Biology of fungi on plants in the sub-Antarctic.

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BIOLOGY OF FUNGI ON PLANTS IN THE SUB-ANTARCTIC

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SUMMARY

A study of microfungi growing on three major plant species on the island of South Georgia was made during the Antarctic summer of 1979 - 1980. Commonly isolated species included *Botrytis cinerea*, *Chaetophoma* sp., *Chrysosporium pannorum*, *Cladosporium sphaerospermum*, *Penicillium* spp. and *Mucor hiemalis*, plus a large number of sterile mycelia.

As leaves aged, their water contents decreased and levels of soluble carbohydrates were reduced. On falling to the litter layer, leaf carbohydrate levels declined further, but water contents were increased. The litter mycoflora was markedly different from that of standing dead leaves.

All of the most commonly isolated phylloplane species were capable of cellulose oxidation and all showed protease activity. Pectinase activity was exhibited by all of the phylloplane species tested. Only *Botrytis cinerea* appeared to be capable of lignin degradation. Significant cellulase activity continued at 1°C, with an optimum around 20°C.

Studies on the effects of temperature and relative humidity on germination and growth have shown relative humidity to be an important factor in determining the germination percentage. Substrate water activities below 0.90 caused a cessation of growth in all of the species tested except for *Cladosporium sphaerospermum*. All species tested had temperature optima for growth at around 20°C, but were capable of growth at 1°C. Germination of *Chaetophoma* sp. was restricted to temperatures above 10°C, but all other species tested showed germination at 5°C.

When placed in water, moribund and dead leaves showed leakage of up to 90% of total available reducing sugars in a few hours. No effect could be detected of freeze-thaw cycles upon leakage.

Linear extension of *Botrytis cinerea* was inhibited by volatile factors released from other colonies, and hyphae of this fungus were often observed to grow helically around hyphae of *Chaetophoma* and *Cladosporium sphaerospermum*.

ANTARCTIC/PHYLLOPLANE/LEAF LITTER/LEAKAGE/SUCCESSION

A C K N O W L E D G E M E N T S

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FALKLAND ISLANDS, SOUTH GEORGIA,
SOUTH SANDWICH ISLANDS AND BRITISH ANTARCTIC TERRITORY

Scale of Statute Miles (Latitude 60°)
Edge of Ice Shelf
Approximate Coastline
Heights in Feet

75° 70° 65° 60° West of Greenwich 55° 50° 45° 40°

WEST FALKLAND
EAST FALKLAND
STANLEY
FALKLAND ISLANDS
Lively I.
Sea Lion I.
Beauchêne I.
ARGENTINA
Punta Arenas
Rio Grande
TIERRA DEL FUEGO
I. Desolacion
I. Santa Ines
C. San Diego
I. de los Estados
I. Nueva
I. Navarino
I. Wollaston
I. Hermite
Cabo de Hornos
Shag Rocks
SOUTH GEORGIA
Grytviken
Clerke Rocks
DRAKE PASSAGE
SOUTHERN OCEAN
SOUTH SHETLAND ISLANDS
Elephant I.
Clarence I.
Gibbs I.
King George I.
Greenwich I.
Smith I.
Deception I.
Astrolabe I.
Trinity I.
d'Urville I.
Joinville I.
Dundee I.
James Ross I.
Snow Hill I.
Robertson I.
Jason Peninsula
Anvers I.
Brabant I.
PALMER ARCHIPELAGO
Adelaide I.
Marguerite B.
GRAHAM LAND
Coronation I.
Signy I.
Laurie I.
Inaccessible I.
SOUTH ORKNEY ISLANDS
SOUTH SANDWICH IS.
Leskov I.
Zavodovski I.
Visokoi I.
Candlemas I.
Saunders I.
Montagu I.
Bristol I.
Southern Thule
BRITISH ANTARCTIC TERRITORY
BRITISH ANTARCTIC TERRITORY
BELLINGSHAUSEN

55° 60° 65° 50° 55° 60°

GENERAL INTRODUCTION

Many studies have been made of Arctic and Alpine microfungi, but relatively little is known about microfungi in the sub-Antarctic regions (Tubaki, 1961; Corte and Daglio, 1963, 1964; Heal *et al.* 1967; Latter and Heal, 1971). Information regarding the species composition and the activity of these fungi, particularly during cold periods and under snow cover, is vitally important for an understanding of nutrient cycling in the tundra ecosystem.

As part of an ongoing project by the British Antarctic Survey, studies have been made of productivity and of nutrient levels in communities of the more important plant species in a tundra ecosystem. The project is studying the ecosystem of South Georgia, and much detailed information has been gathered of levels of inorganic nutrients in plant leaves and litter on the island. Models have been proposed for organic matter production and cycling rates in the more important plant communities (Walton, 1973; Walton *et al.* 1975; Smith and Stephenson, 1975).

Studies of the mycoflora of leaf surfaces in temperate climates have shown characteristic species assemblages to occur on a variety of host plants, and it is now well-established that a succession of phylloplane species will occur as a leaf senesces and falls to the litter layer (Pugh, 1958; Webster and Dix, 1960; Hudson, 1968).

Reasons proposed for this succession have included changes in relative humidity and nutrient levels in the host, antagonism between species, and the relationship of the fungal enzyme complements to the substrate. It is probable that combinations of such factors, and other climatic effects, together determine the species composition on a phylloplane. The species successions appear to be attributable mainly to substrate changes and water availability as the leaves senesce.

The island of South Georgia is comparable in many ways to other maritime sub-Antarctic islands, and yet possesses many introduced plant species (Headland, pers. comm.). Ships periodically visit the island, raising the possibility of large influxes of fungal propagules from temperate and tropical climates, but there is reason to suppose that limitations are imposed upon the survival of such species because of environmental factors resulting from the location of South Georgia. It would be expected that those species which are active in the ecosystem will be adapted, either in their growth or morphology, to survival under the relatively harsh climatic conditions.

This study was undertaken to provide basic information on the species composition of the South Georgian phylloplane mycoflora. It is essential, for a better understanding of nutrient cycling within the tundra ecosystem, that detailed information is available concerning mycoflora activity in the early stages of the decomposition process.

CHAPTER 1

INTRODUCTION TO SOUTH GEORGIA

The sub-Antarctic island of South Georgia ($54^{o}55'S$, $36^{o}38'W$) is situated approximately 2,000 kilometres east of Tierra del Fuego. The island is roughly 160 kilometres long, varying in width from 5 to 40 kilometres (pp. 4 - 5) (Pegler *et al.* 1980). Much of the island lies above 1,000 m height, and most of the island is covered by extensive permanent ice, with many large glaciers reaching the sea at the head of fiords (Smith and Walton, 1975).

Smith and Walton (1975) provide a more detailed description of the island and its history, together with a summary of climatic data up to 1973. As South Georgia lies south of the Antarctic Convergence*, the climate is much colder than at a comparable latitude in the Northern Hemisphere. It is a cold oceanic climate with prevailing westerly winds (French, 1974). Winter and summer seasons are clearly defined, subzero mean monthly air temperatures occur from May to October, and frosts and snowfalls are frequent during the summer. Fig. 2 and Fig. 3 show the maximum and minimum daily air temperatures, respectively, at the meteorological station on South Georgia during the 1979 - 1980 summer season, and Appendix 1 gives tabulated climatic data for this period.

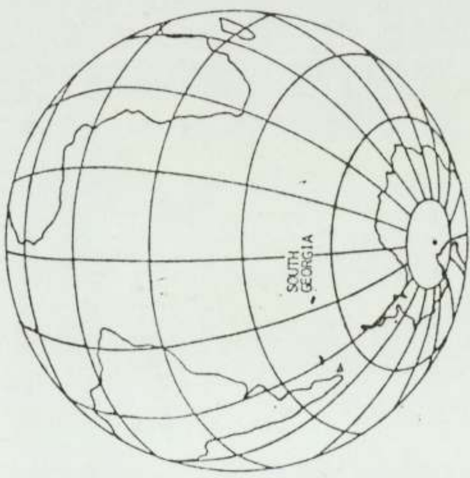
* The Antarctic Convergence is the area of mixing of cold, less saline water resulting from the ice cap, with the warmer waters of the South Pacific and Atlantic Oceans.

Figure 1: South Georgia Research Sites at Grytviken.

1. Research Base.
2. *Acaena* sampling site (South Georgia Research site [S.G.R.S.] 3).
3. *Festuca* sampling site (S.G.R.S.1).
4. *Poa* sampling site.
5. Disused whaling station.
6. Moss sampling site (S.G.R.S.2)

(Contours in feet)

Dark areas indicate freshwater lakes.


54°00'S
54°30'S
38°00'W
37°00'W
36°00'W
Willis Is.
Bird I.
Cape North
Cape Buller
Bay of Isles
Ice Fjord
Cape Demidov
King Haakon Bay
Shallop Cove
Jossac Bight
Prince Olav Harbour
Possession Bay
Antarctic Bay
Fortuna Bay
Stromness
Husvik
Leith
Stromness Bay
Larsen Point
expanded on p.5
Cumberland Bay
King Edward Point
Grytviken
Barff Point
Ocean Harbour
St. Andrews Bay
Royal Bay
Gold Harbour
Cooper I.
Drygalski Fjord
Cape Disappointment
Diaz Cove
Undine South Harbour
Mount Paget
A L L A R D Y C E
Pickersgill Is.
Annenkov I.
Newark Bay
S O U T H G E O R G I A
FALKLAND ISLANDS DEPENDENCIES
0 10 20 30 km
Contour interval 250m
SOUTH GEORGIA

