is illustrated in Figure 3.

Two points become apparent. Firstly, the peak of pollen release does not take place at the same time of the day for different species, thus showing that different plants can respond earlier or later to the same meteorological factors, a point made earlier. Secondly, pollen release dispersal (dehiscence) and pollen do not correspond, in fact there is an appreciable lag for <u>A excelsa</u>. This emphasises the fact that pollen release and pollen dispersal are not the The assumption therefore. same processes. "...that the variation in concentration of pollen close to the source (of release) reflects the daily pattern of pollen release is not always true" (Reddi & Reddi, 1985:113). Liem and Groot

(1973) also found no correlation between pollen release and pollen concentrations in the atmosphere. Although release may have taken place, unless there is a sufficiently strong wind at the same time as release, pollen dispersal will not take place. However Figure 3 does illustrate the main point that pollen release occurs when temperatures are increasing and relative humidity is decreasing, usually in the early morning. This relationship has beeen confirmed by many researchers (Steel, 1983: Fairley & Batchelder, 1986; Fischbach, 1986; Sutra et al., 1987). The peaking of the pollen release period is often referred to as diurnal periodicity (Steel, 1983) and has been referred to earlier on in this chapter.

In a more general sense, with the onset of spring and summer and the beginning of the pollen period one would expect an increase in the mean daily minimum and maximum temperatures which should correlate with increased pollen production. Many authors have found this to be the case, including Hyde (1950), Chen & Huang (1980), Bringvelt et al. (1982), Mercuri et al. (1982), Frenquelli et al. (1983), Ballero et al. (1985), Kumar (1985), Spieksma et al. (1985). As regards precipitation, besides raising the relative humidity and thus preventing dehiscence, rain also has a 'washout effect'. On days of rain the pollen is simply of the atmosphere. washed out lowering concentrations dramatically. This is a well known fact and is documented by many authors (Hyde & 1950; Williams, 1945; Hyde. Sreeramulu & Ramalingam, 1964; Ramalingam, 1971; Satpute et al., 1983; Dutta & Rao, 1983; Ballero et al., 1985; Kumar, 1985).

4O

#### b) <u>Spores</u>

The 🐇 relationship between spores and meteorological factors is extraordinarly complex (Sneller, 1984) and from the available literature it is difficult to find a pattern in the response of different spores to these factors. Moreover, unlike pollen, very few authors have found evidence that atmospheric factors months ahead of . spore can influence directly spore release concentrations in the atmosphere. However it does that atmospheric factors can play an SPPM indirect role early stage iΠ the at an moulds. development of Moulds develop on a substrate. This substrate in the case of many moulds and fungi is vegetation. Thus, if climatic factors are not condusive to vegetative growth, it follows that there will also be a poor mould thus low atmospheric spore growth and concentrations.

"Moisture content of the atmosphere influences the airspora by controlling the growth of surface vegetation which determines the nature and amount of substrate available to fungal organisms." (Verma et al., 1981:61)

Factors such as preciptation, temperature and relative humidity play an indirect role in the early stages of fungal development. Although some fungi such as Aspergillus and Penicillium (Verma occur seasonally, al., 1981) most occur et throughout the year. Further to this, many also global scale defying occur on а any climate. Boldly classification according to speaking, it thus seems that many moulds have adapted to a variety and sometimes contradictory If set of *meteorological* variables. this hypothesis is correct, we must assume that a universal dominant such as <u>Cladosporium</u> responds

differently to different climatic factors. For instance a change in temperature may cause spore release in one region but in another region with . consitently high temperatures it may be a change relative humidity in which causes spore discharge. This adaptabilty amoungst many of the known spores makes for a myriad of permutations when trying to isolate patterns of response to meteorological variables. A further consideration involves the 'damp-air spora' and the 'dry-air spora' (Pennycook, 1980; Mallaiah & Rao, 1982; Sneller, 1984). These are found seasonally and seem to be correlated with humidity regimes. Notwithstanding the cautious approach above it seems to be more sensible to concentrate on three case studies of known spores to illustrate the meteorological influences.

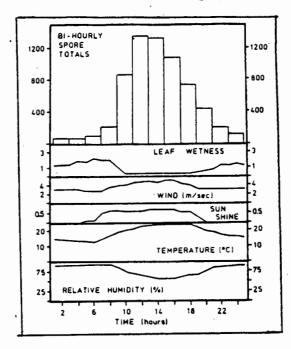
## i) <u>Alternaria</u>.

According to Sneller (1984) this mould is globally distributed, although many researchers have shown that it has a seasonal occurrence (Pennycook, 1980; Sneller et al., 1981; Verma et al., 1981; Infante et al., 1987). This ubiquitous fungi uses rotting vegetation of all descriptions as a substrate for growth. From the point of view Alternaria has definite of human relevance, allergenic properties. (Simmons, 1967; Yunginger 1976; Solomom, 1978; Sneller et et al., al., 1981; Schultze-Werninghaus et al., 1987). Hoffman (1984) notes that in North America there are 14 different listed strains of Alternaria and he most studied of mould claims that it is the allergens.

Sneller et al., (1981) studied the <u>Alternaria</u> mould in Tucson, Arizona. They noted a dramatic five-to-tenfold increase in the concentrations of this organism in the atmosphere over the last 20

years. This coincided with a dramatic increase in the number of trees in the city as a result of a city beautification programme. These trees act as a substrate for the mould. Leach (1975) has shown conclusively that the formation of Alternaria the conidia occur, dark in hours when the relative humidty is high. The division of the cells apparently requires moist conditions. The of the conidia release occurs through photomechanisms when the warmth of the day The photomechanical response has begins. been recorded by other authors for different spores. Hilderbrand & Sutton (1982); eq MacHardy & Gadoury (1986) and Royes (1987). Therefore, at daybreak the decrease in relative humidity and the increase of temperature lead to spore discharge. Leach (1975) tried to separate these factors experimentally. He was able to show that if the other two factors were kept constant, spore release would be triggered by a decrease in relative humidity from near saturation. by increasing the relative humidity from lower to higher levels and by exposing conidiphores to light. Thus, it seems that Alternaria release can be triggered by many factors, a point alluded to earlier. In terms of a seasonal peak, Sneller et (1981) were able to show that in summer, al. precipitation increased and an associated combined increase in relative humidity with average monthly temperatures increasing are correlated strongly with a surge in atmospheric Alternaria conidia. In particular, the rise in relative humidity was considered to be conducive to the growth of <u>Alternaria</u> conidia. The authors, however, do not mention that these factors also provide for increased vegetative growth of therefore providing an abundance plants, of substrate for the mould.

Figure 4 The mean bi-hourly conidia total for <u>Pyrenophora teres</u> trapped above the barley canopy as compared to leaf wetness, temperature, relative humidity, sunshine duration (h) and windspeed at the time of trapping. Values are the mean values for the period of 31 July to 31 August, 1981 (Martin & Clough, 1984:109).



#### ii) Pyrenophora teres

Moving from a spore with allergenic properties we now look at the spore Pyrenophora teres, which is a pathogen responsible for net blotch development on barley in North America. This pathogen has studied by a number of researchers been for example. Shipton et al., (1973), Matthews & Hampton (1977), Hampton (1980), Jordan (1981) and Martin & Clough (1984). Pyrenophora teres uses barley leaves as a substrate for development and thus experiences a seasonal periodicity dependent on the growth and maturation of the barley crop.

From Figure 4 it is apparent that this spore experiences a diurnal liberation with a peak at midday. Spore liberation begins increasing shortly after sunrise. Increasing temperatures, decreasing relative humidity and decreasing leaf wetness are associated with the increase in atmospheric spore content. Leaf wetness is the amount of moisture on a leaf as a percentage of full saturation, often expressed on a scale from 1-3. Thus 1 would be 33%, 2 would be 66% and 3 would be 100%, one assumes. Many authors believe this is an important factor in the production and release of spora (Gottwald & Bertrand, 1982: Petzoldt et al., 1983; Shenoi & Ramalingam, 1983; Martin & Clough, 1984; Savary, 1986). At night in relative humidity, with an increase leaf wetness also increases. A high degree of leaf apparently for the wetness is necessary of the conidia. After production sunrise. increasing temperatures tend to dry the leaves and an increase in atmospheric spore content is immediately obvious. However it is not clear from this paper what is responsible for the release of of If content the spores. the spore the atmosphere increases at sunrise then there is perhaps some photomechanism involved similar to that mentioned for Alternaria. Certainly it was not an increase in wind speed because the authors note that equal number of conidia were trapped at speeds ranging from 2.5 ms<sup>-1</sup> to 6.5 ms<sup>-1</sup>. The authors noted further. that excessively high peaks were associated with prolonged leaf wetness well into the day. A 16hr leaf wetness period was almost always followed Бу extremely high concentrations of conidia in the atmosphere. One can perhaps surmise that this prolonged leaf wetness period result in a larger than usual production of spores which when finally released caused high concentrations in the atmosphere.

46

## iii) <u>Eutypa armeniacae</u>

(

The last case study looks at another fungal Eutypa armeniacae. This is a wound pathogen, pathogen of the commercially important deciduous fruits, grape and apricot and has thus been studied extensively (Carter, 1957; English - & Davis, 1965; Moller & Carter, 1965; Carter & Moller, 1977; Pearson, 1980; Trese et al., 1980; Petzoldt et al., 1983). In the Petzoldt et al. (1983) study, these ascospores showed two well defined release seasons, one in autumn and one in while winter spring, and summer were not associated with high concentrations of this pathogen. These authors were able to show that ascospore discharge is caused by the mechanical effect of falling rain. It would seem that the splash effect of rain tends to dislodge the spores. This finding has support from Moller & Kasimatis (1971), Ramos et al. (1975), Schneideret al. (1986) (1987).Christians and Royes Petzoldt and his associates found that irrigation effect. could have the same A futher two variables, duration of surface free moisture and days from previous release, were introduced into the equation. These two variables are explained by way of the 'exhaustion factor'. After rainfall

which causes a release of ascospores, a period of time is required which should be moisture-free in E. armeniacae can reproduce. order that The longer this period, the greater the release of spores when next rain falls. Further to this, if should fall shortly after the rain previous downfall, then very few spores will be released because the fungus has not had enough time to (the exhaustion factor). It reproduce is interesting to note that the conditions causing high concentrations of the ascospore in spring and autumn, did not cause high concentrations in summer and winter. Petzoldt and his associates were not able to explain this contradiction. However, they were able to show that spore to average concentration correlated was temperature during the release period. It is possible that the average temperature in fact was associated with the production of the spores. In autumn and spring the average temperatures would have been similar, while in winter and summer, the extremes in temperature would not have been conducive to the growth of the pathogen. Thus no amount of precipitation will release non-existent ascospores during these two periods. However this hypothesis needs to be tested carefully.

In summarising this section it is apparent that spores have a far more diverse reaction to atmospheric factors than do pollen. In terms of their production some prefer dry weather while still others prefer moist conditions for reproduction. Release mechanisms are also diverse. Some conidia are released through the mechanical action of rain while others are released through the interplay of temperature and relative humidity. Yet others are released through photomechanical responses to sunlight. Extremely high concentrations in the atmosphere can be the result of an extended period of

production where atmospheric factors such as leaf wetness, and temperature which are conducive to production, persist excessive spore for an unusually long period. When conditions prevail of large for the release the Spore, concentrations are then experienced. It does seem that it is necessary to have an in depth knowledge of each mould/fungus, its production, release and dispersal and the manner in which these j s affected each one of stages bγ atmospheric factors before making any conclusions about spores as a whole group.

### 2.2.6.4 Atmospheric stability

In this instance there is no known literature to that spores and pollen suggest respond differently to temperature inversion layers, therefore pollen and spores will studied be together. Many authors have noted that pollen concentrations are not constant during a 24-hour period and that there is a diurnal variation with pollen concentration lower in the early a and a higher one in the afternoon morning, (Assem, 1973; Hyde, 1973; Fuckreider, 1976; Raynor et al., 1976; Singh & Babu, 1980; Kapyla, 1981; and Mullins, 1981). However, a number of researchers have noted that there is occasionally a high concentration of spores after midnight. (Rempe, 1937, Hirst et al., 1967; Ljungkvist et al., 1977; Bringfelt et al., 1982; Spieksma, 1983; Kapyla, 1984; Keynan et al., 1983b; Steel, 1986; Leuschner et al., 1987; Mandrioli, 1987). These same authors researched the possibility that this high concentration at night was the result of stable atmospheric conditions which, when they develop, occur mainly at night.

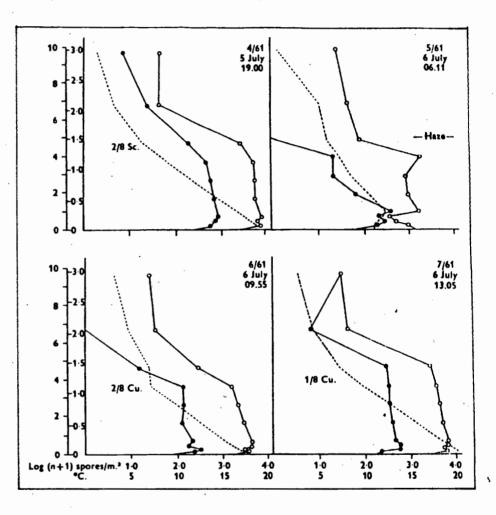
In theory, high concentrations of pollen, when an inversion layer has developed, are possible. The

mechanisms involved are as follows. The turbulent nature of the boundary layer (that layer of atmosphere closest to the ear th) the is most important characteristic of the atmosphere as regards the dispersion of large particles and is wind speed and wind generated by direction (Mandrioli, 1987). During the day the sun heats the surface of the earth. The earth in turn, through long wave radiation, heats the air in the boundary layer. As a result, vertical convection currents. occur in the boundary layer which becomes unstable. Rempe (1937) first theorised pollen earlier released is dispersed by that the the turbulence in this boundary layer and held in suspension by the convection currents. This theory has received added impetus recently by He theorises Green (1986). that particles are kept aloft through 'convective levitation', which processs similar is a in nature to that which keeps hailstones, suspended until their terminal velocities exceed that of the vertical turbulence. Accordingly, the concentration of the pollen would be uniform over a large area. In practice this is possible so long as the vertical turbulent velocities greatly exceed the terminal speed of the pollen (Steel, 1983). Only or fall when convection ceases does the fall speed of the its influence. suspended pollen exert Once thermal turbulence ceases the pollen is released into a shallow stable layer close to the earth's which turbulent surface, in vertical velocities are small. Should unstable conditions develop during the night leading  $\mathbf{to}$ temperature а inversion above this now narrow boundary layer, the pollen will become effectively trapped and will be forced to stay in this narrow mixing laver, or sediment out attheir fall speeds. (about 3 cm s<sup>-1</sup> for grass) thus increasing the atmospheric pollen concentrations close to the earth's surface. A number of researchers have

attempted to test this theory. Hirst et al. (1967) were interested in how changes in the atmospheric temperature gradient would affect the airspora profile over altitude. According to the unstable conditions researchers, in where convection was active during the day, diffusion of airspora may be such that in the first 250 the concentrations increase and metres then gradually decrease with altitiude. This is illustrated in Figure 5 (4/61). Should conditions become stable through the introduction of an inversion layer (5/61 and 6/61) such that mixing does not take place above this altitude then the concentrations rapidly decrease with altitude above the temperature inversion while remaining homogeneous in the mixing layer.

certainly Figure 5 seems to confirm the hypothesis of Hirst and his colleagues. However very few authors have been able to confirm Hirst's results. Kapyla (1984) suspected the same but was not able to demonstrate the relationship of inversion layer to high airspora an concentrations conclusively. Ljungkvist et al. (1977) and Bringfelt et al. (1982) were not able relationship to confirm this either. Only Leuschner et al. (1987) were able to demonstrate that an inversion layer caused high pollen concentrations at night. Lastly Spieksma (1983b) made a thorough study of the phenomenon. In order to prove beyond doubt that inversion layers did in fact cause higher ambient pollen levels, he argued that it should be shown that the stronger the inversion the higher the pollen concentrations in the atmosphere. Spieksma was not able to demonstrate this. In fact Spieksma demonstrated that high pollen concentrations occured on nights when there was no temperature inversion.

Figure 5 Profiles οŕ temperature 👘 (dotted) concentration of pollen (solid circles) and Cladosporium spores (hollow circles) in a sequence of ascents over Farnborough on 5 & 6 July 1961 (Hirst & Stedman, 1967:340)



Concluding this section, it seems that Spieksma is right in asserting that other factors needed to elucidated to explain high be pollen concentrations at night. Certainly there is enough evidence to suggest that nightly inversions do play a part, but other factors like the current days pollen concentrations, and relative humidity which could cause an increase in the mass of the particle, hence causing it to fall out more rapidly than its normal fall speed, need to be studied.

# 2.2.7 <u>Concluding remarks on atmospheric pollen and</u> spores

From the foregoing discussion a number of general points can be made regarding the quantitative aspects of airspora, and the relationship between airspora concentrations and meteorological variables.

According to Davidson (1941), each species sheds a) its pollen regularly at its own time of day, and often with clock-like precision. This pronouncement points to a deterministic cycle where each species will shed its pollen at a particular time of the season. As already has been argued, this is not rigidly true for spores especially and as far as pollen is concerned, there are many that believe that meteorological factors have a stronger influence on production and release of pollen than may first meets the eye. The point is, however, that each species should be looked at individually. Its production and dispersal habits should be studied carefully meteorological variables and should, where possible, be simulated in the laboratory to find out how organisms respond to the stimuli.

- b) Within this framework, the research cited to date indicates that selected meteorological variables do affect the quantity of airborne pollen and spores. This can be pre-seasonal, in terms of the quantity of rainfall which will affect the pollen producing capability of the plant, or daily, variables where such factors as relative humidity and temperature have pronounced effects on the daily concentration of airspora.
- c) A final consideration is the question of time and space in airspora monitoring. It is apparent that at any particular location on the earths surface, the proportions between spores and pollen change on a daily, seasonal and annual basis, sometimes quite dramatically. Moreover, there is not much consistency over space, with large changes according to dominant natural vegetation, quality of substrate, and dispersal agents at work. From this we can conclude that any predictive models built on the basis of data from a chosen location are restricted in applicability to that place.

3. REVIEW OF MATERIALS AND METHODS USED IN AEROPALYNOLOGY

#### 3. REVIEW OF MATERIALS AND METHODS USED IN AEROFALYNOLOGY

3.1 Particle behaviour in still and moving air3.2 Sampling devices : Advantages and disadvantages

3.2.1 The gravity method3.2.2 The impaction method3.2.3 The filtration method3.2.4 The impingement method

3.2.4.1 Liquid containing impingers
3.2.4.2 Slit sampler impingers
3.2.4.3 Sieve impingers
3.2.4.4 Solid-surface impingers

3.3 The Burkard Volumetric Sampler

3.3.1 Description of the Burkard system

3.3.1.1 The Burkard Sampler 3.3.1.2 Hidden components of the Burkard sampler

a) The sampling drumb) The timerc) The vacuum pump

3.3.1.3 The Burkard stand 3.3.1.4 The galvanised iron box 3.3.1.5 The flex

3.4 Airspora concentrations - a sampling artefact

3.5 Sampling : Distance distortion

3.6 Sampling : Vertical distortion

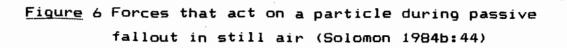
3.7 Plates showing Burkard system

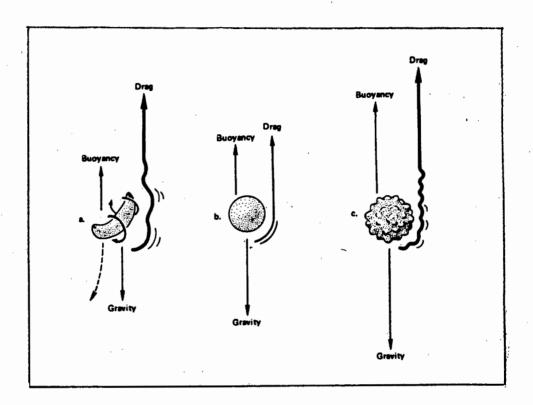
## 3. REVIEW OF MATERIALS AND METHODS USED IN AEROPALYNOLOGY

Direct sampling of the air has not yet been replaced as the most effective way of measuring quantitative airspora content of the atmosphere. This is particularly pertinent when one considers, in the case of fungi, that a bewildering array of species can be observed in an agar culture, yet most will contribute little to atmospheric loads. This is also the case for pollen, where only a limited number of species rely on atmospheric dispersal for the purpose of sexual reproduction. A review of current literature quite clearly indicates that there is no universally "ideal" or accepted strategy for the collection of aerosols and, more specifically, airspora. This observation is corroborated by Solomon (1984a:143) who states that "...it is clear that each approach 'selects' a characteristic spectrum of biogenic agents for investigative attention." Apparently then the type of research being conducted determines the selection of a suitable method. If one adopts this attitude then collection techniques only differ insofar as one is more appropriate for the particular task at hand than another. In this sense it would be best to discuss the relative advantages and disadvantages of each collecting device, particularly as the many misconceptions that exist in aerobiology, Solomon (1984a) points out, can be traced to deficient or misapplied collection techniques. It is necessary, however, to enter a short discussion on the nature of particle behaviour in still and moving air. Once the basic theory of particle behavior has been elaborated upon, one is in a better position to discuss the relative merits of the different collecting techniques and qualify why the Burkard sampler was chosen for this research.

## 3.1 Particle behavior in still and moving air

Starting with the ideal but unrealistic notion that air is still, a particle if introduced to the air will experience <u>gravitational acceleration</u>. Combating this acceleration would be the combined effect of <u>drag from</u> the <u>surrounding air molecules</u> and the <u>innate buoyancy of</u> the particle itself. As fall velocity increases these 3





vector forces reach an equilibrium, the particle acceleration ceases and it continues towards the surface at a fixed velocity. This model assumes a uniform shaped particle and a still atmosphere.

If we now introduce the concepts of volume and shape the picture becomes more complex. The volume of a particle, affects the degree of bouyancy experienced by that particle such that the smaller the volume of the particle, the more bouyant that particle. Shape on the affects the drag which the air molecules other hand an accelerating particle. Thus ideal an apply to particle would be spherical with a smooth surface which allows it to "slip" through the air with little interference from air molecules. Figure 6 demonstrates which the equilibrium the manner in between acceleration. bouyancy and oravitational drao is affected by volume and shape of the particle. Drag is minimal for an idealised smooth spherical unit (b) but increases with greater surface roughness (c). the tumbling motion of an asymmetric particle (a) raises drag. In addition, such particles tend to "yaw" during free fall, as the dashed line indicates.

However, it is possible to determine the terminal velocity of a particle by invoking Stokes' settling velocity, mentioned earlier. The settling velocity is important because it is a determinant of fallout potential and further it can also predict behaviour at vertically placed obstructions. In both cases this has implications for the use of gravity collectors and the vertical impaction collectors. The following equation, as modified by Solomon (1984b) can be considered to be Stokes' law.

Vs \_ Settling Velocity
D = Particle Diameter
g = 980 cm/sec<sup>e</sup>
 part = particle density
 f air = density of still air
 4 = viscosity of still air

Theoretically, a spherical particle of 20 microns will fall out 100 times more rapidly than a particle of 2 microns, assuming they have an equivalent density and surface area. It is evident from the equation that the overriding factor on settling velocity is particle size, (particle diameter, D), as mentioned by Solomon (1984b). This has important repercussions for any collector which relies on gravity because any form of turbulence in the atmosphere must mean that the smaller particles have less chance of reaching the collector. At this point it necessary to introduce the concept of a is thus turbulent atmosphere and the horizontal acceleration caused by wind. If an instantaneous horizontal acceleration is applied to a particle it will stop ("stop distance") after gravity and drag have acted on it. This "stop distance" is proportional to the unit's initial velocity and to Vs which is the product of Vs/g and velocity. This relationship would be adjusted to reflect drag forces but in general, with increasing particle size, stop distance increases exponentially. Practically speaking, this means that particles with relatively large stop distances are less likely to change direction for an obstacle. Thus, large particles are less likely to avoid interception by a vertical impact collector while smaller particles are more

readily redirected by air flow around obstructions, thereby tending to avoid interception. However, it can also be said that smaller particles will also change direction to enter a suction device, such as a Burkard volumetric sampler, far more easily than larger particle types. This line of argument poses the interesting hypothesis that on a windy day small particles are less likely to be intercepted by a suction device, while on a still day with little turbulence, big particles, with their greater fallout potential, will sink rapidly to the ground while the smaller particles will remain bouyant in the atmosphere for a longer period and show a greater potential for being intercepted by a suction device.

#### 3.2 Sampling devices : Advantages and disadvatages.

Bearing in mind the above discussion on particle behaviour, it is now necessary to define clearly what was required of a the sampler in this research. In this project the following regirements were projected:

- a) The sampler was reqired to sample the atmospheric pollen and spores.
- b) The sampler was required to sample a fixed, volume of air in order that atmospheric pollen and spore concentrations could be computed.
- c) The sampler was reqired to be an all-weather model, capable of resisting a damp and cold Cape Town winter.
- d) Wind velocity and wind direction were considered to be important atmospheric variables, therefore the sampler was required to be sensitive to these variables. It therefore, needed to be in possession of a wind vane. Further to this, the collecting orifice of the device had to point into the wind (thus making it sensitive to wind speed) and

therefore, the collecting orifice could not be located at the top of the machine ie. vertically orientated in nature.

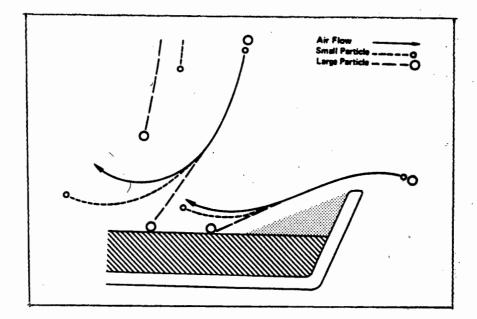
- e) The sampler as far as possible was required to be sensitive to the vagrancies of particle behaviour viz. there different sizes, terminal velocities and stop distances (all of which have been defined and discussed above). The reason for such a broad requirement can be found in the nature of the research undertaken. If one of the fundamental aims is to construct an airspora spectrum, then it is necessary to sample all possible atmospheric spores and pollen.
- f). Lastly, the chosen sampler was required to operate continuously for a weekly interval, without supervision or servicing. The reason for this was to reduce travelling time to the collecting site and therefore also to reduce operating costs.

Bearing in mind the above requirements and the discussion on particle behaviour it is now necessary to compare the available devices. According to Keynan et al. (1986) there are three ways of sampling airspora. Solomon (1984b) adds a fourth method, the impingement method.

- a) Gravity
- b) Impaction
- c) Filtration
- d) Impingement

What follows is a brief description of each of the different methods emphasizing the methods strength and weaknesses. This is followed by a more in-depth analysis of the sampler used in this project - the Burkard Volumetric sampler, justifying its selection and explaining its operation.

Figure 7 Deposition of "air spora" on an agar surface by a moving air stream. The relationship of increasing particle size to progressively greater fallout potential is suggested at the left. Toward the right a moving air stream deposits particles, from eddies, in accordance with their stop distances so that mainly larger units are recovered. With rapid air motion the lip of the culture plate creates a particle free "shadow" (stippled area) over part of the collection surface (taken from Solomom 1984b : 47)



#### 3.2.1 The gravity method

The gravity method, as the name implies, relies upon the action of gravity on airspora, forcing the earth's surface where they them to are collected on a slide or in a dish of culture media. It is the most simple and least costly of all the methods and has been in use since the time of Miquel and Pasteur (Gregory, 1973). The advantages of gravity collectors lies in their simplicity, independence of power sources and they are generally maintenance free. However, the fundamental limitation of the gravity collectors is their inability to determine the volume of air that contributes particles to a sample. Further, it is fact that the real atmosphere a is constantly in a state of flux and deposition of particles is often the result of eddies caused by turbulent air in the atmosphere or turbulence caused as the result of the margins of the slide itself. Without a volumetric common denominator it impossible to differences is assume in recovery (Solomon : 1984b). These factors are particularly prevalent where the open culture are used. The larger "walls" dishes of the collecting dish create deposition "shadows" that vary with air velocity and turbulence and reduce particle recovery (See Figure 7). Figure 7 also demonstrates how this collecting method is biased favour of larger particles. As mentioned in earlier in this discussion, settling velocity has an exponential relationship with particle size, so that any method which relies on gravity as a means of collection must be biased toward the larger particles. This assessment is iп fact corroborated by Sayer et al. (1969) who were able to show the size dependent bias of the gravity compared collectors when they the Andersen volumetric sampler to the gravity culture plate

Figure 8 Hyde's gravity sampler (Hyde, 1950: 398). The slide is held horizontally in a metal clip and is protected from rain by the disk above it. A certain amount of turbulence is required in order for the airspora to be 'captured' on the slide

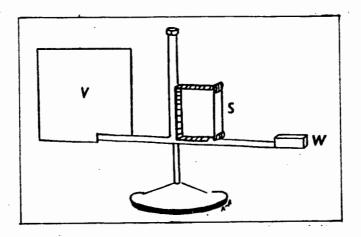
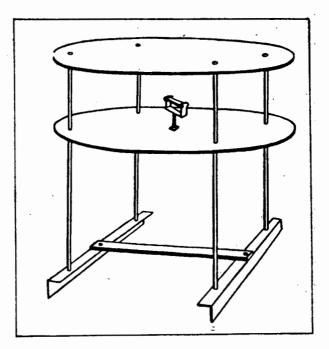


Figure 9 Hyde's aeroscope. S: Slide held on wind vane in vertical position. V: wind vane; W: counterpoise. (Hyde & Williams, 1945:87)

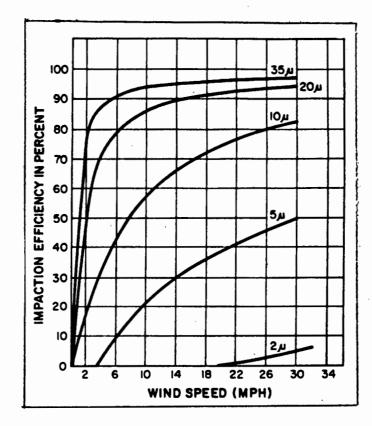


method. A futher criticism of the method lies in the size of the collections. Typically they are rather small and sampling error is thus usually substantial. such that the less abundant spores may be absent from a sample. Figure 8 illustrates one of the earliest gravity samplers used by Hyde (1950) in 1942.

This method is still used extensively today, particularly where the emphasis is on sampling the different type of pollen and spores in the atmosphere. The nutrient dish or petri plate method seems to be particularly popular amoung researchers who sampling are spores. The following list indicates that the gravity method is still in use amoungst researchers. (Gravesen, 1972; Stalker & Moore, 1972; Collins-Williams et al., 1973; Lumkins et al., 1973; Al-Doory et al., 1980; Al-Tikriti et al., 1980; Sneller et al., 1981; Verma et al., 1981; Chaubal & Kotmire 1982; Shenoi & Ramalingam, 1983; Chen, 1984; Lawande & Onyemelukwe, 1984; Fitt et al., 1985; Hurtado & Riegler-Goihman, 1986a; Okhuoya, 1986; Reid et al., 1986; Eversmeyer & Kramer, 1987; Hurtado & Reigler-Goihman, 1987; Infante et al., 1987)

#### 3.2.2 The impaction method

There appear to be two methods of impaction in common use. The first method employs a vertically standing slide which is pointed into the wind by a vane. This method relies upon wind velocity to impact airspora onto the slide. The apparatus required to achieve this task is similar to that illustrated in Figure 9 and is often referred to as a "slide impactor", or an "aeroscope".Several workers have indicated that the probability of Figure 10 Theoretical impaction efficiencies for unit density particles encountering a surface in air moving at different speeds. The probability of impaction is quite low for the smallest unit and increases rapidly with particle diameter and stop distance (Harrington et al. 1959)



impaction is dependent on the terminal velocity and relative air speeds of particles, and varies the width of the target inversely with (Harrington et al., 1959; May, 1967; Gregory, 1973). Although volumetric potential is difficult compute and more sophisticated methods  $\mathbf{to}$ probably give more accurate results, it is still possible to compute volumetric potential for the aeroscope. A totalizing anemometer (wind speed gauge) is used in conjuction with the aeroscope to measure the wind velocity during exposure periods. The volume of air processed may be calculated as follows:

Volume = average wind speed (cm sec<sup>-1</sup>) X time (sec) X sampling area (cm<sup>2</sup>)

Where :

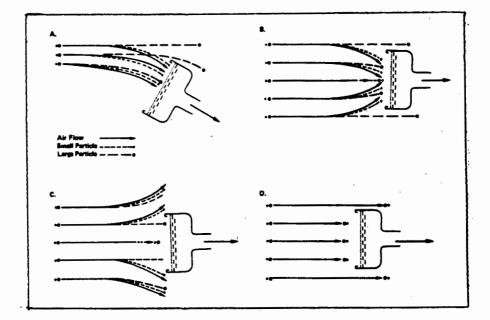
Area = slide width (cm) X diameter of the slide (cm)

The efficiency of this method increases with both particle size and wind velocity, as Figure 10 illustrates. Advantages of the aeroscope are its low cost and simple serviceability, while its greatest disadvantage is its dependence on wind velocity and its bias toward sampling larger particles. This has obvious implications for aerobiology where there is a broad spectrum in sizes of airspora. Further, on still days one assumes that few airspora would be captured and this would not necessarily be representative of the quantitative status of the airspora in the that time. Nonetheless, the atmosphere at aeroscope has seen recent use by researchers such as Pateria & Sahu (1982), Satpute et al. (1983), and Kumar (1985).

The second impaction method, a volumetric method, relies upon the impaction of airspora onto a

sticky surface through the regular movement of a rotating arm. This method is now referred to as the "rotoslide" method and was developed by Ogden & Raynor (1967) and improved upon in 1970 where it was modified and called the "swing-shield sampler" (Ogden et al., rotoslide 1974). A similar innovation, and in wider use according to Solomon (1984a), is the "rotorod sampler" which operates on the same principle as the rotoslide sampler. A simple rod that is coated with a silicon adhesive is whirled rapidly in a circular path to standardize air speed and to eliminate wind direction as a variable. The rods are 1,3 mm in width and are rotated at 2400 rpm by a DCpowered engine. These electric engines have timers incorporated that facilitate intermitent operation for 1 minute out of every 10. Solomon arques that this method produces the most consistent results provided wind speeds remain below 15mph. However the air streams caused by the movement of the rods through the air tend to divert particles away from the catching surface. Thus, regardless of wind velocity impaction of particles is strongly correlated with particle diameter. According to Solomon (1984b), particles that are smaller than 5 to 8 m in size are not consistently trapped by the rotorod sampler. Studies focusing on more minute spores need to rely on the superior collection characteristics of the suction devices. The rotoslide/rotorod devices are, however, popular with researchers. They are relatively easy to operate, are little affected by wind speed and wind direction and they capture consistenly all but the smallest particles. The rotoslide device has been used by Salvaogio & Seabury (1971), Stalker & Moore (1972), Reiss & Kostick (1976), and Fairley & Batchelder (1986). However the rotorod device has proved to be more popular with the following researchers making use of this device. Salvagio

Figure 11 Behaviour of small and larger particles at the intake of a suction device. A. Orifice is misaligned with respect to wind direction. B. Intake is windorientated, but intake speed is well above that of the free stream. C. Intake is wind orientated, but intake speed is well below ambient rate. D. Intake is wind orientated, and speeds at intake and in free stream are equivalent(isokinetic) (Solomon 1984b:53).



et al. (1971), Anderson, et al. (1978), Buck & Levetin (1982), Mallaiah & Rao (1982), Reddi & Reddi (1985), Fischbach (1986), Okhuoya (1986), Savary (1986), Dhorranintra et al. (1987), Eversmeyer & Kramer (1987) and Tilak & Babu (1987).

## 3.2.3 The filtration method

This method relies on air being drawn through a filter by a pump for a measured period. The filters are microscopically then viewed Ьу dissolving the filters and processing the resuspended material. As with other suction devices, the filter surfaces must be orientated towards air flow. Solomon & Gilliam (1970) were able to show that filter samplers directed at right angles to air flow collected no more than a small percentage of the spores collected by a wind orientated unit. Inequalities in air speed between the filter inlet and and the surrounding flow (wind) also affects collection air efficiency. This is illustrated in Figure 11.