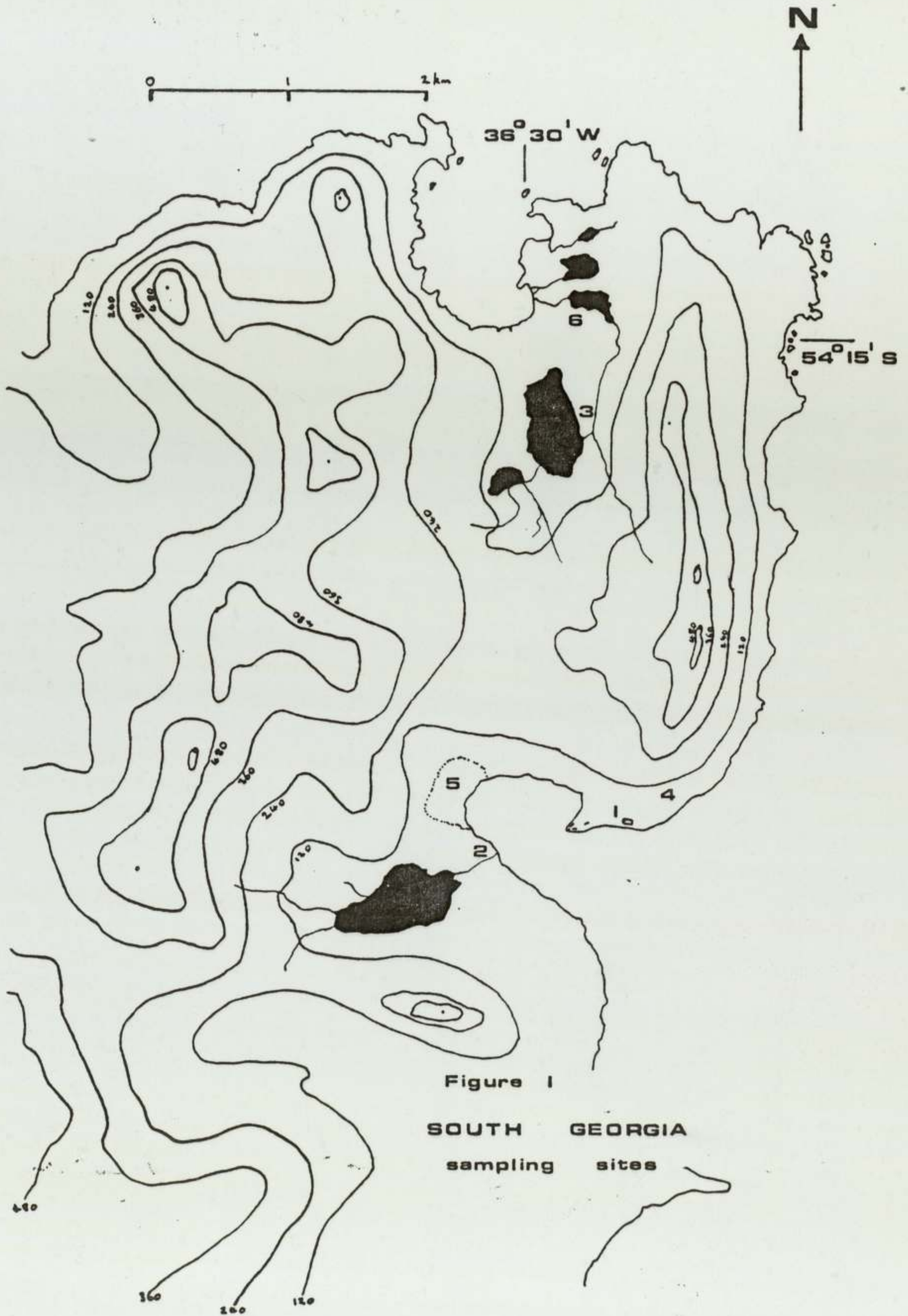
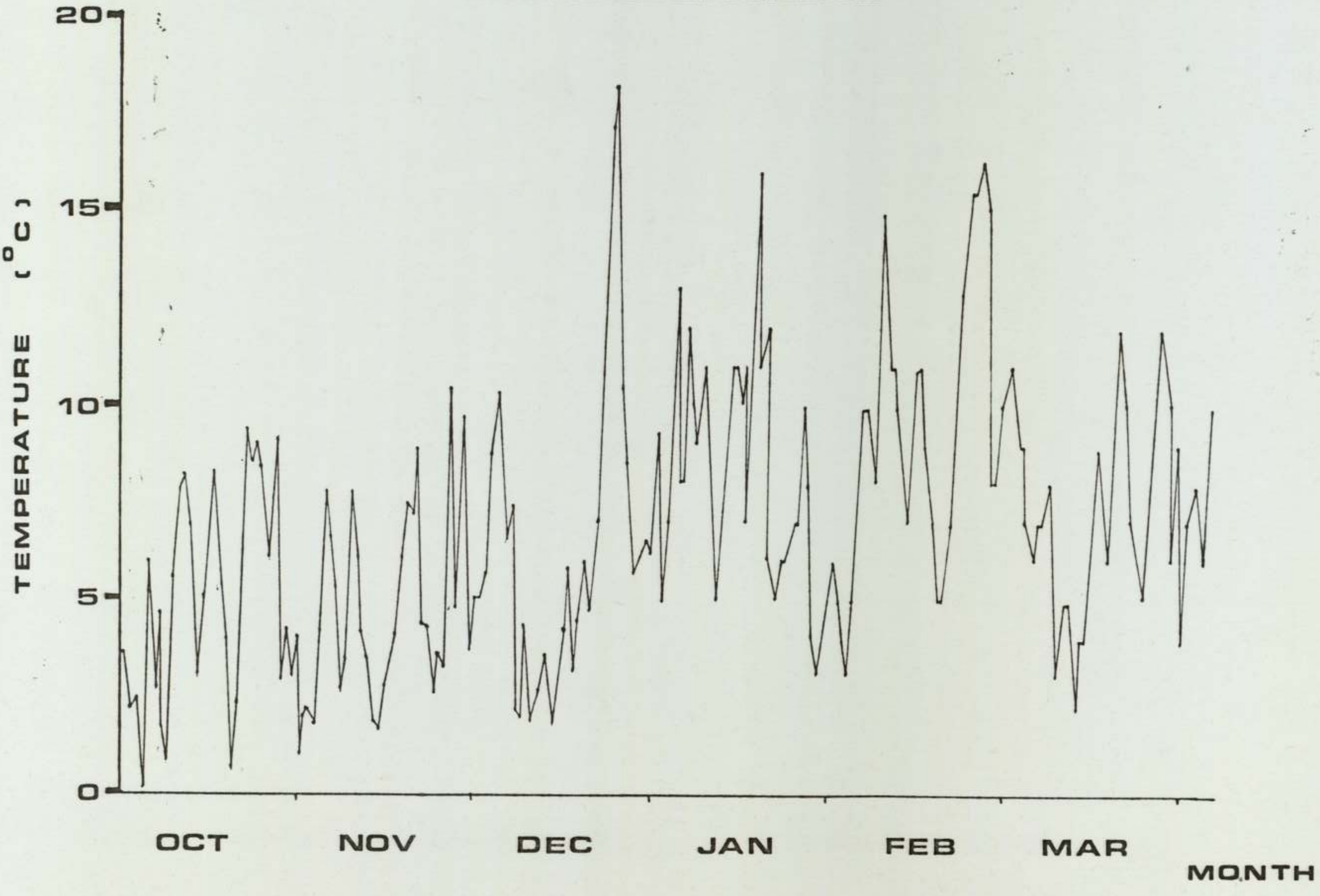


Figure I

SOUTH GEORGIA

sampling sites

Fig. 2. Maximum daily air temperature, King Edward Point, 1979 - 80



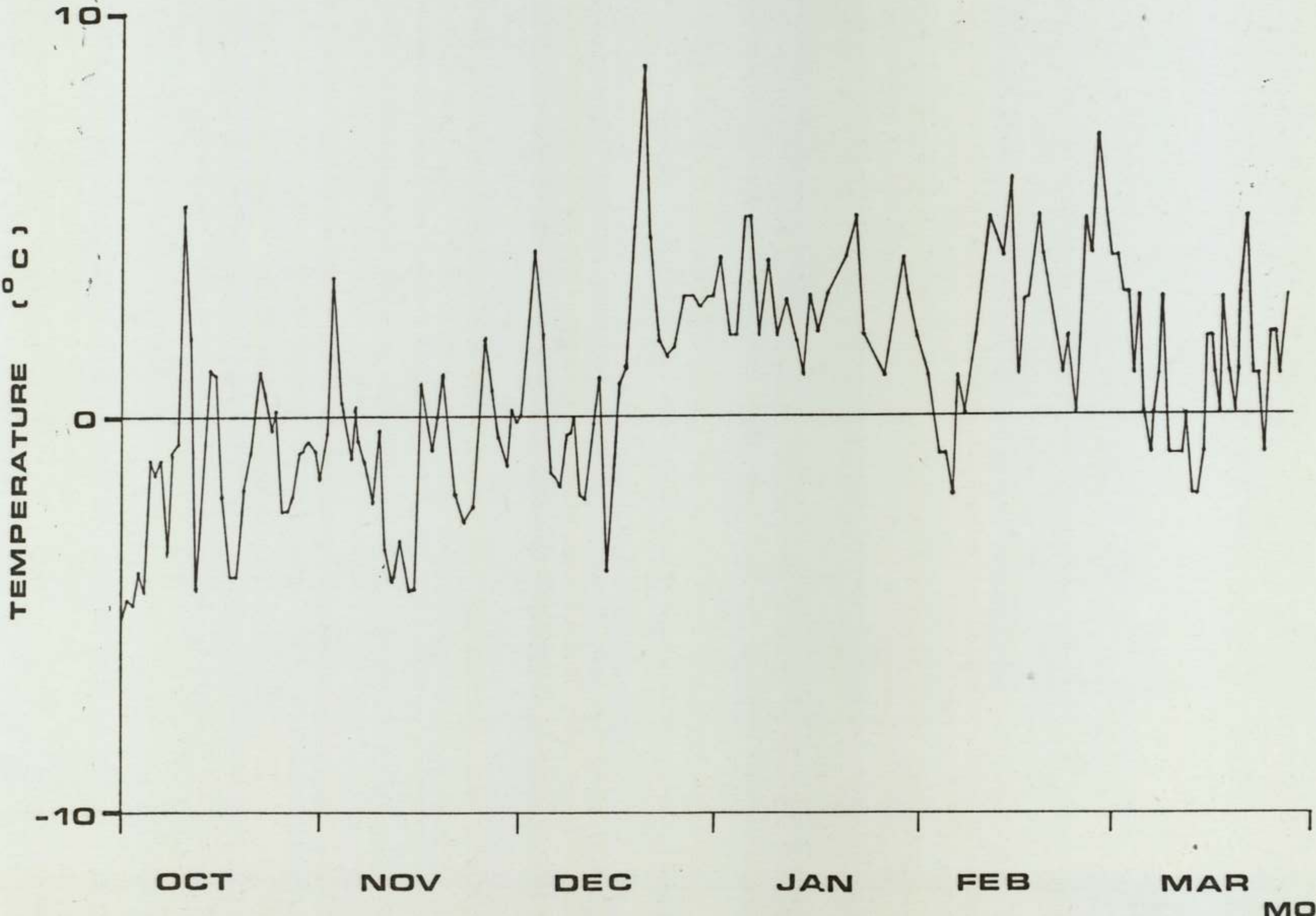


Fig. 3. Minimum daily air temperature, King Edward Point, 1979 - 80

A steady rise in maximum daily temperatures occurred until around mid-January. This corresponds roughly to the onset of the short summer season and was accompanied by a sharp rise in the minimum daily recorded temperature (Fig.3) such that, between December 20th and January 30th, temperatures remained above freezing point. Subsequent frosts in early February were followed by more mild temperatures, but during March the minimum daily air temperatures regularly fell below $0^{o}C$.

Temperatures within vegetation during summer are normally well above ambient and exceed $40^{o}C$ in the litter layer of *Festuca contracta* grassland (Walton, pers. comm.). Near surface temperatures in moss banks seldom fall below $0^{o}C$ in summer, or for periods in winter when covered by snow.

Severe frosts can occur in late autumn and early spring after the melt, and during this latter period new plant growth is particularly susceptible to frosts. The temperature in warm air pockets beneath the snow may reach $5^{o}C$ above ambient (Smith and Walton, 1975). The range of values for pH, loss on ignition, moisture content and mineral contents for each of the principal soil types on South Georgia are given by Smith and Walton (1975).

There are no indigenous grazing mammals in South Georgia, beetles being the largest native herbivore.