Figure 11 reveals that the ideal situation can be found in diagram D. Here inlet and ambient air speeds are equal and the flow lines are thus not distorted at the mouth of the collector. Thus the full range of air particles can now be colected. This is, however, a difficult situation to reach because the inlet air speed is set at a fixed rate by the pump, while the ambient air speed varies according to weather conditions. Clearly, the ideal of matched ambient air speeds and inlet speeds cannot be met unless some method can be devised whereby inlet air speeds vary with the natural variation in ambient air flow. In diagram A of Figure 11 particles are lost because the monitor is not aligned with the wind flow. In diagram B, the inlet speed exceeds the wind speed

and thus flow lines tend to converge on the orifice. The smaller particles tend to diverge from the original flow paths thus giving higher concentrations while the larger particles are not diverted, tending to maintain their predetermined directions. In diagram C, inlet speed is below ambient air speed. The orifice region thus stagnates and the smaller particles tend to follow the deviated flow lines and thus escape collection, the larger while particles with longer stop distances proceed in a straight line into the orifice, causing artificially high recoveries. To minimise these errors Solomon (1984b) suggests that the device should at all times be orientated into the wind, inlet speeds should be approximated such that they match average wind speeds and orifices should have relatively large collecting areas.

An example of a filtration device is the AISI sampler which was tested by Keynan et al. (1986). The sampler sucks air at a rate of 7 litres per minute through a strip of filter paper. According to Keynan and his associates the advatages of the sampler are :

- a) The sampler is fully automatic and can be operated for a period of over a month without servicing.
- b) The number of pollen grains trapped in each sample spot, even for short periods of time, is high.
- c) The sampler retains the composition of pollen mixture in spite of the variation of size and pollen number.

The sampler, however, suffers the same airflow problems that affect most devices using an

orifice to direct air flow. Further, should 24 hr samples be required, the machine would have to be serviced everyday.

#### 3.2.4 The impingement method

This method, according to the literature surveyed, appears to be the most popular method adopted by researchers. Most of the devices which use the impingement method adopt a two-stage process. Initially a measured volume of air is sucked into the device. As the particles enter the orifice they are accelerated and impact onto a suitable collecting surface. The probability of is dependent the particles being captured on particle size, acquired speed and the strength of the adhesive properties at the impingement point. As with the filter devices the factors affecting the intial stage of collection are identical.(see Figure 11). Solomon (1984b) comments that due to the acceleration of the particles through the orifice, impingement regardless of size of particle, takes place in most cases. There are 4 types of impingement methods currently in use. Each is separately and briefly discussed before embarking on a more detailed analysis the of Burkard volumetric sampler.

## 3.2.4.1 Liquid-containing impingers

These impingers are perhaps the least expensive and simplest to operate. Air is drawn in through an orifice where it is accelerated through a narrow glass pipe into a well-mixed and defined volume of fluid. Once collected the particles may be viewed microscopically. However since the inlets of these devices lie in a horizontal for use out plane, they are not suitable of. doors. As yet no attempt has been made to make these impingers wind responsive and thus they are best suited to calm air applications. One of the disadvantages of the liquid impingers can be found in the stresses placed on clusters of spores and pollen due to the acceleration of the particles in the glass tube and subsequent immersion in the fluid. These clusters then tend to break up into seperate spores and pollen which tends to hide an important indicator of spore dispersal.

## 3.2.4.2 Slit samplers

In this method, particles are impacted onto a suitable sterile medium. Growth points are used to estimate prevalence as colony-forming units (CFU) per unit volume of processed air (Solomon, 1984b). Particles pass through a narrow intake slit and are then accelerated onto a medium in a rotating culture dish. The turntable rotates at a fixed speed and can be varied from one rotation in 30 seconds to one in 5 minutes. This method has been adopted in 3 different slit samplers :

a) The BIAP slit sampler (Larson, 1981)

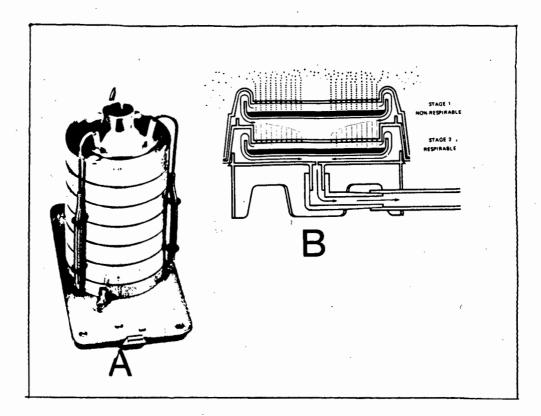
- b) The FOA slit sampler (Bergstrom, 1975)
- c) Casella slit sampler (Henningson et al., 1981)

As is the case with other suction devices, the airflow problems described in Figure 11 are also applicable to the slit samplers.

#### 3.2.4.3 Sieve impingers

This method was originally introduced by Andersen (1958). The sampling device consists of a series

Figure 12 Andersen Sieve impingers. A. Six-stage device. B. Air and particle behaviour during operation of the two-stage device. (In Solomon, 1984b : 61)



of stainless steel plates with holes of diminishing size stacked serially. (See Figure 12 Diagram A and B). Anderson's sampler consisted of

eight, six ot two sieve plates as indicated in Figure 12. Beneath each plate ("stage") a culture dish may be positioned to capture approaching particles.

The theory behind the sieve impinger is based on the fact that different particles have different stop distances. In Figure 12, diagram B, the larger particles with longer stop distances are accelerated through the holes of the first sieve and because of the larger stop distances defy the airflow to the next sieve and impact onto the first plate. Because the holes in the first plate are large, the smaller particles are not accelerated to the same extent as the larger particles and thus pass through the holes and follow the air flow to the next sieve. (Their stop distances are smaller and thus they avoid the first plate as well). This process is repeated at the next sieve and so on until the last sieve. The end result is a gradation of particles, with the smallest particles in the bottom culture dish and the largest particles in the top dish.

It is claimed by Andersen (1958) that retention of the particles by the multistage sieves is high (>95%). However, a definite disadvantage of these collectors is the time required to "read" each microscopically. This problem dish may be overcome using a limited number of sieves and dishes. Further, like other samplers with fixed orifices, the intake efficiency of these devices is jeopardised if exposed to rapidly moving air, although apparently there has been an attempt to mount the device on a wind vane with the orifice

pointing into the wind (Solomon, 1984b). The Andersen sampling device has been successfully used recently by Beaumont et al. (1985).

## 3.2.4.4 Solid-surface collectors

This particular form of impingement relies on a fixed volume of air being drawn across a sticky surface by an air pump. However, before assessing the Burkard sampler, (a solid surface collector), it is necessary to study its principle oppositon in this category - the Kramer-Collins sampler. The Kramer-Collins drum sampler samples air at a fixed rate of 101 min<sup>-1</sup>. Inside the device is a drum, similar to the Burkard drum to be discussed later, which has a tape atttached to it, coated with petroleum jelly. The drum rotates once evey 7 days after which the tape is removed, processed and then read microscopically. It is very similar to the Burkard volumetric sampler and like the Burkard sampler, it is able to give aispora counts in concentrations  $m^{-3}$ . As far as this author is aware it is not attached to a wind vane and is thus not sensitive to the vagrancies of air flow, a serious flaw if sampling is taking place out of doors. It certainly has not proved to be as popular as the Burkard device but has been used by Kramer et al. (1976), Hilderbrand & Sutton (1982), Martin & Clough (1984) and Massey & Fournier-Massey (1984).

#### 3.3 The Burkard Volumetric Sampler

Having elaborated on the samplers which were not chosen for this research on the basis that they did not meet the requirements listed in paragraph 3.2, it is now necessary to study at some length the Burkard volumetric sampler which is a sampler belonging to the solid surface impinger category. This sampler has its origins in the sampler first developed by Hirst (1952). The

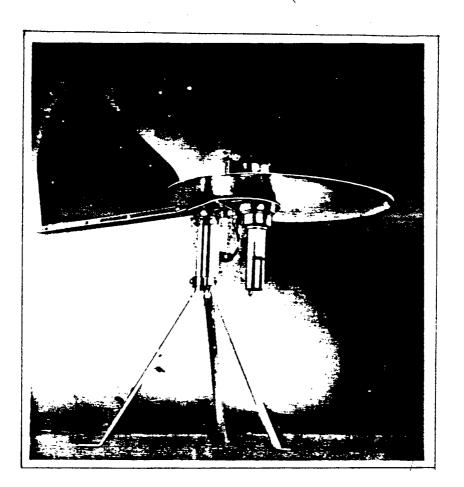


Figure 13 Hirst Spore Trap (Hirst, 1952:258)

priginal model sampled a fixed volume of air at an approximate rate of 101  $\min^{-1}$  for a 24 hour period. It was then possible to calculate the number of airspora m-⇒ of air given the area of the slide. Figure 13 illustrates the early model. This sampler has seen recent use by Mcdonald (1980), Reddi et .al. (1980), Mallaiah & Rao (1982), Spieksma (1983), Steel (1983), Halwagy & Halwagy (1984), Schneider-Christians et al. (1986), Spieksma & Tonkelaar (1986), Macchia et al. (1987) and Royes (1987). Notwithstanding its continued use, the original Hirst spore trap has a Severe limitation in that it was capable of sampling for only a period. The subsequent Burkard volumetric 24 hour sampler (Solomon, 1984b) which is an improvement on the Hirst spore trap and operates on the same principle is able to collect for a 7 day period and according to the literature surveyed proved to be the most popular machine. This sampler will now be dicussed in detail.

Bearing in mind the criticisms of Solomons (1984a) and Keynan et al. (1986), the Burkhard trap remains the most popular type of sampler. Ljungkvist et al. (1977), Pennycook (1980), Bai & Reddi (1981), Kapyla & Penttinen (1981), Nilsson & Persson (1981), Bringvelt et al. (1982), Gottwald & Bertrand (1982), Mercuri еt al. (1982), Nilsson & Palmberg-Gotthard (1982), Frenquelli et al. (1983), Petzoldt (1983), Shenoi & Ramalingham (1984), Kapyla (1984), O'Rourke & (1983), Hasnain Lebowitz (1984), Anderson (1985), Ballero et al. (1985), Spieksma et al. (1985), Machardy & Gadoury (1986), Spieksma (1986), Al-Eisawi & Dajani (1987), Galan et al. (1987), Logo & Cristofolini (1987), Schutze-Werninhaus al. (1987) and Sutra et al. (1987) have a11 et successfully demonstrated the reliability and applicability of this sampling device. However in the context of this study it reliably fulfills at least 5 of the criteria listed in paragraph 3.2. Firstly, it samples both pollen and spores (Anderson, 1985). Secondly, it samples a fixed volume of air such that volumetric concentrations of airspora can be computed

(Solomon, 1984b). Thirdly, it is a sturdy construction, designed in such a manner that it is watertight in the and particularly in the airspora mechanical areas collecting area. It is thus suitable for use in the wet winters of the Cape. Fourthly, the sampler has a wind vane thus making it sensitive to changes in wind sampling orifice direction. The also lies at right angles to the general airflow, making it sensitive to changes in the wind speed. Fifthly, it is not known at this stage whether the Burkard samp]er tends to be biased toward any pollen or spore type in terms of selectively sampling some airspora while ignoring others. Reference is made to the aerodynamics of the collecting orifice which influence the type of airspora can collected, depending on wind velocity and the speed at is been drawn through the orifice. (This which air paragraph 3.1 it problem was discussed in and is acknowledged that the problems referred to in that section are doubtless also applicable to the Burkard sampler). Sixthly, the Burkard sampler is capable Df sampling continuously at 24 hr intervals for a 7 day period without requiring serving. What follows 15 а detailed description of the Burkhard sampler, its intallation and its operation.

## 3.3.1 Description of the Burkard system

Two Burkard volumetric samplers were used in this research project. (The reasons for this are outlined in the next chapter dealing with the and design methodology research of this dissertation). The sampler located in Epping (Plate 1) is battery operated while the sampler at Bothasig (Plate 2) is a 'mains' operated sampler. Besides these differences the two identical. The Bothasig sampler is samplers are placed on a simple stand 1,5 meteres high and is connected to the mains of an adjoining house, while the Epping sampler proved more difficult to

The Burkard system encompasses the four main components of the Epping battery operated Burkard sampler. These four components are illustrated in Flate 1. The sampling device (A) is mounted on (B), the stand. A galvanised iron box (C), containing an adaptor, trickle charger and 12 volt battery is connected by two terminals to the Burkard sampler. The trickle charger is connected to a security light via a flex (D).

#### 3.3.1.1 The Burkard sampler

Plate 3 illustrates that there are 6 component parts to the Burkard sampler itself. The wind vane (A) keeps the device pointing into the wind. Within the casing (B) are found the sampling drum The sampling orifice (C) and timer. has a dimension 14 X 2 mm and directs the air flow onto the sampling drum. A plate (D) prevents rain from falling directly onto the nozzle and being sucked into the drum casing (B). The casing (E) acts both as a protective shield to the electric motor and an exhaust for the air sucked through the casing (B). The rotating drum is secured to a timer located on (F) which extends into the casing (B). The Legs (G) secure the sampler on the stand.

## 3.3.1.2 Hidden components of Burkard sampler

#### a) The sampling drum

The sampling drum (Flate 4) has a circumference of 345 mm. Melinex tape is fitted onto the circumference of the drum. Vaseline is then painted onto the melinex tape. This is the medium in which the airspora are caught. The drum is secured to the timing device with a retaining nut (C). The drum must be aligned such that the mark (A) is under the pointer. The mark (B) will then

(C). The drum must be aligned such that the mark (A) is under the pointer. The mark (B) will then be facing the inlet nozzle. The timer will turn 355 degrees over a 7 day period in a the drum clockwise direction. The remaining 5 degrees is a precautionary measure; should the operator arrive after the 7 days the drum will cover the remaining 5 degrees in four hours before intruding on the first days sample.

b) The timer

is a simple clock mechanism with The timer an extruding shaft onto which the drum is attached. To the left of the timer (Plate 6) is a small rotational disk which covers the fine adjustment setting. This enables the operator to speed up or retard the timer so that the drum rotates the required distance. In Plate 7 the key is used to timing device, thus making i t wind the operational for a 7 day period.

## c) The vacuum pump

Plate 8 and 9 show the two different vacuum pumps which constitute the major difference between the In both cases the protective two samplers. exhaust coverings have been lowered to facilitate a clearer view of the two motors. The electric motor (Epping sampler) runs off a 12 volt motor To the left of the motor (A) is vehicle battery. potentiometer (B), which can increase the а revolutions or decrease the revolutions of the motor, thus altering the volume of air being drawn in by the motor. The motor operates a small fan which inhales the air. This fan is hidden in the cowling above the motor. In Plate 9 the mains operated motor is bigger and does not have а potentiometer attached to it. Instead the volume of air sampled is calibrated through the use of a

## 3.2.1.3 The Burkard stand

The stand (Plate 1) for the Epping sampler was especially designed for the Burkard battery operated sampler in Cape Town conditions. It is standard piece of equipment. not .3 The construction is built of angle-iron that has been welded together and painted with an anticorrosive paint. It stands 1,65 metres high and weighs 45 kilograms. The construction is pyrimidal, thus facilitating triangular and mobility and strength. The "feet" are held to the ground by tent pegs, preventing the structure from toppling over in gale force winds. The stand used for the Bothasig sampler is on loan as is the sampler, from the Cape Town Municipality. The stand is made out of galvanised steel and is less sturdy but easier to move about.

## 3.2.1.4 The Galvanised iron box

The box housing, (A) the adaptor, (B) battery charger and (C) 12 volt wet cell battery, are galvanised, thus preventing rusting and they are also water-proofed with bitumen to prevent rain the electrical circuits entering causing dangerous short circuiting. The adaptor connects the security light to the Tesla battery charger. The security light operates on a photo cell and thus switches on automatically at sunset and off at sunrise. The current is 220 volts and is transformed into 12 volts by the charger, while the amperage can be varied by the adjustor at the bottom of the charger. The battery, a 12 volt Sabat model, is connected by two terminals to the

the box is locked to prevent theft of its contents.

#### 3.3.1.5 The flex

The flex of the Epping sampler is a 3 core type, bound with an ultra-violet resistant plastic. It is connected to the base of the security light and lies on the lawn of the site. (see Plate 1).

#### 3.4 Airspora concentrations - A sampling artefact?

The conclusion to be drawn from studies of different sampling devices is an important guiding principle. Clearly, the effiency of the different samplers is not homogeneous. Some samplers are biased towards sampling the larger particles while some of the samplers are capable of capturing larger concentrations of particles. This has broad implications for building predictive models, especially where the aim is to quantitatively assess critical values of allergenic pollen concentrations. If different devices give different concentrations. then how is it possible to take seriously critical values of atmospheric inhalants when the samplers are not in agreement on atmospheric concentrations of the inhalant? Henningson et al. (1981:159) summarise their research on a comparative study of apparatus for sampling airborne microorganisms:

"The conclusion of this study is that careful planning is necessary before sampling airborne micro-organisms in various enviroments to ensure that relevant and reliable results are obtained. This work also shows the importance of choosing the right ... samplers for each occasion and of carefully considering their limitations." <u>Table</u> 3 Comparisons of mean spore concentrations m<sup>-3</sup> air sampled during three 10 day periods at each site in 1976. (Kramer & Eversmeyer, 1984:120)

> Means for each 10-day period are based on counts made for eight 3-hr periods for each day for the ten days. Means in the same row followed by the same letter are not significantly different (p=0.05) based on Duncan's multiple range test; comparisons between 10-day periods are not made.

	Upland Prairie		City Park		New Suburban		River Bottom		% Between-sit differences	
Cladosporium						:			11	
		-				/				
Period 1	10	277bc	21	062a	12	671bc	17	283ab		/
Period 2	10	120a	9	570a	11	067a	13	466a	•	
Period 3	4	068a	4	600a	4	456a	3	138a		
Ascospores									17	
Period 1	2	548a	3	734a	1	600a	3	016a		
Period 2		914a	1	070a	1	854a	2	152a		
Period 3		380ъ	1	813a	1	578a	1	887a		
Basidiospores									17	-
Period 1	2	402a	5	609a	1	994a	4	339a		
Period 2		978a	2	064a	1	876a	1	312a		
Period 3		81b	1	425a	3	162a	1	289a		

٩.

A further consideration when using samplers is there applicability over space in both the horizontal and vertical plane. The next two paragraphs deal with this problem

#### 3.5 Sampling : Distance distortion

Kramer & Eversmeyer (1984) make the point that often only one spore trap is used to make inferences about the airspora concentrations of the surrounding area. Their study concentrated on the validity of these inferences. They chose sites in and around Manhattan, Kansas, ranging in distance from 3 km - 25 km from the city centre. Table 3 represents their results for spore concentrations for four sites. The results demonstrate conclusively that it is an erroneous practice attempting to apply airspora concentrations to too broad an area. Studies by Hyde (1952), Davies (1969), Raynor et al. (1973), Stix & Ferritti (1974), Raynor et al. (1975), Brown & Jackson (1978), Leuschner & Boehm (1979). Spieksma (1980) and Strandhede & Wihl (1981) show that concentrations can vary over a matter of metres. Notwithstanding the above, it is of interest to note that the study by Strandhede and Wihl shows that the pollen counts for Malmo and Copenhagen tend to differ quantitatively although the same type of airspora were findings have fairly measured. These serious implications for the construction of predictive models in the area of allergy related forecasting. Eversmeyer and Kramer (1987b) were not able to find any significant diferences in mean spore concentrations for samplers at the same height but 2 metres apart. They conclude:

"A single volumetric sampler will adequately measure spore concentrations for powering simulation models. but care must be taken todetermine the location of the sampling unit in relationship to spore source and ecological and environmental conditions.". (Eversmeyer 8 Kramer, 19875:109)

The work by Spieksma (1980) is also very illuminating in this regard. He clealy accepts that distances tend to distort the consistency of the pollen catch but also, with reservations, states that one sampler is adequate for simulation models.

"Nevertheless, the general seasonal pattern for the pollen types found in relatively high concentrations shows a great similarity, at least between places within a region with the same climate. This similarity becomes more distinct when the daily pollen count is transformed into a '10-day running mean' presentation.". (Spieksma, 1980:600)

It seems then that horizontal differences occur both over limited and extended distances, although the work by Eversmaeyer & Kramer (1987b) tends to contradict this. However, the two quotations above perhaps put into perspective the relevance of these horizontal differences. Provided that the region for which the simulation model is being prepared, has a reasonably homogeneous climate, then any daily differences tend to average out over a day period when converted into mean scores. This seems to be the key to the applicability of simulation models. It can be concluded that the region being sampled must have a homogeneous climate; quantitative differences in airspora concentratiopns will then average out, Bias caused as result of ecological and environmental conditions, such as dense stands of wind pollinated Acacia in the Cape Town case, should be reduced by placing the sampler so that these conditions are minimised.

## 3.6 Sampling : Vertical distortion

The earlier literature review indicates that research has shown vertical gradients in airspora concentrations is dependent on atmospheric stability. That airspora

concentrations vary over vertical distance is beyond dispute. Even within the first 30 metres of atmosphere (1984a) found significant differences in Lyon et al. spore concentrations. Earlier work by Raynor et al. (1973) indicated that ragweed pollen concentrations vary to a height of 108 m but the variation is negligible over long periods of time. However, the work by Lyon et al. (1984a) and Raynor at al. (1973) indicated that the vertical placement of the sampler cannot be left to . random choice, but needs to be carefully considered. The day to day variation was apparently dramatic. Other work by Reddi et al. (1980) and Eversmeyer & Kramer (1987b) tends to substantiate the impression that concentrations vary with height. These findings must have significant implications for allergenic "high-rise" dwellers, where concentrations are often more pronounced at higher altitudes. Two important points become apparent, bearing in mind the above discussion.

- a) Follen and spore concentrations are indicators of the type of sampling device as much- as they are indicators of particular meteorological conditions.
   A researcher should be aware that the estimated concentrations are not absolute. Depending on equipment used, they may be significantly different.
- ь) Pollen and spore concentrations are distorted over thus exceeding caution must be shown space. in determining the spatial range (horizontal and vertical) of the estimated concentrations. In order to successfully achieve this task, a researcher should sample carefully the pre-determined range and the data<sup>)</sup> with the then correlate central site concentration. No other method seems available to predict with confidence the airspora concentration from a large area.

## 3.7 Plates showing Burkard sysytem

Plate 1 The Burkard system Plate 2 The Burkard system (Epping)

(Bothasig)

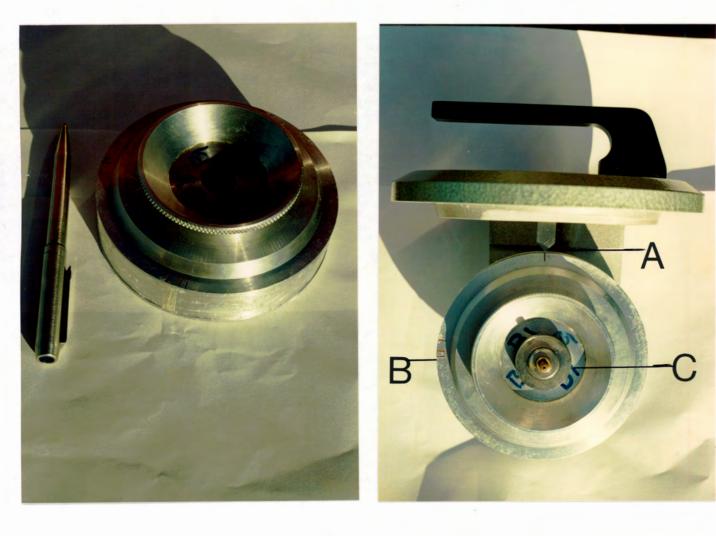


## Plate 3 The Burkard sampler



## Flate 4 Sampling drum

Plate 5 Sampling drum installed on timing device



# Flate 6 The timer

# Plate 7 Timer : Winding mechanism





<u>Plate 8</u> Vacuum pump (battery operated)

Flate 9 Vacuum pump (mains operated)



Plate 10 Components of box on Epping sampler stand.



# 4. RESEARCH METHODOLOGY

## 4. RESEARCH METHODOLOGY

- 4.1 Sampling location
- 4.2 Sampling strategy
- 4.3 Laboratory method and microscope Work

4.3.1 Method 1 : The non-chemical process

- a) Traverse along the length of the slide
- b) Transverse traverses
- c) Microscope fields
- 4.3.2 Acetolysis and the weighted vial method4.3.3 Airspora identification
- 4.4 Statistical method

4.5 Plate showing distorting vegetation at the Epping site

#### 4. RESEARCH METHODOLOGY

#### 4.1 Sampling location

In choosing a site for the research, four factors need to be considered :

a). Regionality and centrality.

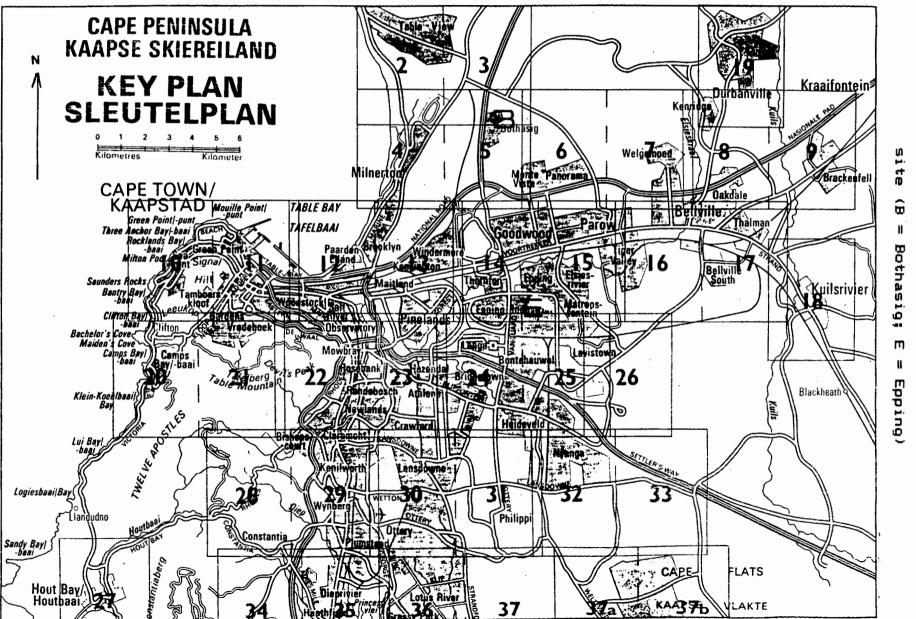
b). Accessibility of site.

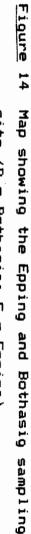
c). Security.

d). Proximity to a meteorological station.

In this type of research, where one of the aims is to quantitatively measure the airspora of the region, it is important to account for bias in terms of location. A location in the Southern suburbs would introduce a bias toward garden exotics, while a site on Table Mountain would be biased toward Fynbos pollen. However, it is necessary to point out that Cape Town in well known for its different climatic belts and any location will favour the adopted climatic belt. Thus it is important to admit that one sampling site cannot be construed to adequately sample the entire peninsula region. This job would require at least 5 different samplers in each of the 5 known climatic belts. Notwithstanding the previous point, in this project two samplers were used in order to give some idea of the extent of the difference in the airspora spectrum between two places on the Cape Flats.

A site no more than 15 km distant from the university and easily reached Ьγ motor vehicle was campus adequate to meet the demands of considered accessibility. Moreover it was important that the owners of the site be receptive to the installation of the equipment and its operation for a two year period as well as being tolerant of a once weekly visit to service the sampling unit and collect the sampling data. A





committment to this end was necessary to prevent the movement of the sampler to another site thus breaking the uniformity of the collected data. More important however, was a committment on the part of the owner of the premises to ensure the security of the sampler. Lastly it was necessary that the chosen location be in close proximity to a meteorological station so that the correlation between meteorological data and airspora concentrations would be representative of the site.

The first site is located in an industrial area, Epping Industria, Site 4. The site is located inside the grounds of a beveraging company. The sampler stands on an open piece of lawn surrounded by warehouses to the N (Plate 1) and security fences to the E, W and S (Plate 11). It would be unrealistic to expect the site to be free of vegetation which may distort the quantitative results of the investigation. Referring to Plate 11, grass, <u>Acacia</u> trees, palm trees and succulents were evident in the pollen concentrations counted.

As can be seen from the map (Figure 14), the site is very central to the Cape metropolitan area. The journey from the university campus to the site via Settlers Freeway is 14km, thus making the site accessible while the straight line distance to DF Malan meteorological station is 8km. The meteorolgical data, according to the resident meteorologist at the meteorological station, would be representative of the Epping site over the distance. The site owners, Amalgamated Beveraging & Canning were willing to co-operate with us for a period of 2 years. Their resident electrician installed the flex from the security light to the Burkard sampler while their security guards kept "an eye" on the apparatus for the full 24 hour period. Thus it is evident that the Epping site fulfilled the four criteria mentioned at the beginning of paragraph 4.1.

The second site is located in Bothasig, a garden suburb, to the North of Cape Town. The sampler is installed in

°₽

the back garden of a property owned by the Cape Town Municipality and used for community health purposes. The sampler was loaned to the university by the Cape Town Municipality for the duration of this research. One of the conditions attached to this loan, however, was that the sampler was not to be moved from the site at Bothasig. To this end therefore, there was little option but to use the site provided. The sampler is located behind the dwelling on the site and one suspects that this offers unwanted protection from the wind in particular and probably rain as well. Moreover it is pollen surrounded by suburbia and thus exotic was expected to be found in the atmosphere in fairly high concentrations. Notwithstanding the above, the sampler was relatively secure, not being obvious from the road and in terms of distance was approxiamtedly 20km from the university campus. Unfortunately, the site is not close enogh to the DF Malan meteorological station for supplied by that station to be of any the data relevance. Therfore no attempt was made to correlate the Bothasig site with meteorological data from variables.

## 4.2 <u>Sampling strategy</u>

The Epping and Bothasig sampling tapes were collected every week on a Monday morning. The Epping tape was analysed for airspora on a 24hr basis, working from OhOO until 24h00, thus coinciding with the weather data from the DF Malan office. The Bothasig tape was analysed for airspora on a 7 day basis. The data from these tapes was used in the following way.

- a) The daily Epping data (pollen and spores at the family taxonomic level) was recorded and the mean daily concentrations for the airspora was computed. From this data the mean weekly, mean monthly and mean annual airspora concentrations were also computed.
- b) The mean weekly Bothasig data (pollen and spores at the family taxonomic level) was recorded. From this

data the mean monthly and mean annual airspora concentrations were computed.

- c) The Bothasig and Epping data were compared on a weekly, monthly and annual basis in order to establish quantitative differences between the sites.
- d) The DF Malan meteorological station supplied the necessary daily meteorological data at the end of each month. This was then compiled and stored on a computer.
- e) The mean daily Epping airspora concentrations were then correlated with meteorological data from the DF Malan meteorological station in an effort to establish realtionships between the airspora and meteorological variables, leading to the creation of a predictive model.

#### 4.3 Laboratory Method and Microscope Work

Literature on aeropalynolgy indicates that there are numerous techniques for the laboratory preparation of pollen leading to accurate counting under the microscope. Before deciding whether the pollen will be laboratory processed in the and made ready for microscope work, one must first decide which technique of pollen counting and volumetric analysis will be used.

According to Kapyla & Penttinen (1981:131) "...it is impossible to count the whole slide and that is why estimation methods are needed ". To this end there are two methods available to the aeropalynolgist. The first method requires that the sampling tape be placed directly under the microscope and transverses are made across the whole tape. This completed, a complicated formula is applied to determine the concentration of airspora in that 24 hour period. The second method involves the chemical treatment of the airspora in а known as acetolysis, leading to the final process calculation of airspora concentrations. Both these methods will be studied in some detail.

#### 4.3.1 Method 1 : The non-chemical process

Most aeropalynolgists favour this method as it is time-effective (Anderson et al., 1978; Kapyla & Penttinen, 1981; Bringfelt et al., 1982; Halwagy 1984; Kapyla, 1984; Fairley & & Halwaqy, 8, Batchelder, 1986). The Burkard manufacturers operating instructions suggest that this method is the most time-effective and should be adopted for estimating airspora concentrations when using the Burkard volumetric sampling device. Before reading the slide the following procedure is usually adopted.

a) The tape is divided into 7, 24 hour sections.

b) A section representing a 24 hour period is placed on the slide.

c) A staining solution is made up containing :

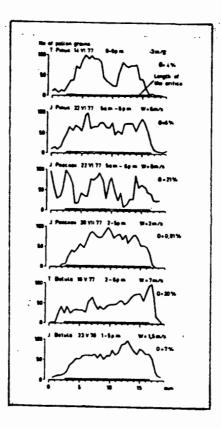
35 g Gelvatol 100 ml distilled water 50 ml Glycerol 2 g Phenol

d) Two drops of this solution are placed on the tape and a cover slip is placed over the tape.e) The slide is now ready for reading.

Kapyla & Penttinen (1981) list 3 types of sampling units that can be used in this method.

#### a) Traverse along the length of the slide

This method is used by the aerobiologists Davies et al. (1963) and Stix & Grosse-Braukmann (1970). The method is appropriate for estimating short term concentrations and diurnal variations but apparently often gives unrelaible estimates, if only done once or twice, because of the irregularities in the Figure 15 The distribution of particles along the length of the orifice from normally operated slides. Jyvaskyla Airport, sucking speed 101 min<sup>-1</sup> (Kapyla & Penttinen, 1981:133)



of the transverse variation particle concentrations on the slide. "The nowadays common practice to use one or two traverses along the length of the slide may give unreliable estimates because of the irregular variation" transverse (Kapyla & Penttinen. 1981:140). Figure 15 indicates how the transverse distribution of particles on the tape varies with both species and wind speed.

#### b) <u>Transverse traverses</u>

This method has been adopted by aerobiologists in the USA (Ogden et al., 1974) and in Sweden (Kotzamanidou & Nilsson, 1977). In this method a single traverse is made transversely, i.e. across the width of the tape - thus traverse variation is eliminated. Kapyla & Penttinen (1981) suggest that 12 such transverse traverses are enough for a relaible estimation of airspora concentrations.

### c) <u>Microscope fields</u>

This method has been used by the Finnish Aerobiology Group. It tends to take into account both the transverse variation (Figure 15) and lengthwise variation of particles on the tape. A large number of sampling units is needed and a method using random sampling with replacement is accepted as suitable.

Kapyla & Penttinen (1981) argue that their research indicates that the transverse . method gives the most traverse accurate estimate of the mean daily concentration while the microscope field method is the least time consuming and offers a formula to calculate of the estimator the of the error mean

provided a sample size of > 100 is used (see Kapyla & Penttinen, 1981:139 for formula)

Nevertheless, none of the literature sited points out two of the most dramatic shortcomings of these methods. The volumetric samplers take in airspora as well as pollutants, insects, sand and scraps of vegetation. These, if not separated from the airspora, tend to cover the pollen and spores making it difficult to accurately identify the organism. Further pollen is particularly difficult to identify in its natural state. These two problems are dealt with in the second method.

#### 4.3.2 Acetolysis and the weighed vial method

Erdtman (1960), Faegri & Iverson (1975) and Moore & Webb (1978) are consistent in argueing that a process, known as acetolysis, should be adopted to remove the cellulose of pollen. Once the it cellulose of the pollen is removed is an easier task to identify the pollen. They argue that without this process of acetolysis it is difficult to identify pollen, especially if the intention is to 'key' the pollen down to species level. The exine features are perhaps the best clue to the identity of a pollen grain and acetolysis tends to make the features of the exine readily observable. The method used in this research project incorporates acetolysis and the following paragraph deals with the method in greater detail.

Cellulose is a polysaccharide and can be removed by acid hydrolysis (acetolysis). The protoplasm is also removed and the pollen grain is rendered translucent. Once the cellulose has been removed the exime of the pollen grain is more apparent and thus the pollen is more readily identifiable. Acetolysis involves the mixing of glacial acetic anhydride mixed with concentrated sulphuric acid

in a ratio of 9:1. Further to acetolysis the airspora are treated in hydrofluoric acid. This chemical is responsible for the removal of silica sample. This is particularly necessary from the when using samples from the Cape Flats, because in summer the abundant sand on the Cape Flats is the atmosphere by the SE wind and is blown into apparent in the samples. At the microscope stage of the process, the presence of sand in a sample will prevent the cover slip from lying flush on the slide. According to Moore & Webb (1978) and Faegri & Iverson (1975) these chemical treatments can have an adverse effect on the airspora. When using acetolysis spores apparently can show signs of damage if prolonged treatment (ie. more than three minutes) occurs.(1) Further, grain size is hydrofluoric reduced when using arid. these Notwithstanding reservations, in both authors opinions, the advantages of using these chemical treatments outweigh the disadvantages.

The calculation of the dailv volumetric concentration of airspora, once the chemical process has been completed, is derived from the method used by Jorgensen (1967:489) which relies on counting "...all pollen in weighted aliquots of the samples...". Once the airspora have been stored chemically processed they are in preweighted vials. The volumetric concentration is based on the changing weight of the vial after the sample has been removed for counting on the microscope (See Appendix 1 for full description of this process).

The acetolysis and weighted vial method was adopted for this research for the reasons oulined above viz, the removal of silica from daily samples after treatment by HF, the removal of cellulose after acetolysis, making

<sup>1</sup> Experience in the laboratory during the research period tends to confirm this point of view

4.3.3 Microscope methods and airspora identification

The counting method used is explained in Appendix 1, steps 21-23. Pollen was identified by making reference to the pollen collection held in the υf Cape Towns." University Biogeography' Department. Further reference was made to Moore & Webb (1978). Spore identification was based on the colour plates found in Gregory (1973). Nilsson (1983) and Hurtado & Riegler-Goihman (1986b). When it was not possible to identify a pollen or spore, the organism was categorised as "pollen other" or "spore other" respectively.

#### 4.4 Statistical methods

Predictive models were based on the statistical test of forward and backward multiple regression analysis. This test was administered using the BMDP2R programme. Regressions were used to quantify the relationship when the value of one variable between variables was the values of the other affected by changes in variables. The affected variable in this research was pollen and spore concentrations while the meteorological variables were the independent variables. In the forward and backward stepping multiple regession analysis, at each step the meteorological variable with the highest F-to-enter value is included in the equation, the object being to improve the Fratio score. In the backward stepping, meteorological variables are removed that least affect the F ratio at any step. The strength and weaknesses of this type of statistical modelling will be examined in the discussion chapter of this research.

- 4.5 Plate showing distorting vegetation at the Epping site
- <u>Plate 11</u> The Epping sampling site showing distorting vegetation



#### RESULTS 5.

#### 5. RESULTS

5.1 The airspora spectrum : Bothasig and Epping > 5.2 Spores and pollen : annual variation

- 5.2.1 Poaceae and Pinaceae
- 5.2.2 Amaranthaceae and Chenopodiaceae
- 5.2.3 Ericaceae and Proteaceae

5.2.4 Fungal spores

- 5.3 Relationship between airspora concentrations and meteorological factors
  - 5.3.1 Introductory investigation
  - 5.3.2 Single regressional analysis
    - a) Total pollen
    - b) Pinaceae
    - c) Poaceae
    - d) Total spores
    - e) <u>Alternaria</u>
    - f) Basidiospores

5.3.3 Stepwize regressional analysis

5.4 Wind direction

#### 5. RESULTS

The results of this research are divided into four sections. The first section deals with the quantitative aspects of the airspora spectrum for Epping and Bothasig. The second sections deals with the annual variation in the airspora spectrum. The third section deals with the relationship between airspora concentrations and meteorological variables and in this section an attempt is made to construct some predictive models for spores and pollen in the Epping area. the fourth sections analyses the effect of wind direction on airspora concentrations.

#### 5.1 The airspora spectrum : Bothasig and Epping.

In this section the general trends are initially identified followed by a more detailed analysis of the airspora spectrum for Bothasig and Epping. An attempt will be made to compare the two spectrums wherever possible.

Figure 16, shows that in both Epping and Bothasig spores are the most dominant component of the atmosphere. Pollen as a yearly total, represents 20.3% and 23% of the airspora spectrum for Epping and Bothasig respectively, while spores represent 79.7% and 77% for the two places respectively.

In Figure 17 it is clear that grasses (Family Poaceae) are the most dominant pollen in the pollen spectrum for both Epping(40.4%) and Bothasig(48%). In Epping the next most dominant pollen is Pinaceae(5.9%) and at Bothasig, Restionaceae(7.4%) is the next most dominant pollen family. Comparing Bothasig with Epping, Fabaceae, Cyperaceae and Asteraceae have similar representation at sites suggesting that the pollen of these these two three families are evenly spread in the collecting area. One of the most noticeable differences in the two

signific	pollen spectrum ance according ations (m <sup>-3</sup> ).	in decending order of to mean daily
POLLEN MEAN	DAILY CONC. ( M-3)	% OF POLLEN SPECTRUM
		· ·
Poaceae	3.479	40.320
Pinaceae	0.509	5.908
Restionaceae	0.468	5.430
Fabaceae	0.365	4.235
Asteraceae	0.358	4.153
Cyperaceae	0.330	3.832
Proteaceae	0.293	3.404
Ericaceae	0.281	3.264
Caryophyllaceae	0.194	2.251
Thymelaeaceae	0.169	1.968
Myricaceae	0.151	1.756
Acacia	0.110	1.275
Amaranthaceae	0.101	1.174
Sterculiaceae	0.096	1.120
<u>Myriophyllum</u>	0.091	1.057
Chenopodiaceae	0.084	0.984
Amaryllidaceae	0.057	0.664
Nymphaeaceae	0.053	0.620
Plantaginaceae	0.042	0.490
Liliaceae	0.036	0.434
Betulaceae	0.033	0.386
Rosaceae	0.024	0.281
Aizoaceae	0.021	0.254
Euphorbiaceae	0.021	0.244
Apiaceae	0.019	0.229
Boraginaceae	0.019	0.223
Plumbaginaceae	0.019	0.231
Seliginaceae	0.014	0.170
Bruniaceae	0.012	0.150
Balsaminaceae	0.011	0.136
Cupressaceae	0.011	0.134
Typhaceae	0.011	0.128
Polygonaceae	0.010	0.118
Ranunculaceae	0.010	0.115
Rutaceae	0.006	0.079
Umbelliferaceae	0.006	0.071
Urticaceae	0.006	0.071
Rhamnaceae	0.005	0.065
Santalaceae	0.005	0.060
Campanulaceae	0.005	0.057
Crassulaceae Cannabaceae	0.002 0.002	0.034
Labiaceae	0.002	0.028
Ladiaceae	0.002	0.026
Polygalaceae	0.002	0.010
Pollen Other	1.061	12.300
Pollen Total	8.6277	100.00

111

ί,

# <u>Table 5</u> Bothasig pollen spectrum in decending order of significance according to mean daily concentrations $(m^{-3})$ .

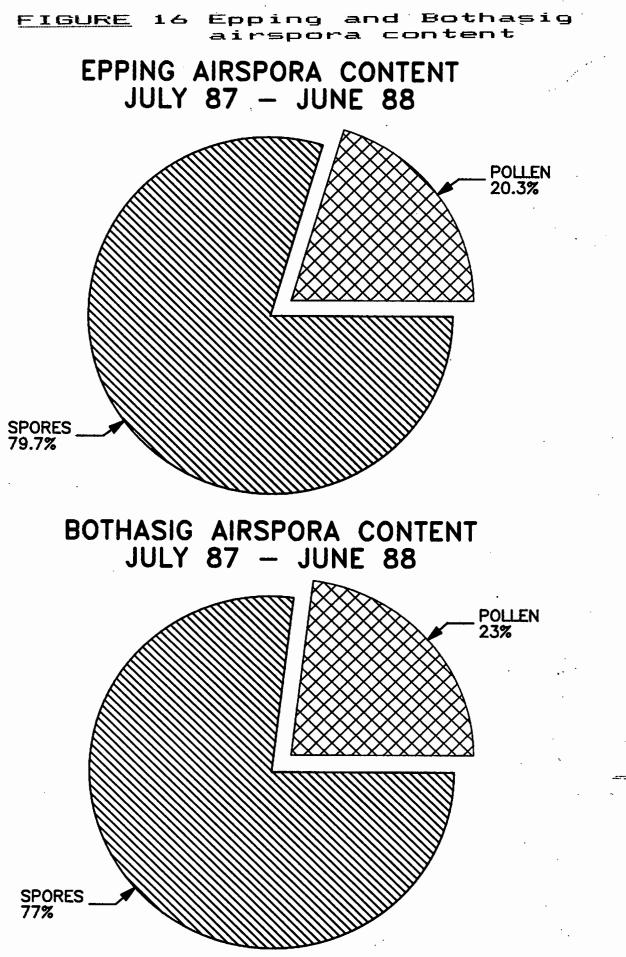
POLLEN	MEAN DAILY CONC. ( M-3)	% OF FOLLEN SPECTRUM
Poaceae	4.770	48.020
Restionaceae	0.739	7.445
Fabaceae	0.538	5.424
Asteraceae	0.359	3.620
Myriophyllum	0.324	3.267
Cyperaceae	0.300	3.023
Pinaceae	0.216	3.141
Carophyllacea		1.858
Ericaceae	0.167	1.708
Proteaceae	0.168	1.692
Thymelaeaceae		1.628
Amaryllidacea		1.505
Acacia	0.115	1.164
Myricaceae	0.108	1.090
Chenopodiacea		0.729
Amaranthaceae	0.052	0.523
Betulaceae	0.051	0.517
Plantaginacea		0.499
Apiaceae	0.037	0.382
Sterculiaceae	0.019	0.199
Aizoeaceae	0.018	0.188
Boraginaceae	0.018	0.182
Crassulaceae	0.013	0.131
Polygonaceae	0.012	0.127
Urticaceae	0.012	0.125
Plumbaginacea		1.119
Rosaceae	0.011	0.117
Labiaceae	0.008	0.090
Euphorbiaceae		0.088
Campanulaceae	0.007	0.080
Rutaceae	0.006	0.065
Cupressaceae		0.045
Seliginaceae	0.003	0.036
Rhamnaceae	0.003	0.034
Nymphaeaceae	0.003	0.032
Malvaceae	0.003	0.030
Buxaceae	0.002	0.026
Ranunculaceae	0.002	0.026
Typhaceae	0.002	0.024
Geraniaceae	0.001	0.012
Bruniaceae	0.001	0.010
Pollen Other	1.185	11.920
Pollen Total	9.937	100.000

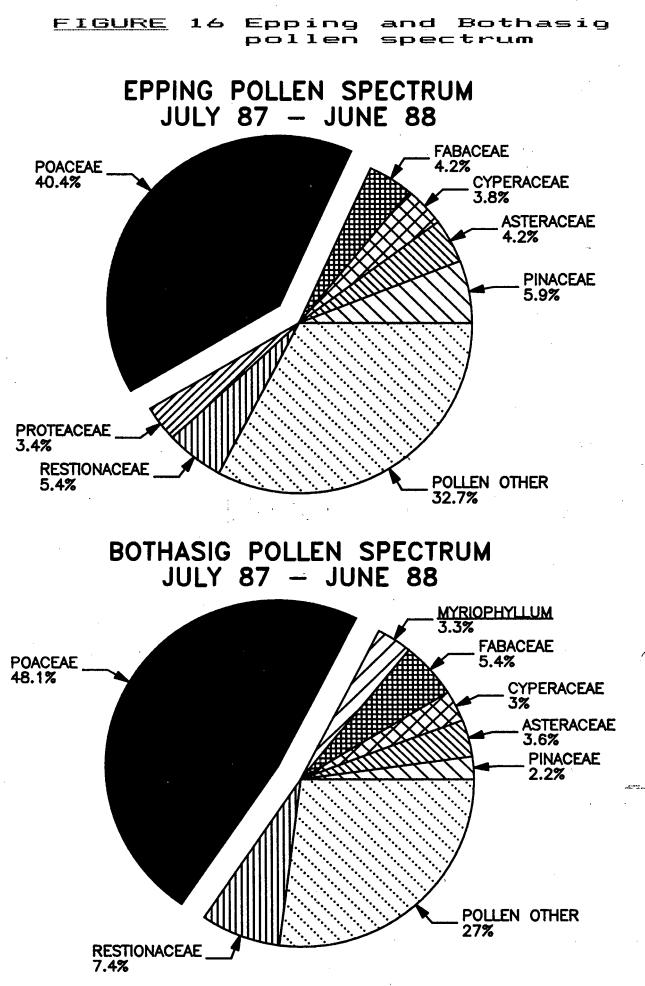
<u>Table</u> 6 Epping spore s significance a concentrations(m <sup>-3</sup>	according	
SPORE MEAN DAILY CO	<u>)NC. ( M-3</u> )	% OF POLLEN SPECTRUM
Basidiospore	15.705	46.269
Alternaria	1.334	3.932
Pithomyces chartarum	0.857	2.526
Drechslera	0.460	1.356
Fern Spore	0.185	0.546
Cordana musae	0.152	0.448
Torula herbarum	0.147	0.434
Chaetomium	0.145	0.429
Ascospore	0.120	0.356
<u>Cladosporium</u>	0.120	0.356
<u>Melanomma pulvis-pyrius</u>	0.090	0.265
Conidia	0.063	0.187
<u>Paraphaeosphaeria michotti</u>	0.044	0.130
<u>Helminthosporium</u>	0.037	0.110
Sporangiospore	0.020	0.060
<u>Tetraploa aristata</u>	0.015	0.045
Asterosporium	0.013	0.038
Leptosphaeria	0.013	0.038
Periconia	0.007	0.023
<u>Stemphylium</u>	0.004	0.012
<u>Tetracoccosporium paxianum</u>		0.013
Monodyctuś	0.003	0.010
Spore Other	14.395	42.407
Spore Total	33.944	/ 100.000

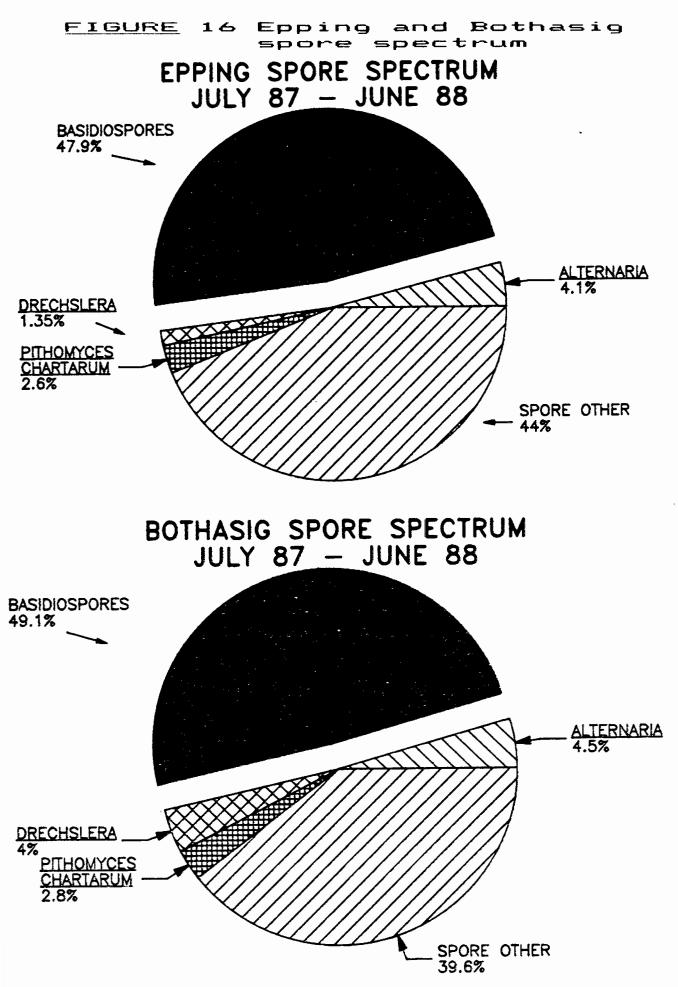
Table	7	Bothasig	spore	spec	tru	m in	decen	ding	order	of
		significance	accor	ding	to	mean	daily	conce	entrati	ons
		(m∽₃).								

SPORE MEAN DAILY CON	<u>IC. ( M-3</u> )	% OF POLLEN SPECTRUM
Basidiospore	16.335	49.099
Alternaría	1.501	4.512
<u>Drechslera</u>	1.328	3.994
Fithomyces chartarum	0.940	2.825
Fern Spore	0.306	0.920
Chaetomium	0.180	0.542
<u>Cordana musae</u>	0.154	0.463
<u>Torula herbarum</u>	0.149	0.439
Melanomma pulvis-pyrius	0.053	0.161
Ascospore	0.052	<b>0.156</b> 7
<u>Tetraploa aristata</u>	0.039	0.118
<u>Cladosporium</u>	0.034	0.104
<u>Paraphaeosphaeria michotti</u>	0.028	0.086
<u>Periconia</u>	0.028	0.084
<u>Tetracoccosporium paxianum</u>	0.027	0.081
Asterosporium	0.020	0.060
Leptosphaeria	0.018	0.057
Helminthosporium	0.014	0.042
Monodyctus	0.008	0.026
<u>Stemphylium</u>	0.002	0.008
Conidia	0.001	0.004
Spore Other	12.040	36.190
Spore Total	33.269	100.000

)





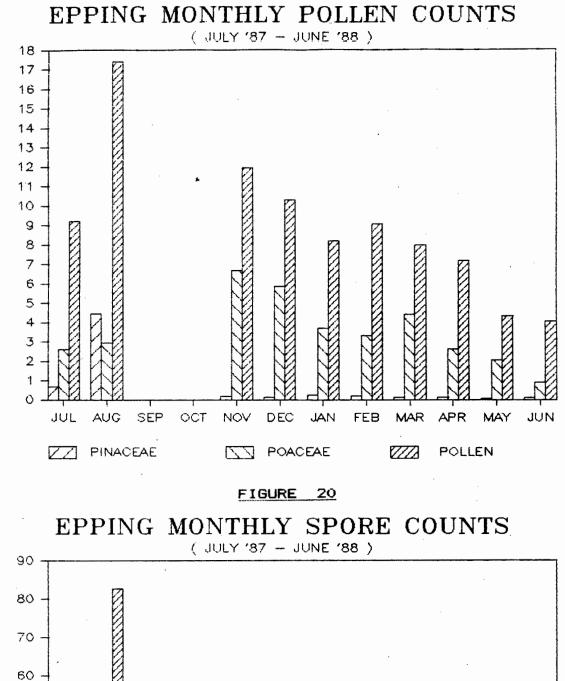


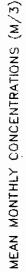
respective spectrums, is the comparative dominance of Myriophyllum in the case of Bothasig and the family Proteaceae in the case of Epping. At Epping Myriophyllum appears in negligble guantities, while at Bothasig. Proteaceae. although evident, is seen in only small quantities. The segment labelled "other pollen" in both cases is the total of the remaining identifiable pollen Tables 4 & 5 show the full and unidentified pollen. pollen spectrum for both Epping and Bothasig. It is of interest to note that both the weeds (Amarathaceae and Chenopodiaceae) and Fynbos families (eq. Ericaceae and Proteaceae) are above average contributors to the pollen spectrum.

Basidiospores are the most dominant fungal spore in both the case of Bothasig(49.1%) and Epping (47.9%). (See Figure 18). <u>Alternaria</u> is the second most dominant fungus in the atmosphere for both Bothasig(4.5%) and Epping(4.1%). In both cases, <u>Drechslera</u> and <u>Pithomyces</u> <u>chartarum</u> are well represented in the spore spectrum. "Spore other" is made up of the total of the remaining identifiable and unidentifiable spores.

Table 6 and Table 7 show the full spore spectrum for Bothasiq. With the exception of, both Epping and Chaetomium, Paraphaeosphaeria michotii, Leptosphaeria, Ascomycetes), the (which belong to fern spores, ascospores and basidiospores, all the other genera come Deuteromycetes 🕺 (fungi from the dominant class imperfecti). Although the basidiospores are numerically dominant, the class Deuteromycetes is better represented in the spore spectrum. These observations hold true for both Epping and Bothasig.

#### FIGURE 19

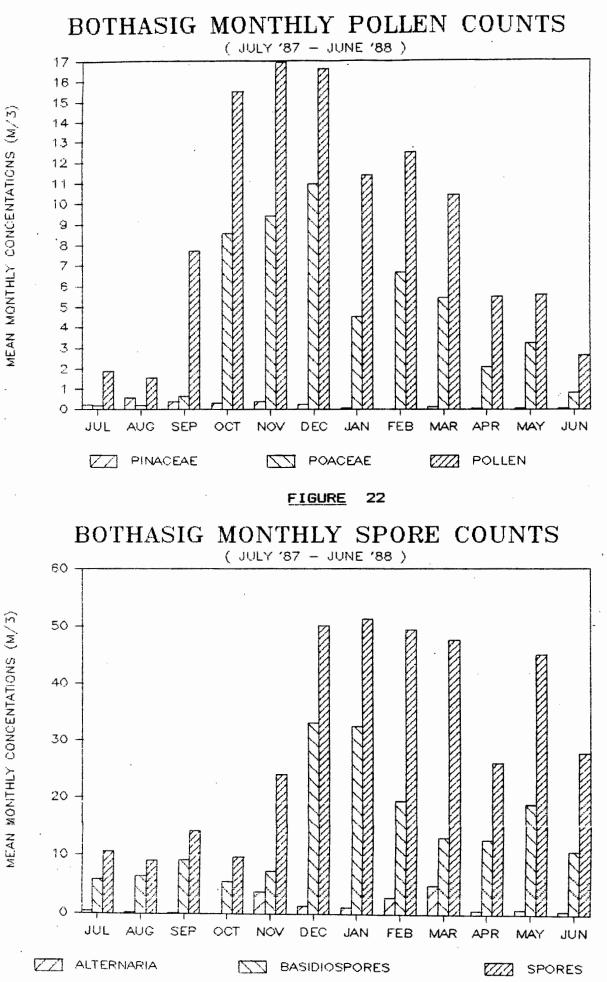


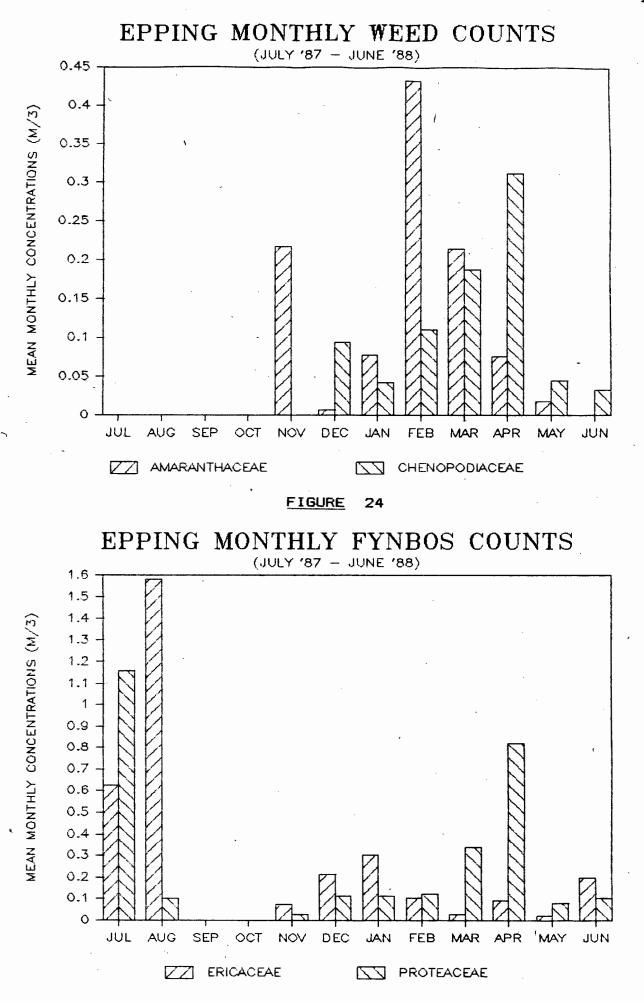


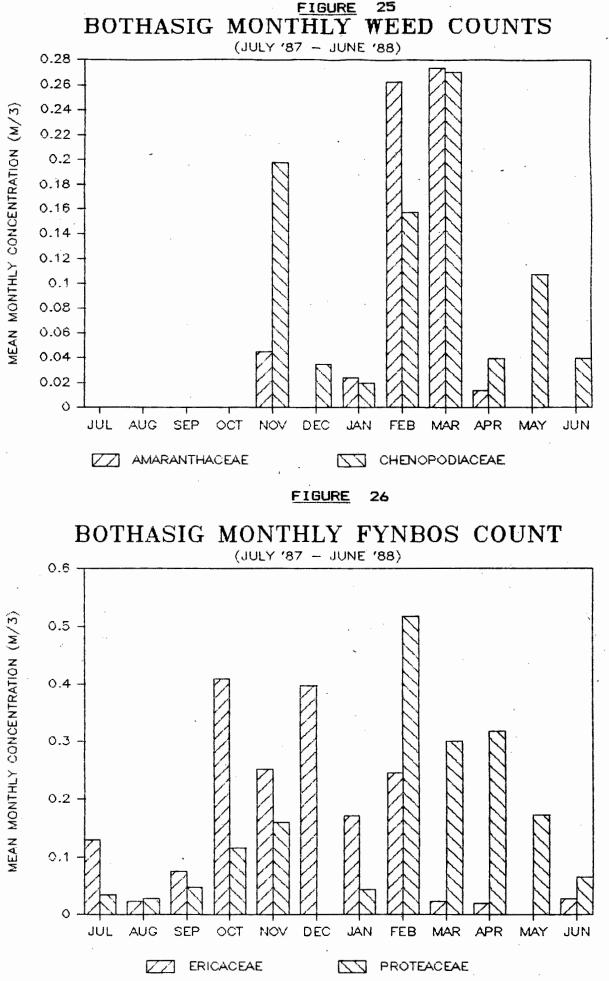
MEAN MONTHLY CONCENTRATIONS (M/3)

50 40 30 -20 10 0 APR JUN JUL AUG SEP OCT NOV DEC JAN FEB MÁR MAY Z ALTERNARIA BASIDIOSPORES  $\overline{Z}$ SPORES  $\overline{\}$ 

#### FIGURE 21







#### 5.2 Spores and pollen : Annual variation

#### 5.2.1 Poaceae and Pinaceae

Figures 19 & 21 show the monthly concentrations for Pinaceae, Poaceae and total pollen for Epping and Bothasig respectively. Unfortunatley there is a gap in the data for Epping during September and October because of a technical problem with the Burkard volumetric sampler. However, both figures indicate a peak in pollen production over spring and the early summer period (September-December) after which pollen production begins to decrease. It appears that this pattern begins earlier in the case of Epping (August). Poaceae tends to remain at relatively high levels throughout the year, peaking in the spring and summer period, while Pinaceae, at least in the case of Bothasig, peaks in spring and then virtually disappears for the rest of the year. This would fit in with the picture whereby anthesis in the grasses tends to while the continue beyond spring and summer, Pinaceae flower only in spring and early summer.

#### 5.2.2 Amaranthaceae and Chenopodiaceae

Figures 23 & 25 show the Epping and Bothasig monthly weed concentrations respectively. The weed families referred to are Amaranthaceae and Chenopodiaceae. Both figures indicate a seasonal high during Febuary and March. It is of interest to note that the Epping location indicates higher concentrations of these famalies than does Bothasig.

#### 5.2.3 Ericaceae and Proteaceae

Figures 24 & 26 show the Epping and Bothasig monthly Fynbos concentrations. Ericaceae and Proteaceae were chosen as good indicators of this plant kingdom Epping shows an unusually high concentration of these two families in July and August, 1987. Notwithstanding these two months. the Epping and Bothsigs site show remarkable parity. The seasonal presence of these two poller types in the atmosphere, shows a summer dominance from roughly October until May, with peaks in the late summer (April for Epping and Febuary for Bothasig).

#### 5.2.4 Fungal spores

Figure 20 and Figure 22 show the mean monthly spore concentrations for the two major spore types, Basidiosporea and Alternaria, at Epping and Bothasig respectively. There is no seasonal spores in is dominance of spring as there in pollen. Bothasig shows higher spore counts in the middle and late summer, while Epping, although showino a large basidiospore peak in early spring, also seems to show fairly consistent levels throughout the year. (The gap in the Epping data, as with the pollen, is the result of mechanical problems with the Epping collector.) It is difficult to find any kind of seasonal for either pattern Alternaria DT. the Basidiospores, suffice is to say that in the case of Bothasig, the Basidiospores seem to peak from the end of Febuary, while Alternaria December to seems to be more prevalent from December through to the end of March.

## 5.3 <u>Relationship between airspora concentrations and</u> <u>meteorological factors</u>.

#### 5.3.1 Introductory investigation

Initially, 7 meteorological varaiables were chosen for comparison with the mean daily airspora concentration from Epping. The monitoring period lasted 6 weeks during the winter period of 1987. The variables chosen were wind velocity and direction, relative humidity, mean temperature, precipitation, sun hours and the presence or absence of temperature inversions. These factors were correlated with pollen and spore concentrations in simple, single correlations. the results are tabulated in Table 8.

Table 8 indicates that pollen concentrations are positively correlated with total daily sun hours and mean daily temperature, and negatively correlated with mean daily relative humidity. From these relationships it was concluded, very generally, that warm, sunny and dry conditions were conducive to high pollen concentrations in the atmosphere in the winter and early spring months.

In the case of spore concentrations a negative correlation with mean daily wind velocity and a positve correlation with sun hours seems to suggest that calm sunny days are related to high spore concentrations in the atmosphere in winter.

Multiple regressional analysis was then conducted on the same data set (Table 9). These multiple regression equations then act as a first step toward a more complex predictive model. It should <u>Table 8</u> Correlation coefficients (r) of dependent variables (pollen and spores) versus independent variables. MDWV = Mean daily wind velocity MDRH = Mean daily relative humidity MDT = Mean daily temperature S.H. = Total daily sun hours A.P. = Atmospheric pressure

		*				
	AIRSPOR	AIRSPORA				
/ METEOROLOGICAL	POLLEN	SPORE				
	194	321*				
MDRH	543****	285				
MDT	<b>-</b> 416**	. 206				
Precip	153	225				
S.H.	<b>.</b> 451***	<b>.</b> 490 <del>***</del> *				
A.P.	.0 <b>89</b>	. 268				

Asterisks indicate the level of significance in the probability of a greater absolute value of r under the HD : r = 0 (no asterisk = not significant; \* = 0,05; \*\* = 0.025; \*\*\* = 0,01; \*\*\*\* = 0.002)

Table 9	Multiple Regressional Equations for poll	en and		
	spores showing $\mathbb{R}^2$ , F ratio and P.			
	TP = Mean daily temperature			
	HD = Mean daily humidity			
	Sun = Total daily sun hours			
	WV = Mean daily wind velocity			
AIRSPORA	A EQUATION	R²	F ratio	۴
Pollen	Y=1.66320+.03738.TP02124.HD+.05384.Sun	. 6227	5 067	.00
		.022/	0.007	
Spore	Y=.63401+.09619.Sun+.0067.WV	.4902	5.062	.01

(

be noted that these equations were built on only 6 weeks data over the winter/early spring period. Further, some of the factors such as temperature and realative humidity are probably autocorrelated and the equations generated, do not take this into consideration. The full results of this preliminary study are reported in Hawke & Meadows (1989)

The full years data is taken from the 01/07/87 -30/06/88. Besides the meteorological factors considered in the initial pilot study, other factors were also introduced so that as many factors as possible could be considered in the final stepwise multiple regression equations. The other factors considered were: maximum temperature, **ຫ**ໍາກໍາ**ຫ**ບຫ temperature. difference in temperature, maximum relative humidity, minumum relative humidity, difference in humidity, maximum hourly wind velocity, a.m. inversion level and p.m. inversion level. These factors were chosen for the following reasons. The earlier literature review indicated that many researchers consider temperature and relative humidity to be important variables in not only influencing the growth of flora, but also in affecting the release and dispersal of airspora. Further, it was also apparent in the survey that extremes in temperature and relative humidity, measured as the difference between max and min temperature and relative humidity were thought to be associated with the release of airspora. Wind velocity is clearly associated with the dispersal of pollen and spores and since many believe that a critical wind speed is required to disperse pollen, maximum hourly wind velocity was incorporated a]so variable. as а Atmospheric pressure is listed by few researchers as an important variable. Notwithstanding this fact, it was incorporated because this factor has not been studied in the S.A. context. Lastly, it is apparent from the literature review that there is some controversy over

the role of atmospheric inversions on airspora concentrations. For this reason, atmospheric inversion levels were included (This variable was measured as the height in metres at which the inversion was apparent. a.m (OhOO) and p.m (12hOO) readings were provided by the DF Malan weather station). The steps in trying to isolate the most important variables were as follows:

- 1. Single regressional tests were performed on each variable in order to establish whether that variable was correlated with either spores or pollen. Futher to this, single regressional tests were also performed for basidiospores, <u>Alternaria</u>, Poaceae and Pinaceae, the commonest individual pollen. In this way it was possible to get an idea of how individual types responded to various meteorological factors.
- The different factors were then subjected to stepwise multiple regression analysis in order to eliminate all but the most important variables.
- 3. Having completed the regression equations, those factors that appear to be the most important were plotted graphically against one another in order to get a clearer visual idea of the relationship between these variables and the airspora concentrations.

Table 10 Correlation coefficients (r) of dependent variables (Pinaceae, Poaceae, total pollen, Alternaria, Basidiospores, total spores) versus independent variables.

MT1 = Maximum daily temperature	DH = Difference between min
MT2 = Minimum daily temperature	and max temperature
MT3 = Mean daily temperature	AP = Atmospheric pressure
DT = Difference between maximum and	PP = Precipitation
minimum daily temperature	MW1 = Mean daily wind velocity
MH1 = Maximum daily relative humidity	MW2 = Max hourly wind velocity
MH2 = Minimum daily relative humidity	IA = A.M. Inversion level
MH3 = Mean daily relative humidity	IP = P.M. Inversion level

#### AIRSPORA

MET FACTOR PINACEAE POACEAE T POLLEN ALTERNARIA BASID T SPORE

MT1	.017	.335***	.408***	.231**	.091 .266***
MT2	306**	.199***	.083	.177*	188**099
мтэ	144	.326***	.294***	.200***	031 .135*
DT	.311**	.106	.287***	.031	.274*** .347***
MH1	077	222***	193***	125	013014
MH2	391***	210***	376***	084	179**261***
MHB	336**	254***	385***	143	157*209***
DH	.387***	.085	.275***	.008	.173** .256***
AP	.184	.144-	079	187*	.204*** .048
<b>P</b> P	182	201***	271***	.004	071154**
SH	.059	<b>.</b> 368***	.402***	.154*	.119 .215***
MW1	201	<b>.</b> 194***	.121*	.172*	171**140*
MW2	128	.169**	.129*	.271***	161**143*
IA	.105	.080	051	.053	178**207***
IP	.051	.067	129	.056	094144*
					•

Asterisks indicate the level of significance in the probability of a greater absolute value of r under the HO : r = O (no asterisk = not significant; \* = 0.05; \*\* = 0.01; \*\*\* = 0.001)

#### 5.3.2 Single regressional analysis

Table 10 indicates the following:

- a) Total Pollen : Pollen is positively correlated with temperature (MT1 & MT3), sunshine hours, difference in relative humidity and wind velocity (MW1 & MW2) and negatively correlated with MH3) relative humidity (MH1, MH2 & and precipitation. Thus warm, sunny, dry days with some wind are associated with high atmospheric pollen levels, while damp, cold days with relatively little wind are associated with low atmospheric pollen levels. It is of interest to note that the greater the difference in maximum and minimum daily humidity, the higher the atmospheric pollen content.
- b) Pinaceae : Pine appears to have a negative association with minimum temperature (MT2) and min and mean relative humidity (MH2 & MH3) while it is positively correlated with the difference between minimum and maximum temperature (DT) and relative humidity (DH). Broadly speaking days which see big differences between maximum and minimum temperatures and relative humidity such that minimum temperatures are not too high are associated with high Pinaceae concentrations. These days in fact would be spring days and Figure 19 indicates that for Epping, at any rate, Pine pollen is at its highest concentration during the spring months. The same is apparent for Bothasig (Figure 21).
- c) <u>Poaceae</u> : Poaceae accounts for about 45% of the pollen spectrum (see Figure 17 ). It is thus not surprising that the associations with

meteorological factors are similar to the general pollen pattern. Thus warm, dry, sunny days with wind are associated with high grass concentrations. Grass pollen also has a weak association with atmospheric pressure such that high atmospheric pressures are associated with low atmospheric grass pollen concentrations. This is unexpected in that few researchers have found any correlation between atmospheric pressure and pollen.