Fig. 4 THE HOST PLANT SPECIES

a) *Acaena magellanica*

b) *Festuca contracta*

c) Lichens and mosses in a *Festuca* sward

d) *Poa flabellata*

a

b

c

d

Introduced rats are widespread in many coastal areas, where they may eat roots or rhizomes of some plant species. The introduction of reindeer between 1909 and 1924 has resulted in serious damage to vegetation by grazing and trampling in those areas to which they are confined (Kightley and Smith, 1973). Deposits of wet acidic peat beneath some vegetation types suggest that decomposition is slow, particularly under anaerobic conditions where microbial and invertebrate populations are small (Smith and Walton, 1975). However, personal observation of basidiomycete sporophores growing in a waterlogged bryophyte community suggests that this fungal group may be of some importance in the decomposition of such inhospitable substrates. Pegler *et al.*(1980) reported the incidence of several higher fungi from marshy ground near rivers and in wet bryophyte mats.

The three predominant higher plant species which are native to the island are:

a) *ACAENA MAGELLANICA* (Lam.) Vahl., a dwarf shrub. (see Fig. 4a)

b) *FESTUCA CONTRACTA* T. Kirk, a meadow grass. (see Fig. 4b)

c) *POA FLABELLATA* (Lam.) Hook., a tussock grass. (see Fig. 4d)

Greene and Walton (1975) and Smith and Walton (1975) give brief notes on these species and their distribution in the sub-Antarctic.

The two grass species exhibit growth forms which result in relatively low ground cover, with distances of up to 60cm between plants in *Festuca* communities, and often of a number of metres in *Poa* communities. *Acaena* communities in lowland areas usually exhibit almost complete ground cover, the leaflets forming a dense canopy (Fig. 5a) with seed heads held above the leaves.

The extensive and thick root systems of all three host plant species confer stable anchorages against wind action, and also provide adequate access to moist underlying soil layers during the periods of cold, dry weather which occur frequently under such climatic conditions (Headland, pers. comm.).

The flexible petioles of *Acaena* are relatively immune to wind damage, whilst the grasses have sufficiently rigid leaf structures to maintain erect those leaves which are photosynthetically active. Dead leaves eventually become susceptible to damage by the frequent high winds, and fall to the litter layer at the bases of tussocks.

Levels of sugars are relatively high in new leaves of *Acaena* and *Poa* (ch. 2), and root systems are known to contain carbohydrate reserves for the production of new leaf material early in the spring. *Poa* is known to pre-initiate growing points and new leaves in autumn for growth early in the following season (Gunn, 1976; Walton, pers. comm.).

Acaena communities are usually monospecific. The dense canopy of green leaves prevents germination and growth of other plant species, and a dry litter layer up to 5 cm deep during summer may hinder seed germination. The shrub is a member of the Rosaceae, growing up to 30 centimetres in height. Although deciduous in that most leaves are lost before the beginning of winter, the leaves are not shed as in most deciduous species, remaining attached to the stem whilst the lamina decays *in situ*. The petioles may remain on the stem and take several years to decay (Walton, 1977). A simulation model of dry-matter production in a mature community of *Acaena magellanica* on South Georgia showed a very high annual turnover of biomass, almost all of which was attributable to the leaves (Jones and Gore, In press).

Festuca contracta appears to be an early coloniser of the more recently formed mineral soils derived from the breakdown of shale fragments. It can grow in conjunction with other species, but mainly exists in a mixed community with lichens and mosses as the predominant partners (see Fig. 4c).

Dry matter production appears to be relatively low, and litter accumulation away from the small tussocks is negligible. Dead leaves may remain upright for at least one year, eventually being flattened by wind or snow damage. Standing dead leaves comprise around 70% of *Festuca - Acaena*

communities (Walton *et al.* 1975), and up to 85% of dense *Festuca* grassland. Decomposition within the tussock is slow and the dry matter resulting from several years of growth is often still present. (Smith and Stephenson, 1975).

Poa flabellata is a tall monocotyledon, with inflorescences reaching a height of up to 2 metres from the base of the plant. It grows as large tussocks of up to 60 centimetres diameter, in which new, dying and standing dead leaves are held (see Fig. 5). The base of each tussock is surrounded by fallen dead leaves with varying moisture contents, those nearest the soil being the oldest and usually having the highest moisture contents. *Poa flabellata* has a high proportion of overwintering green leaves and, since the rate of decomposition is similar to that of *Festuca* material (Smith and Stephenson, 1975), the return of nutrients to the substratum is likely to be relatively low. The standing crop of living leaves can be as high as 15 kg m^{-2}, with dead material at up to 10 kg m^{-2}, but mean values are roughly 7.5 kg m^{-2} and 5 kg m^{-2} respectively (Smith and Walton, 1975). There is very little permanent litter accumulation under *Acaena magellanica* stands because of rapid decomposition of foliage during the winter months (Walton, 1977). Over 90% loss of dry weight occurs within 14 weeks, (Walton, 1973; Smith, 1976). Smith and Stephenson (1975) showed that dry weight loss from litter bags of *Festuca contracta* was around 56% per year, whilst standing dead

Fig.5 Sections through the leaf canopy of *Acaena*, and tussocks of *Festuca* and *Poa*.

a). Section through a leaf canopy of *Acaena magellanica*, showing growth habit, petioles, and dense leaf canopy.

b). Section cut through a small tussock of *Festuca contracta*, showing a preponderance of standing dead leaves and low levels of litter accumulation.

c). Section through a tussock of *Poa flabellata* showing accumulation of litter material at the base of the tussock.

a

b

C

material losses ranged from 20% to 25% per annum. Losses of *Poa flabellata* standing dead material ranged from 12% to 30% per annum. These are probably over-estimates, since under natural conditions it takes several years before standing material is incorporated into the litter of a *Festuca* grassland ecosystem (Smith and Walton, 1975).

Dominant soil conditions are peats, brown well-drained soils and mineral soils on glacial deposits. Active forms of nonsorted patterned ground are common as a result of frost heaving, although no permafrost exists (Brown and Veum, 1974).

Studies of a similar ecosystem, that of Marion Island in the sub-Antarctic, have led to conclusions that the major sources of nitrogen input to the system are; bird excreta (the main source on Marion Island); salt spray; and rain deposition of ammonia. The latter two sources are of relatively minor importance on Marion Island, but may be more significant on South Georgia in those areas which are infrequently visited by birds. Rock degradation provides a negligible input of nitrogen, and nitrogen fixation, although apparently of major importance in some tundra ecosystems is not considered at present to be of major importance on South Georgia (Alexander, 1974; Lindeboom, 1979; Walton, pers. comm.).

Further information on Arctic and Antarctic tundra ecosystems and their characteristics regarding decomposition processes is given by Holding *et al.*(1974).

CHAPTER 2

LEAVES AND LEAF SURFACES

INTRODUCTION - The Nature of the Phylloplane.

The leaf surfaces of higher plants are covered by non-cellular cuticles which are non-living and heterogeneous in chemical composition. Their major components are waxes and cutin, the latter being a biopolymer of fatty and hydroxy-fatty acids (Holloway, 1971). Cutin is the chief structural component, with wax embedded in the membrane and exuded on its surface (Martin and Juniper, 1970).

An examination of many species has shown that leaf waxes may be present in a wide range of forms, e.g. as tubes, plates, fibrils, granules or flat sheets. Some wax types may form a complete cover on the leaf surface so that not even stomata are apparent on surface replica studies. In some cases wax is absent from guard cells, or the cuticle itself may be completely free of wax. Wax production is influenced by light. Peas, for example, produce very little if any wax in the dark, but following 24 hours illumination, will produce minute crystalline wax projections (Martin and Juniper, 1970). Wax production in peas apparently continues until the cessation of leaf expansion. When leaves attain maximum size, wax deposits tend to decline, presumably as a result of weathering or abrasion (Holloway, 1971).

Other components of the cuticle include cellulose and pectin, which occur in the membrane where it merges with the epidermal cell walls. The cuticle has an important

influence on processes taking place on the plant surface (Hull, 1970). Its most important function is probably to waterproof the plant surface, possibly to prevent leaching from the epidermal cells.

The physical properties of a leaf surface are markedly affected by their roughness. The most obvious feature contributing to this is the modification of the cuticle surface by the underlying venation. The prominence of veins is generally more pronounced on the lower surface. The more xeromophic a leaf, the less conspicuous is the venation (Stokes, 1963). On many monocotyledonous leaves, venation appears as a series of prominent, parallel-running ridges along the leaf length. The topography of the cuticle surface is governed by the shape and size of underlying cells, which is itself frequently different between adaxial and abaxial surfaces.

Trichomes are the microscopic uni- or multicellular projections from the epidermis, which are also covered by the cuticle. They are generally more abundant on midribs and veins of a leaf. Their frequency is related to age, the number of trichomes per unit area decreasing with senescence due to abrasion and weathering (Stokes, 1963).

The most important influence of the physiochemical properties of the leaf surface is upon their wettability. The upper surface of most leaves is usually more hydrophobic, although this is not always true. The primary factors governing leaf wettability are the nature of the

chemical groups exposed in the cuticle, and the surface roughness. Differences in wettability are not wholly accounted for by differences in surface wax composition (Holloway, 1971). Water repellency is greatest on leaf surfaces which possess roughness in the form of crystalline wax deposits. These trap air films between the leaf surface and water droplets. Flattened wax deposits are generally more wettable and do not involve air films (Challen, 1962).

The parallel ridges of venation systems also trap air films. Leaf wettability is therefore a characteristic affected by a number of complex factors. The resistance to wetting is probably an adaptation of the leaf which is designed to reduce leaching.

Tukey (1971) defines leaching as the removal of substances from plants by the action of aqueous solutions, such as rain, dew, mist and fog. Substances leached from plants include a great variety of materials (Morgan and Tukey, 1964). Inorganic nutrients leached include all the essential minerals, and also large amounts of organic substances have been noted, including free sugars, pectins and sugar alcohols. These may accumulate on leaf surfaces following alternate short periods of wetting and drying, and hence act as a substrate for fungal and bacterial activity.

All of the plant amino acids and many organic acids have been found in leachates. Work has shown that gibberellins and vitamins can also be leached (Kozel and Tukey, 1968). Organic substances, mainly carbohydrates, account for the major quantity of leached materials (Tukey, 1971). Losses as large as 800 kilogrammes of carbohydrates per acre per year have been reported from *Malus domestica* (Dalbro, 1955). Tukey *et al.* (1958), found that up to 6% of the dry weight equivalent could be leached from leaves of *Phaseolus vulgaris* in 24 hours.

A variety of factors influence the composition and quantity of leachates from foliage. Young, actively growing tissue is relatively immune to leaching, but susceptibility increases with age, peaking at senescence (Arens, 1934). This is probably a result of an increased number of "sinks" in young and active leaves where cell expansion and hence ion uptake are prevalent. Leaves from healthy and vigorous plants are much less susceptible to leaching than are leaves which are damaged, whether damaged by micro-organisms, insects, climate or mechanical means (Tukey and Morgan, 1963).

Light intensity and temperature increase leaching, possibly via increases in photosynthesis and lipid permeability. Leached cations have been shown to originate from "free spaces" within the lamina (Tukey *et al*.1965). Exchange reactions have been shown to be involved between sites on the cuticle and hydrogen ions within the leaching solutions. Cations can move directly from the translocatory

stream into the leaching solution by diffusion and mass flow through areas devoid of cuticle.

Leachates originating from the above-ground parts of plants can be reabsorbed directly by leaves or roots of the same plant, adjacent plants, or by phylloplane microorganisms. Such cycling may provide an important nutrient source for fungal activity on the phylloplane and in litter.