- d) Total spores : Spores in general are positively correlated with temperature (MT1, MT3 & DT) and sunshine hours while humidity (MH2, MH3 & DH), wind velocity (WS1 & WS2), precipitation and atmospheric inversion level (IA & IP) are negatively correlated with spores. This presents the picture of warm, dry, sunny and windless days being conducive to high spore concentrations in the atmosphere. However, there are two other important observations. In inversion conditions, where the mixing level is extremely low, spore concentrations seem to increase. Further. the greater the differences in maximum and minimum temperatures and humidity, the higher the levels of ambient atmospheric spores. Thus days showing extremes in temperatures and relative humidities to be associated with Seen higher spore concentrations.
- e) <u>Alternaria</u>: <u>Alternaria</u> has a positive association with temperature (MT1, MT2 & MT3), sunshine hours, atmospheric pressure and wind velocity (MW1 & MW2). The picture here is one of warm, sunny, and windy days being associated with high <u>Alternaria</u> concentrations. These conditions are associated with summer days and Figure 22 indicates that <u>Alternaria</u> concentrations at the

Bothasig site peak during the summer months, thus confirming this relationship. However, this relationship is not as evident at the Epping site (Figure 20). Dampness in the form of rain or relative humidity does not show any signicant relationship with <u>Alternaria</u>.

f) Basidiospores : Since basidiospores constitute 49% of the total spore spectrum, it can рб expected that similarities exist in the manner in which basidiospores respond to meteorological to compared tota] spores. factors when Basidiospores are positively correlated with the difference between maximum and ຫາມມາຍແມ່ temperatures, maximum and minimum humidity, and atmospheric pressure, while they are negatively temperature (MT2), correlated with relative humidity (MH2 & MH3), wind velocity (MW1 & MW2) and inversion level (IA). The general picture for enhanced basidiospore concentrations is thus one of days where the minimum temperature must remain comparatively low must minimum relative 35 humidity. This enhances the possibility of larger differences between max and min temperature and max and min relative humidity which is apparently conducive to high basidiospore concentrations. However, the days should be comparatively windfree with reasonably high atmospheric pressure. The weak negative correlation with a.m. inversion levels seems to suggest that a low morning mixing level also enhances basisiospore concentrations. These conditions are mainly achieved in winter, the season when the highest peaks in basidiospore concentrations occur at Epping (Figure 20). Interestingly, this relationship is not confirmed in the case of Bothasig. Figure 22 indicates that basidiospores show a peak during the summer period.

<u>Table</u> 11 Forward and backward stepwise regression analyses of daily airspora concentrations in terms of meteorological data for Cape Town, 1987 - 1988.

MT1	=	Maximum daily temperature	DH	= Difference between minimum
MT2	=	Minimum daily temperature		and maximum temperature
MT3	=	Mean daily temperature	AP	= Atmospheric pressure
DT	Ξ	Difference between maximum and	PP	= Precipitation
		minimum daily temperature	MW1	= Mean daily wind velocity
MH1	=	Maximum daily relative humidity	MW2	= Max hourly wind velocity
MH2	=	Minimum daily relative humidity	IA	= A.M. Inversion level
MH3.	=	Mean daily relative humidity	IP	= P.M. Inversion level

	AIRSPORA					
STEPS IN REGRESSION	PINACEAE	POACEAE	T POLLEN	ALTERNARIA	BASID	T SPORE
STEP 1	MH2	MT3	MT1	 AP	DT	DT
R <sup>2</sup>	-16	.10	.16	.05		.07
F RATIO	7.24	18.03	31.55	5.76	3.47	12.58
STEP 2	AP	MW2	MW2	MW2	AP	IA
R <sup>2</sup>	.19	.12	.20	.07	.06	
F RATIO	4.38	10.68	21.50	3.97	5.67	9.58
STEP 3	MW2	SH	MH2	<b>MW</b> 1	IA	MT3
R <del>2</del>	.22	.13	.22	.10	.07	.12
F RATIO	3.56		15.84		4.19	7.42
STEP 4	DT	IP	IP	MT1	IA <del>*</del>	MT2
R2	.27		.24		.06	
F RATIO	3.39		12.94	3.66		6.65
STEP 5	IP	IP*	IA	AP+	AP	PP
R²	.31	.14			.05	.14
F RATIO	3.31	7.80		4.87	8.47	5.54
STEP 6	MH2+	SH	MT3	MW 1		DT+
R2	.31	.12	.25			.14
F RATIO	4.02	10.68	9.08	5.15		6.92
STEP 7	IP	MW2	MT1+	MT1		PP
R <sup>2</sup>	.27		.25			.13
F RATIO	4.63	18.03	10.91	5.38		8.79
STEF 8	MW2		IA			IA
R <sup>2</sup>	.19		.24			.11
F RATIO	4.35		12.91		1	10.17
STEP 9	AP	`	IP		,	MT3
R <sup>2</sup>	.14		.20			.01
F RATIO	6.24		15.16			1.71
STEP 10			MW2			
R <sup>2</sup>			.18			
F RATIO			18.55			
STEP 11			MH2			
R2			.13			
F RATIO			25.38			•
REMAINDER	DT	MT3	MT3	MW2	DT	MT2

 $R^2$  = Multiple  $R^2$  for each step

<u>Table 12</u> Regression equations based on the results of the stepwize regression analysis

t

MT1 = Maximum daily temperature
MT2 = Minimum daily temperature
MT3 = Mean daily temperature
DT = Difference between maximum and minimum
daily temperature
MH2 = Minimum daily relative humidity
AP = Atmospheric pressure
SH = Total daily sun hours /
MW1 = Mean daily wind velocity
MW2 = Maximum hourly velocity
IA = A.M. Inversion level
IF = P.M. Inversion level

AIRSPORA	EQUATION	MULT R <sup>2</sup>	FRATIO
	Y =34.56 + .043DT002.MH2 + .033.AP		
	+ .054.MW2	.27	3.39
POACEAE	Y =272 + .027.MT3 + .014.SH +.011.MW2		
	+.156.IP	.14	6.10
T POLLEN	Y = .299 + .027.MT1004.MH2 + .028.MW2	,	
	247.IP	.24	12.94
ALTERN	Y = 3.71 + .015.MT1004.AP051.MW1		
	+.057.MW2	.12	3.66
BASID	Y = -11.52 + .018.DT + .012.AP246.IA	.07	4.19
T.SPORES	Y = 1.11057.MT2 + .067.MT3010.DT		
	481.IA	.14	6.65

l

#### 5.3.3 Stepwize regressional analysis

.

The next step in the process of establishing which of the factors are the most important contributing variables lies in the use of forward and backward stepwize multiple regression analysis, the results of which can be seen in Table 11.

From Table 11, the following general picture emerges. Temperature for spores and pollen seems to be the dominant variable. In the case of Pinaceae and Poaceae, maximum humidity and mean temperature are the first variables to be added into the equation respectively. In the case of total pollen, it is maximum daily temperature that is the first variable to be added into the equation.

Atmospheric pressure and difference in max and min temperature in the case of <u>Alternaria</u> and basidiospores are respectively the first variables to be added into the equation, while in the case of total spores difference in max and min temperature is added into the equation first. Again these variables are not necessarily the most important variables but they are the most reliable predictors.

From the stepwise regressions the equations in Table 12 were computed which, with a degree of circumspection, may be used for predictive purposes. These equations are based on the first four steps of the forward addition of variables in the regression analysis. It should be born in mind that the Multiple  $R^{e}$  values were relatively low, as with the r values in the previous simple correlation analysis. Thus the predictive value of the equations should be treated with caution.

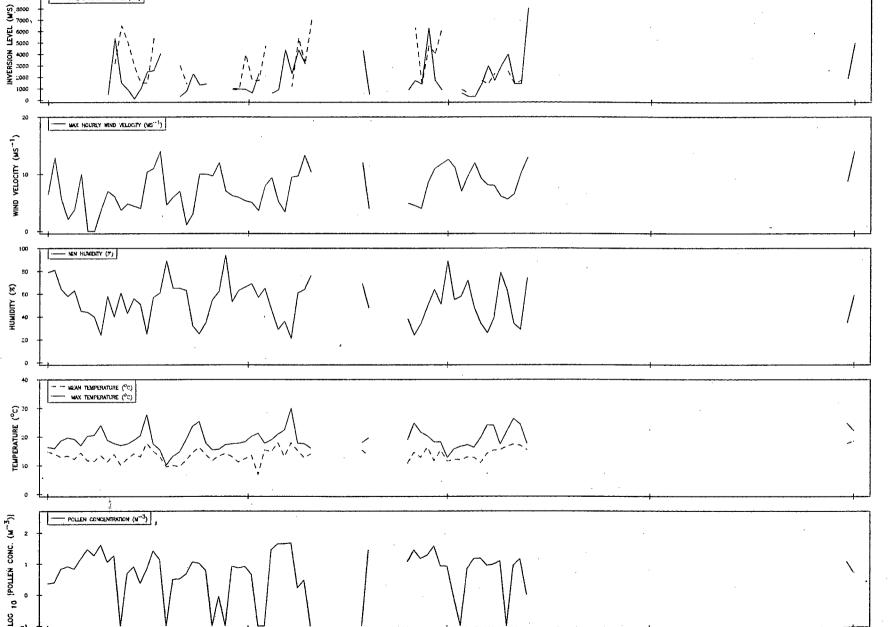
Considering the equations in Table 12, as noted already, temperature and humidity play important roles as a predictive elements when considering levels atmospheric the of airspora concentrations. However, it is evident that maximum hourly wind speed (gusts) is strongly implicated in the equations, particularly in the case of pollen and <u>Alternaria</u> (see Table 12).

In Figures 27a to 28c the independent variables chosen by the statistical programme, BMDP2R, to be regressed against each of the six dependent variables in the stepwise regression, are plotted graphically for the full year that data was collected. This was done in order to achieve a visual impression of the relationship between the independent variables and the dependent variables (only total pollen and total spores were used for this task). The red line in Figure 28a-28c has no special significance, save to make the graph more visable. From these graphs peak pollen and spore concentrations were pinpointed which helped in identifying the peaks used in the analysis of the effect of wind direction on peak spore and pollen concentrations. Figures 27a - 28c aré referred to again in the discussion chapter of this dissertation.

#### 5.4 Wind direction

Up to this point nothing has been said with regard to wind direction. Wind direction is not quantifiable in the sense that a direction such as SE cannot be given a which has numeric number meaning. Thus it is not possible to include wind direction in a regression analysis where the numbers used to indicate wind direction (the 16 points of the compass) are nominal in





SEP 87

~

OCT 87\_

AUG 87

λ.

-1

JULY 87

•

٠.

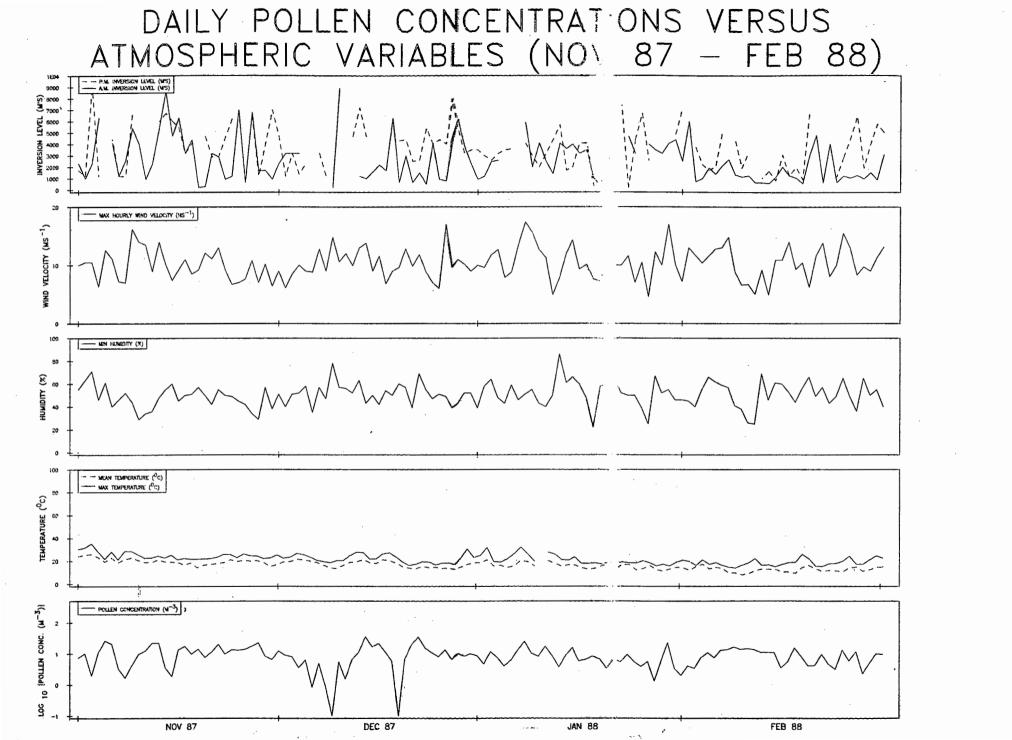
1/38

η

IGURE

N

٧ ù

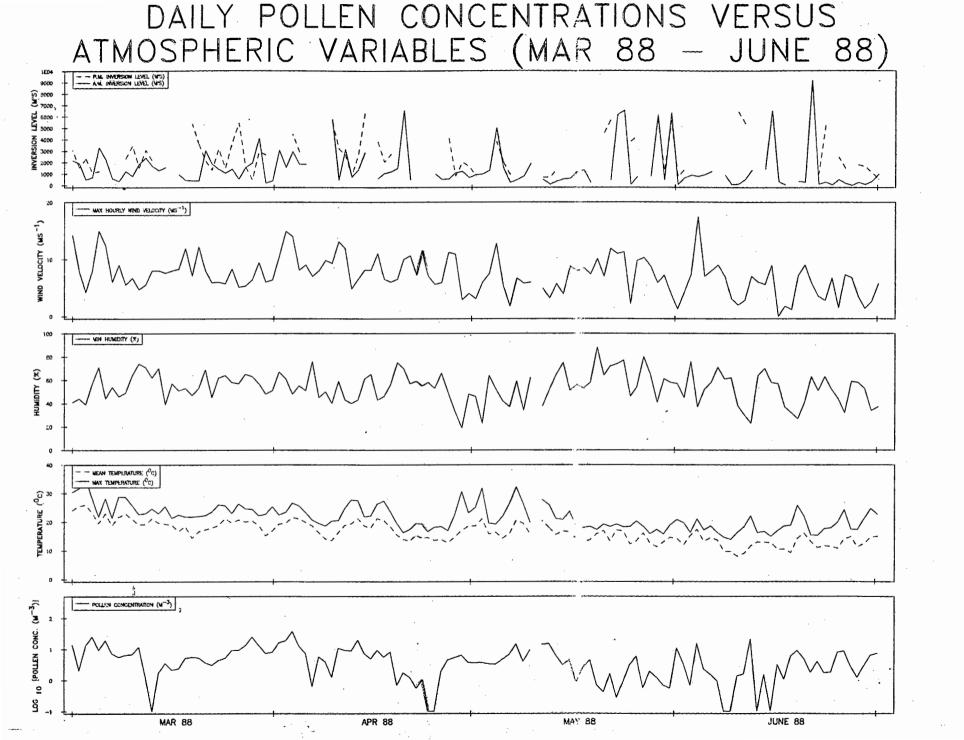


139

T

H

NVD



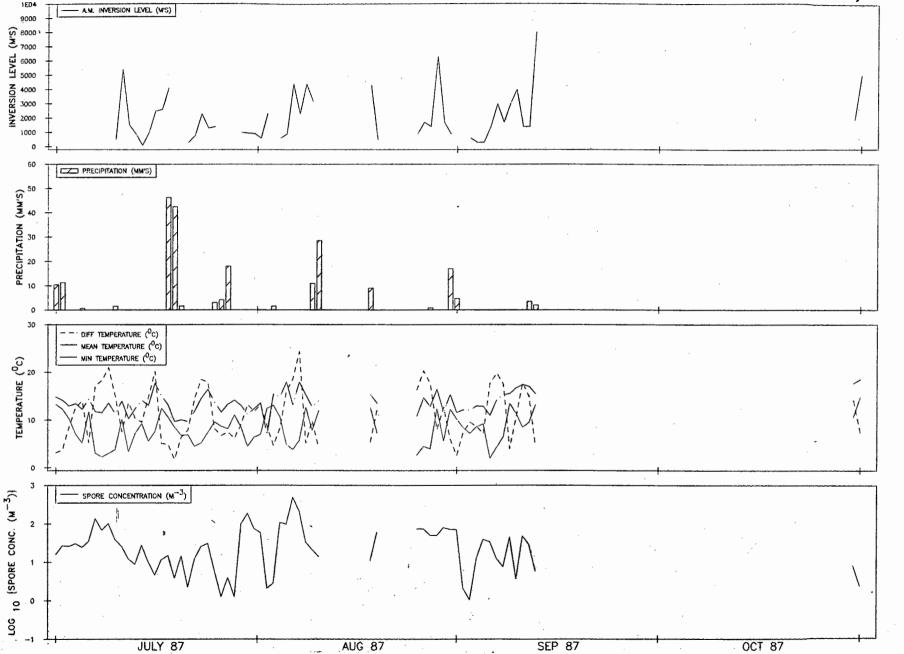
140

NNN

Т

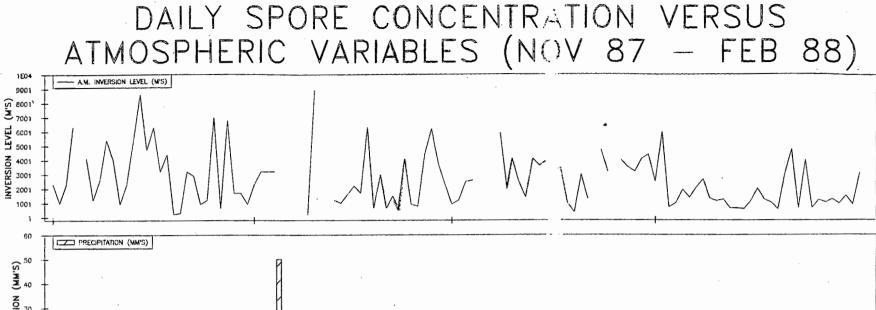
IGURE

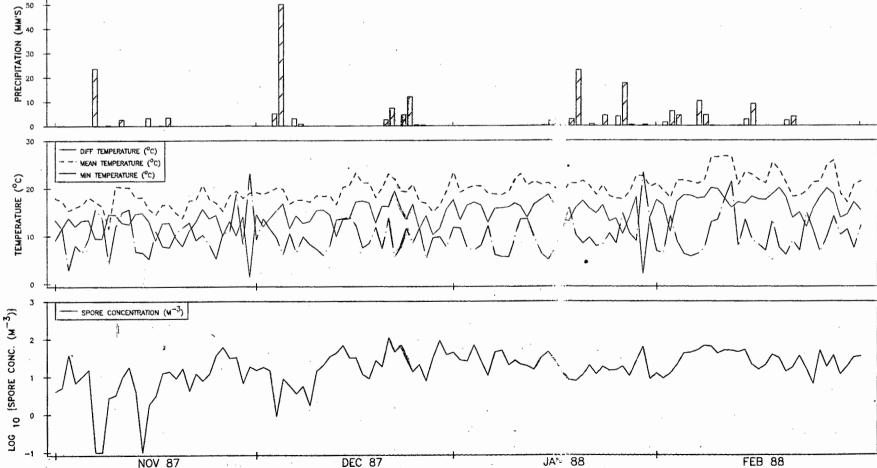




GURE 28a

Т



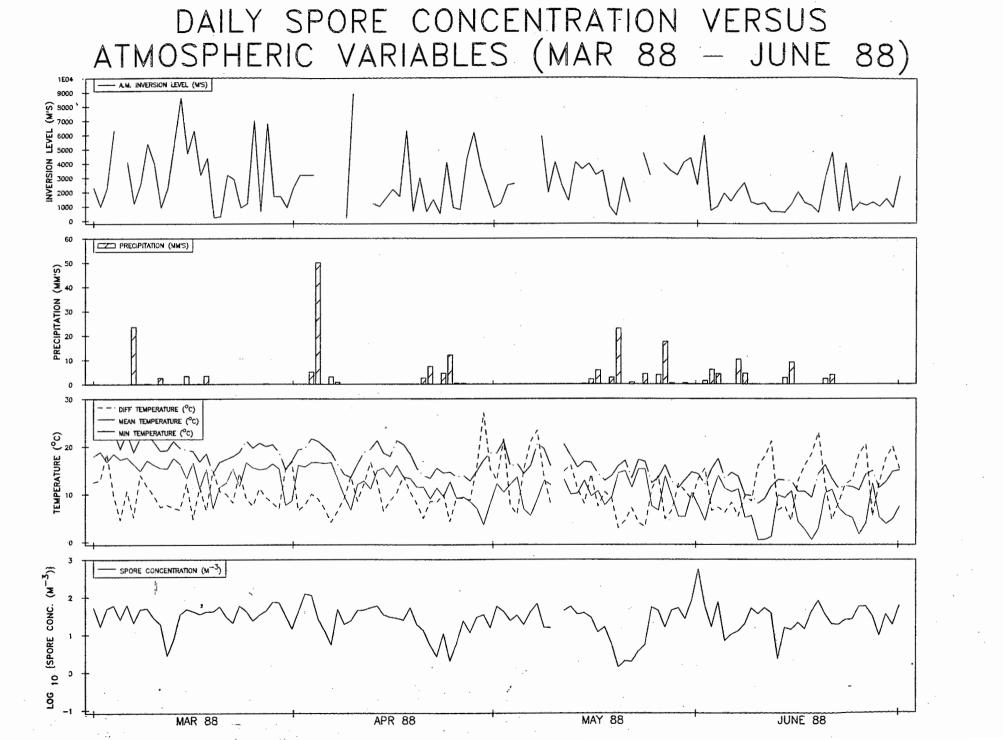


DEC 87

NOV 87

IGURE 

T



143

GURE 280

T

m

nature. Another method has to be found to analyse the signifcance of wind direction. Figure 29, 30 and 31 are wind roses that were employed to make an anlysis of the relationship between direction and airspora wind concentrations. These wind roses were designed in the following way. In Figure 29 the number of days during the sampling period that experienced wind were totalled. Following this, the days on which a particular wind direction predominated (eq. SE) was totalled. This was done for all recorded wind directions. Finally the number of days that the wind blew in a particular direction was calculated as a percentage of the total days receiving wind during the sampling period. These figures were then plotted on a wind rose (Figure 29). In Figure 30, the number of occassions where more than 25 pollen  $m^{-3}$  of air were sampled during a given wind direction, were totalled. The number of days where more than 25 grain  $m^{-3}$  of air were evident was calculated as a percentage of the total number of days that the wind 31 blew in that direction. In Figure the same calculations were made for spores. In this case, however, 60 spores  $m^{-3}$  of air was the adopted figure. The reason for adopting these concentrations will be explained in the next paragraph.

Figure 29 indicates that the S - SE wind is the most dominant summer seasonal wind while the NW - N wind is the dominant winter wind direction. This is a well known fact in the Peninsula with the southerly winds resulting from the south Atlantic high which dominates in summer and the NW winds resulting from frontal systems which originate deep in the South Atlantic and which dominate the winter weather patterns. Between these two basic wind directions, they account for nearly 73% (see Table 13) of the wind received by the Feninsula during the course of the year under observation. In order to clarify whether wind direction has any part to play in the peaks that are the daily airspora apparent in

Wind Direction	Days Occuring	<u>% Of Total</u>
N	17	5.6
NNE	16	5.4
NE	8	2.6
ENE	Ō	o
E	0	ò
ESE	0	0
SE	0	0
SSE	14	4.7
S	101	33
SS₩	27	8.9
S₩	27	8.9
WSW	2	. 0.7
W ć	2	0.7
WNW	<b>o</b>	ο
NW	50	16.5
NNW .	29	9.6
Calm days	7	2

Table 13 Wind direction during the sampling period

(It should be noted that on 72 days the wind direction was not recorded because the Burkard trap was not functional on these days and thus the data would have been irrelevant in the research) t

sampling period

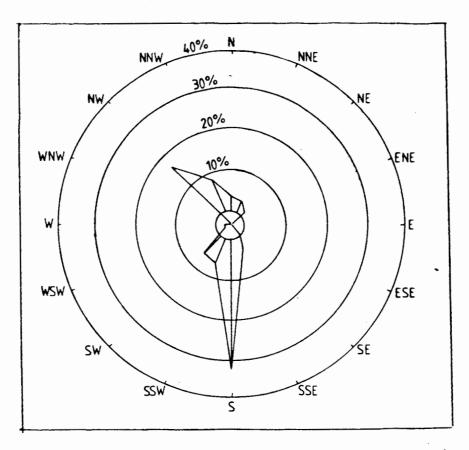
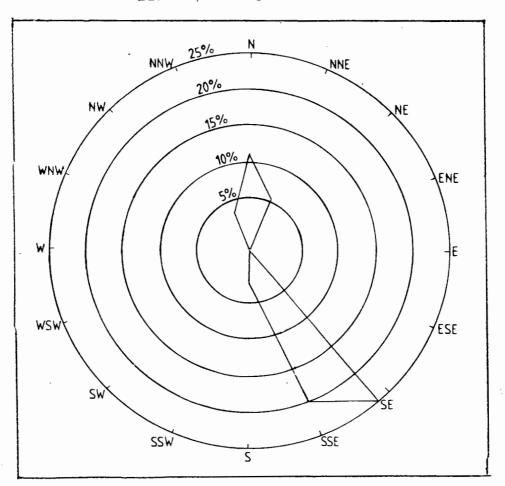
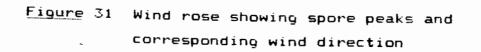
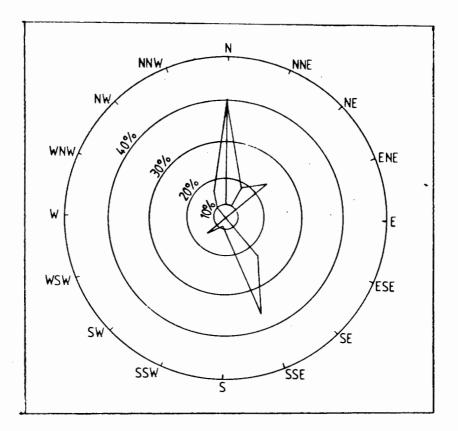


Figure 30 Wind rose showing pollen peaks and corresponding wind direction





:



j

concentrations the two roses, Figure 30 and Figure 31, were constructed according to the explanation offered in the previous paragraph. For pollen, a peak was considered to be 25 grains or more  $m^{-3}$  of air while a peak for spores was considered to be 60 grains or more  $m^{-3}$  of air. These figures were estimated simply by looking at the size of the peaks in the daily data (Figures 27a-28c) and estimating the average figure above which a peak would occur. Figure 31 shows the between wind direction and relationship spore concentrations. This gives some insight into the relationship, because the windrose shows the percentage of days when the wind was blowing in a specific direction and a peak above 60 grains  $m^{-3}$  was collected. By way of example, on days when the wind was blowing north, 41% of these days were associated with peaks of  $60 \text{ m}^{-3}$  or more. Studying Figure 31 it is apparent that the N and SSE wind are responsible for the peaks in the spore concentrations. It is of interest that this does not tally with the two dominant wind directions, S and NW respectively (Figure 29). At this point the best explanation that can be offered is that the N - NEsector is inland of the peninsula and therefore the wind has an opportunity to collect and hold spores in suspension over some distance before the wind reaches the peninsula. In the case of Figure 30, it is again apparent that the N and SSE - SE wind are responsible for the peaks in pollen. Again the explanation although only tentative is equivalent to that offered for spore peaks.

# 6. DISCUSSION

## 6. DISCUSSION

- 6.1 Quantitative differences in the Airspora spectrum
  - 6.1.1 The balance between pollen and spores in the atmosphere
  - 6.1.2 Follen
  - 6.1.3 Spores
- 6.2 Epping and Bothasig : Between site differences
- 6.3 Seasonal trends

6.3.1 Pollen 6.3.2 Spores

6.4 Relationship between meteorological variables and airspora concentrations

6.4.1 Wind velocity

6.4.2 Relative humidity, temperature and precipitation

6.4.3 Sunshine hours

6.4.4 Atmospheric pressure

6.4.5 Temperature inversion levels

6.4.6 Wind direction

- 6.5 The Use and abuse of regression equations in model building
- 6.6 Synoptic charts : An alternative route in model building?

#### 6. DISCUSSION

# 6.1 <u>Ruantitative differences in the airspora spectrum</u>

# 6.1.1 The balance between pollen and spores in the atmosphere

The previous chapter indicates that spores are the dominant partner in the airspora spectrum. This is confirmed by the work of authors such as Anderson (1985) and Kumar (1985). Further to this the exact nature of the balance between spores and pollen is remarkably consistent with the results of other work world-wide. For example, Satpute et al., (1983) show that spores represent 72% of all airspora while pollen represents 22%. (the balance of 6% consisted of other organisms such as insect parts etc.) This compares favourable with an average of 78% for spores and . pollen for the two sites in Cape Town. 22% for However the work of Royes (1987) indicates that this figure is not consistent worldwide. He established that spores represented 97.73% of the spectrum while pollen comprised only 0.4%. Ofspecial interest is the recent S.A. study by Cadman (1988)on the airspora of the Witwatersrand. Cadman (1988) found that 6% of the made up of pollen, while 93% airspora was was Notwithstanding made up of spores. these differences, it is to be expected that there will be a variation in the airspora spectrum according to the resident flora in the region.

### 6.1.2 Pollen

Looking at the pollen spectrum exclusively, the dominance of Poaceae in the spectrum of both Bothasig and Epping is not reflected world-wide, although in some countries, for example the British (Hyde, Isles 1950), it is dominant.

Whereas the average for the two collecting sites in Cape Town is 44%, Frenquelli et al (1983) found that grass pollen only representes 17.47% of the spectrum in Italy. This is an interesting difference because the climate DT Italy, Frenguelli et al. (1783)notes, is sub-Mediterranean and therefore similar to the Peninsula's climate and it is suspected that the Peninsula figures should have been closer tn given by Frenguelli et al. (1983). those figures Cheng & Huang (1980) found that Poaceae 22.7% of the pollen represented spectrum in Taiwan and Al-Doory et al (1980) put Poaceae at of the pollen spectrum in Washington only 5.4% DC, USA. The climate of these two locations do bear any resemblance to the Peninsula's not climate. Again in the S.A. context, of special interest is the study of Cadman (1988) who found that grasses made up approximately 46% of the while weed and tree pollen spectrum pollen 7% respectively represented 9% and in the Witwatersrand. The Poaceae figure compares favourably with that of the Bothasig and Epping sites but the weed and tree percentage is much higher than the Cape Town figure. It would thus appear that the Cape Town figures for Poaceae are unusually high when compared to the rest of the keeping with world but are in that of the Witwatersrand. As Poaceae has well known allergenic properties, the dominance of Poaceae in the pollen spectrum shou]d be noted by researchers working in the field of allergies. Referring to the total pollen spectrums for Epping and Bothasig, the occurence of other species such as Restionaceae, Fabaceae. Proteaceae and Cyperaceae Asteraceae, Ericacea, is frequent as these taxa are known to exist not only in urban gardens in the Cape but are also constituents of the Cape Fynbos kingdom. However in а more general sense Amaranthaceae,

Chenopodiaceae, Cupressaceae, Asteraceae, Ericaceae, Pinaceae Cyperaceae, and Plantaginaceae are all represented in both the Epping and Bothasig pollen spectrum and this is in keeping with spectra in the rest of the world. Particularly noticeable is the dominance of the (Amaranthaceae & Chenopodiaceae)in weeds the spectra of both Epping and Bothasig. This is a phenomenon which occurs elewhere in the world (Anderson et al., 1978; Singh & Babu, 1982; Halwagy & Halwagy, 1984). Acacia is prevalent at both collecting sites. The long-leafed wattle, Acacia longifolia, is a widespread alien across the Peninsula and flowers profusely in the early months of spring. In all likelihood it is responsible for the relatively high <u>Acacia</u> concentrations at both sites.

# 6.1.3 Spores

The Deuteromycetes class (eg Alternaria, Fithomyces chartarum, Drechslera) is the best represented class in both the Epping and Bothasig is consistent with the spectra. This spore observations made by Lacey (1981) and Al-Doory (1984). However, in a quantitative sense, it is the Basidiomycotina that are dominant which is contrary to the findings of the above two authors.(These authors found that Cladosporium and then <u>Alternaria</u> were the most dominant). In very few cases does basidiospore dominance occur but there are recordings which show basidiospore dominance. for example Salvaggio (1970) and et al. (1984). Both found Hasnain that basidiospores showed massive dominance in the spore spectrum. It is not clear why this is the exception rather than the rule, but both authors mention that the substrate for the formation of the basidia was abundant in their collecting locations. However referring to Tables 6 and 7,

it is clear that Alternaria is second in the order of merit, after the basidiospores. This is also an organism that has well known allergenic properties. (Simmons, 1967; Solomon, 1978; Yunginger, 1980). In Cape Town and probably in the rest of South Africa, it is deserving of more thorough investigation. Finally, other dominant spores such as Pithomyces chartarum, Drechslera, Cladosporium, and Chaetonium (Tables 6 and 7) to mention but a few, are all found in the spectrums of Epping and Bothasig. The only major omision is Fusarium which appears in large concentrations in the rest of the world (Al-Doory, 1984). ٥f special interest in the S.A. context is the work of Ordman & Etter (1956) who invetigated fungi on the Witwatersrand. Their investigations show that Cladosporium contributed nearly a third of the colonies appearing on a culture dish during the collecting period. The next commonest fungi was Alternaria(12%) then Penicillium(10%). and order of merit in terms Although the of concentrations for Epping and Bothasig is not typical of many other global locations, i t is also clear that the findings of authors such as al. (1964) are not necessarily Sandhu et in keeping with broad trends in the rest of the world either. However, as mentioned earlier, this when one considers is to be expected the diversity of flora found in different regions of the world.

#### 6.2 Epping and Bothasig : Between site differences

It is necessary to briefly discuss the quantitative differences between the Epping and Bothasig airspora spectrums. Epping, with few exceptions seems to have the same types of pollen in its spectrum when compared to Bothasig. For two locations that are at the most 15km apart this would be expected. Significant differences, however, can be found in a quantitative comparison of

the two sites. Firstly in terms of mean daily pollen concentrations, Bothasig experiences approximately a 15% higher level of atmospheric pollen when compared to Epping. Although it is speculation attempting to explain this difference, the fact that Bothasig is a garden suburb and Epping an industrial site, probably goes some way in explaining the difference. Further to this, significantly Epping has a higher mean daily concentration of Pinaceae pollen. This is perhaps due to the fact that Pinelands, a suburb dominated by pines and proximity to Epping, is responsible in close for increasing the levels of atmospheric Pinaceae pollen concentrations in this area.

There is very little difference between the spore spectrums of Epping and Bothsig. With a few minor exceptions, for example Epping has small quantities of sporangiospores, both locations display similar spectra. Quantitatively, an extraordinary similarity exists in mean daily atmospheric spore concentrations between the two sites. At Epping the mean daily spore concentration is 33.94 spores  $m^{-3}$  of air while for Bothasig the figure 33.27 m<sup>−s</sup> of is spores air, а difference of It approximately two percent. is also apparent that there is a large degree of uniformity in the order of merit, with the most dominant spores being basidiospores, Alternaria, Drechslera, Pithomyces chartarum, fern spores, Chaetomium, Cordana musae, and Torula herbarum in that order. The exception is found in Bothasig where Drechslera and Pithomyces chartarum are reversed. The greater degree of conformity in the spore spectrum of the two sites, when compared to the pollen spectrum of the two sites seems to confirm the theory discussed in the earlier literature review that spores are more evenly spread over space and tend to be more global in distribution. Pollen concentrations on the other hand, although capable of long distance dispersal tend to be representative of the local flora. If this is the case then the conformity in the spore spectra is understandable, while the differences in the pollen

spectra reflect, perhaps, the different floral make up of the two sites.

Lastly something needs to be said about the glaringly low mean daily concentrations in both the pollen and spore spectrums, even amongst the dominants, Poaceae and basidiospores. That these figures are low js beyond dispute when compared to work done in the rest of the There occasions daily world. were when spore concentrations were above 550  $m^{-2}$  of air in Epping but this was a rare occurrence. Daily pollen concentrations never breached 50 grains m<sup>-a</sup> at Epping. By way of example, Kapyla (1984) was able to show that pines in Scandinavia, when in season, were producing well over 500 grains m<sup>-a</sup> of air and likewise for birch trees. Hasnain et al. (1984) was able to show that in New Zealand, basidiospore concentrations during peak periods often reached as high as 5000  $m^{-3}$  of air and regularly passed 2000 m<sup>-9</sup> of air. In trying to explain the low counts in Cape Town cognizance should be taken of two factors. Firstly, it is possible that the acetolysis preparation which involves many decantations method of may imply the loss of a significant number of spores and pollen grains. Certainly it is possible that the chemical treatment alone may destroy the less resistant and more delicate spores. As was mentioned earlier, mostresearchers place the Melinex tape from the collector straight onto the slide. stain the vaseline and then read the slide. In this way no grains are lost through A second consideration which cannot processing. bP ignored, is the possibility that there simply is not a high ambient level of airspora in the atmosphere of Cape Town. One may hypothesize that this is either the result of persistently high seasonal wind velocities which would tend to clear the atmosphere (there is no evidence in the literature that suggests anyone has tested this hypothesis) or alternatively a floral and mould constitution which does not produce and release vast multitudes of grains. These hypothesis' remain to Ъe tested. A suggested avenue of investigation to deal with

this problem would be first to adopt the more popular method of processing the airspora. Should this render, on average, significantly higher counts, then the problem would be part way to being solved. A further alternative would be to move the samplers to another location in order to test for uniformity. Should this yield higher concentrations of airspora, then the low counts for the current stations could be considered to be representative of the local conditions.

### 6.3 Seasonal trends

# 6.3.1 Pollen

Figures 19 & 20 and Figures 23 - 26 indicate the expected, namely that pollen peaks occur in spring and summer. is This in keeping with done Cadman (1988)research bу on the Witwatersrand, and elewhere in the world (Hyde, 1950; Nillson & Muller, 1981). A perplexing Table 24. The problem occurs in Fynbos concentrations (Ericaceae and Amaranthaceae) to be disproportionately high for the appear winter season. No valid explanation be can this occurence. offered for An interesting observation from the point of view of the two Fynbos families (Figures 24 26) & is the staggered peaking of these two families. While Ericaceae concentrations seem to peak iп October/November, the Proteaceae concentrations seem to peak later in the summer in Febuary and March/April. This appears to be the case for both Epping and Bothasig. From the point of view of the weed families (Figure 23 & 25), Amaranthaceae and Chenopodiaceae, both seem to peak in the late summer period (Febuary/March)

# 6.3.2 Spores

There is not the same degrees of seasonal dominance amongst the spores as there is in pollen (see Figures 20 & 22). This finding i 5 confirmed by many researchers eg. Sneller (1984). An interesting fact arises out of the work of Sneller (1984). that He argues Alternaria concentrations increase in the spring and summer months because in spring plant debris begins to increase with the rapid growth in plants during the growth season. The amount of substrate available to the Alternaria fungus increases and thus the concentrations of the fungus increase as well. In this study, although there is a slight Alternaria concentration in the increase in spring and summer months, this is not pronounced. Perhaps the reason for this is simply due to climate. In the Peninsula many trees and shrubs vegetation keep their foilage throughout the year. Thus there is substrate available for the fungus Alternaria throughout the year, Snellers' (1984) research is based in North America which experiences winter conditions such that plants stop growing and the necessary substrate ceases to exist leading to a decrease in Alternaria concentrations during the winter period.

# 6.4 <u>Relationship between meteorological variables and</u> airspore concentrations.

## 6.4.1 Wind velocity

Table 10 indicates that wind velocity, both in terms of the mean daily wind velocity (MW1) and maximum hourly wind velocity (MW2) are signifcant variables, although not consistently so for the different pollen and spores measured. Further to

different pollen and spores measured. Further to this, the level of correlation is low, although significant, which suggests that the relationship between wind and airspora concetrations is tenuous at best. Moreover, the regressions in Table 12, based on the stepwise regression analysis in Table 11 suggest that maximum hourly velocity is particularly important in а predictive sense. Although the warning is taken seriously that one cannot use repression analysis to elaborate on the causal effect of a parameter it seems that maximum hourly velocity which the wind speed refers to qusts іп may be for dislodging pollen from responsible the argued by Reddi et al. (1980).anther. as velocity is without doubt However, wind а variable asociated with fluctuations in pollen and spore levels and this assessment has support from various other authors, for example Kumar (1980) and Fischbach (1986). It is of interest to note that the relationship with wind velocity is not uniform for all types. Referring to Table 10, i t is apparent that <u>Alternaria</u> is positively correlated with maximum hourly wind velocity, while basidiospores show a negative correlation with this factor. The same is true for mean daily wind velocity. This is possibly explained by the fact that Alternaria is a summer type. Summer in the Peninsula is dominated by "blustery" SE winds. This perhaps explains why this spore is correlated with the factor, maximum hourly wind velocity.

#### 6.4.2 Relative humidity, temperature and precipitation

As has already been mentioned in the literature review, it seems senseless discussing these factors seperately because they are so closely related to one-another. Temperature and relative humidity were measured in terms of the maximum,

mean and daily figures. Table minimum, 10 indicates that in the case of relative humidity and temperature, these factors show a definite of relationship with most the six dependent and variables measured these various relationships have been outlined in the results section of this dissertation. The findings that some or all of these meteorological factors are sigificant is in keeping with research work 1977: elsewhere (Ljungkvist, Mercuri et al.. 1982; Kapyla, 1984; Ballero et al., 1985). Again one feels tempted on the basis of the offer a causal correlations to try and explanation for some of these relationships, but as has been pointed out this would be pure speculation at this point. However looking at the regression equations in Table 12, it is apparent that temperature and relative humidity do play an important role in a predictive sense. As in the case of wind velocity, however, the role of these factors is not uniform and this is apparent in Table 10. By way of example, minimum temperature has a weak positive relationship with Poaceae but a reasonably strong negative relationship with Pinaceae. However it is interesting to note that case of relative humidity, all in the six dependant variables show a negative relationship with 3 measurements of relative humidity (MH1, MH2 and MH3). Thus an increasing maximum, minimum and mean relative humidity is associated with decreasing airspora concentrations. However, it is apparent that an inceasing difference between and minimum humidity is positvely maximum associated with Pinaceae and basidiospores. Again one is tempted to try and explain the causal reasons for this. Precipitation generally should show a negative relationship with airspora. This is in fact the case (see Table 10). According to authors such as Hyde (1950) this is becasue the rain washes the airspora out of the atmosphere,

significance and is left out of the regression equations in Table 12. Perhaps this is because the variable is being masked by other variables, or because rain is a phenomenon which occurs only occasionally and thus does not influence airspora markedly on a daily basis. Notwithstanding this observation, there are authors (Petzoldt et al., 1983) who suggest that rainsplash can in fact dislodge spores into the atmosphere and thus increase volumetric concentrations in the atmosphere. However the evidence in this research does not lend support to this point of view.

#### 6.4.3 Sunshine hours

Hyde (1950) argued that increased sunshine hours led to increased grass pollen concentrations in the atmosphere. There are in fact any number of possible reasons for this. Associated increase in temperature could have inspired anther growth in the flowers, or the photoaction of the light may have triggered pollen release. In any event there is no doubting that increased sunshine hours is associated with increased temperatures and thus it is difficult to separate the causal components of these two variables. Lyon et al. (1984b) suggested that ascospores and basidiospores responded positively to increased sunlight hours. In the current study Table 10 indicates that sunshine hours is a significant variable for both spores and pollen. For example, Poaceae and Alternaria show a positive relationship with sunshine hours. Notwithstanding the effect – of sunshine hours, in the regression equations in Table 12, it is clear that temperature has greater predictive value than sunshine hours. The only exception to this is Poaceae. In this regard, it must be pointed out that Liem & Groot (1973) have shown experimentally that anthesis in grasses is strongly influenced by light. Perhaps,

(1973) have shown experimentally that anthesis in grasses is strongly influenced by light. Perhaps, then, it is not surprising that temerature is included in the regression equations.

# 6.4.4 Atmospheric pressure

Very few authors have been able to show a link atmospheric between pressure and airspora concentrations, although Ljungkvist et al. (1977) claimed that a sudden decrease in pressure seemed to increase the pine pollen count. In Table 12 atmospheric pressure is included in the regression equation for Pinaceae, therefore it must be assumed that it does have some predictive significance. In Table 10 it is evident that a significant but weak positive Poaceae has atmospheric relationship with pressure. The explanation for this may be that the summer season in the Peninsula is associated with fine weather caused by the southward movement of the South Atlantic high pressure cell. This is a summer phenomenon and therefore the possibilty of a relationship between high atmospheric pressure and Poaceae concentrations does exist. Table 10 suggests a significant positive relationship between atmospheric pressure and basidiospore concentrations. The correlation is, however, very weak as it is for the other 5 dependent variables. Strangely, perhaps, it does seem to have some predictive significance being included in the regression equations for Pinaceae and basidiospores. Very few authors however recognise it as a really significant variable.

#### 6.4.5 Temperature inversion levels

As the literature review pointed out, there is considerable disagreement on the effect of temperature inversions on atmospheric airspora

concentrations. Steel (1983) and Mandrioli (1987) attempted to explain the theory behind increased concentrations by implicating a low inversion level with increased atmospheric airspora concentrations. Rempe (1937) and Leuschner et al. (1987) have argued that their research shows that this relatioship exists. Spieksma (1983) argues in fact, that this relationship was not proved by his research and further there were occasions when the contrary was in true. This project has failed to demonstrate conclusively that any relationship existed between atmospheric airspora concentrations and inversion levels. Table 10 seems to suggest that morning inversion levels are negatively correlated with spore concentrations. Thus increasing height in the mixing level is associated with decreasing spore levels and the explanation for this phenomenon offered by Mandrioli (1987) and discussed at length in Chapter, may be applicable. However, the correlation is weak and should not be used as evidence in support of the assertions made by Steel (1983) and Mandrioli (1987). Interestingly, morning inversion levels do form part of the regression equation for spores and thus a. m inversion levels does have some predictive significance.

#### 6.4.6 Wind direction

The wind roses (Figures 29-318) indicate that there is not a relationship between the peaks in the airspora spectrum and the domiant seasonal wind direction. These dominant winds ( S and NW) as has already been mentioned are responsible for 73% of wind received during the course of the year studied (see Table 13). It is important to notice that the peaks in the mean daily airspora concentrations are associated with winds that do not form part of the seasonal picture.

before reaching Cape town (N - NE sector). Hyde (1950), Bagni et al. (1977), McDonald (1980) and Spieksma & Tonkelaar (1986) all found that peaks in airspora concentrations occurred when winds blew offshore. However the peaks that occurred with the wind blowing from the SSE to SE (Figures defy rational explanation 30 & 31) if wind an important parameter, because direction is winds from this sector defnitely blow over the ocean before reaching Cape Indian Town. Notwithstanding the above point, i t j 5, а the 20km of land that possibility that а southerly wind would blow over before reaching Epping/Bothasig, would allow the wind to gather pollen and spores before reaching these two sites.

# 6.5 The use and abuse of regression equations in model building

A11 the relationships between airspora and meteorological factors, with the exception of wind direction, discussed in the results section of this research are based on correlations and regression analysis (Tables 8-12). It is clear from the literature available on the subject that regression analysis, although a powerful tool, needs to be treated with caution.

"If the laim is to use correlation and regression analyses for making predictions, it ïs not know anything about the causal necessary to relationships (even if this is useful). Naturally, in theory, if we exactly know all the causal factors leading to a certain phenomenon, we can predict exactly its development in the In practice, we calculate future as well. experimental multiregression equations, and these are certainly of very great value in estimating what will be the pollen and spore situation in

the future. But it must not be imagined that indicate **biological** these (always) true dependence, or true causal relationships, and they do not (necessarily) form any basis for the mathematical modelling – of the biological phenomena...In other words. а numerical (mathematical) explanation is not necessarily a biological (causal) explanation." (Makinen, 1977 : 153)

From the above the point should be clear that it 15 scientifically immoral to make any conclusions about the biological causes of high or low atmospheric airspora concentrations using regressional analysis. This stands if one considers the discussion to reason in the previous chapters. Temperature, for instance, effects the development of the plant at various stages in its phenology. During the growth stage, during the stage of during dehiscence filament extension. and also, When during pollen release. possibily, using regressional analysis it is not at all clear which of these stages is being measured, therefore it would be incorrect, for instance, to claim that high temperatures pollen concentrations. Perhaps cause high high temperatures are in fact causing massive and rapid filament extension which perhaps creates the potential a later for high atmospheric pollen concentrations at stage. A further consideration is the autocorrelation of the independent variables. In aerobiological studies several factors participate which are autocorrelated. By way of example, spore concentrations are dependent on on humidity, temperature, on rain and and these meteorological factors are mutually dependent. It is verv difficult to separate them out and thus categorically state that one is more important than the other. Moreover, researchers have strongly emphasized the biological nature of the pollination process. The following quotation from Makinen (1977 :152) 15 illuminating in this respect.

following quotation from Makinen (1977 :152) is illuminating in this respect.

"If we wish to find a meteorological cause for the occurrence of allergenic <u>Alnus</u> pollen in the air ..., it is not much use studying simple or multiple, linear or non-linear correlations between pollen frequencies and meteorological data alone ... allergenic pollen in the air is the result of numerous development stages which may occur interdependently or independent of each other."

Many studies apparently neglect the phenology of particular taxa. By way of example, we may state that there is a high correlation between the incidence of Pinus pollen, increased wind velocity and decreased humidity, but we may not infer that these variables cause the pollen peak. The peak may in fact depend on other unknown factors and certainly other genetic factors are at work during the life cycle of the pine pollen. Each stage will have special requirements for meteorological and other conditions. Makinen maintains that this is as pertinent for fungal spores for as pollen. Bringfelt et al. (1982) also argue that the predictive models which are born out of multiple regression analysis cannot be used to offer any causal explanation because biological factors also play a role. Lyon et al. (1984b) distinguishes between the factors influencing the release of spores into the atmosphere factors influencing and the the number of spores remaining in the atmosphere. Accordingly, they agree with Makinen and Bringfelt and his associates that spore release involves primarily biological factors but also meteorological factors, while the spores remaining in the atmosphere depend largely on meteorological factors. This agrees with the argument put forward in previous chapters, that for pollen the process of anthesis and dehiscence are primarily genetic mechanisms which are meteorological influenced by considerations while

spore production release and dispersal is not at all clear. What the findings of the scientists emphasize is that meteorological factors cannot be seen as singularly causal. The high spore content may be correlated with high relative humidity but this may be influencing both production and release of spores. Moreover other factors are also at work such as leaf wetness and precipitation. These factors in turn are sometimes autocorrelated and are difficult to separate. Without labouring the point any further, it is safe only to say that there is a <u>relationship</u> between meteorological factors and airspora and that this relationship is not necessarily causal.

If the aim is to build a model for prediction purposes then any relationship inferred can only be mathematical. components of the relationship may only be The causal hinted at and this, in any case, will be speculation. Nevertheless. the most important reason for not inferring causal relationships from correlations can be found in the basic distiction between correlational methods and true experimental studies (Plutchik, 1974). Firstly, when correlating the independent variables with the dependent variables, no attempt is made in correlational studies to manipulate or change the conditions. For example, in this study no attempt was made to manipulate wind speed (by locating the sampler protected spot) to observe whether this would in a effect aispora concentrations. In experimental studies, the independent varaible is manipulated in order to measure the effect this would have on the dependent variable thus establishing the cause and effect principle. Secondly, time sequence in correlational studies has no particular relevance. Ιt matters not whether, for instance, temperature is measured first and or vica versa. then airspora concentrations, In an independent experimental study, the variable (temperature) is always set and measured accurately first and then only is the response of the dependent variable measured (airspora concentration). A third distinction is that in correlational studies if two

variables turn out to have a high and reliable correlation then this relationship can be used for prediction. However, correlational studies do not tell us which of the independent variables used is in fact causing the response of the dependent variable, or which of the independent variables measured, influences the dependent variable to the greatest extent. Fourthly, in experimental design, the an experiment should be repeatable ie. the conditions being known and controlled should be duplicated exactly a second time. This allows for the inital test results to be checked and rechecked. Clearly this is not possible in the type of study undertaken in this reasearch. One years meteorological data cannot be duplicated a second time nor for that matter can airspora concentrations. The differences that have been described do not mean that correlational studies are of no value. For the type of research undertaken here. where atmospheric conditions are difficult and costly to copy in a controlled laboratory situation, the correlational method represents an acceptable method of investigation. In addition. correlational studies frequently suggest hypotheses that may be tested at a later stage by means of experiments (Plutchik, 1974). One need not apologise for using regressional analysis, provided it is used circumspectly and with caution. That regression analyses/correlation analyses have been used successfully in establishing associations between meteorolgical factors and airspora concentrations and to build predictive models is beyond dispute.(Eversmeyer & Burleigh, 1970; Burleigh et al., 1972; Eversmeyer et al., 1973; Reiss & Kostic, 1976; Makinen, 1977; Ljungkvist et al., 1977; Andersen, 1980; Bringfelt et al., 1982; Savary, 1986)

A further consideration in this research is the low r values for the single correlations between aispora concentrations and meteorological factors and relatively low  $R^{e}$  for the regression equations. This brings to mind the warning of Gilbert (1986). "...the problems manifest themselves in applied work in terms of...wrong signs, insignificant coefficients and so forth. The term 'wrong' is telling - we know what the right sign is; the estimates give us the wrong sign; and the...response to these pathological manifestations is to respecify his equation in some way - to add or subtract variables, change the definition of variables and so forth - until, eventually, he gets an equation which has all the corect signs, statistically significant coefficients..., a relatively high R<sup>e</sup> and so forth" (Gilbert 1986:284)

Although stepwize regression analysis was used in this research implying a certain manipulation of the data to "improve" the R<sup>e</sup> of the chosen equations, very little manipulation of data of the type listed in the above quotation took place. Had variables been "added and subtracted" and the definition of variables changed, it distinct possibility that that the R<sup>e</sup> values could is a have been stronger. The fact that the r and R<sup>e</sup> values were not particularly strong, calls into question whether the correlational method can actually be used for quantifying relationships where the relationship between flora and weather is so complex. In this regard it should be said that weak correlations may in fact be a strength, and a warning that these factors have a complex relationship with the genetic and physiological properties of flora. Thus any single factor is not seen to act consistently because the plant is influenced by so many other factors at one or other time during its life cycle. If this line of reasoning is correct then perhaps one should not în fact expect strong correlations.

Another possibility is to employ a method of modelling which does not rely on regressions. Washington (pers. comm.) has suggested that an alternative to predictive models, which essentially rely on computer modelling, would be the use of a synoptic chart coupled with the the viual aid of graphs as seen in the Figures 27a-28c.

models, which essentially rely on computer modelling, would be the use of a synoptic chart coupled with the the viual aid of graphs as seen in the Figures 27a-28c. The argument behind this approach lies in the fact that greater forces are at work in the release and distribution of daily airspora than simply the fluctuations of selected factors and that a more holistic approach is required in order to arrive at a broader definition of weather patterns which produce above average or below average concentrations in the atmosphere. Although this dissertation does not intend following this line of investigation thoroughly, it was felt that an initial attempt should be made to relate peaks and troughs in the graphs (Figures 27a-28c) to sub-continental scale weather patterns and in so doing attempt to isolate some general trends in the weather which would be helpful in creating an alternative model for predictive purposes, where the emphasis is on the broad picture rather than the day to day fluctuations in various parameters.

# 6.6 Synoptic charts : An alternative route in model building?

In adopting this method of investigation it was decided that two peak and trough periods would be analysed from each of the winter and summer seasons. The first winter period selected was from the 07/07/87 - 14/07/87. This identified as showing unusually large period was fluctuations in atmospheric levels of airspora (see Figures 27a & 28a ). Studying the synoptic charts for the period (Appendix 3.1-3.8) the following pattern emerges. The first three days represent high levels in atmospheric pollen and spores. The synoptic charts for the period show a high pressure zone to the South of the country with approaching cold fronts. A dominant feature is the presence of berg winds which blow from the interior of the subcontinent. This is an important feature since these winds have the opportunity to pick up pollen and spores from the interior, thus increasing

their concentration in the atmosphere. However, it is noticeable that as the fronts approach the subcontinent and pass over it, the wind direction turns to the South and then the North West. The wind in both cases is now blowing off the sea and this is associated with drops in both pollen and spore concentrations (11/07/87 14/07/87). This association between wind direction and airspora concentrations is documented by a number of authors already mentioned in the literature review (eg McDonald, 1980). It is of interest to note however, that the pollen levels drop to zero  $m^{-3}$ , the spore whereas levels, although decreasing in volume still remain at relatively high volumes.

The second winter period studied was from 29/05/88 -(Appendix 3.9-3.16). The 05/06/88 period shows remarkably high spore peak on the 31/05/88 (Figure 28c) followed by a trough, while pollen concentrations which by this time of the year have very low atmospheric levels anyway, also shows a small peak on the same day. Both pollen and spores experience a trough on the 02/06/88, a considerable increase on the 03/06/88. followed by another trough for the next 5 days. Studying the synoptic charts for the period, it is evident that a cold front has just passed over the peninsula on the 26/05/88 bringing rain and resulting in low pollen and spore counts. The following day the weather began to clear and this continued until the 28/05/88. As one would expect, pollen and spore concentrations recovered to average levels with winds blowing from the South The approach of a further cold front on the 29/05/88 bringing slight drizzle reduced airspora levels once more. However with the passing of this front a high pressure system ridged in on the west coast bringing conditions (9.6 sunshine) and the hrs. wind fine direction swings to NNE (30/02/88). Although a coastal low develops the following day, the wind direction remains NE. These conditions are associated with a 100% increase in pollen concentrations and a 70% increase in spore concentrations. Again the general picture seems to

should be emphasized that this is of great significance because the seasonal winds, which the cape experiences, the SE wind in and the NW summer wind in winter, originate from the Indian and Atlantic oceans respectively. Thus these winds do not collect airspora before reaching the coast. Should the wind however blow from the sector N to NE, then there is the possibility that the wind will collect airspora from the earth's surface. This tends to confirm the argument for airspora peaks in the discussion on wind direction (Paragraph 6.4.6 and Figures 29-31). It is of interest to note that as the next cold front approaches on the 01/06/88, airspora levels begin to drop once more. The wind direction swings to NW - NNW, thus originating from the Atlantic. In these conditions it is impossible for the wind to pick up large concentrations of pollen (at most the wind will blow over only 15 km's of land before reaching the collection point) and also unlikely that it will pick up many spores.

The period 10/12/87 - 16/12/87 (Appendix 3.17-3.23) is the first of two summer periods to receive attention. The first three days of this period show unusually low pollen concentrations while spore concentrations are moderate. From the 13/12/87 levels in both pollen and spores increase and remain at high levels for the next three days. The synoptic charts for the period show an approaching cold front on the 10/12/87 which passes over the Peninsula on the 11/12/87 bringing cool damp weather, although rain was not recorded at the D.F. Malan weather station. Wind direction during the two days has swung from NW to S, thus originating from the Atlantic and Indian oceans respectively. Although this is a summer period the trend seems to be the same as the winter frontal pattern. A cool airmass in the form of a frontal system passes over the subcontinent. This is associated with winds originating from the seamass and in turn there is a corresponding reduction in airspora. The period from the 13/12/87, marking an increase in airspora levels, is associated with typical summer

in turn there is a corresponding reduction in airspora. The period from the 13/12/87, marking an increase in airspora levels, is associated with typical summer conditions. The high pressure system to the south of the country remains in place for a four day period. This system is associated with the seasonal SE wind and sunny, fine weather. Although these winds have their origin over the Indian ocean, it is evident that the relatively high levels of airspora cannot be explained by wind direction. Strictly speaking, one would have expected lower levels if wind direction was a dominant variable. However, it is worth noting that the airspora levels are not excessively high and seem to be in keeping with other days where the high pressure system is dominant and the SE wind prevails.

The second summer period which received attention was the period 26/02/88 - 02/03/88 (Appendix 3.24-3.29). This period iniatally shows a depressed pollen and spore count followed by a recovery in both spores and pollen to reasonably high levels (Figures 27b, 27c, 28b, 28c). The synoptic charts indicate that on the 26/02/88 a frontal system approachs the sub continent and passes over the peninsula on the 27/02/88. Cooler damp weather with a NW wind is associated with low airspora levels. As the frontal system moves eastwards, the South Atlantic high ridges in behind it bringing fine weather with S to SE winds. At this point the airspora levels recover to reasonably high levels once more.

In summary it is apparent that synoptic charts may be useful as predictive tools because, in the short analysis above, some definite trends do emerge.

a). It apparent that excessive fluctuations is in aispora concentrations (above 100 grains $^{-3}$  and less than 5 grains  $m^{-3}$ ) are a feature of the winter and eàrly spring season, while in summer the fluctuations, while apparent, are certainly not as extreme.

b). Troughs in the airspora in both winter and summer seasons seem to be associated with frontal systems and damp weather. In winter, peaks seem to be associated with winds which have their origin in the interior of the country while in summer peaks tend to be more recovery periods from a trough probably caused by frontal systems which has wandered further north than expected.

Further to this it would be speculation to try and infer any further relationship between airspora levels and information gleaned from a synoptic chart. Suffice to say at this point that it appears to be a worthy avenue of endeavour and perhaps the correct approach would be a combination of computer modelling and the use of synoptic charts in an effort to provide a more realistic and balanced approach to a field of study which by all accounts remains unchartered territory. Certainly a holistic approach which incorporates broad trends in the weather, plant physiology and daily fluctuations in certain meteorological factors would be the ideal approach.

In conclusion, a further criticism of this research can be found in the lack of an hourly record of airspora concentrations and meteorological factors. The correlations in this study are based on the mean daily concentrations of airspora and mean daily meteorological factors. Thus sudden changes in weather for example at sunset and sunrise (mainly temperature and relative humidity) are not taken into account. What is probably now required is an hourly record of the fluctuations in airspora concentrations and likewise for meteorological factors. In that way it is possible to plot the exact nature of a pollen or spores response to hourly changes in meteorological variables. It should be said at this point that this requires amount an enormous of microscope work and it was felt that initially it would

. 1

# 7. CONCLUSION

## 7. CONCLUSION

7.1 Pitfalls in aeropalynological research

7.2 The way forward

ł

### 7. CONCLUSION

### 7.1 Pitfalls in aeropalynological research

It should be said that the results in this research should not be treated as a definitive study on airborne pollen and spores in the peninsula. There are a number of reasons for this. Firstly, the collecting period was one year and it is clear from the literature that at least a five year monitoring period is required in order to establish a reliable predictive model for an area. Secondly, it should be born in mind that Cape Town covers a vast area with an extraordinary diversity in climate depending on which side of table mountain one is at any point in time. Simply, Cape towns climate is not homogenous. Further, although no confirmation of this is hand. it is strongly suspected that vegetation at diversity is extreme, depending where one is in relation to the Fynbos plant kingdom. It is thus not possible to apply models built on data from one collecting station (Epping) to the whole of the peninsula. The following points also need to be carefully considered:

- a). Any significant correlation should not be seen as an indication that there is a causal relationship between the two variables. Rather it should be seen as an association of two variables.
- b). The results of the research are applicable to the vicinity of Epping and Bothasig and probably become less dependable as one moves away from these two areas.
- c). It should also be acknowledged that different sampling devices and a different method of laboratory treatment of the collected pollen and spores may well have led to different results.

Besides these principles there were other problems encountered during the research which ultimately must

affect the accuracy of the results. The reliability of the Burkard sampler at Epping was dependent on a constant supply of 12 volts from the battery. If the supply varied it would mean the device would not sample a constant volume of air, thus giving highly suspect volumetric concentrations of airspora. Initially, it was uncertain how long the electric motor would take to exhaust the battery, thus setting the trickle charger at the correct amperage was guess work. Needless to say the battery did run flat on the 2nd and 3rd of August 1987. At night the security lights would automatically trigger the trickle charger and the sampler would have run direct from this source for approximately 12 hours for these two days. A very rough estimate was thus made for the two days lost readings. A far more serious problem lay with the electric motor operating the pump. The Figures 27a & 28a indicate a huge gap in the Epping data from 13/09/07 - 29/10/87. This represents a period of almost two months during the crucial spring period. The reason for the gaps in the data during this period is simply that the eletric motor failed completely. After contacting the Burkard manufacturers in Britain, it was apparent that a replacement engine would take 3 months to reach us. A decision was then taken to install a cheap Japanese engine which was acquired from a toy shop, the original purpose of the engine being to power model boats. This engine proved to be very efficient and served the collector without complaint for the remainder of the monitoring period. However, there can be no doubt that the two month gap in the data, particularly as it spring period, would have affected was in the the outcome of the regressional analysis and the predictive equations that were built on the regression analysis. This is another reason explaining why the predictive capabilities of the equations should be treated with some caution.

A further gap in the data can be seen from 11/08/89 -24/08/89. This gap was caused as a result of failure of the clock which is responsible for turning the collecting drum. Again as there are no Burkard dealers in S.A. a local clock maker had to repair the device, after which it operated without further mishap. It needs to be said that the battery operated Burkard collector, in this research at any rate, did not prove itself to be particularly reliable.

#### 7.2 The way forward

- a) In the first instance, to receive a clearer picture of the airspora spectrum for Cape Town as a whole, at least another three monitoring stations need to be set up. Suggestions in this regard would be Fishoek, Camps Bay and Newlands. These areas represent different aspects of the total climate picture in Cape Town and are located closer to the local Fynbos and would thus give a clearer picture of the broader airspora spectrum.
- b) As has already been mentioned, it is necessary to collect data for at least five years before attempting to build a suitable predictive model for the region.
- c) Serious consideration should be given to adopting the more conventional approach when processing the airspora on the melinex tape. The approach adopted in this research. (the prior treatment of the airspora under the process of acetolysis), allowed a close comparison of the pollen and spores with the pollen reference collection held in the Biogeography section of the Department of Evironmental and Geographical science at U.C.T. Although this had its advantages, it was a costly exercise in time and the approach of placing daily or more conventional hourly sections of the melinex tape under a cover slip and then reading the slide directly under the microscope would in retrospect save an enormous amount of time. In fact it is probably true to say that if an hourly or bi-hourly analysis of airspora

is attempted, the conventional system is the only one that is really pratical.

- d) Fortunately there was a reasonably comprehensive pollen slide and photograph collection which could be referenced in the case of an unknown pollen. However, the case of spores was more difficult. For the most part Gregory,s (1973) book contains a section where a number of well known spores are illustrated. This served as a guide when attempting to name an unknown spore, but it was felt that this was unsatisfactory and explains in part why there is a large "spore other" component to the aispora spectrum (Tables 6 & 7). Since the spores are so the airspora spectrum it dominant in is thus necessary to build up a spore reference collection in order to make identification more accurate.
- e) Lastly, closer co-ordination with the medical fraternity would make this type of research more relevant particularly in the light of the fact that spores are so dominant, and in fact receive very little attention in this country.

8. REFERENCES

1

18i

#### REFERENCES

- Al-Doory, Y. 1984: Airborne Fungi. In <u>Mould Allergy</u>. Edited by Al-Doory, Y and Domson J.F. Lea and Febinger : Philadelphia.
- Al-Doory, Y., Domson, J.F., Howard, W.A. and Sly, R.M. 1980: Airborne fungi and pollens of the Washington, D.C. Metropolitan area. <u>Ann. Allergy</u>, 45: 360-367.
- Al-Eisawi, D. and Dajani, B. 1987: A study of airborne poller grains in Amman, Jordan. Grana, 26: 231-238.
- Al-Tikriti, S.K. Al-Salihi, M. and Gaillard, G.E. 1980: Pollen and mould survey of Baghdad, Iraq. <u>Ann. Allergy</u>, 45: 97-99.
- Alexopoulos, C.J. and Mims, C.W. 1979: <u>Introductory Mycology</u>. 3rd Ed. John Wiley and Sons : New York.
- Andersen, A.A. 1958: New sampler for the collection, sizing and enumeration of viable airborne particles. <u>J. Bact</u>., 76: 471-478.
- Anderson, E.F., Dorsett, C.S. and Fleming, E.D. 1978: The airborne pollens of Walla Walla Washington. <u>Ann. Allergy</u>, 41: 232-235.
- Anderson, J.H. 1985: Allergenic airborne pollen and spores in Anchorage, Alaska. <u>Ann. Allergy</u>, 54: 390-399.
- Anderson, S. 1980: Influence of climatic variation on pollen season severity in wind-pollinated trees and herbs. <u>Grana</u>, 19: 47-52.
- Assem, A. Van Den. 1973: Daily census of airborne pollen in the Netherlands especially in relation to hay fever. In <u>Airborne</u> <u>transmission and airborne infection</u>. Edited by Hers and Winkler. Dosthoek : Utrecht.

- Aylor, D.E. and Lukens, R.J. 1974: Liberation of <u>Helminthosporium</u> <u>maydis</u> spores by wind in the field. <u>Phytopathology</u>, 64: 1136-1138.
- Bagni. N., Davies, R.R., Mallea, M., Nolard, N., Spieksma, F.Th. and Stix, E. 1977: Sporenkonzentrationen in stadten der Europaischen Gemeinschaft (E.G.).II. <u>Cladosporium</u> und <u>Alternaria</u> sporen. <u>Acta Allergol.</u>, 32: 118-138.
- Bai, A.J. and Reddi, C.S. 1981: The fungus airspora of Visakhapatnam. <u>Proc. Indian Natn. Acad</u>., B47, 5: 731-747.
- Ballero, M., Piu, G. and Sechi, P. 1985: A pollen calendar for Oristano (Central Sardinia): Preliminary data. <u>Bol. Soc.</u> <u>Brot. Ser</u>., 58: 57-62.
- Beaumont, F., Kauffman, H.F., Van Der Mark, T.H., Sluiter, H.J. and De Vries, K. 1985: Volumetric aerobiological survey of conidial fungi in the North-East Netherlands. <u>Allergy</u>, 40: 173-180.
- Bergstrom, B. 1975: Bruksanvisning for FOA slit sampler modell B-74 och A-73. FOA rapport C40017-B4.
- Blackley, C.H. 1873: <u>Experimental researches on the cause and</u> <u>nature of Catarrhus Aestivus</u>. Balliere, Tindall & Cox, London.
- Bringfelt, B., Engstrom, I. and Nilsson, S. 1982: An evaluation of some models to predict airborne pollen concentrations from meteorological conditions. Stockholm, Sweden. <u>Grana</u>, 21 : 59-64.
- Brown, H.M. and Jackson, F.A. 1978: Aerobiological studies based in Derby. III. A comparison of simultaneous pollen and spore counts from the East coast, Midlands and West coast of England and Wales. <u>Clin. Allergy</u>, 8: 611-619.