The microclimate of leaf surfaces is an important factor contributing to the ecology of phylloplane microorganisms. Surrounding each leaf, and modifying energy exchange on the phylloplane, is the boundary layer (Sutton, 1953). This is a thin air-layer influenced by the properties of the leaf surface. Broad leaves have a thicker boundary layer than do narrow leaves, and ridges or trichomes can also increase the depth of the layer.

The temperature at a leaf surface is determined by an equilibrium where heat losses equal the heat gain by radiation. As previously mentioned, in a high-radiation environment with still air, as happens often in the sub-Antarctic, litter surface temperatures may be as high as 40°C, and leaf surface temperatures may reach 45°C (Walton, pers. comm.)

The humidity at the leaf surface is dependant upon the rate of transpiratory water loss across the boundary

layer. Relative humidity follows a diurnal cycle, being highest at night and lowest during daytime. At night, as humidity approaches saturation, radiative cooling of the leaf frequently causes condensation on the surface. This may be more important for fungal growth than are relative humidity levels (Burrage, 1976).

In the boundary layer most gaseous exchange occurs by simple diffusion. Further from the lamina, exchange is caused by air movement. The size of the boundary layer determines rates of diffusion, and hence trichomes or undulations on the leaf surface will decrease gaseous transfer and increase the localised humidity of the boundary air layer.

Evidently, the phylloplane environment is affected by a complex variety of factors, which combine to provide a situation which may or may not be amenable to fungal growth. Unless specific inhibitory substances are present on the leaf surface, then the fungal conidium or propagule may germinate utilising its own carbohydrate store. The humidity levels in a boundary layer of an actively transpiring leaf are often great enough to support germination directly, although precipitation and condensation are probably the more important sources of water to a developing mycelium.

MATERIALS AND METHODS

1) Scanning Electron Micrographs of Host Plant Leaves

Using leaf material excised from active plants on South Georgia and returned to England in cold storage (5°C), 2 - 3cm lengths of young grass leaves, and leaflets of *Acaena* were cut and dried in a desiccator. Pilot experiments using an alcohol drying series and osmium tetroxide fixative had revealed no visible difference between this method and air drying.

Dried leaf pieces were attached to specimen stubs using Electrodag 915 high conductivity paint, gold coated using a vacuum coating unit and finally examined by a Cambridge Stereoscan S150 Scanning Electron Microscope. Photomicrographs were made of various features of each host plant leaf surface (adaxial and abaxial).

2) Leaf Organic Matter, Water Content and pH.

At monthly intervals throughout the summer season (Dec. - Apr.), random samples were taken of leaves from each host plant and age category (see ch. 3). Litter was taken from the bases of grass tussocks and from beneath the canopy of *Acaena* . Leaves were cut into pieces and weighed fresh. Pieces of leaf were then dried at 60°C to constant weight, and reweighed to determine water content. Dried leaves were then carbonised in a muffle furnace at 480°C and reweighed when cool to determine the organic matter content.

pH of leaves and litter was determined by homogenising 25g of fresh leaves in a Waring blender, with 25cm^3 distilled water. The pH of the resulting filtrate was measured.

3) Gas Liquid Chromatography of Leaf Material

Weighed amounts of dried leaf material were refluxed for fifteen minutes in $15cm^3$ of 80% ethanol, decanted and refluxed for a further two changes of ethanol, making a total of forty-five minutes reflux time, in $45cm^3$ ethanol. The ethanol fraction was filtered through Whatman No.1 filter papers and then dried down under a vacuum in a rotary evaporator using a water bath at 40^oC. The dried residue was resuspended in $5cm^3$ ethanol,centrifuged to remove particulate debris, and to it were added one spatula tip of each of IR120(H) and IR45(OH) ion exchange resins. The sample was then left on a shaking tray for thirty minutes to allow adsorption.

$1cm^3$ of the sample was thoroughly dried under vacuum in a $5cm^3$ pear-shaped flask at 40^oC, and silylated by addition of $0.35cm^3$ pyridine (anhydrous),$0.1cm^3$ hexamethyldisilazane and $0.05cm^3$ trimethylchlorosilane . Silylation was for at least three hours. A standard solution of arabitol, fructose, glucose and sucrose at 2.5mg cm^{-3} was prepared, and $0.4cm^3$ dried down and silylated with each set of samples. Samples were analysed for carbohydrates using a Pye Unicam Series 304 chromatograph and CDP1 computing integrator.

4. Effect of Freeze-Thaw Cycles on Metabolite Leakage from Leaves

20cm^3 aliquots of distilled water were dispensed into 100cm^3 conical flasks and covered with loose metal caps. Flasks were autoclaved at 121°C for fifteen minutes and allowed to cool. Leaves of *Acaena magellanica* and *Poa flabellata*, which had been returned to England at +5°C and were therefore not fresh, were cut into lengths of approximately 5cm (*Acaena* separated into leaflets), and serially washed to remove surface yeasts and leachates. Leaves were then immersed in 1% w/w mercuric nitrate solution for one minute to kill any remaining mycelia, rewashed in sterile water, and finally placed into the conical flasks, roughly 300mg of leaf pieces per flask. Flasks were incubated at +5°C, -5°C and in a twenty-four hour freeze-thaw cycle of +5° to -5°C (one freeze and one thaw per day) to simulate field conditions. At intervals of twenty-four hours flask contents were filtered, the dry weight of leaves was determined for each flask, and the leakage medium was analysed for potassium, phosphate and reducing sugars. Four replicate flasks were made for each measurement.

Potassium was estimated by flame photometry against a calibration curve of KCl solution. Phosphate concentrations were estimated by a colour reaction involving addition of 1cm^3 of 0.5M trichloroacetic acid and 1cm^3 of colour reagent (Appendix 2) to 1cm^3 of filtrate. The

absorbance of the resulting blue colour was measured at a wavelength of 660 nm against a reagent blank using a Beckman DB spectrophotometer. Concentration was calculated from a previously constructed calibration curve.

Reducing sugars were measured using the dinitrosalicylic acid colour reagent assay. $3cm^3$ aliquots of colour reagent (Appendix 2) were added to $1cm^3$ samples of filtrate in test tubes. Tubes were shaken thoroughly and placed in a boiling water bath for fifteen minutes. The absorbance of the resulting colour was measured at 540 nm against a reagent blank using a Beckman spectrophotometer, and concentrations were calculated from a previously constructed calibration curve using fructose as the standard.

5. Effect of Wetting on Leakage of Leaf Metabolites

$20cm^3$ aliquots of distilled water were dispensed into $100cm^3$ flasks and autoclaved. Leaf pieces of *Acaena* and *Poa* were prepared according to the method of the previous section. Flasks with leaves were incubated at 15^oC with occasional stirring. At intervals, flasks were removed from incubation and leaves were separated from the water by filtration. The dry weight of leaves was determined, and the filtrates were analysed for phosphate, potassium and reducing sugars according to the methods of the previous section. Four replicate flasks were made per efflux time measurement. After all leaves were har-

vested, each sample was refluxed in 80% ethanol for fifteen minutes, decanted and refluxed a further two times. The resulting solution was filtered and analysed for reducing sugar concentration as previously described. From this was calculated the total available amount of reducing sugar for each sample of leaves.

RESULTS

1. Scanning Electron Photomicrographs

Fig.6 shows various features of *Acaena magellanica* leaf surfaces. The topography of both adaxial and abaxial surfaces was similar, but venation was pronounced on the lower surfaces of the leaf, where trichomes were more common. The lower surfaces had a higher density of stomata per unit area, these being uncommon on the adaxial surfaces. The stomata appeared to be bounded by ridges of raised epidermis effectively forming sunken stomata. Wax extrusions were common on both sides of leaves, and could be washed off by brief immersion of leaves in chloroform or diethyl ether. The ridged effect of the two epidermal layers was not an artefact, as observations of fresh leaves by light microscopy had also shown this feature.

Leaves of *Festuca contracta* were rolled under field conditions, i.e. presenting the abaxial surface to the atmosphere and allowing only a relatively small air gap between the edges of the lamina (Figs. 7 and 8).

The abaxial surfaces were relatively smooth and featureless, whilst the internal surfaces were densely covered by trichomes. Stomata were generally more common on the internal, adaxial surface, where they would presumably be protected from the atmosphere by the existence of a large boundary layer within the confines of the edges of the rolled leaf.

Fig.9 shows leaf surfaces of *Poa flabellata* . The adaxial surfaces were densely covered by trichomes and globose protrusions in ordered rows, the trichomes being concentrated mainly over the parallel venation ridges. It appears that stomata were present on the veins, between trichomes on the adaxial surface. The abaxial surfaces were relatively smooth with few trichomes. Stomata were apparently as numerous on the abaxial surface as on the adaxial, and were oriented transversely to the venation. Older leaves of *Poa flabellata* had rougher abaxial surfaces, possibly due to weathering of wax or cutinaceous material.

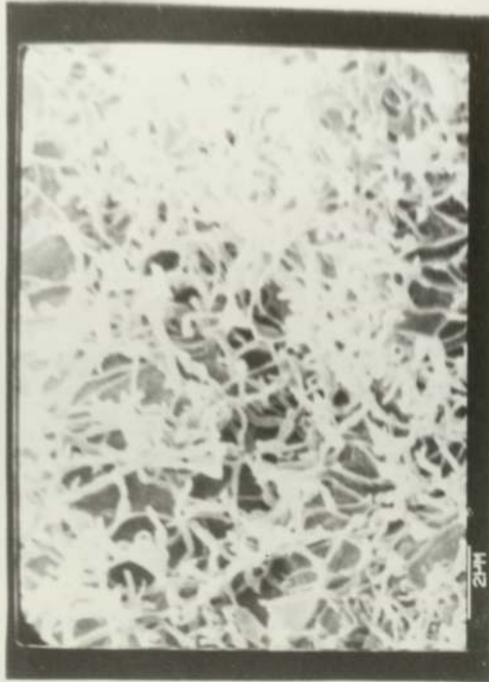
Fig. 6

a) *Acaena* upper epidermis.

b) *Acaena* upper epidermis showing wax structures.

c) *Acaena* upper epidermis showing trichome.