- Buck, P. and Levetin, E. 1982: Weather patterns and ragweed pollen production in Tulsa, Oklahoma. <u>Ann. Allergy</u>, 49: 272-275.
- Burge, H.A. 1986: Some comments on the aerobiology of fungus spores. <u>Grana</u>, 25: 143-146.
- Burleigh, J.R., Eversmeyer, M.G. and Roelfs, A.P. 1972: Development of linear equations for predicting wheat leaf rust. <u>Phytopathology</u>, 62: 947-953.
- Cadman, A. 1988: Atmospheric monitoring in the PWV: Results for 1987. Allergy Update, 4: 4-6.
- Carter, M.V. 1957: <u>Eutypa armeniacae</u>, an airborne vascular pathogen of <u>Prunus armeniacae</u> L, in Southern Australia. <u>Aust. J. Bot.</u>, 5: 21-35.
- Carter, M.V. and Moller, W.J. 1977: Eutypa canker and diebacks of apricots. <u>Eur. Plant Prot. Organ. Bull</u>., 7: 85-94.
- Charpin, J., Boutin, C., Aubert, J. and Barre, A. 1973: Correlations between atmospheric studies and clinical data in pollinosis. In <u>Airborne transmission and airborne</u> <u>infection</u>. Edited by Hers, J.F.Ph and Winkler, K.C. Oosthoek Publ. Co., Utrecht.
- Chaubal, P.D. and Kotmire, S.Y. 1982: Aerobiological studies at Kolhapur. <u>Acta Botanica Indica</u>, 10: 100-102.
- Chen, S-H. 1984: Aeropalynological study of Nanking, Taipei (Taiwan). <u>Taiwania</u>, 29: 113-119.
- Chen, S-H. and Huang, T. 1980: Aeropalynological Study of Taipei Basin, Taiwan. <u>Grana</u>, 19: 147-155.
- Collins-Williams, C., Kuo, H.K., Garey, D.N., Davidson, S., Collins-Williams, D., Fitch, M. and Fischer, J.B. 1973: Atmospheric mould counts in Torornto, Canada. <u>Ann. Allergy</u>, 31: 69-71.

- Craigie, J.H. 1945: Epidemiology of stem rust in Western Canada. Scient. Agric., 25: 285-401.
- Cunningham, D.D. 1873: <u>Microscopic Examination of Air</u>. Government Printer, Calcutta.
- Davidson, R.L. 1941: A note on anthesis in some grasses near Johannesburg, and the relation of anthesis to collection of pollen for medical purposes. <u>J.S. Afr. Bot.</u> 7: 145-152.
- Davies, R.R. 1969: Aerobiology and the relief of asthma in an alpine valley. <u>Acta Allergol</u>, 24: 377-395.
- Davies, R.R., Denny, M.J. and Newton, L.M. 1963: A comparison between the summer and autumn air spores at London and Liverpool. <u>Acta Allergol</u>., 18: 131-147.
- Davies, R.R. and Smith L.P. 1974: Weather and the grass pollen content of the air. <u>Clin. Allergy</u>, 3: 263-267.
- Dhorranintra, B., Bunnag, C. and Limsuvan, S. 1987: Survey of Atmospheric Pollens in various Provinces of Thailand.In <u>Advances in Aerobiology; Proceedings of the 3rd</u> <u>International conference on Aerobiology</u>, Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Dixon, W.J., Engelman, L., Frame, J.W., Hill, M.A., Jemrich, R.I. and Toporek, J.D.(Eds.) 1983: <u>BMDP statistical software</u>. University of California press : Berkeley.
- El-Ghazaly, G and Fawzy, M. 1987: A study of airborne pollen grains of Alexandria, Egypt. In <u>Advances in Aerobiology;</u> <u>Proceedings of the 3rd International conference on</u> <u>Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- English, H. and Davis, J.R. 1965: Apricot dieback fungus found on Western chokeberry. <u>Plant dis. rep.</u>, 49: 178-183.

- Erdtman, G. 1960: The Acetolysis method. <u>Svensk. bot. tidshr</u>.,, 54: 561-564.
- Eversmeyer, M.G. and Burleigh, J.R. 1970: A method of predicting epidemic development of wheat leaf rust. <u>Phytopathology</u>, 60: 805-811.
- Eversmeyer, M.G., Burleigh, J.R. and Roelfs, A.P. 1973: Equations for predicting wheat stem rust development. <u>Phytopathology</u>, 63: 348-351
- Eversmeyer, M.G. and Kramer, C.L. 1987a: Components and techniques of integrated pest management threshold. Determinations of aerial pathogens. <u>Plant Disease</u>, 71: 456-459.
- Eversmeyer, M.G. and Kramer, C.L. 1987b: Single versus multiple sampler comparisons. <u>Grana</u> 26: 109-112.
- Faegri, K. and Iverson, J. 1975: <u>Textbook of Pollen Analysis</u>. Blackwell scientific publications, Oxford : London.
- Fairley, D. and Batchelder, G.L. 1986: A study of oak pollen production and phenology in Northern California: Prediction af annual variation in pollen counts based on geographic and meteorological factors. J. Allergy Clin. Immunol.,, 78, 2: 300-307.
- Fischbach, F.A. 1986: Biophysical factors in ragweed pollen. Grana, 25: 221-233.
- Fitt, B.D.L., Creighton, N.F. and Bainbridge, A. 1985: Role of Wind and Rain in Dispersal of <u>Botrytis Fabea</u> Conidia. <u>Trans.</u> <u>Br. Mycol. Soc</u>. 85: 307-312.
- Frenguelli, G., Mincigrucci, G., Romano, B. and Bricchi, E. 1983: Census of airborne pollen grains in the atmosphere of Ascoli Piceno (Central Italy). <u>New Phytol</u>., 95: 147-151.

- Fuckreider, K. 1976: Der graspollengehalt der luft in mittel-Europa. Berichte 9/76; Umweltbundesamt, Munchen.
- Galan, C., Ruiz de Clavijo, E., Infante, F. and Gallego, G. 1987: Annual and daily variation of pollen from <u>Olea Europaea L.</u> in the atmosphere of Cordoba (Spain) along two year of Sampling. In <u>Advances in Aerobiology; Proceedings of the 3rd</u> <u>International Conference on Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Gilbert, C.L. 1986: Frofessor Hendry,s econometric methology. Oxford Bulletin of Economics and Statistics, 48: 283-305.
- Gottwald, T.R. and Bertrand, P.F. 1982: Patterns of diurnal and seasonal airborne spore concentrations of <u>Fusicladium</u> <u>effusum</u> and its impact on a Pecan scab Eeidemic. <u>Phytopathology</u>, 72: 330-335.
- Gravesen, S. 1972: Identification and quantification of indoor airborne micro-fungi during 12 months from 44 Danish homes. <u>Acta Allergol</u>., 27: 337-354.
- Green, J.S.A. 1986: Levitation by convection, or does what goes up necessarily come down? <u>Weather</u>, 41: 110-114.
- Gregory, P.H. 1973: <u>The Microbiology of the Atmosphere</u>. Leonard Hill : Bucks.
- Halwagy, M., and Halwagy, R. 1984: Seasonal distribution of pollen grains in the atmosphere of Kuwait. <u>Pollen et Spores</u>, 36, 2: 201-216.
- Hampton, J.G. 1980: The role of seed-borne inoculum in the epidemiology of net blotch of barley in New Zealand. <u>N. Z. J. Exp. Agric.</u>, 8: 297-299.
- Harrington, J.B., Gill, G.C. and Warr, B.R. 1959: High efficiency pollen samplers for use in clinical allergy. <u>J. Allergy</u>, 30 : 357.

- Harz, C.D. 1904: Bakteriologische untersuchungen der freien atmosphare mittels luftballons, nebst bemerkungen urber den atmospharischen staub. <u>Jb. Dtsh. Luftsch Verb.</u> 1904: 147-170.
- Hasnain, S.M., Newhook, F.J., Wilson, J.D. and Corbin, J.B. 1984: First report of <u>Ganoderma</u> allergenicity in New Zealand. <u>New</u> <u>Zealand Journal of Science</u>, 27: 261-267.
- Hawke, P.R. and Meadows, M.E. 1989: Winter airspora spectra and meteorological conditions in Cape Town, South Africa. <u>Grana</u> (In press).
- Hemming, R. 1908: Uber die arten und die verbreitvag der lebensfahigen mikroorjanismen in der atmosphaire. <u>Z.Hyg.</u> <u>Infeckt kr.</u>, 58: 345-385.
- Henningson, E., Roffey, R. and Borallius, A. 1981: A comparative study of apparatus for sampling airborne micro-organisms. <u>Grana</u>, 20: 155-159.
- Hilderbrand, P.D. and Sutton, J.C. 1982: Weather variables in relation to an epidemic of onion downy mildew. <u>Phytopathology</u>, 72: 219-224.
- Hirst, J.M. 1952: An automatic volumetric spore trap. <u>Ann. Appl.</u> Biol., 39: 257.
- Hirst, J.M. and Stedman, D.J. 1967: Long distance spore transport. Methods of measurement. Vertical spore profiles and the detection of immigrant spores. <u>J. gen. micobiol</u>. 48: 329-355.
- Hoffman. D.R. 1984: Mould Allergens. In <u>Mould Allergy</u>. Edited by Al-Doory, Y and Domson, J.F. Lea and Febinger : Philadelphia.
- Hotchkiss, M., Scherago, M. and Chaplin, I. 1963: A weekly mould survey of the air in Indianapolis, Indiana. <u>Ann. Allery</u>, 21: 563.

Hubbard, C.E. 1968: Grasses. Penguin Books Ltd., Harmondsworth.

- Hubert, K. 1932: Beobachtungen uber die verbreitung des gelbrostes being kunstlichen feldinfektion. <u>Foitschr. Landw</u>. 7: 195-198.
- Hurtado, I. and Riegler-Goihman, M. 1986a: Air-sampling studies in a tropical area. <u>Grana</u>, 25: 63-68.
- Hurtado, I. and Riegler-Goihman, M. 1986b: Air-sampling studies in a tropical area II. Fungus spores. <u>Grana</u>, 25: 69-73.
- Hurtado, I. and Riegler-Goihman, M. 1987: Air sampling in a tropical Area. Four year results. In <u>Advances in</u> <u>Aerobiology: Proceedings of the 3rd International conference</u> <u>on Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Hyde, H.A. 1949: The phenology of British hayfever plants and its significance to allergists. <u>Br. med. J</u>., 1: 897.
- Hyde, H.A. 1950: Studies in Atmospheric Pollen. IV A survey of pollen deposition in Great Britain made in 1943. Part I, The influence of situation and weather. <u>New Phytologist</u>, 49: 398-406.
- Hyde, H.A. 1952: Grass pollen in Great Britain. <u>Acta Allergol</u>, 5: 98-112.
- Hyde, H.A. 1969: Aeropalynology in Britain an outline. <u>New</u> <u>Phytologist</u>, 43: 579-590.
- Hyde, H.A. 1973: Atmospheric pollen grains and spores in relation to allergy. II. <u>Clin. Allergy</u>, 3: 109-126.
- Hyde, H.A. and Williams, D.A. 1944: Studies in atmospheric pollen. I. A daily census of pollens in Cardiff, 1942. <u>New</u> <u>Phytologist</u>, 43: 49-60.

- Hyde, H.A. and Williams, D.A. 1945: Studies in atmospheric pollen 11. Diurnal variation in the incidence of grass pollen. <u>New</u> <u>Phytologist</u>, 44: 83-94.
- Infante, F., Ruiz de Clavijo, E., Galan, C. and Gallego, G. 1987: Occurrence of <u>Alternaria</u> in indoor and outdoor habitats in Cordoba (Spain). In <u>Advances in Aerobiology; Proceedings of the 3rd International conference on Aerobiology. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.</u>
- Janzon, L. 1981: Airborne pollen grains under winter conditions. Grana, 20: 183-185.
- Jordan, V.W.L. 1981: Actiology of barley net blotch caused by <u>Pyrenophora teres</u> and some effects on yield. <u>Plant Pathol.</u>, 30: 77-87.
- Jurgenson, S. 1967: A method of absolute pollen counting. <u>New</u> <u>Phytologist.</u>, 66: 489-493.
- Kapyla, M. 1981: Diurnal variation of non-arboreal pollen in the air in Finland. <u>Grana</u>, 20: 55-59.
- Kapyla, M. 1984: Diurnal variation of tree pollen in the air in Finland. <u>Grana</u>, 23: 167-176.
- Kapyla, M. and Penttinen, A. 1981: An evaluation of the microscopial counting methods of the tape in Hirst-Burkard pollen and spore trap. <u>Grana</u>, 20: 131-141.
- Keynan, N., Shomer-Ihan, A., Goren, C., Geller-Berenstein, C., Beljerano, A., Neuman, I. and Waisel, Y. 1986: The use of the AISI air sampler for pollen sampling airborne pollen. Pollen et Spores, 28: 97-104.
- Kotzmanidou, P and Nilsson, S. 1977: On the pollen incidence and phenology of some trees in southern and central Sweden, 1974-1975. A preliminary study. <u>Grana</u>, 16: 195-198.

- Kramer, C.L. and Eversmeyer, M.G. 1984: Comparisons of airspora concentrations at various sites within a ten kilometre radius of Manhattan. Kansas, U.S.A. <u>Gr</u>ana, 23: 117-142.
- Kumar, S. 1985: A survey of aero-allergenic pollen and spores in the urban environment of Bareilly (India). <u>Geophytology</u>, 15: 188-198.
- Lacey, J. 1981: The aerobiology of conidial fungi. In <u>Biology of</u> <u>Conidial Fungi</u>. Edited by Cole, G.T. and Kendrick, B. Academic Press : New York.
- Larson, E.S. 1981: A three year survey of microfungi in the outdoor air of Cpoenhagen, 1977-1979. Grana, 20: 197-198.
- Lawande, R.V. and Onyemelukwe, G.C. 1984: Airborne fungi during Harmattan in Zaria, Nigeria. <u>Ann. of Allery</u>, 52: 47-49.
- Leach , C.M. 1975: Influence of relative humidity and red-infrared radiation on violent spore release by <u>Drechslera turcica</u> and other fungi. <u>Phytopathology</u>. 67: 879-895.
- Leuschner, R.M. and Boehm, G. 1979: Investigation with the "Individual Pollen Collector" and the "Burkard Trap" with reference to hay fever patients. <u>Clin. Allergy</u>, 9: 175-184.
- Leuschner, R.M. and Boehm, G. 1981: Special weather conditions, air pollution, pollen and allergy attacks of patients. (abstr.) - Proc. 4th Charles Blackley Symp., Nottingham pp. 15-16.
- Leuschner, R.M., Boehm, G. and Brombacher, Chr. 1987: Influence of inversion layers on the daily pollen count and on the allergic attacks of patients. In <u>Advances in Aerobiology:</u> <u>Proceedings of the 3rd International conference on</u> <u>Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Lewis, W.H. and Imber, W.E. 1975: Allergy epidemiology in the St. Louis area. IV Weeds. <u>Ann. Allergy</u>, 35: 181-187.

- Liem, S.N. and Groot, J. 1973: Anthesis and pollen dispersal of <u>Holcus Lanatus L</u>. and <u>Festuca Rubra L</u>. in relation to climate factors. <u>Rev. of Palaeobot. and Palynol</u>., 15: 3-16.
- Ljungkvist, S., Bringfelt, B. and Fredriksson, U. 1977: Correlation between the pollen content of the Stockholm air and meteorological data. <u>Grana</u>, 16: 145-146.
- Longo, L.R. and Cristofolini, G. 1987: Airborne pollen sampling in Trieste (Italy). <u>Grana</u>, 26: 91-96.
- Lumpkins, E.D., Corbit, S.L. and Tiedeman, G.M. 1973: Airborne fungi survey. I. Culture-plate survey of the home environment. <u>Ann. Allergy</u>, 31: 361-370.
- Lyon, F.L., Kramer, C.L. and Eversmeyer, M.G. 1984a: Vertical variation of airspora concentrations in the atmosphere. Grana, 23: 123-145.
- Lyon, F.L., Kramer, C.L. and Eversmeyer, M.G. 1984b: Variation of airspora in the atmosphere due to weather conditions. <u>Grana</u>, 23: 177-181.
- Macchia, L., Alaini, M., Caiaffa, M.F., Carbonara, A.M., Gatti, E., Iacobelli, A., Strada, S., Casella, G. and Tursi, A. 1987: Monitoring of atmospheric conditions and forecast of olive pollen season. In <u>Advances in Aerobiology: Proceedings</u> of the 3rd International conference on <u>Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- MacHardy, W.E. and Gadoury, D.M. 1986: Patterns of ascospore discharge by <u>Venturia inaequalis</u>. <u>Phytopathology</u>, 76: 985-990.
- Makinen, K. 1977: Correlation of Atmospheric spore frequencies with meteorological data. <u>Grana</u>, 16: 149-153.

- Mallaiah, K.V. and Rao, A.S. 1982: Aerial dissemination of urediniospores of groundnut rust. <u>Trans. Br. Mycol. Soc</u>., 78: 21-28.
- Mandrioli, P. 1987: Biometeorology and its relation to pollen count. In <u>Advances in Aerobiology</u>; <u>Proceedings of the 3rd</u> <u>International conference on Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Martin, R.A. and Clough, K.S. 1984: Relationship of airborne spore load of <u>Pyrenophora teres</u> and weather variables to net blotch development on Barley. <u>Canadian Journal of Plant</u> <u>Pathology</u>, 6: 105-110.
- Massey, D.G and Fournier-Massey, G. 1984: Airborne pollen sampling in Manoa Valley, Hawaii: Effect of rain, humidity and Wind. <u>Annals of Allergy</u>, 52: 333-337.
- Matthews, D. and Hampton, J.G. 1977: A survey of New Zealand certified barley seed for the presence of pathogenic Drechslera species. N. Z. J. Exp. Agric., 5: 331-334.
- May, K.R. 1967: Physical aspects of sampling airborne microbes. In <u>Airborne Microbes</u>. Edited by Gregory, P.H. and Monteith, J.L. Cambridge University Press : Cambridge.
- McDonald, M.S. 1980: Correlation of air-borne grass pollen levels with meteorological data. <u>Grana</u>, 19: 53-56.
- Mercuri, L., De Dominicus, V., Cresti, M., Sarfatti, G. and Gampolini, F. 1982: Airborne pollen concentrations in the atmosphere of Siena and correlation with meteorological data. <u>Pollen et Spores</u>, 24: 315-319.
- Meredith, D.S. 1962: Some components of the air spora in Jamaican banana plantations. <u>Ann. Appl. Biol</u>. 50: 577-594.
- Mims, C.W. 1984: Classification of fungi. In <u>Mould Allergy</u>. Edited by Al-Doory, Y. and Domson, J.F. Lea. and Febiger : Philadelphia.

- Moller, W.J. and Carter, M.V. 1965: Production and dispersal of ascospores in <u>Eutypa aremeniacae</u>. <u>Flant Dis. Rep</u>., 62: 254-258.
- Moller, W.J. and Kasimatis, A.N. 1978: Dieback of grapevines caused by <u>Eutypa armeniacae</u>. <u>Plant Dis. Rep.</u>, 62: 254-258.
- Moore, P.D. and Webb, J.A. 1978: <u>An illustrated guide to pollen</u> <u>analysis</u>, Hodder and Stoughton: London.
- Mullins, J. 1981: Diurnal variation in grasspollen count. Abst. Proc. 4th Charles Blackley Symposium, Nottingham.
- Mullins, J., Warnock, D.W., Powell, J., Jones, I. and Harvey, R. 1977: Grass pollen content of the air in the Bristol Channel region in 1976. <u>Clinical Allergy</u>, 7: 391-395.
- Nilsson, S. (ed.) 1983: <u>Atlas of airborne fungal spores in</u> Europe. Springer-Verlag : Berlin.
- Nilsson, S. and Muller, J. 1981: Tree pollen spectra in the Stockholm region (Sweden) 1973-1980. <u>Grana</u>, 20: 179-182.
- Nilsson, S. and Palmberg-Gotthard, J. 1982; Pollen calendar for Huddinge (Sweden), 1977-1981. <u>Grana</u>, 21: 183-185.
- O'Rourke, M.K. and Buchmann, S.L. 1987: Follen yield of two CVS. of <u>Olea europaea</u> 1. In <u>Advances in Aerobiology; Proceedings</u> <u>of the 3rd International conference on Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- D'Rourke, M.K. and Lebowitz, M.D. 1984: A comparison of regional atmospheric pollen with pollen collected at and near homes. <u>Grana</u>, 23: 55-65.
- Ogden, E.C. and Raynor, G.S. 1967: A new sampler for airborne pollen. The rotoslide. <u>J. Allergy</u>. 40: 1-10.

- Ogden, E.C., Raynor, G.S., Hayes, J.V., Lewis, D.M. and Haines, J.H. 1974: <u>Manual for sampling airborne pollen</u>. Hafner Press, New York.
- Okhuoya, J.A. 1986: Seasonal and diurnal changes of two leaf pathogens of Rubber (<u>Hevea brasiliensis Muell. Arg.</u>) in the air of Iyanomo, Nigeria. <u>Acta Mycologica</u> 22: 65-71.
- Ordman, D. 1947: Pollenosis in South Africa. <u>S.A.Med.J</u>., 21: 38-48.
- Ordman, D. 1955: Respiratory allergy in the coastal areas of South Africa: The significance of clima<u>te. S.A.Med.J</u>., 29:173.
- Drdman, D. 1961: Relation of climate to respiratory allergy. <u>Ann.</u> <u>Allergy</u>, 19: 29.
- Ordman, D. 1963: Seasonal respiratory allergy in Bulawayo, Rhodesia: The pollen and fungus factors. <u>S.A.Med. J</u>., 46: 1898-1901.
- Ordman, D and Etter, K.G. 1956: The air-borne fungi of Johannesburg. <u>S.A.Med.J.</u>, 30: 1054-1058.
- Pateria, A.K. and Sahu, T.R. 1982: An analysis of atmospheric allergenic fungal spores at Sagar. <u>Acta Botanica Indica</u>, 10: 152-155.
- Pearson, R.C. 1980: Discharge of ascospores of <u>Eutypa armeniacae</u> in New York. <u>Plant Dis</u>., 64: 171-174.
- Pennycook, S.R. 1980: The airspora of Auckland city, New Zealand 1. Seasonal and diel periodicities. <u>New Zealand Journal of</u> <u>Science</u>, 23: 27-37.
- Peturson, B. 1931: Epidemiology of cereal rusts. <u>Dom. Canada</u> <u>Dept. Agric. Div. Bot. Dom. Botanist</u>, 44-46.

- Petzold, C.H., Sall, M.A. and Moller, W.J. 1983: Factors determining the relative number of ascospores released by <u>Eutypa armeniacae</u> in California. <u>Plant disease</u>, 67: 857-860.
- Petzoldt, C.H. 1983: Factors determining the relative number of ascospores released by <u>Eutypa armeniacae</u> in California. <u>Plant Disease</u>, 67: 857-860.
- Plutchik, R. 1974: <u>Foundations of experimental research</u>. Harper & Row : New York.
- Ramalingham, A. 1971: Airspora of Mysore. <u>Proc. Indian Acad.</u> <u>Sci</u>., 74: 227-240.
- Ramos, D.E., Moller, W.J. and English, W.H. 1975: Production and dispersal of ascospores of <u>Eutypa armeniacae</u> in California. <u>Phytopathology</u>, 65: 1364-1371.
- Raynor, G.S., Hayes, J.V. and Ogden, E.C. 1976: Temporal variability in airborne pollen concentrations. <u>Ann. Allergy</u>, 36: 386-396.
- Raynor, G.S., Ogden, E.C. and Hayes, J.V. 1973: Variation in ragweed pollen concentrations to a height of 108 metres. <u>J.</u> <u>Allergy clin. Immunol.</u>, 51,4: 199-207.
- Raynor, G.S., Ogden, E.C. and Hayes, J.V. 1975: Spatial variability in airborne pollen concentrations. <u>J. Allergy</u> <u>Clin. Immunol.</u>, 55,3: 195-202.
- Reddi, C.S. and Reddi, N.S. 1985: Relation of pollen release to pollen concentrations in the air. <u>Grana</u>, 24: 109-113.
- Reddi, C.S., Reddi, E.U.B., Janaki Bai, A., Raju, K.V.R. and Reddi, M.S. 1980: The ecology of anther dehiscence, pollen liberation and dispersal in <u>Xanthium Strumarium Linn</u>. <u>Indian</u> <u>J. Ecol</u>., 7: 171-181.

- Reid, M.J., Moss, R.B., Hsu, Y-P., Kwasnicki, J.M., Commerford, T.M. and Nelson, B.L. 1986: Seasonal Asthma in Northern California : Allergic causes and efficacy of immunotherapy. J. Allergy Clin. Immunol., 78: 590-600.
- Reiss, N.M. and Kostic, S.R. 1976: Pollen season severity and meteorological parameters in central New Jersey. <u>J. Allergy</u> <u>Clin. Immunol.</u>, 57,6: 609-614.
- Rempe, H. 1937: Untersuchungen uber die verbreitung des blutenstaubes durch die luftstromungen. <u>Planta</u>, 27:93-147.
- Royes, V.I.J. 1987: Some components of the air spora in Jamaica and their possible medical application. <u>Grana</u>, 26: 151-157.
- Salisbury, J.H. 1866: On the cause of intermittent and remittent fevers, with investigations which tend to prove that these affections are caused by certain species of <u>Palmella</u>. <u>Am J.</u> <u>Med. Sci.</u>, 51: 51-75.
- Salvaggio, J. 1970: New Orleans asthma. II. Relationship of climatologic and seasonal factors to outbreak. <u>J. Allergy</u>, 45: 257.
- Salvaggio, J. and Seabury, J. 1971: New Orleans asthma. <u>J.</u> <u>Allergy Clin. Immunol</u>., 48: 82-95.
- Salvaggio, J., Zaslow, L., Greer, J. and Seabury, J. 1971: New Orleans asthma III. Semiquantitative and aerometric pollen sampling, 1967 and 1968. <u>Ann. Allergy</u>, 29: 305-317.
- Sandhu, D.K., Shivpuri, D.N. and Sandhu, R.S. 1964: Studies on the air-borne fungal spores in Delhi. Their role in respiratory allergy. <u>Ann. Allergy</u>, 22: 374.
- Satpute, M., Dutta, B.K. and Rao, R.R. 1983: Contributions to the aerobiology of Shillong. I. Studies on the seasonal variation of atmospheric pollen and fungal spores. <u>Proc.</u> <u>Indian nath. Acad.</u>, B49: 675-686.

- Savary, S. 1986: Relative Humidity and wind velocity associated with diurnal rhythmicity of aerial dispersal of <u>Fuccinia</u> <u>arachidis</u> urediniospores. <u>Neth. J. Fl.Path.</u>, 92: 115-125.
- Sayer, W.J., Dudley, B.S. and Jamshid, G. 1969: Estimation of airborne fungal flora by the Andersen sampler versus the gravity settling culture plate. I. Isolation frequency and number of colonies. J. Allergy, 44: 219-225.
- Schmidt, W. 1918: Die verbreitung von samen und verwandte erscheinungen. <u>Probl. Kosm. Phys.</u>, 7: 1-118.
- Schneider-Christians, T., Fliege, F. and Schlosser, E. 1986: On the release, survival and importance of basidiospores of <u>Corticium salmonicolor</u>, the pathogen causing pink disease. Journal of Plant Diseases and Protection, 93: 397-403.
- Schultze-Werninghaus, G., Levy, J., Bergmann, E.M., Kappos, A.D. and Meier-Sydow, J. 1987: Clinical significance of airborne <u>Alternaria Tenuis</u> spores : Seasonal symptoms, positive skin and bronchial challenge tests with <u>Alternaria</u> in subjects with asthma and rhinitis. In <u>Advances in Aerobiology;</u> <u>Proceedings of the 3rd International conference on</u> <u>Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Shenoi, M.M and Ramalingam, A. 1983: Leaf blight of sorghum : Influence of meteorological factors and crop growth stages on the spread of innoculum and disease. <u>Indian Phytopath</u>., 36: 700-706.
- Shipton, W.A., Khan, T.N. and Boyd, W.J.R. 1973: Net blotch of barley. <u>Rev. Plant Pathol.</u>, 52: 269-290.
- Simmons. E.G. 1967: Typification of <u>Alternaria</u>, <u>Stemphylium</u>, <u>Ulocladium</u>. <u>Mycologia</u>, 59: 67-74.

- Singh, A.B. 1987: Airborne pollen types of allergenic significance in India. In <u>Advances in Aerobiology;</u> <u>Proceedings of the 3rd International conference on</u> <u>Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Singh, A.B. and Babu, C.R. 1980: Studies on pollen allergy in Delhi. Diurnal periodicity of common allergenic pollen. <u>Allergy</u>, 35: 311-317.
- Singh, A.B. and Babu, C.R. 1982: Survey of atmospheric pollen allergens and their atmospheric prevalence. <u>Ann. Allergy</u>, 48:115-122.
- Sneller, M.R. 1984: Mould allergy and climate conditions. In <u>Mould allergy</u>. Edited by Al-Doory, Y. and Domson, J.F. Lea and Febinger : Philadelphia.
- Sneller, M.R., Hayes, H.D. and Pinnas, J.L. 1981: Frequency of airborne <u>Alternaria</u> spores in Tuscon, Arizona, over a 20year period. <u>Ann. Allergy</u>, 46: 30.
- Solomon, W.R. 1978: Aerobiology and inhalant allergens. I. Pollens and fungi. In <u>Allergy: Principles and practice</u>. Edited by Middleton, E., Reed, C.E. and Ellis, E.F. Moşby : St. Louis.
- Solomon, W.R. 1984a: Sampling Airborne Allergens. <u>Ann. of</u> Allergy. 52: 140-147.
- Solomon, W.R. 1984b: Sampling techniques for airborne fungi. In <u>Mould Allergy</u>. Edited by Al-Doory, Y and Domson J.F. Lea and Febinger : Philadelphia.
- Solomon, W.R. and Gilliam, J.A. 1970: A modified application of the Andersen sampler to the study of airborne fungus particles. J. Allergy, 45: 1.
- Spieksma, F.Th.M. 1980: Daily hay fever forecast in the Netherlands. <u>Allergy</u>, 1980: 593-603.

- Spieksma, F.Th.M. 1983a: Airborne pollen concentration in Leiden, The Netherlands, 1977-1981. <u>Grana</u>, 22: 119-128.
- Spieksma, F.Th.M. 1983b: Fluctuations in grass-pollen counts in relation to nightly inversion and air pollution potential of atmosphere. Int. J. Biometeor., 27: 107-116.
- Spieksma, F.Th.M. 1986: Airborne pollen concentrations in Leiden, The Netherlands, 1977-1981. III. Herbs and weeds flowering in the summer. <u>Grana</u>, 25: 47-54.
- Spieksma, F.Th.M., Van Den Assem, A.D. and Collette, J.A. 1985: Airborne pollen concentration in Leiden, The Netherlands, 1977-1981. II. Poaceae (grasses), variations and relation to hay fever. <u>Grana</u>, 24: 99-108.
- Spieksma, F.Th.M. and den Tonkelaar, J.F. 1986: Four-hourly fluctuations in grass-pollen concentrations in relation to wet versus dry weather, and to short versus long over-land advection. Int. J. Biometeor., 30: 351-358.
- Sreeramulu, T. and Ramalingham, A. 1964: Some short period changes in the atmospheric spore content associated with changes in the weather and other conditions. <u>Proc. Indian</u> <u>Acad. Sci.</u>, 6: 154-172.
- Stakman, E.C., Henry, A.W., Curran, G.C., and Christopher, W.N. 1923: Spores in the upper air. <u>J. agric. Res</u>., 24: 599-606.
- Stalker, W.W. and Moore, P.M. 1972: Air-borne pollen and fungus spore patterns in the Birmingham, Alabama, area. <u>Ann.</u> <u>Allergy</u>, 30: 326-334.
- Steel, M. 1983: Grass Pollen in the Atmosphere and its effect on Hay Fever, Weather, 38: 130-139.
- Stix, E. 1977: Grasspollengehalt der luft von Munchen. vorbereitung fur eine vorhersage. <u>Munch. Med. Wschr</u>. 119: 1595-1598.

- Stix, E. and Ferritti, M.L. 1974: Grundwerte des pollen-und sporengehaltes der luft in der Bundesrepublik Deutschland 1967-1970. Deutsche Forschungsgemeinschaft, Kommission zur Erforschung der Luftverunreinigung; Mitteilung, 11: 1-53.
- Stix, E. and Gross-Brauckmann, G. 1970: Der pollen-und sporengehalt der luft und seine tager-und jahres-zeithlichen schwnkungen unter mittel-Europaischen verhaltrissen. <u>Flore</u>, 159: 1-37.
- Strandhede, S. and Wihl, J. 1981: Comparison of pollen counts in Copenhagen and Malmo. <u>Grana</u>, 20: 187-189.
- Street, D. and Hamburger, R.N. 1976: Atmospheric pollen and spore sampling in San Diego, California. I. Meteorological correlations and potential clinical relevance. <u>Ann. Allergy</u>, 37: 32-40.
- Sutra, J.P., Ickovic, M.R., De Luca, H., Peltre, G. and David, B. 1987: Chestnut pollen counts related to patients pollinosis in Paris. In <u>Advances in Aerobiology</u>: <u>Proceedings of the 3rd</u> <u>International conference on Aerobiology</u>. Edited by Boehm, G. and Leuschner, R.M. Birkhaeuser Verlag : Basel, Switzerland.
- Tilak, S.T. and Babu, M. 1987: Aeromycology at Aurangabad. IV. Phycomycetes. <u>Indian J. Bot</u>., 10: 84-88.
- Trese, A.T., Burton, C.L. and Ramsdell, D.C. 1980: <u>Eutypa</u> <u>armeniacae</u> in Michigan vineyards: Ascospore production and survival, host infection, and fungal growth at lower temperatures. <u>Phytopathology</u>, 70: 788-793.
- Verma, A.K. and Kamal. 1982: Airspora studies over an Arhar (Cajanus Cajan) field. The diurnal and seasonal periodicity of certain plant pathogens. <u>Indian J. Mycol. Pl. Pathol.</u>, 12: 40-45.

- Verma, A.K., Gupta, B.K. and Kamal. 1981: Air-spora Studies over Arhar-Seasonal Distribution Fungal Air-Spora as obtained by Gravity Plate Method. <u>Environment India</u>, 4: 56-61.
- Yunginger, J.W., Roberts, G.D. and Gleich, G.J. 1976: Studies on <u>Alternaria</u> allergens. I. Establishment of the radioallergosorbent test for measurement of <u>Alternaria</u> allergens. <u>J. Allergy Clin. Immunol.</u>, 57: 293.

# APPENDIX 1. LABORATORY AND COUNTING METHODS

## APPENDIX 1. LABORATORY AND COUNTING METHODS

- 1.1 Steps in laboratory procedure
- 1.2 Computation of volumetric concentrations
- 1.3 Plates showing steps in laboratory method

#### APPENDIX 1. LABORATORY AND COUNTING METHODS

### 1.1 Steps in laboratory procedure

- Remove the tape from the drum and place it on a calibrated ruler provided by the Burkard company (Plate 12 & 13). Care must be taken to prevent smudging of the vaseline surface with fingers thus severely distorting the sample.
- 2. The tape must now be cut into 24 hr sections. Accuracy is essential as an error of 2mm represents a time error of one hour (Plate 14).
  - 15 ml 3. The daily sections are placed in X-6B is added centrifuge tubes. 5ml of to dissolve the vaseline (X-6B is a product of Shell chemicals. It is misable with alcohols, has 60% aromatics and a FBP of 106 °C - 140°C. Vaseline is easily dissolved by the compound). The tubes are left to stand in a water bath at 40°C fot 24 hr (Plate 15).
  - 4. Remove the melinex tape, slowly washing the remaining sediment off with X-6B.
  - 5. Centrifuge X-6B. Speed 3100 r.p.m. Time 4 mins. A Zeiss Hermle Z320 is used for this procedure (Plate 16). Decant X-6B.
  - Add 6 ml of glacial acetic acid. Centrifuge and decant.
  - 7. Add 6 ml of Acetolysis mixture (mixture consists of glacial acetic anhydride mixed with concentrated sulphuric acid in a ratio of 9:1, as in Erdtman, 1960). Mixture must be agitated and then left standing in water bath for 3 mins. at 80°C (Plate 17). All operations should take place in a fume cupboard.

- 8. Centrifuge acetolysis mixture as in step 5. Decant acetolysis mixture.
- Transfer to plastic tubes using glacial acetic acid as medium.
- 10. Centrifuge and decant glacial acetic acid.
- 11. Add 5 ml of hydrofluoric acid (Plate 18) and place in water bath for 40 minutes at 80°C. (Plastic tubes must be used because HF reacts with glass).
- 12. Centrifuge and decant HF. Add 5 ml of glacial acetic acid and agitate. Decant glacial acetic acid into original centrifuge tubes taking care not to mix the tubes.
- 13. Centrifuge and decant glacial acetic acid and add 8ml of distilled water.
- 14. Centrifuge and decant distilled water. Add 6 ml of distilled water. Add 2 drops of stain (safranin mixed in a 50/50 solution with water).
- 15. Centrifuge and decant stained distilled water. Add 6 ml of distilled water.
- 16. Centrifuge and decant distilled water.
- 17. Weigh a dry vial without the lid accurately to 4 decimal places (scale used : Mettler H33AR. See Flate 19).
- 18. Transfer to vial using 4 ml of distilled water. Centrifuge for 6 mins and decant carefully.
- 19. Add two drops of glycerine. Weigh the vial without the lid.

- 20. Agitate the glycerine solution slowly to achieve a random distribution of pollen grains.
- 21. Using a pipette, place a drop of the glycerine on  $a_{(}$  clean slide. Weigh the vial once more without the lid.
- 22. Place a cover slip on the slide and insert under a microscope (all microscopy was done with a Nikon microscope at 400 X magnification, Plate 20)
- 23. It is necessary that an absolute count is made. The entire slide must be read as in most cases not more than 15% of the original glycerine solution is placed on the slide.

#### 1.2 Computation of volumetric concentrations

The following formula was used to compute the airspora concentrations  $m^{-3}$  of air.

100  
1. 
$$X = Y \times ----- \frac{2}{6} N$$

 $X = Airspora concentrations m^{-3}$  of air.

Y = The pollen count.

N = A constant of 14.4. This is based on volume of air sampled by the Burkard at 101 min<sup>-1</sup> for a 24 hr period.

Z = Percentage of the glycerine read. This figure is computed with the following formula.

**b-c** 100  
2. 
$$Z = --- \times ----$$
  
**b-a** 1

a = vial weight (g)

b = Weight of vial and glycerine solution less glycerine on slide (g). Vial weight : 4,7500 g. Vial and Glycerine (1) 5,2213 g. Vial and Glycerine (2) 5,2202 g. Pollen count = 60 grains.

$$5,2213 - 5,2002 100$$
  
$$Z = ----- x ---5,2213 - 4,7500 1$$

'	0,0211		100
=		×	
	0,4730		1

= 4,48%

 $\begin{array}{r} 100 \\ X = 60 \times ---- \div 14.4 \\ 4.48 \end{array}$ 

= 93 grains  $m^{-3}$ .

.

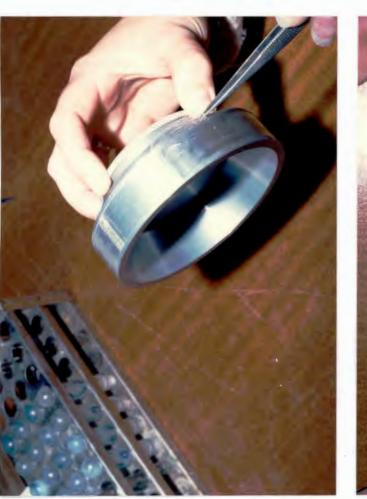


Plate 12 Tape removed from

drum

#### 1.3 Plates showing steps in laboratory method

Plate 13 Tape placed on calibrated ruler

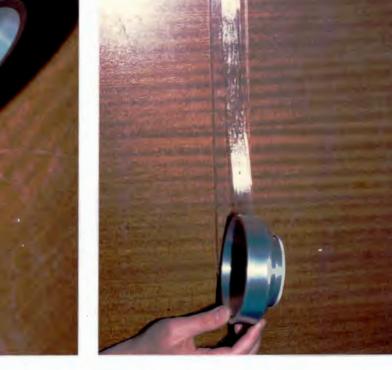


Plate 14 Melinex Tape divided into daily sections





Plate 15 Vaseline dissolved off tapes

Plate 16 Zeiss swing out head centrifuge





# Plate 17 Acetolysis process

Plate 18 Hydrofluoric acid and plastic tubes





# Plate 19 Mettler scale. Vial on Plate 20 Nikon microscope scale plate.

# AFFENDIX 2. PLATES SHOWING POLLEN AND SPORES

-

,

# PLATE 22 Cyperaceae

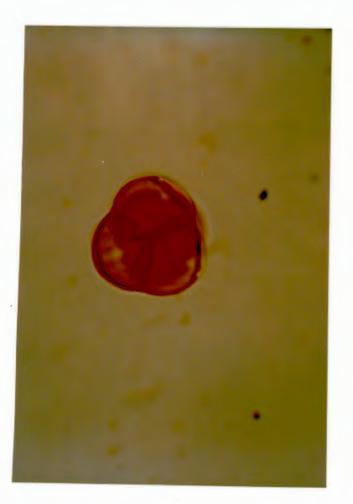




## PLATE 23 Ericaceae

.

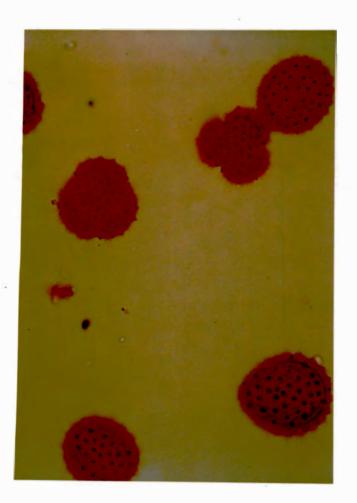
.



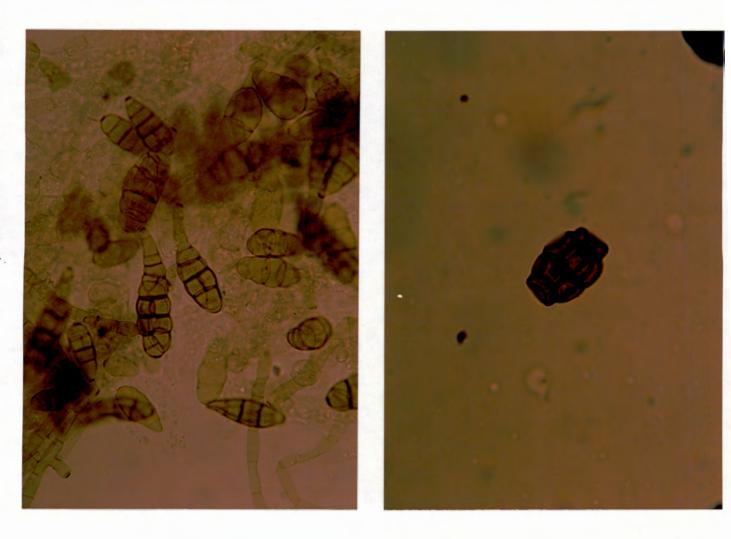


## PLATE 25 Asteraceae

## PLATE 26 Proteaceae



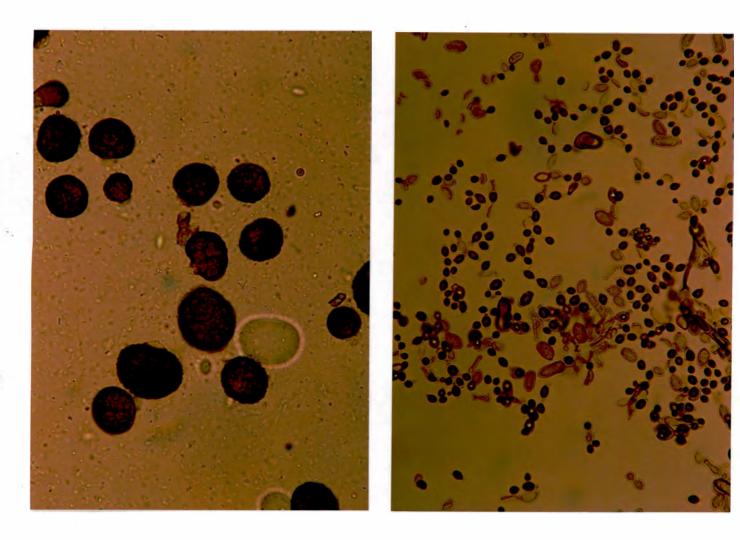




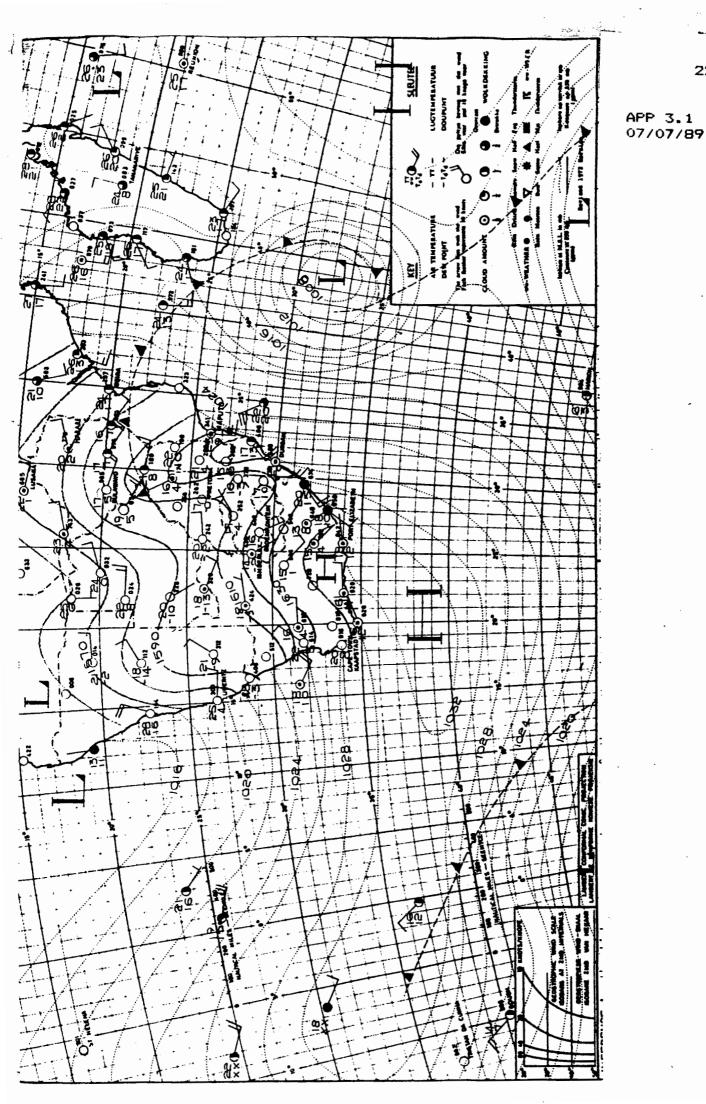
## PLATE 29 Epicoccum

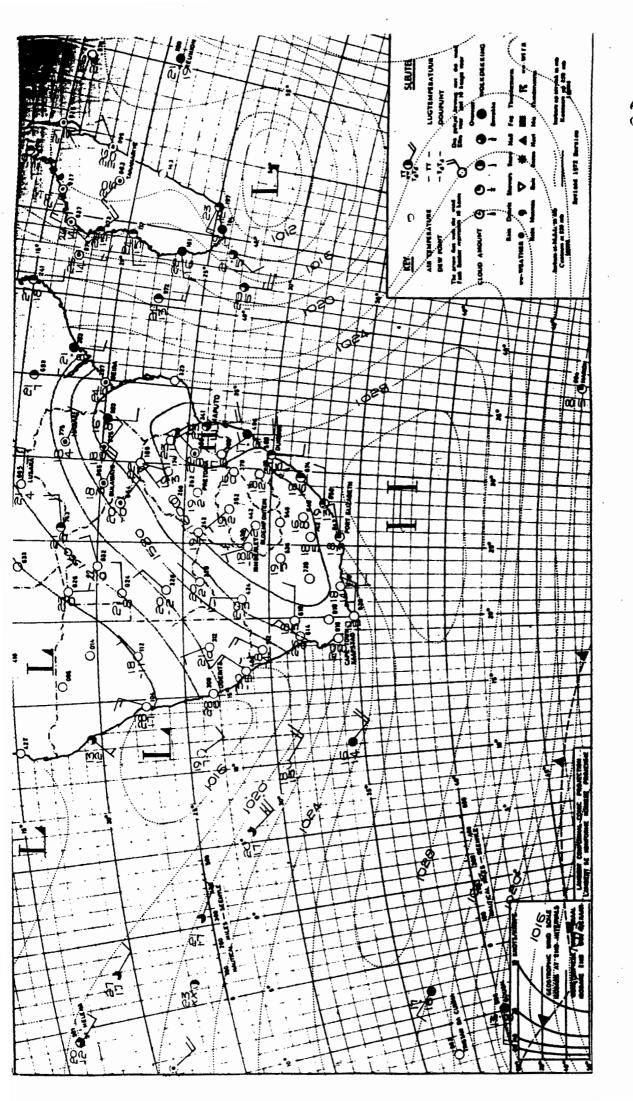
A

## PLATE 30 Cladosporium and <u>Aspergillus</u>

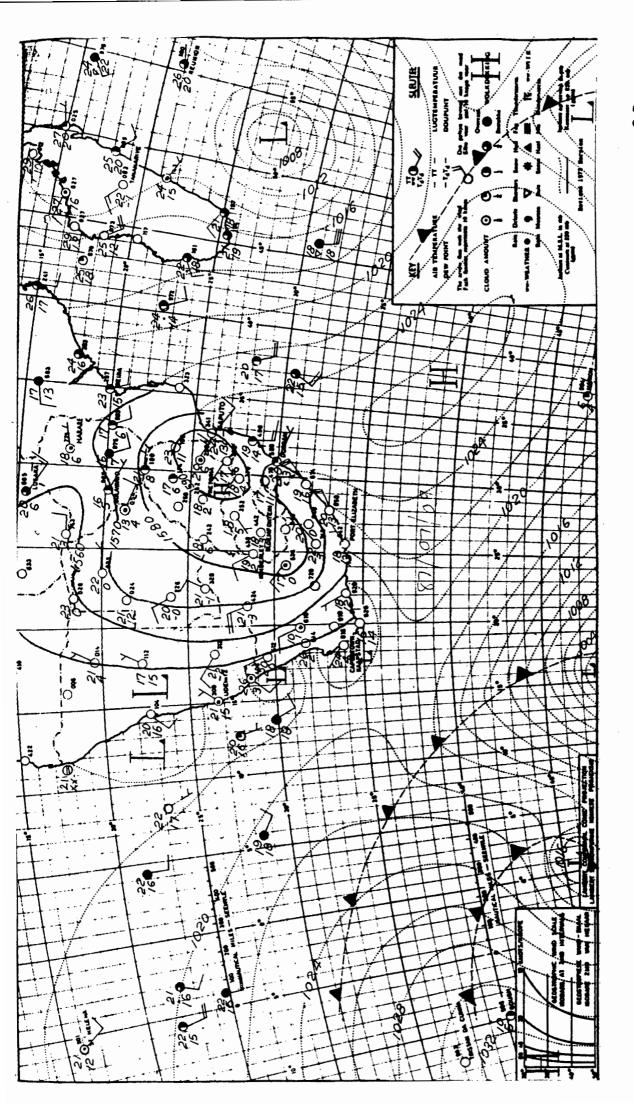


APPENDIX 3. SYNOPTIC CHARTS

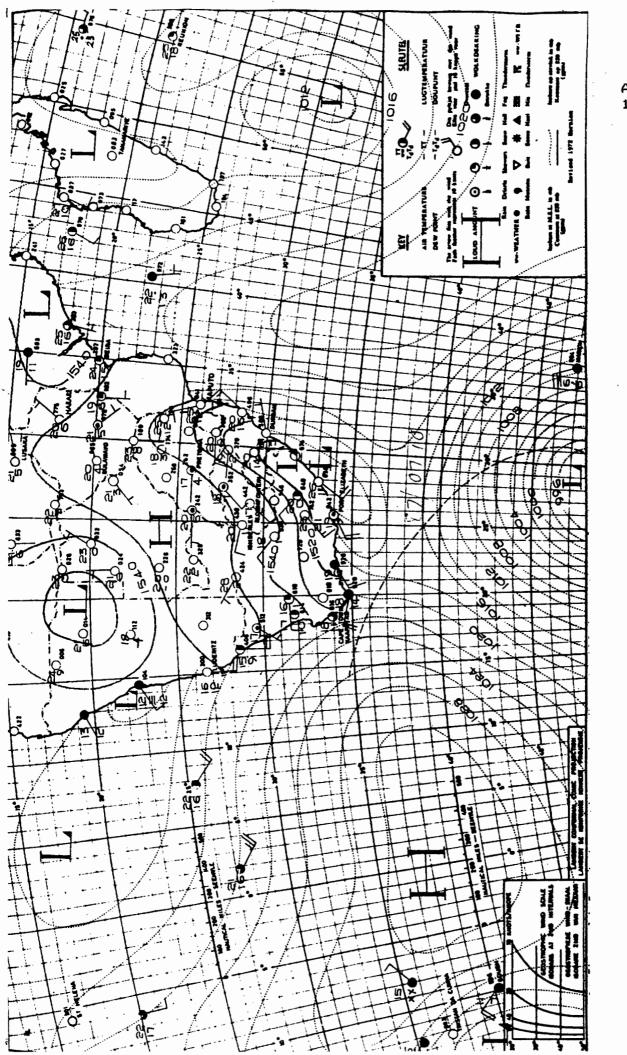




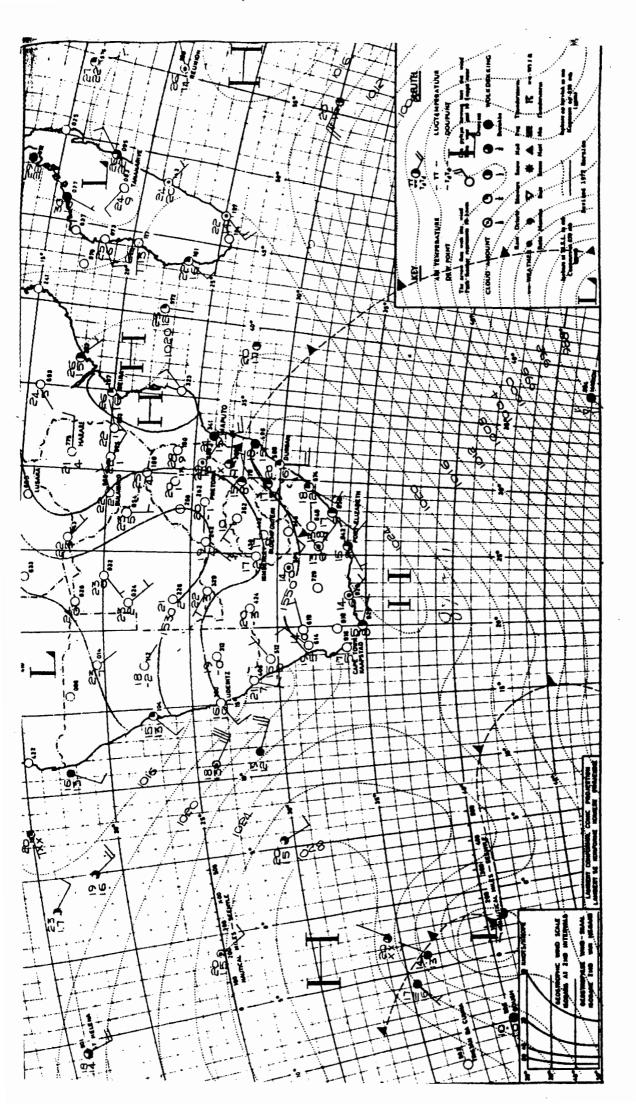
APP 3.2 08/07/87



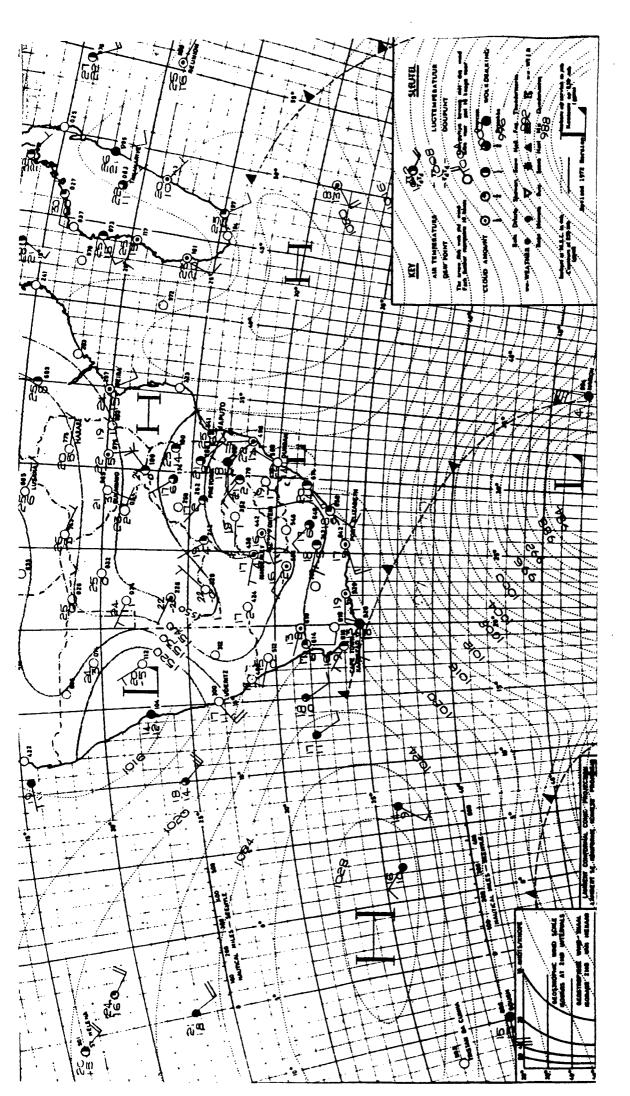
APP 3.3 09/07/87



APP 3.4 10/07/87

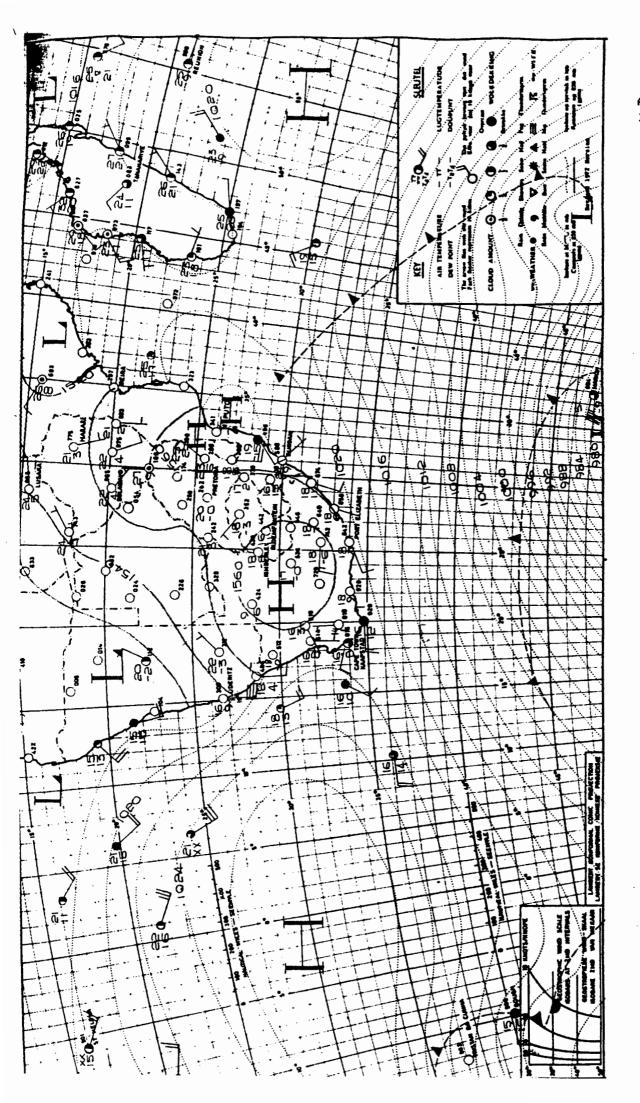


APP 3.5 11/07/87

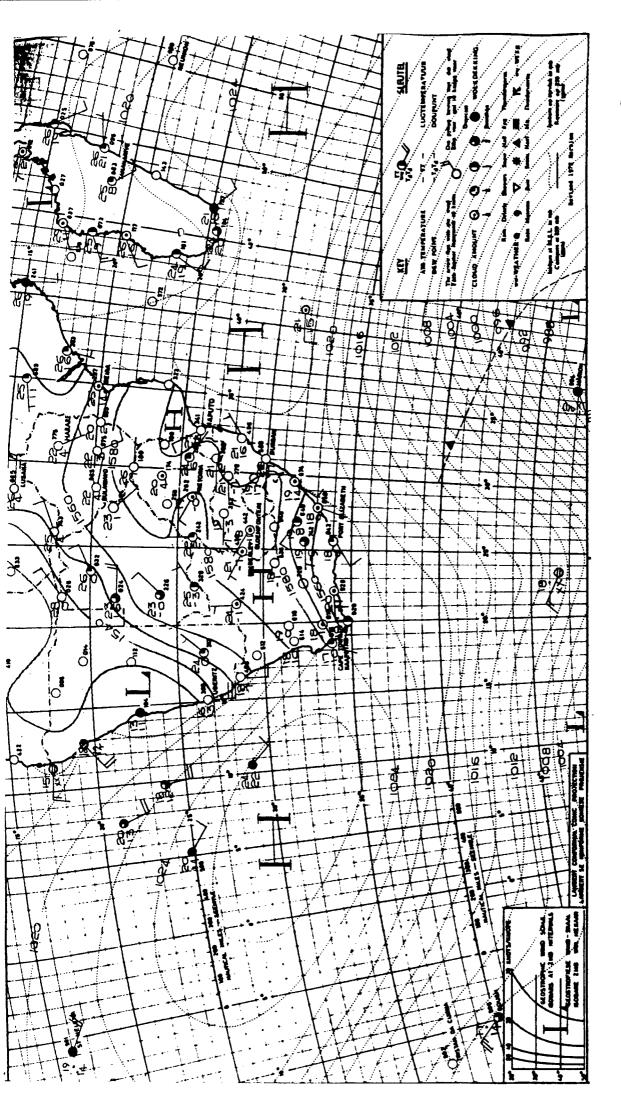




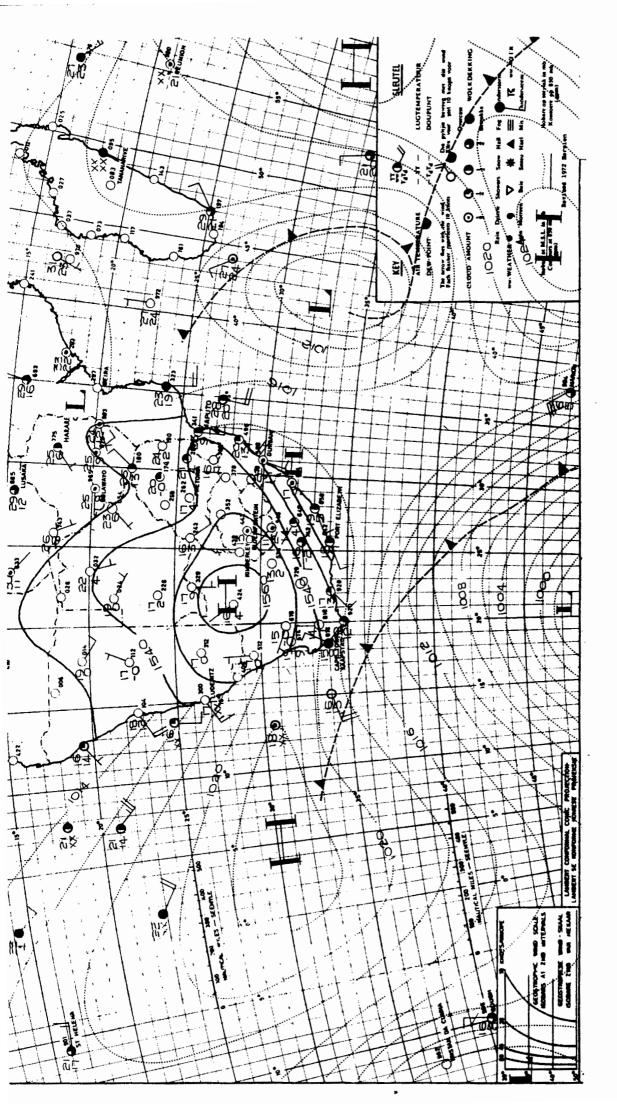
APP 3.7 12/07/87



APP 3.7 13/07/87

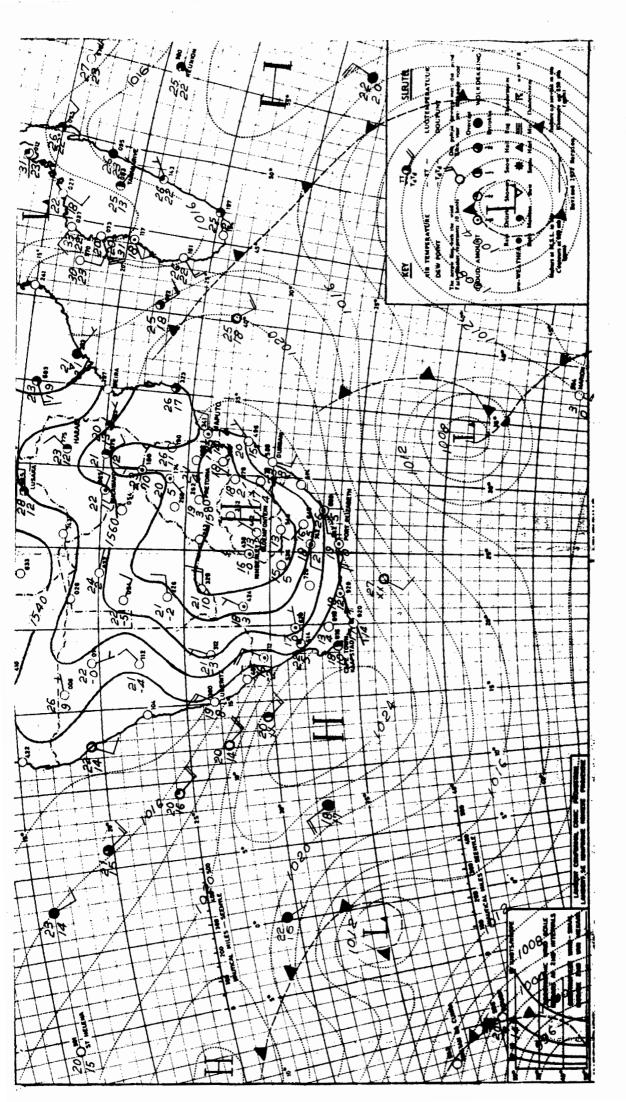


#### APP 3.8 14/07/87

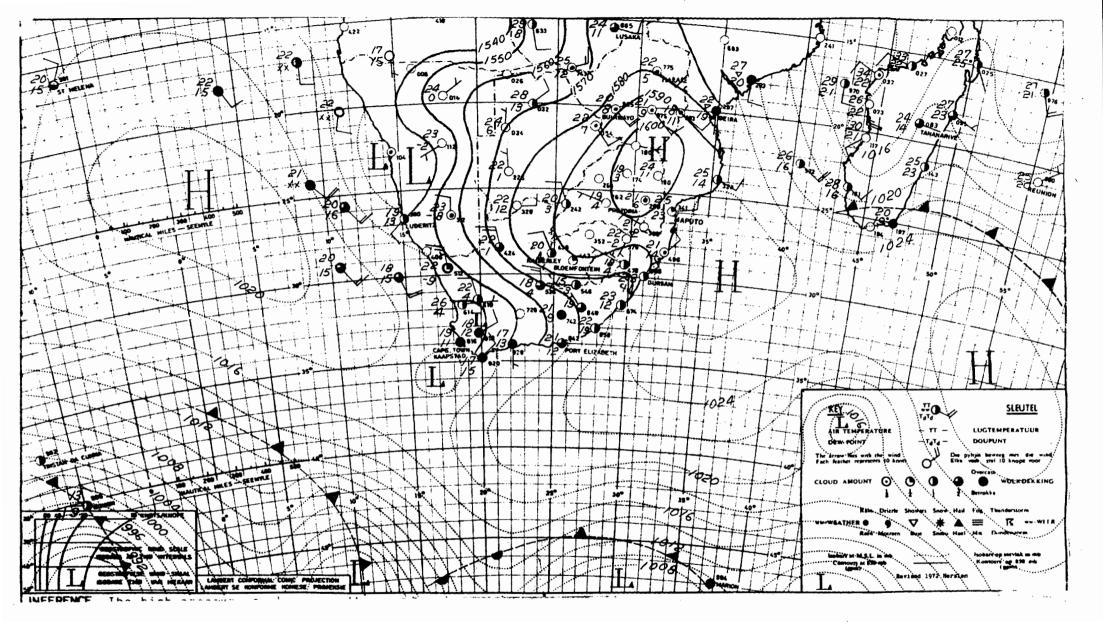


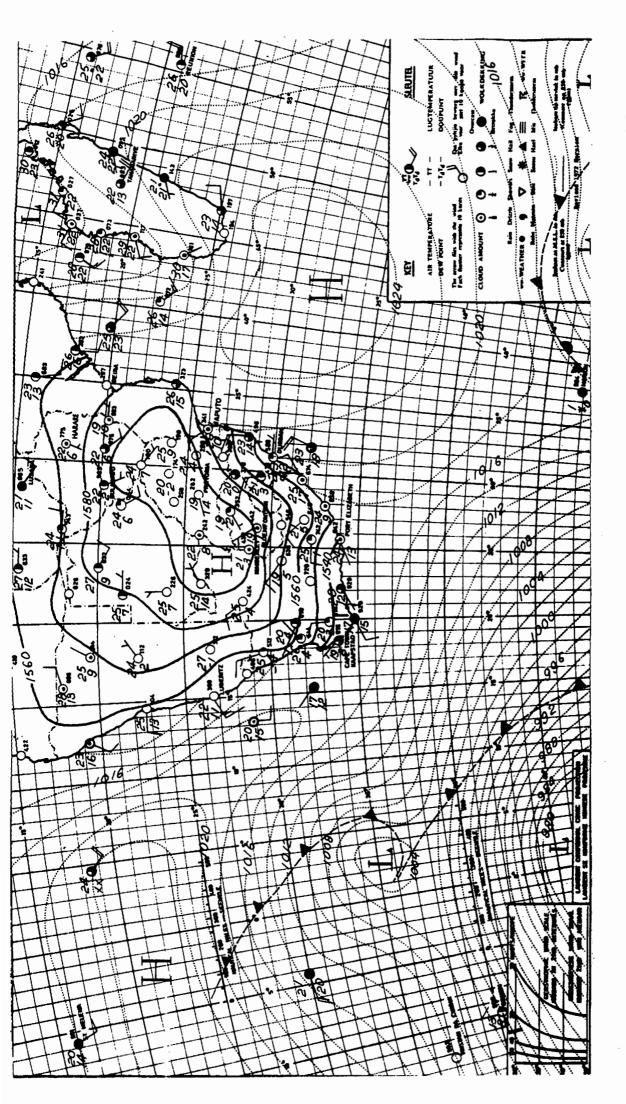
APP 3.9 29/05/88

Anglin 🗤 Langer (17)