d) *Acaena* stoma surrounded by wax platelets.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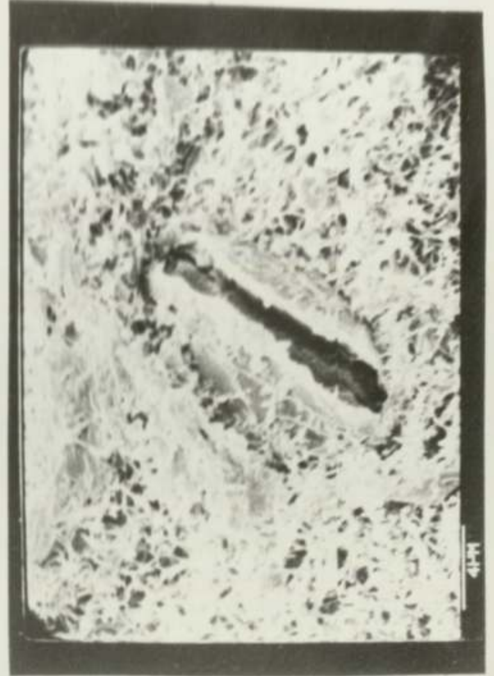
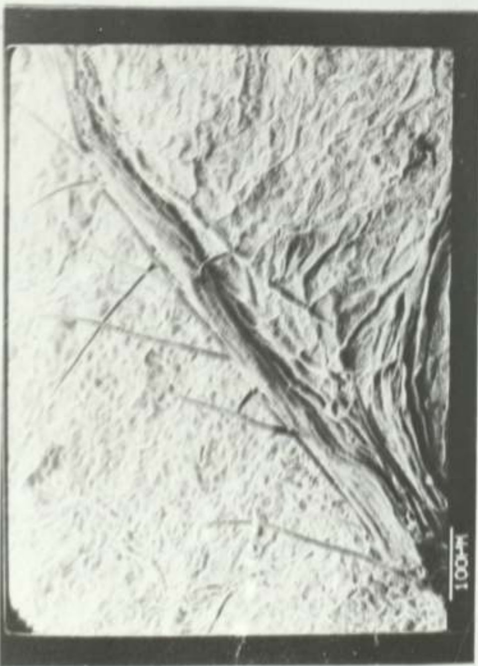

d

a

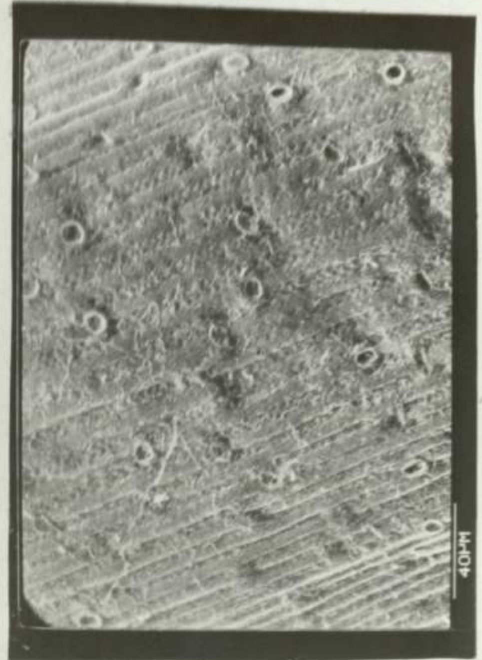
c



a

b

c



d

Fig. 8

a) Close view of stoma on *Festuca* abaxial surface.

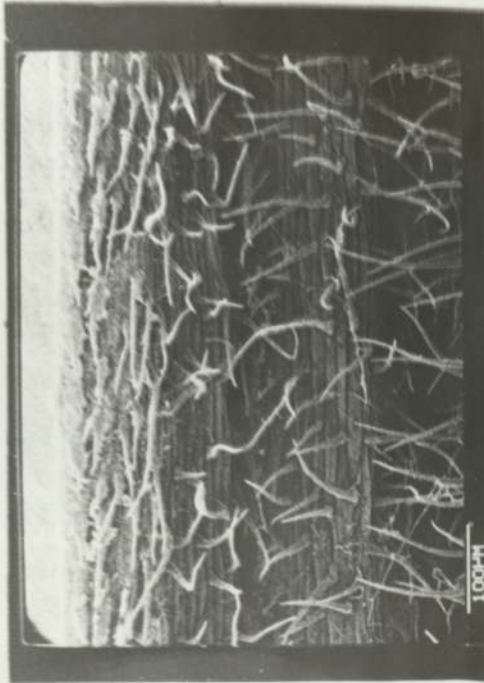
b) Unrolled inner surface of *Festuca* leaf.

c) Hyphae growing between trichomes on *Festuca*.

d) Interior of curled *Festuca* leaf (adaxial surface).

a

b



c

d

Fig. 9

a) Adaxial surface of *Poa* showing covering of trichomes on leaf veins.

b) *Poa* leaf vein and trichomes.

c,d) *Poa* abaxial epidermis showing relatively smooth surface.

a

b

c

d

2. Leaf Organic Matter, Water Content and pH

Results are presented in Fig. 10. No significant changes occurred throughout the season, and therefore the means of all samples tested are given for each result.

Fig. 10. Organic Matter Content, Water Content and pH of Leaf Age Classes (± S.D.).

Leaf age class*		Water Content (% fresh wt.)	Organic Matter (% dry wt.)	pH
ACAENA	1	81.0 ± 2.3	89.0 ± 4.6	6.4
	3	18.9 ± 2.4	96.7 ± 4.3	6.2
	4	75.7 ± 2.9	80.5 ± 4.1	5.7
FESTUCA	1	68.5 ± 3.1	96.0 ± 1.7	6.0
	3	20.4 ± 2.5	95.1 ± 1.1	4.7
	4	78.7 ± 8.8	82.7 ± 4.2	4.2
POA	1	59.9 ± 2.1	96.7 ± 0.5	6.7
	3	20.9 ± 1.7	97.2 ± 0.2	6.5
	4	82.8 ± 3.4	87.7 ± 6.6	5.3

1 denotes New Leaves

3 denotes Standing Dead Leaves

4 denotes Litter

* (Chapter 3)

The pH, water contents and organic matter contents did not vary significantly over the summer season, but variations between age classes and species were significant.

The most significant change was in water content, which dropped to very low levels in all the standing dead leaves ($p > 0.01$). *Acaena* and *Festuca* litter had a water content equal to that of green leaves, but the water is perhaps differentially available, being metabolically incorporated in the living leaves. The *Poa* litter was of a significantly higher water content than green leaves. No significant differences were found between the organic matter contents of age classes of each species. Generally the litter samples possessed a lower pH than the leaves, probably as a result of organic acid production.

3. Gas Liquid Chromatography of Leaf Extracts

Results of the carbohydrate analyses of various leaf age classes of *Acaena magellanica* and *Poa flabellata* are given in Fig.11.

Acaena new leaves contained relatively large amounts of sucrose, and showed total sugar contents of around 13% of dry weight. As leaves aged the amounts of carbohydrates decreased, and litter of *Acaena* had a sugar content one order of magnitude lower than that of the new leaves. Trehalose, a fungal carbohydrate, was found in relatively large amounts in the filtrates from standing dead (class 3) leaves of *Acaena*, indicating a significant amount of fungal presence.

Poa leaves of all ages contained lower amounts of total sugars per unit dry weight than did *Acaena*. Sucrose, fructose, and β-glucose were the more abundant sugars in new leaves. Carbohydrate levels in class 3 leaves were lower than those of *Acaena* litter, and a large proportion of this was trehalose. Carbohydrate levels in *Poa* litter were relatively low, although trehalose levels may raise this slightly. The difference between carbohydrate contents of the two litters may help to explain some of the differences in fungal species composition which were found during sampling (Chapter 4).

Fig.11 Carbohydrate Analysis (µg mg^{-1} dry wt. ± S.D.)

(a) *ACAENA MAGELLANICA*

Carbohydrate	New Leaves	Standing Dead Leaves	Litter
Arabitol	0.00	0.19 ± 0.08	0.00
Fructose	13.90 ± 6.38	0.34 ± 0.19	3.32 ± 1.74
α - Glucose	5.64 ± 2.65	0.67 ± 0.71	4.78 ± 2.48
β - Glucose	6.52 ± 3.20	1.12 ± 0.55	3.07 ± 1.44
Mannitol	0.00	2.51 ± 3.04	0.40 ± 0.19
Sucrose	104.24 ± 56.20	28.68 ± 31.78	3.16 ± 3.71
Trehalose	0.00	5.59 ± 2.99	0.21 ± 0.06
Total:	130.3 ± 14.00	39.92 ± 38.97	14.86 ± 5.96

(b) *POA FLABELLATA*

Carbohydrate	New Leaves	Standing Dead Leaves	Litter
Arabitol	0.00	0.78 ± 0.40	0.00
Fructose	11.60 ± 5.64	1.00 ± 1.41	0.26 ± 0.27
α - Glucose	6.09 ± 3.14	0.71 ± 1.00	0.13 ± 0.13
β - Glucose	10.38 ± 5.07	0.89 ± 1.25	0.42 ± 0.30
Mannitol	0.00	0.18 ± 0.25	0.00
Sucrose	14.42 ± 7.49	0.89 ± 1.25	0.33 ± 0.26
Trehalose	0.86 ± 0.73	2.31 ± 2.06	NT
Total:	43.58 ± 20.98	6.74 ± 7.63	1.45 ± 0.91

NT denotes not tested.

4. Effect of Freeze-Thaw Cycles on Leakage

Results are shown in Figs. 12-13.The concentration of reducing sugar in the medium appeared to be unaltered by increasing numbers of freeze-thaw cycles for both *Acaena* and *Poa* leaves. It was noted that leaves began leakage to the distilled water within minutes of their immersion, and that freeze-thaw cycles of the type used would not affect this leakage significantly. Large numbers of freeze-thaw transitions within a short time period may have a significant effect on leakage of young, active leaves, but at present no effect can be detected.

5. Effect of Wetting on Leakage from Leaves

As a result of observations made during the course of the previous experiment, it was decided that wetting efflux from the leaves should be investigated. Results of the study of leakage from leaves of *Acaena* and *Poa* are shown in Figs. 14 to 19 . Both plants displayed rapid loss of reducing sugars over the first eight to ten hours, but this rate of loss slowed as the amounts of sugars remaining in the leaves declined to around 10% of the original amount. The loss of phosphate and potassium from dead leaves of *Acaena* followed a curve similar to that of the reducing sugars.

Loss of solutes from *Poa* litter was relatively low, reflecting the low amounts of reducing sugars and, presumably, of available ions in the material.

Fig. 12 Effect of Freeze - Thaw Cycles on *Acaena* Leaves

(Concentrations of Leachates Expressed as $mg l^{-1} mg^{-1}$ dry weight $\pm$ S.D.)

a) Freeze - Thaw

LEACHATE	TIME (days)				
	0	2	7	9	12
Reducing Sugars	3.58 $\pm$ 0.24	5.01 $\pm$ 0.48	4.22 $\pm$ 1.03	4.53 $\pm$ 0.18	5.20 $\pm$ 0.71
K^+	1.47 $\pm$ 0.07	1.60 $\pm$ 0.34	1.24 $\pm$ 0.24	1.21 $\pm$ 0.09	1.71 $\pm$ 0.38
PO_4^{3-}	0.24 $\pm$ 0.12	0.34 $\pm$ 0.02	0.31 $\pm$ 0.04	0.38 $\pm$ 0.01	0.36 $\pm$ 0.03

Fig. **12** Effect of Freeze - Thaw Cycles on *Acaena* Leaves

b) Constant 1°C

LEACHATE	TIME (days)				
	0	2	7	9	12
Reducing Sugars	2.33 $\pm$ 0.56	4.82 $\pm$ 0.04	4.42 $\pm$ 0.09	4.48 $\pm$ 0.13	5.02 $\pm$ 0.69
K^+	0.99 $\pm$ 0.09	1.65 $\pm$ 0.13	1.32 $\pm$ 0.20	1.14 $\pm$ 0.80	1.24 $\pm$ 0.70
PO_4^{3-}	0.16 $\pm$ 0.05	0.33 $\pm$ 0.03	0.31 $\pm$ 0.03	0.34 $\pm$ 0.02	0.36 $\pm$ 0.06