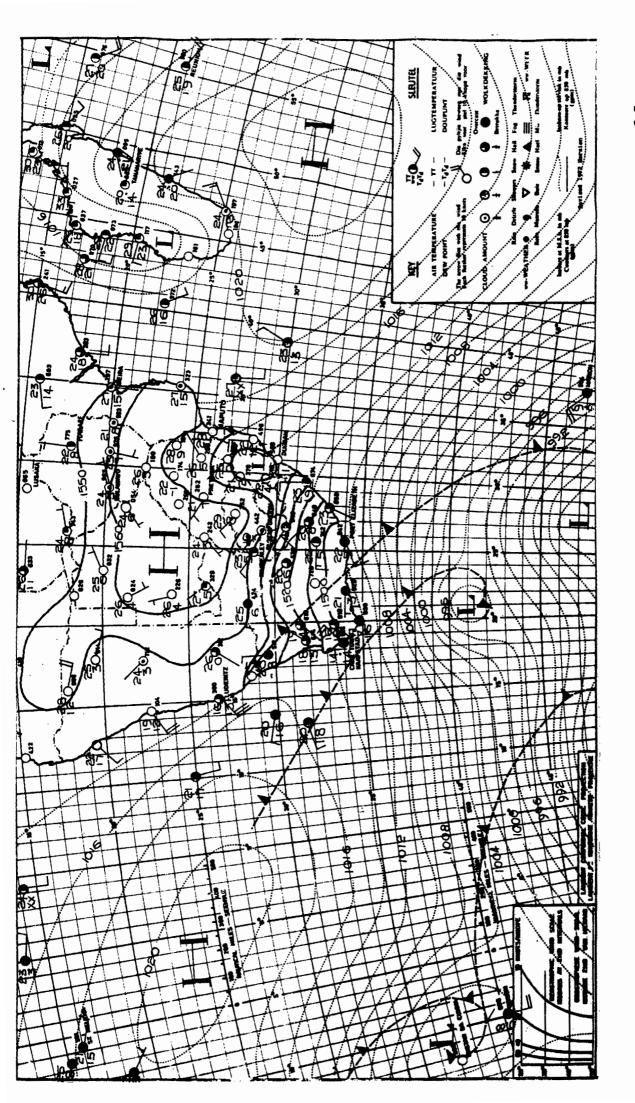


APP 3.10 30/05/88

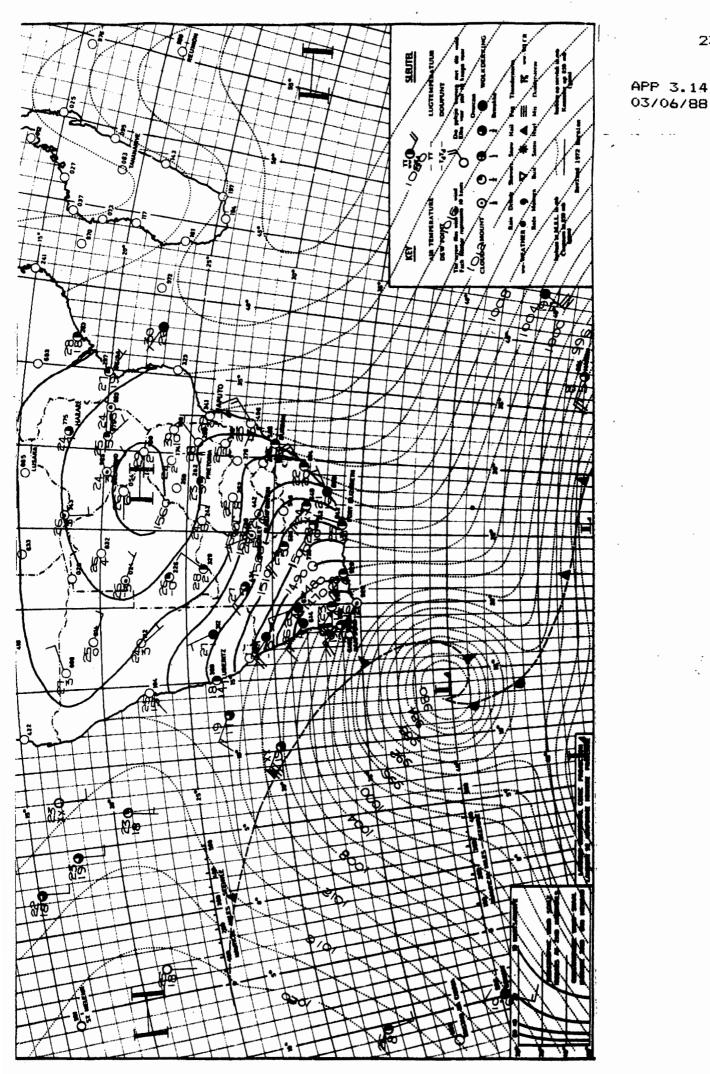




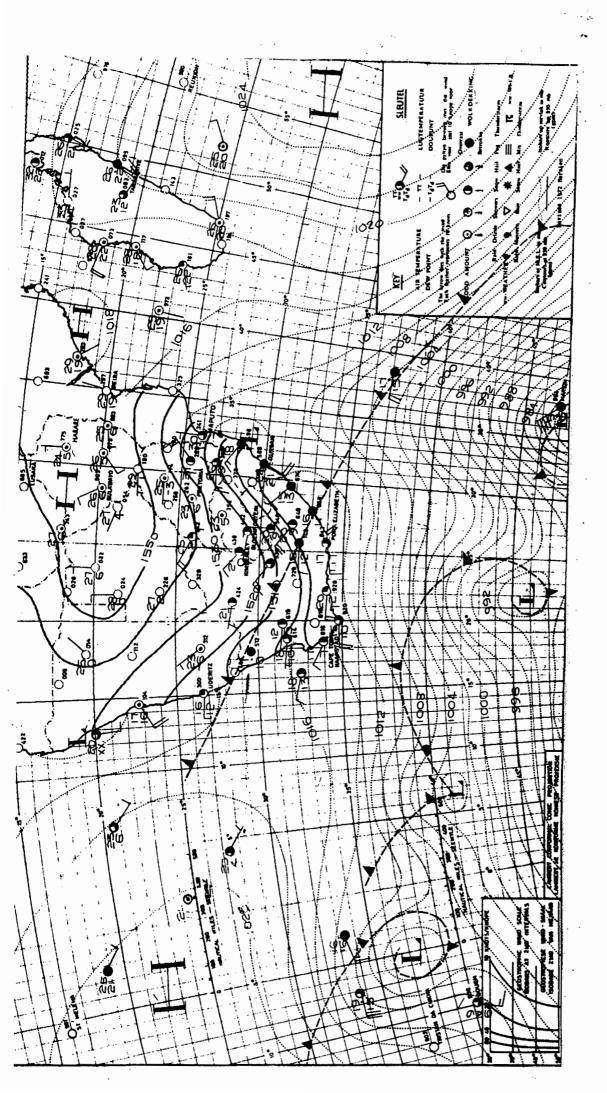
APP 3.12 01/06/88



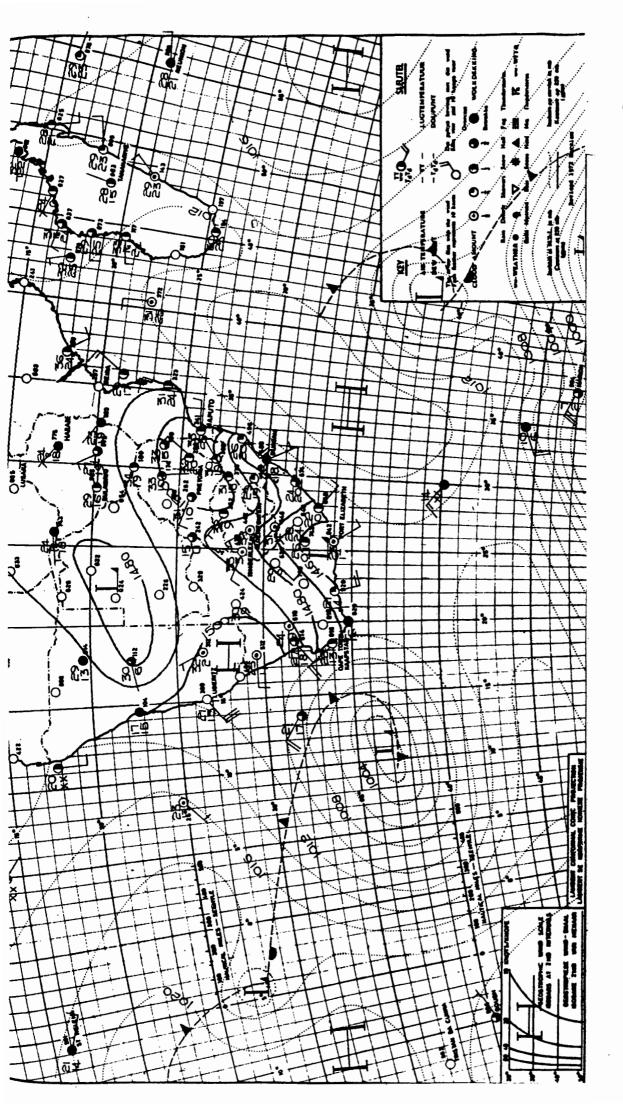
APP 3.13 02/06/88



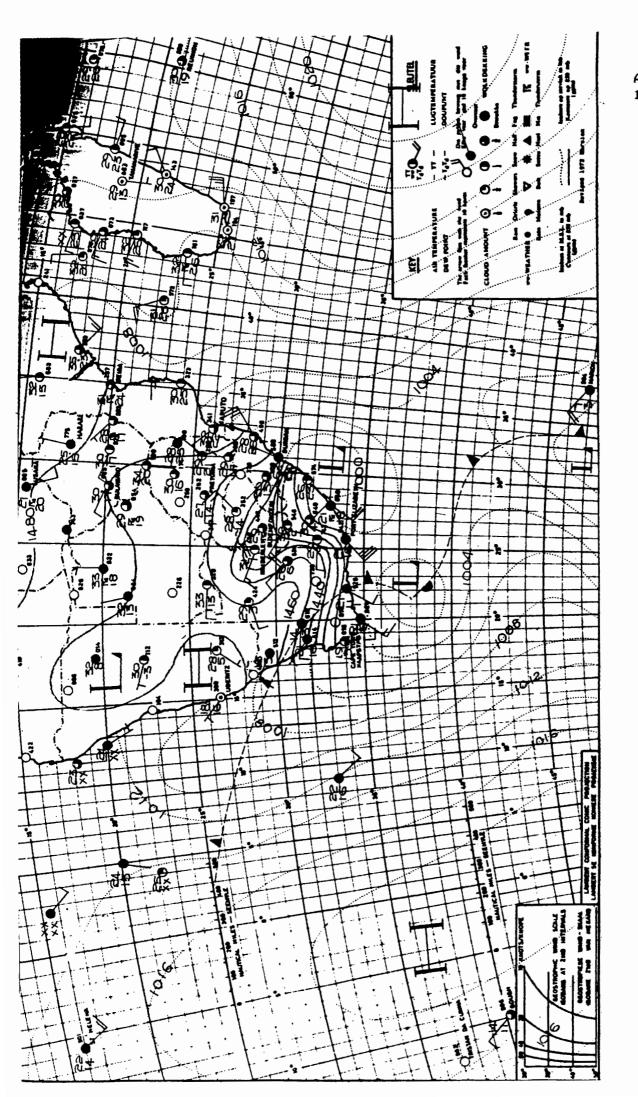
APP 3.14



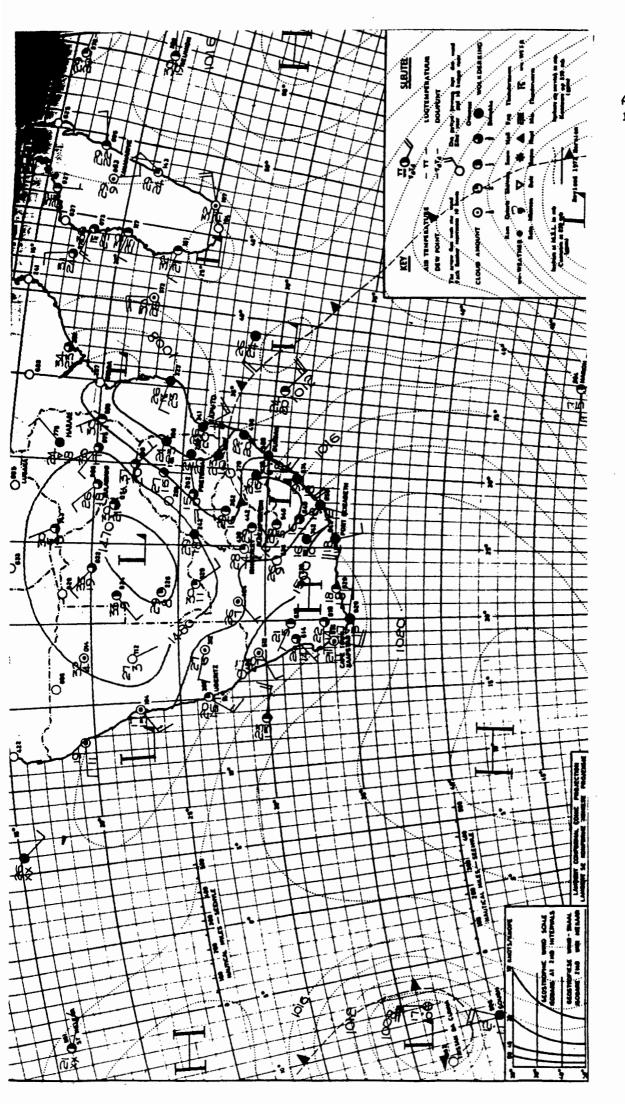
APP 3.15 04/06/88



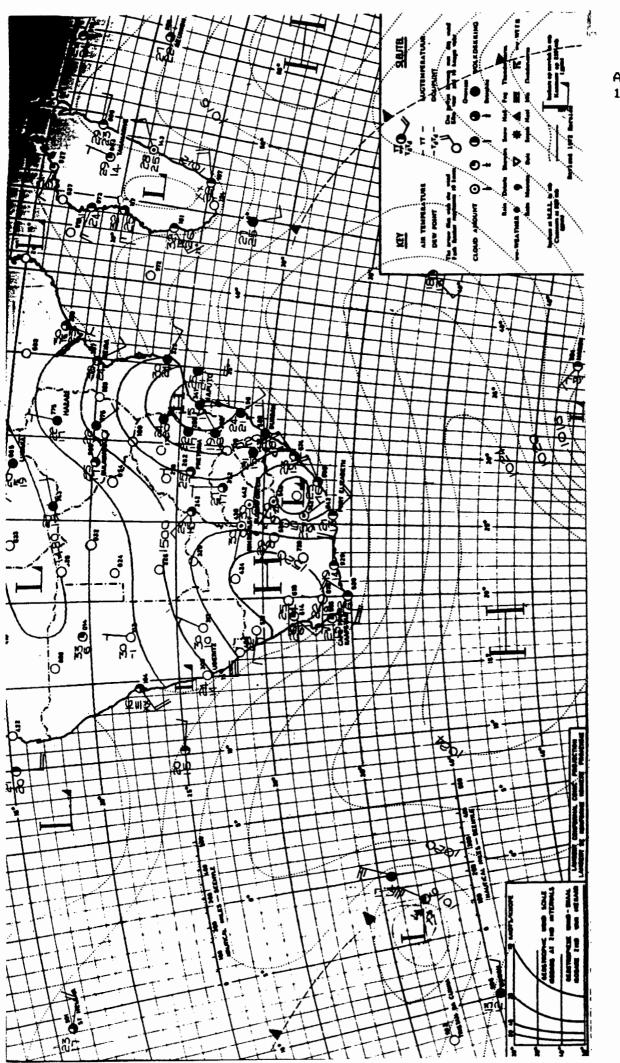
APP 3.17 10/12/87



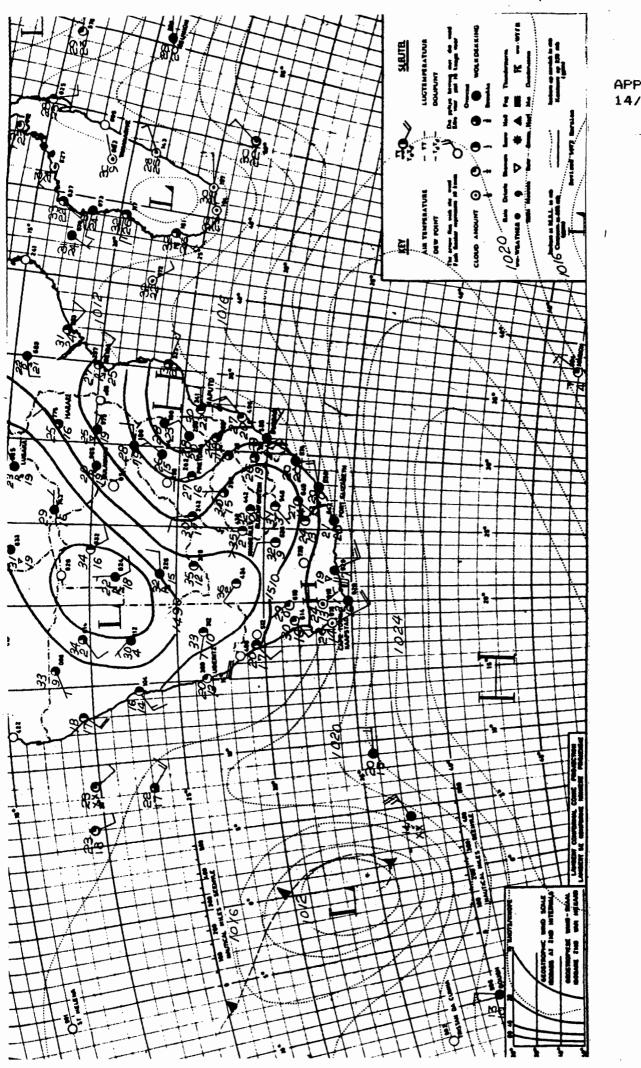
APP 3.18 11/12/87



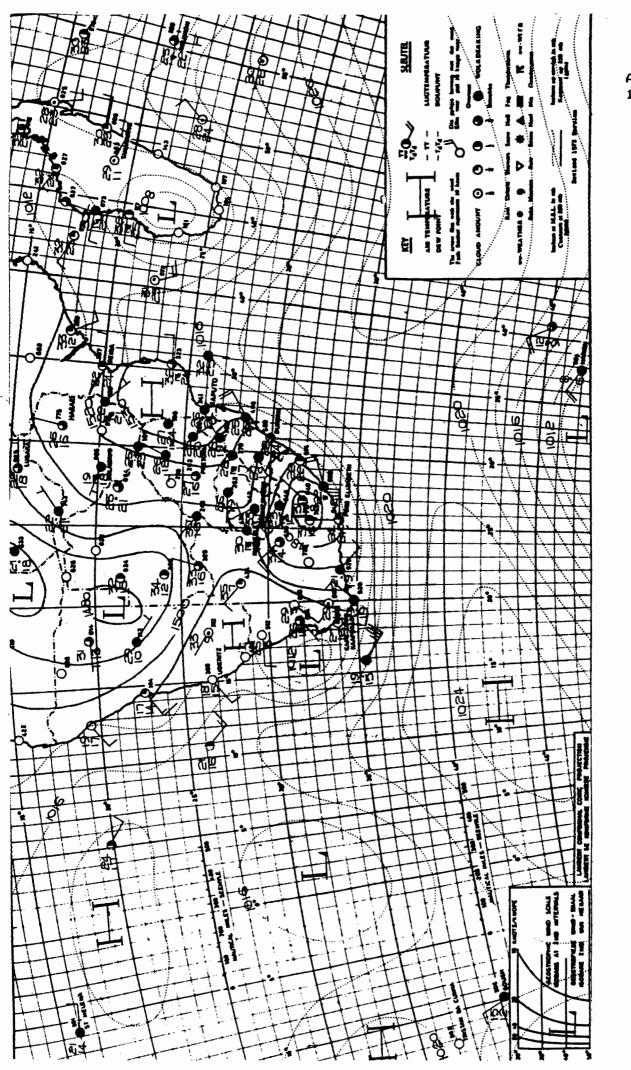
#### APP 3.19 12/12/87



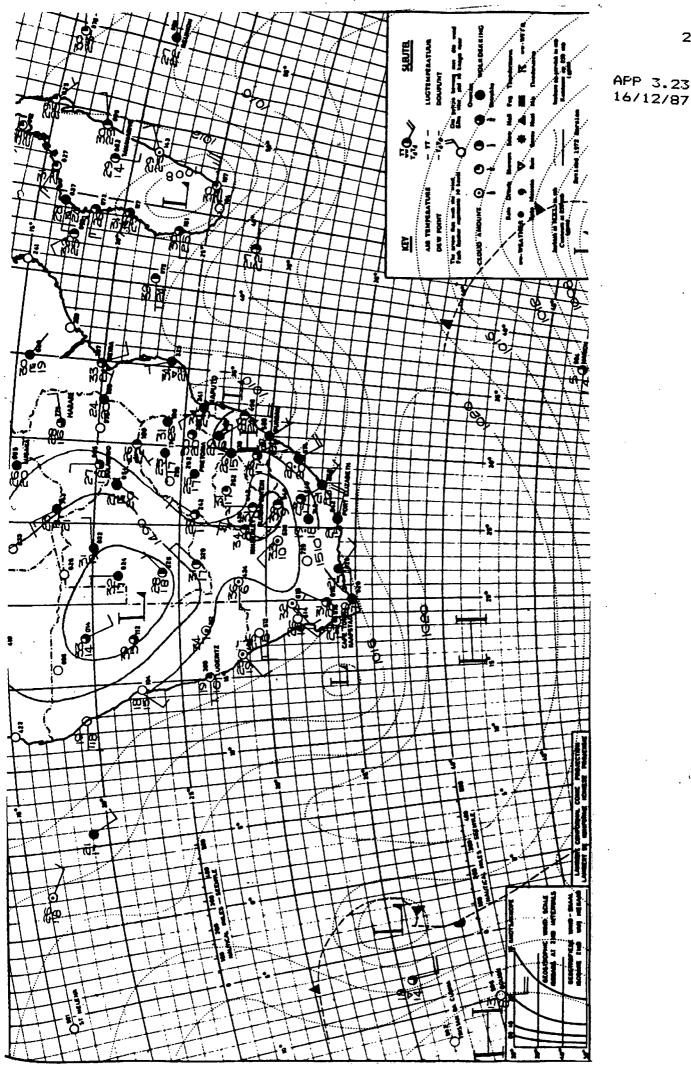
APP 3.20 13/12/87



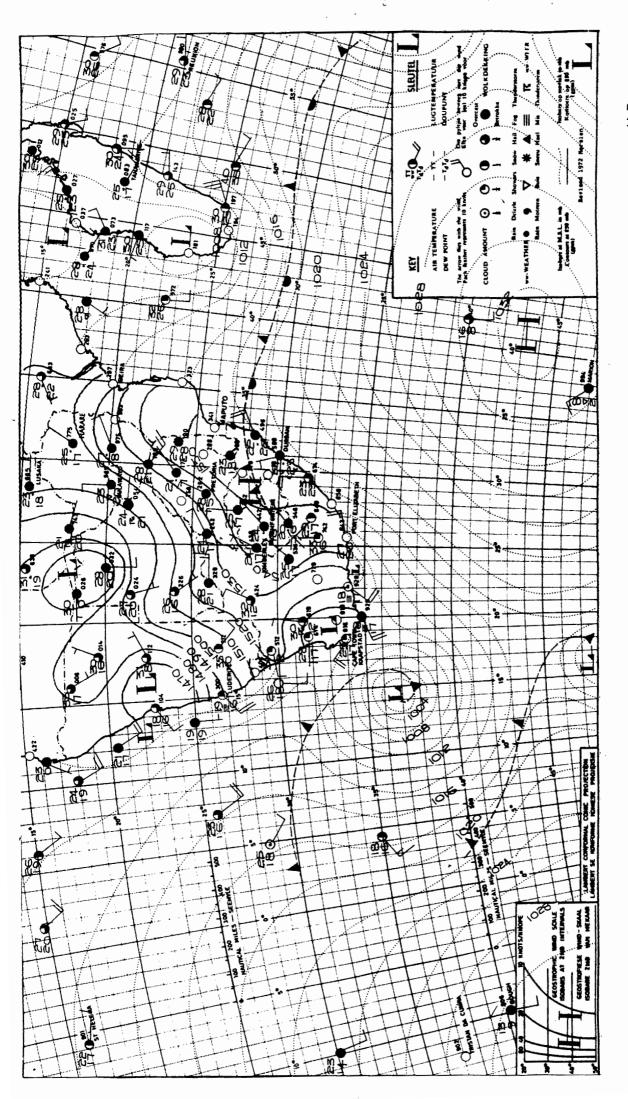
APP 3.21 14/12/87



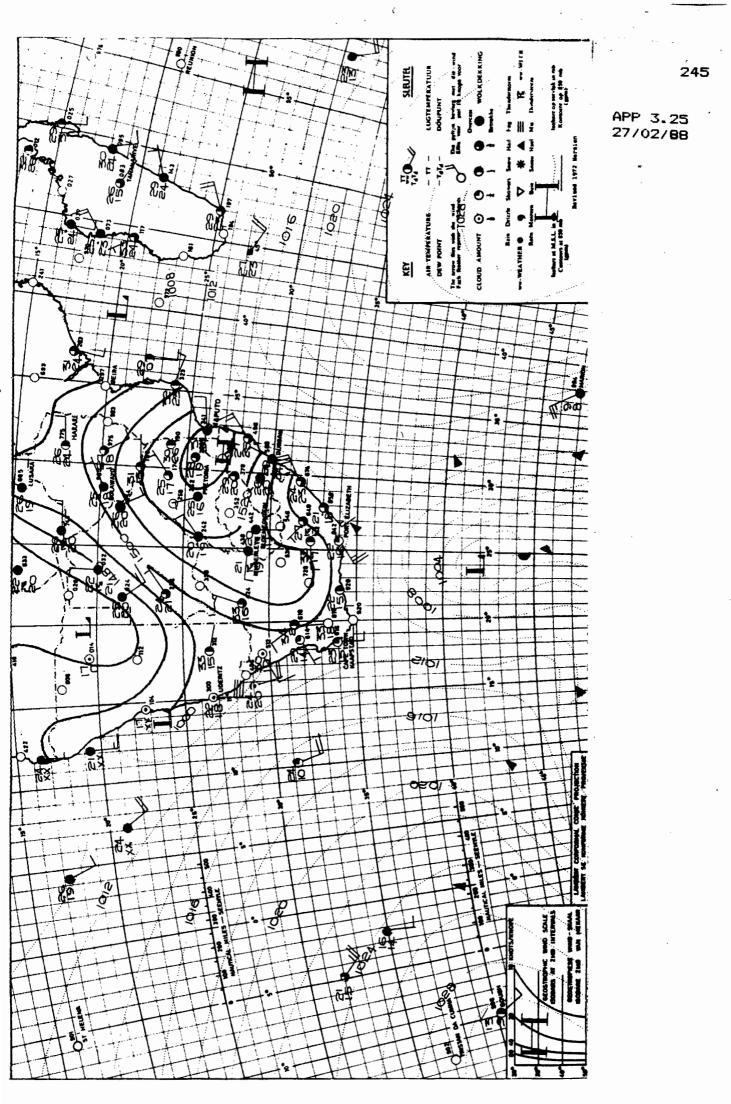
APP 3.22 15/12/87

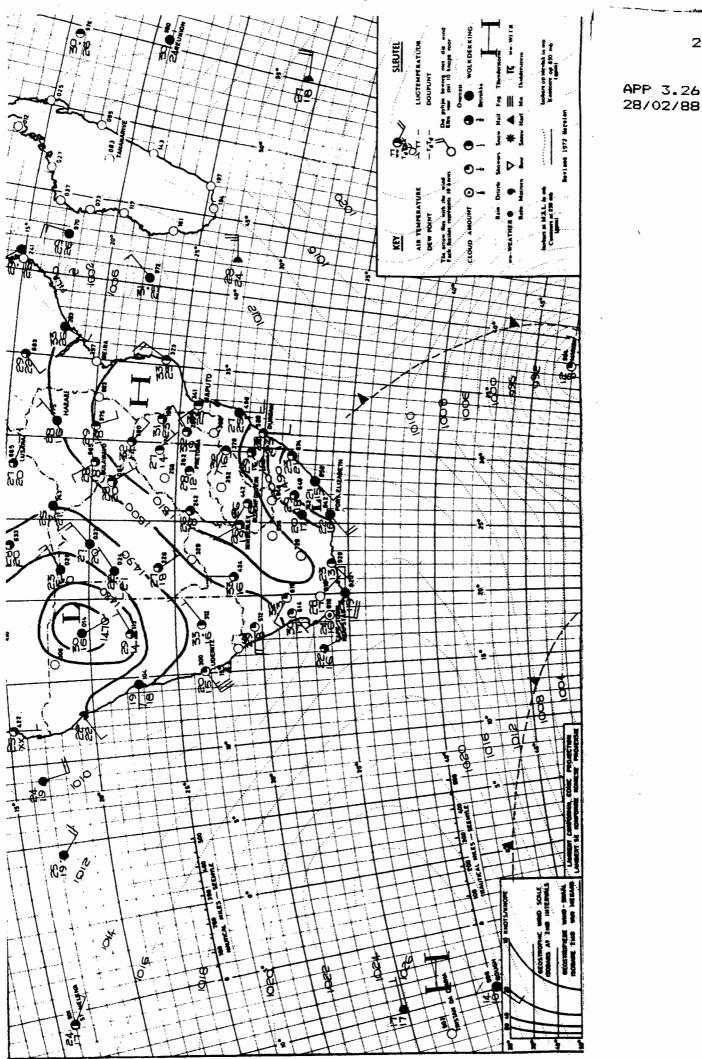


APP 3.23

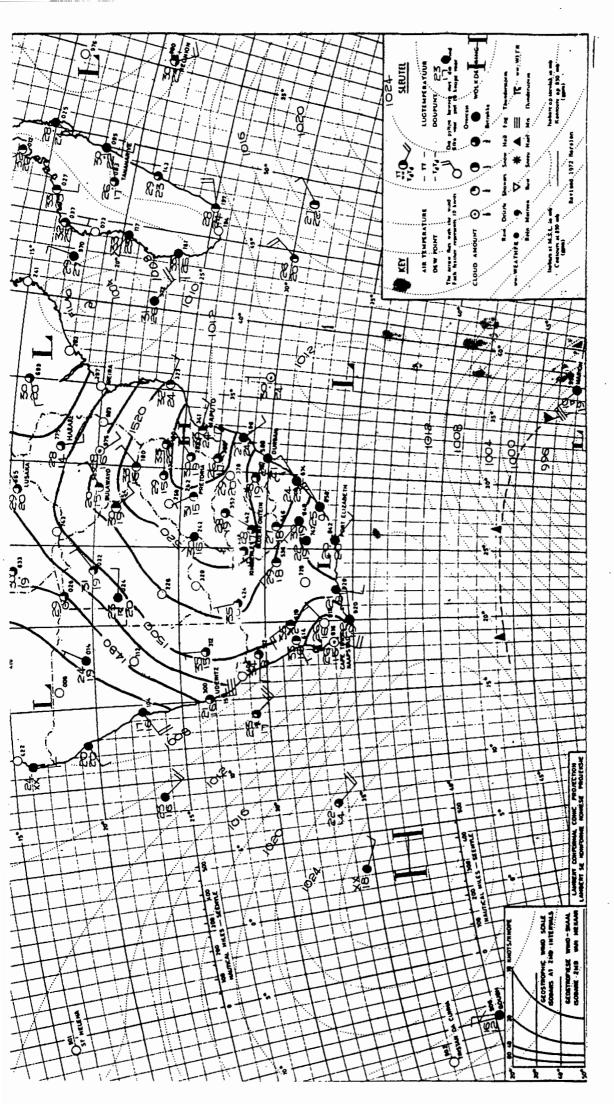


APP 3.24 26/02/88

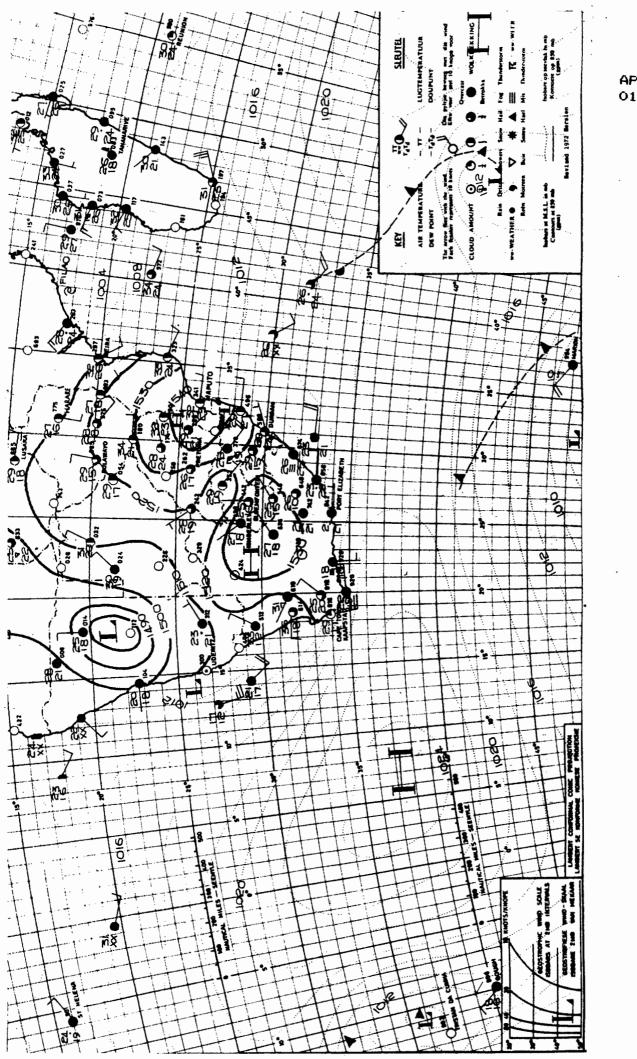




í.

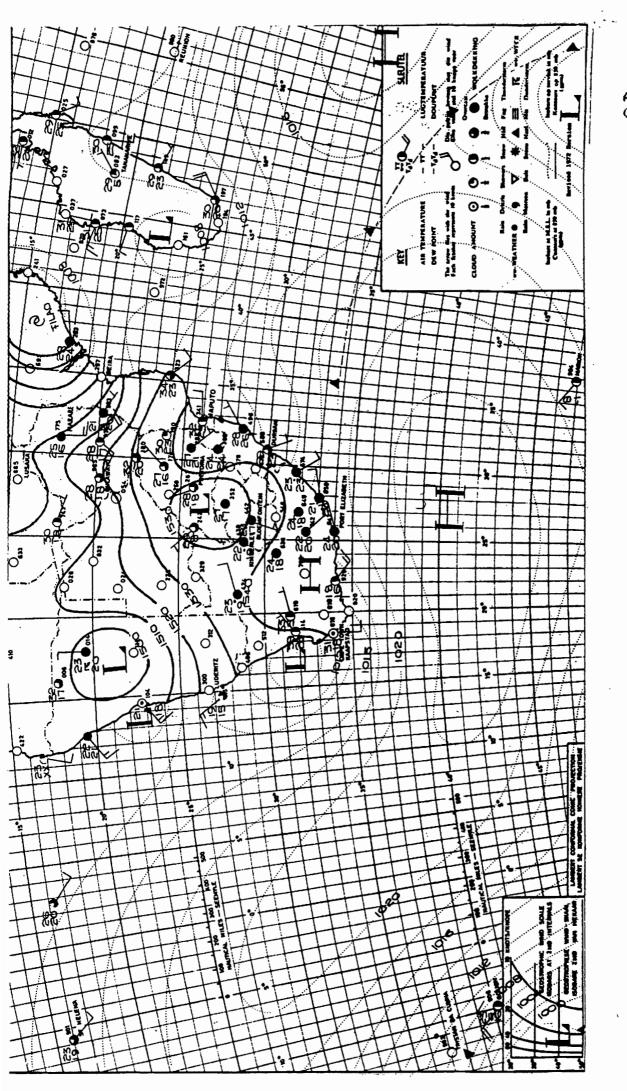


### · APP 3.27 29/02/88



APP 3.28

01.03.88



APP 3.29 02/03/88