Fig. 13 Effect of Freeze-Thaw Cycles on *Poa* Leaves

(Concentrations of Leachates Expressed as $mgl^{-1}mg^{-1}$dry weight $\pm$ S.D.)

a) Freeze - Thaw

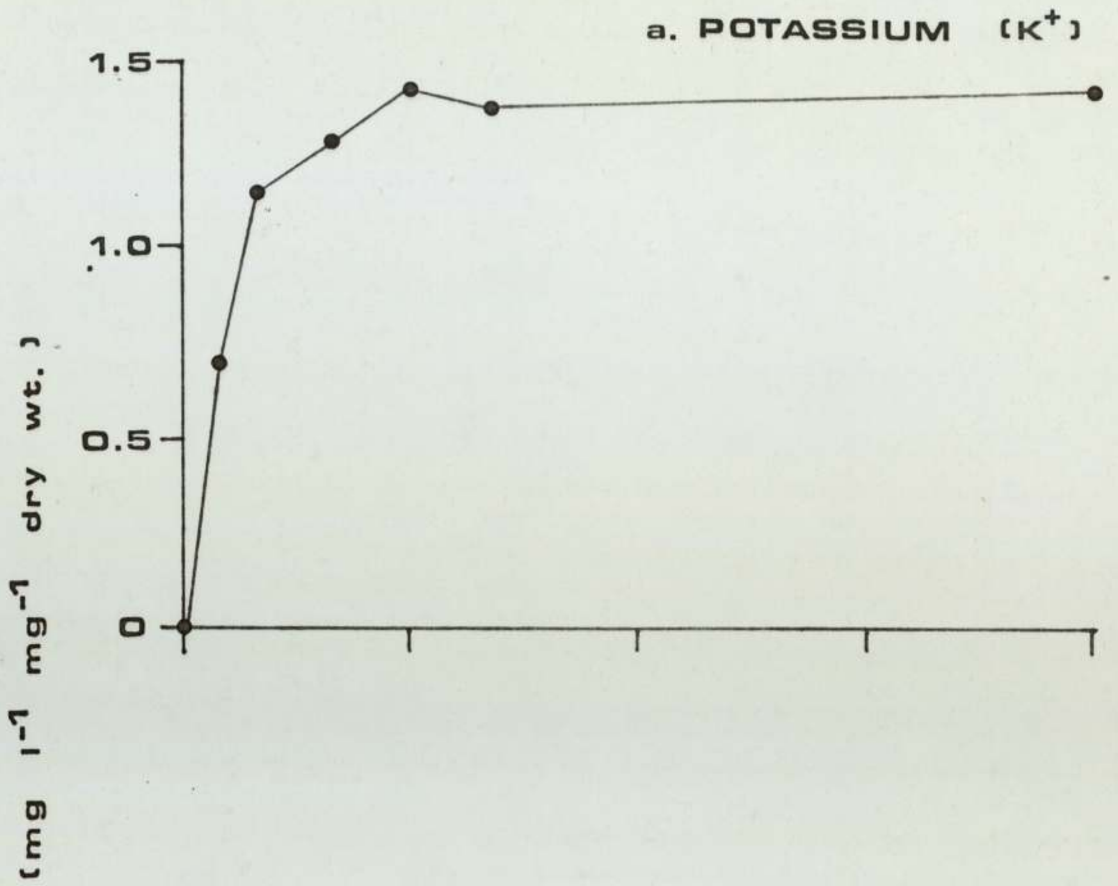
LEACHATE	TIME (days)					
	1	2	3	4	5	7
Reducing Sugars	0.59 $\pm$ 0.44	0.97 $\pm$ 0.54	1.14 $\pm$ 0.21	0.81 $\pm$ 0.52	1.98 $\pm$ 0.78	1.31 $\pm$ 0.72
K^+	0.33 $\pm$ 0.12	0.51 $\pm$ 0.09	0.71 $\pm$ 0.19	0.42 $\pm$ 0.21	0.89 $\pm$ 0.29	0.00
PO_4^{3-}	0.17 $\pm$ 0.06	0.20 $\pm$ 0.09	0.25 $\pm$ 0.15	0.09 $\pm$ 0.01	0.28 $\pm$ 0.03	0.00

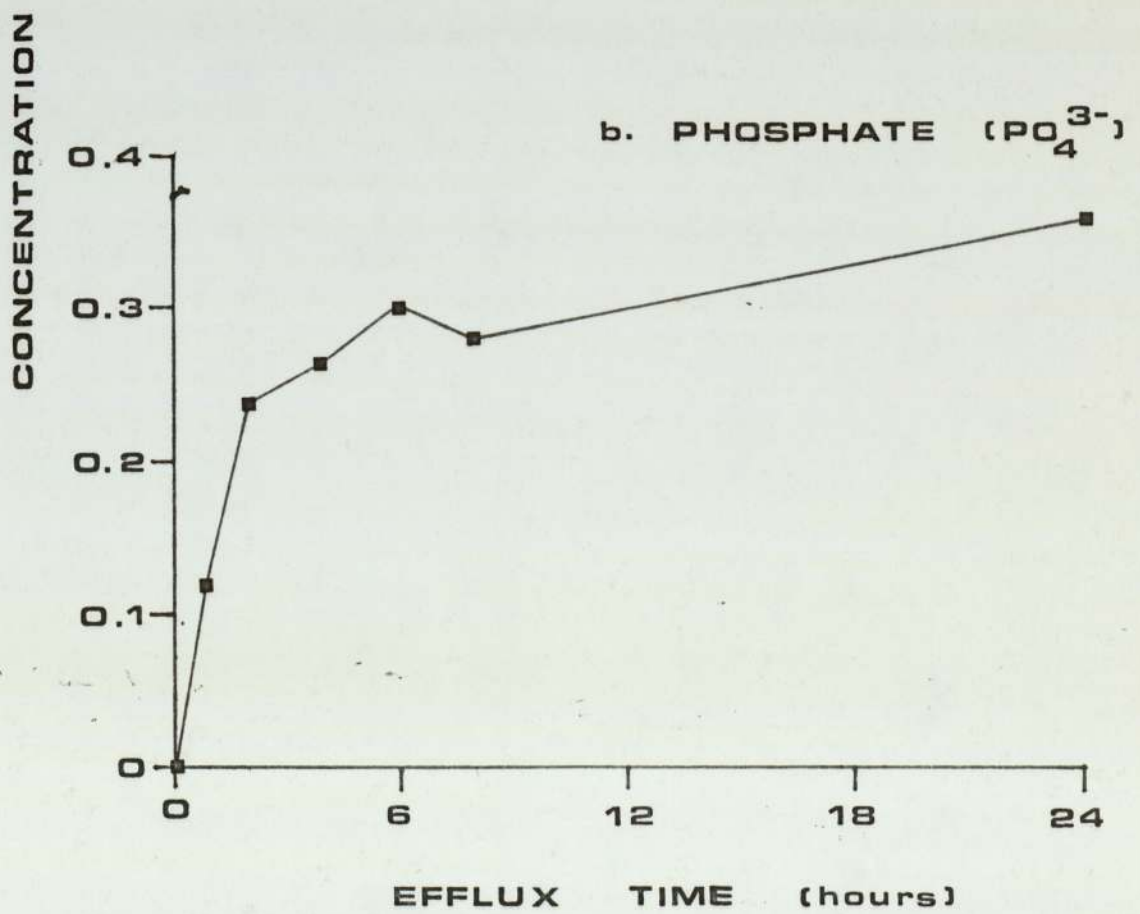
Fig. 13 Effect of Freeze - Thaw Cycles on *Poa* Leaves

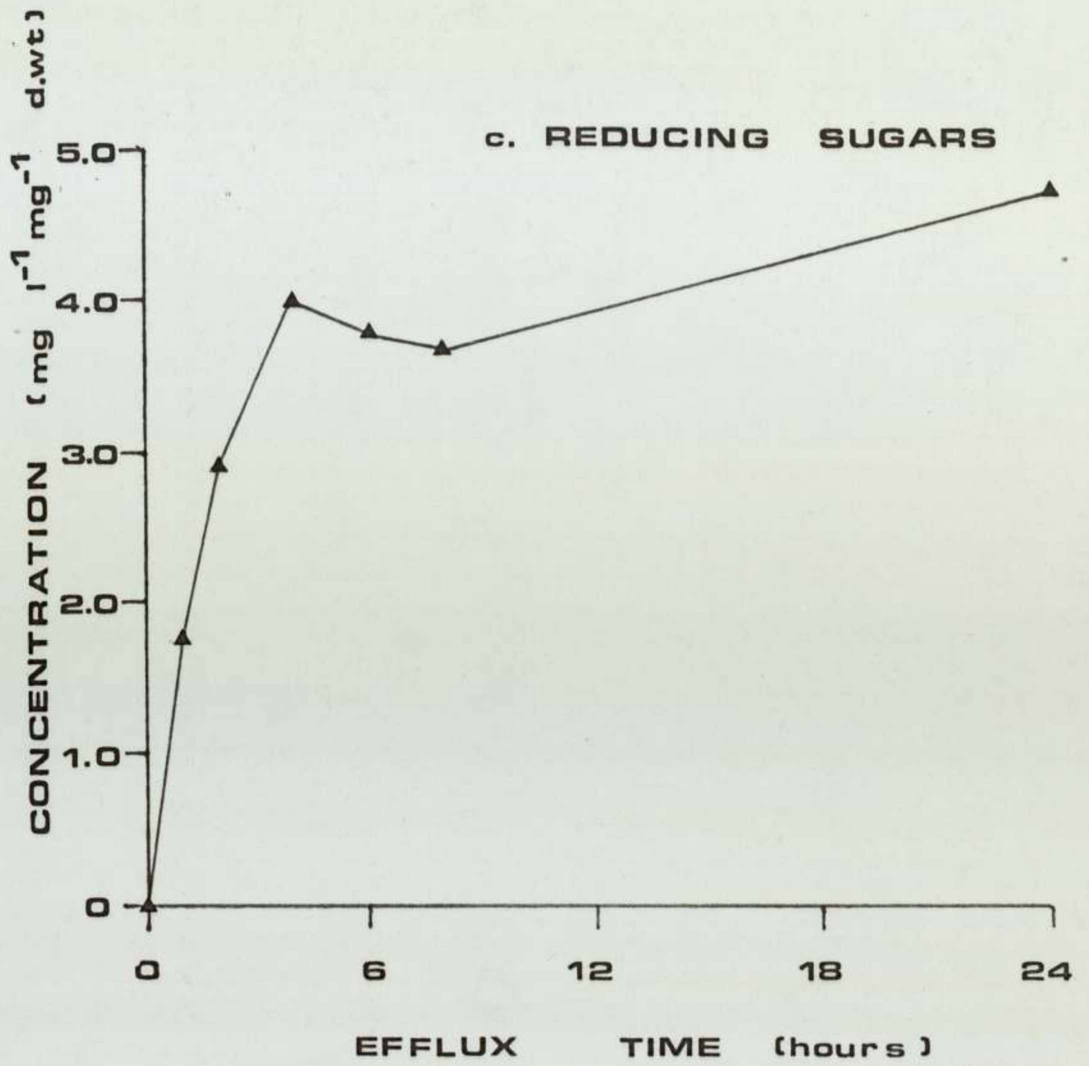
b) Constant 5°C

LEACHATE	TIME (days)					
	1	2	3	4	5	7
Reducing Sugars	1.02 ± 0.42	1.14 ± 0.06	1.67 ± 0.32	1.97 ± 0.36	1.54 ± 0.52	1.77 ± 0.28
K^+	0.47 ± 0.18	0.35 ± 0.01	0.78 ± 0.14	0.77 ± 0.27	0.52 ± 0.14	0.00
PO_4^{3-}	0.24 ± 0.07	0.39 ± 0.02	0.35 ± 0.03	0.30 ± 0.11	0.29 ± 0.10	0.00

Fig. 14. (a - c) Time course of wetting efflux from *Acaena magellanica* litter


a. POTASSIUM (K+)
1.5
1.0
0.5
0
CONCENTRATION (mg l-1 mg-1 dry wt.)


b. PHOSPHATE (PO4 3-)
0.4
0.3
0.2
0.1
0
0
6
12
18
24
EFFLUX TIME (hours)



c. REDUCING SUGARS
CONCENTRATION (mg l^{-1} mg^{-1} d.wt)
5.0
4.0
3.0
2.0
1.0
0
0
6
12
18
24
EFFLUX TIME (hours)

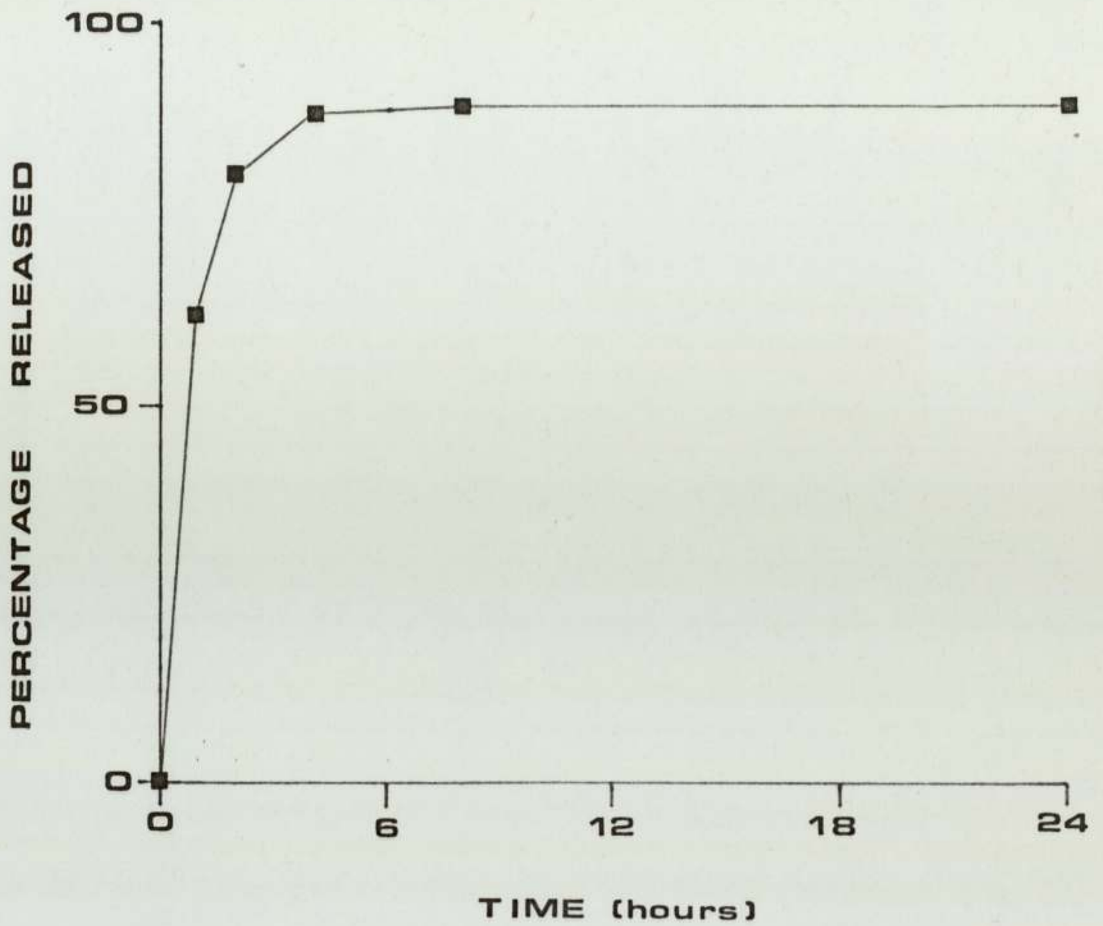


Fig. 15 Effect of wetting upon efflux of reducing sugars from *Acaena*. Expressed as percentage of total available reducing sugars.

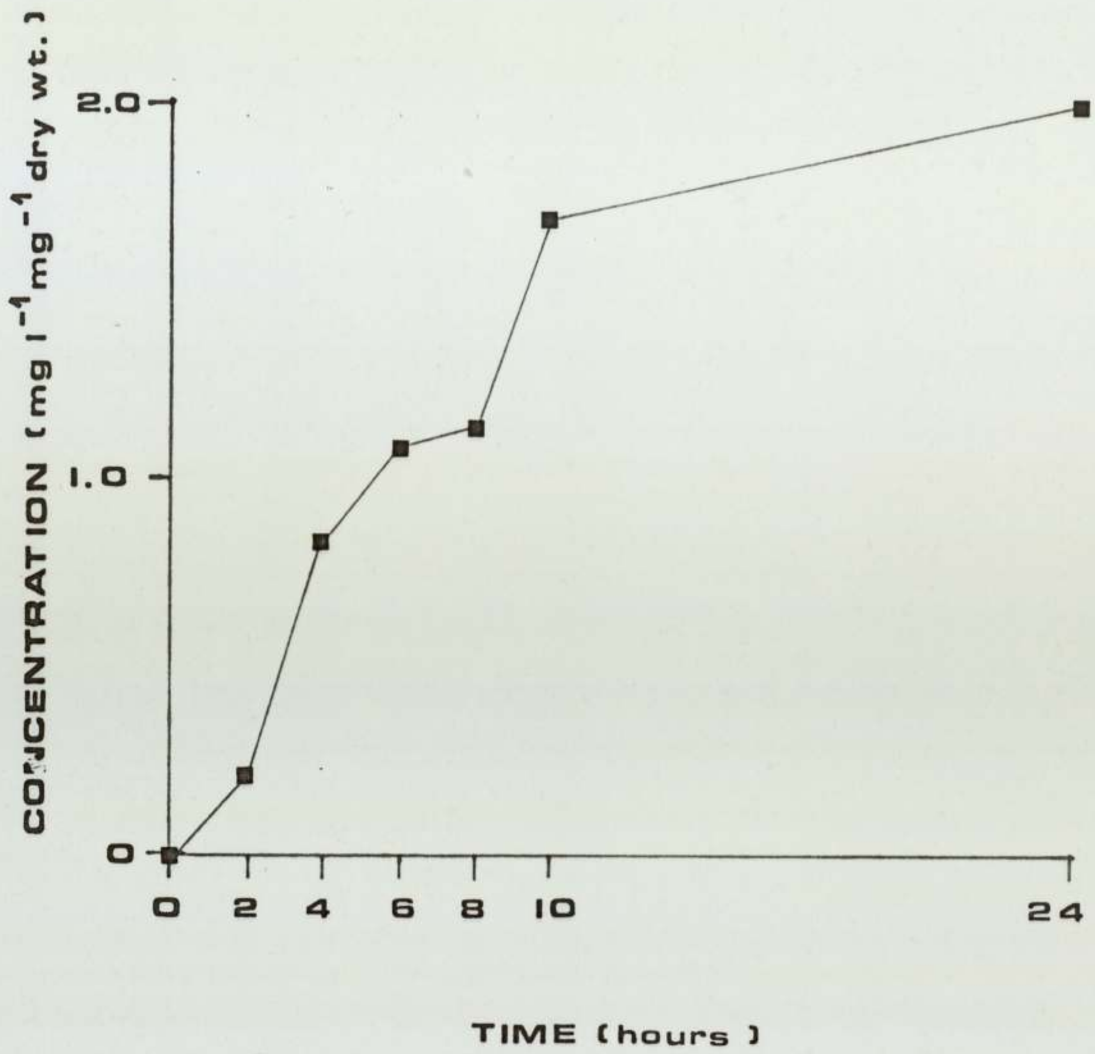


Fig.16a. LEAKAGE OF REDUCING SUGARS FROM *POA* LEAVES

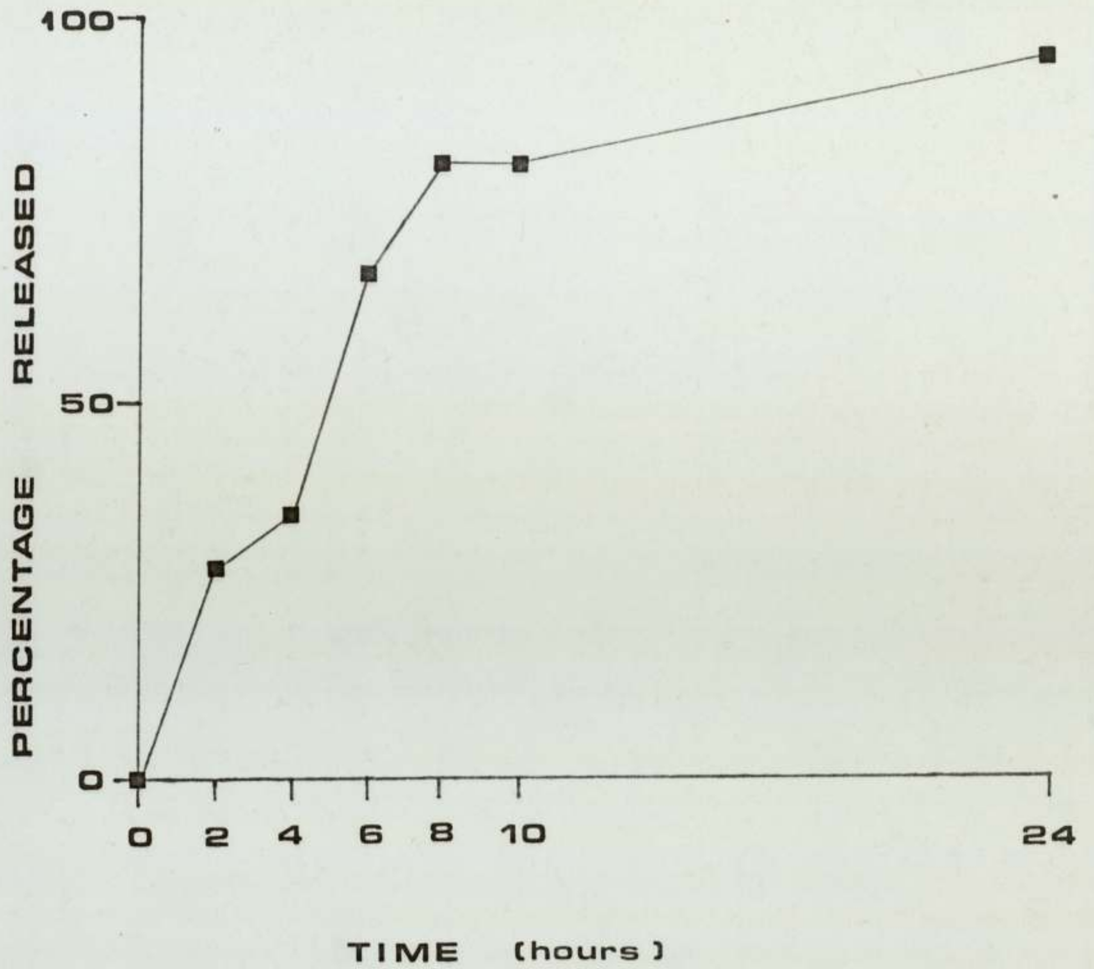


Fig.16b Effect of wetting upon efflux of reducing sugars from *Poa*. Expressed as percentage of total available reducing sugars.

Fig. 17 . Effect of Wetting on Leakage of Class 3 *Acaena* Leaves

a) Efflux of reducing sugars (± S.D.)

Time (h)	Concentration ($mgl^{-1}mg^{-1}$)	Percentage of total available
0	0	0
1	1.78 ± 0.25	61.90 ± 4.35
2	2.91 ± 0.37	80.97 ± 1.20
4	3.97 ± 0.80	88.43 ± 2.14
6	3.78 ± 0.27	89.06 ± 3.35
8	3.68 ± 0.50	89.30 ± 2.84
24	4.70 ± 0.52	89.50 ± 2.78

b) Efflux of Potassium and Phosphate (± S.D.)

Time (h)	Concentration ($mgl^{-1}mg^{-1}$ dry wt)	
	K^+	PO_4^{3-}
0	0	0
1	0.69 ± 0.18	0.11 ± 0.03
2	1.11 ± 0.13	0.23 ± 0.03
4	1.27 ± 0.35	0.26 ± 0.02
6	1.40 ± 0.11	0.30 ± 0.03
8	1.36 ± 0.07	0.27 ± 0.03
24	1.41 ± 0.10	0.35 ± 0.04

Fig. 18 Effect of Wetting on Leakage of Class 1 *Poa* Leaves

Efflux of reducing sugars ($\pm$ S.D.)

Time (h)	Concentration ($mgl^{-1}mg^{-1}$)	Percentage of total available
0	0	0
2	0.21 $\pm$ 0.05	27.84 $\pm$ 6.08
4	0.83 $\pm$ 0.09	34.79 $\pm$ 7.18
6	1.09 $\pm$ 0.31	66.20 $\pm$ 8.49
8	1.34 $\pm$ 0.18	80.64 $\pm$ 7.31
10	1.67 $\pm$ 0.06	80.72 $\pm$ 6.82
24	1.96 $\pm$ 0.12	94.81 $\pm$14.23

Fig. 19 Effect of Wetting on Leakage from *Poa* Class 4 material (litter layer) (± S.D.)

Time (h)	Concentration ($mgl^{-1}mg^{-1}$ dry wt)	
	PO_4^{3-}	K^+
0	0	0
0.25	0.13 ± 0.02	0.10 ± 0.00
0.50	0.14 ± 0.02	0.12 ± 0.01
1	0.13 ± 0.04	0.10 ± 0.01
2	0.14 ± 0.01	0.11 ± 0.00
4	0.17 ± 0.05	0.13 ± 0.03
6	0.16 ± 0.02	0.11 ± 0.01
8	0.11 ± 0.01	0.09 ± 0.01
24	0.14 ± 0.02	0.11 ± 0.02

(Reducing sugar concentration < 0.17 mg 1^{-1} mg^{-1} dry weight)

DISCUSSION

At times during the summer season, water activity of the air within *Poa* leaf canopies can reach relatively low values (Walton, pers. comm.). Plants growing in such an environment have need of physiological or morphological adaptation to ensure their continued survival through periods of water stress. Deep tap roots and rhizomes of all three species confer such an advantage, in addition to the fact that such root systems act as carbohydrate reserves to ensure fast growth during the summer season. The leaves of both *Festuca* and *Poa* appear to be adapted to ensure water conservation, and it is perhaps for this reason that these grass leaves can continue survival from early spring to late autumn. There is evidence that some leaves remain in a dormant, living state beneath snow cover in preparation for growth in spring (Walton, pers. comm.).

The strategy adopted by *Acaena* for survival during winter is the abscission of leaves, although leaves may not fall to the litter until the following year. The abscission may be a response to water stress later in the growth season as much as a response to colder weather.

The dense cover of trichomes on the adaxial grass leaf surfaces could be an adaptation to water stress, but the thicker boundary layers which result from this adaptation will aid fungal survival on the phylloplane.

Propagules are presumably trapped more easily by a trichome-covered surface, and are less likely to be removed by wind and rain action.

The effectively sunken stomata of *Acaena* again indicate a resistance to water stress. The wax platelets of *Acaena* leaf surfaces extend to the edges of the stomata and help to increase the thickness of the boundary layer.

In an environment where air temperatures are often below freezing point, the formation of ice crystals in the atmosphere can decrease relative humidity considerably. Transpiration rates would therefore increase, and plant viability would be affected by wilting unless adaptation by the plants reduced rates of water loss. The leaf surfaces of the three host plant species studied appear to be adapted to ensure survival through dry periods.

The pH, water content and organic matter content of the leaves and litter did not vary significantly during the season, but variations between age classes were present. The most significant change was that of water content, which was relatively low in the class 3 leaves (standing dead) ($p > 0.001$). Living leaves and litter had similar water contents, but water is probably differentially available in these two age classes, water in living leaves being metabolically active and water in litter being freely available (Pugh, pers. comm.).

In general, litters had a lower pH than the leaves, presumably as a result of organic acid production (Hurst and Burges, 1967). Such low pH values as those found in *Festuca* litter (4.2) could have a significant effect upon bacterial and fungal activity, and could help to account for mycoflora changes in the transition from class 3 leaves (standing dead) to litter,

High cellular carbohydrate levels can lead to high levels of carbohydrates on leaf surfaces (Tukey, 1971). High levels of nutrients can help increase competition, and hence change the species composition in a given habitat as good competitive saprophytes are introduced (Pugh, 1980).

The relatively high levels of total carbohydrates in *Acaena* leaves may make these more susceptible to fungal growth. The litter also contained relatively high sugar contents compared with litter of *Poa*. This high sugar level may be one of the factors governing the decomposition of *Acaena* leaves beneath snow cover during the winter, as the majority of litter has been degraded by the spring of the following year. *Poa* litter has very little carbohydrates, presumably what little amounts there are, are quickly utilised by micro-organisms as soon as they are produced. This could explain the predominance of *Chrysosporium pannorum* in *Poa* litter. This species has a strong cellulolytic capacity and yet appears to be unable

to compete under conditions of high nutrient availability with other, more quickly growing species which can take advantage of such an environment.

Contrary to expectations, freeze-thaw cycles were shown to exert no discernable effect upon plant leakage. Leaves leaked significant amounts of metabolites after only a few minutes of immersion in water, and after twenty-four hours had lost up to 90% of the total extractable carbohydrates. Unfortunately, no fresh leaf material was available for this study, and the leaves used in each case were samples which had been collected in early spring on South Georgia, and returned to England at $5^{O}C$.

These leaves were comparable with leaves on the borderline between class 2 (mature) and class 3 (senescent/dead) as described in the following chapter, both in carbohydrate content and degree of damage. Further studies are needed using fresh field material in order to determine rates of leakage from new leaves.

This study indicates that a large proportion of the carbohydrate present in mature leaves and senescing leaves of *Acaena* and *Poa* is available for fungal growth under conditions amenable to leaf leakage.

Cheng *et al.* (1971) have shown that freeze-thaw cycles may increase losses of metabolites from leaves, and further studies of this phenomenon are necessary, again using fresh material, as new leaves may be more likely to produce

more conclusive results because of the damage to viable cells.

CHAPTER 3

SAMPLING METHODS AND FUNGAL ISOLATES

INTRODUCTION

All higher plants produce new leaves during their active growth periods. The leaves continue to function in their designated roles as photosynthetic, storage or protective organs until they become senescent, are abscised by the plant, or are damaged by external factors.

From the moment they are exposed to the atmosphere, new leaves provide a substrate for colonisation by saprophytic and parasitic fungi and bacteria. Initial colonisers will be fast-growing species capable of utilising the carbohydrate and amino acid exudates present on the phylloplane, but as the leaf matures, many factors may combine to alter the original mycoflora population. Environmental factors such as temperature, ultraviolet radiation and Water Activity (A_w) play a part in determining the relative growth rates of the various species, and hence indirectly determining their relative competitive abilities. The fungi themselves may often cause changes on the phylloplane which can affect the subsequent mycoflora. Exudations by leaching and guttation on the leaf surface allow early colonisation by "sugar fungi", usually Mucorales, which are able to grow quickly to exploit this substrate. There will be further nutrient release following the freeze-thaw cycles - see Chapter 2. Eventually this primary carbohydrate

source is depleted, and the initial colonisers decline in numbers, their mycelia providing a further substrate for possible colonisation. Plant nutrition and age affect the quality and quantity of exudates which accumulate. The amount of new leaf tissue available for colonisation also varies with season and nutrient supply. The influence of existing plant damage upon fungal growth is under debate, but pathogen metabolites, pathogen damage and physiological change in the plant may all contribute to an increase in saprophytic growth (Hudson, 1968; Pugh *et al.* 1972).

Studies of the distribution of fungi on leaf surfaces have shown that some patterns can be recognised. A distinct difference exists between upper and lower surface populations on horizontal leaves, whilst vertical leaves have much the same population on each surface (Dickinson, 1976). The primary colonists on monocot leaves have been shown to accumulate at the leaf tip (Pugh, 1958). This is due primarily to the effect of the basal intercalary meristem growth pattern, i.e. the leaf tip is the oldest portion of the lamina. From this it can be suggested that a succession of fungal species should be observable along a monocot leaf blade due almost entirely to leaf age, exposure to the airspora, and climatic effects such as drying.

Of the 100, - 250,000 fungal species known, a minority in normal circumstances appear to require the presence of a living host. These include various mycorrhizal fungi and the obligate biotrophs which parasitize but do not kill their hosts. The remainder of the fungi are facultative saprophytes or obligate saprophytes (Pugh, 1974).

The nature of the leaf surface has long been known to have a profound effect upon the deposition and growth of fungal propagules involved in pathogenesis. Dickinson and Preece (1976) and Blakeman (1981) have reviewed many of the various factors influencing the phylloplane mycoflora.

Most fungal propagules on leaf surfaces derive from the air spora, having been produced elsewhere. Rain splash may also transmit soil micro-organisms to the leaves of plants. It is likely that the relationship between phylloplane morphology and propagule deposition is more complex than is at present realised, as is the effect of small-scale irregularities in aerial plant surfaces (Dickinson, 1976).

As a wind flow encounters an obstruction such as a leaf, it moves to either side of the object due to pressure differentials. A suspended particle will therefore be carried past the obstacle unless it has sufficient momentum to maintain its course in spite of the deviation of the air stream (Merral, 1981). At low windspeeds the trapping efficiency

of leaves is high, but this decreases as windspeed increases. Leaf spore retention is also greater if the leaf surface is wet. Large spores are preferentially selected by both sedimentation and impaction processes. Studies indicate that once captured, spores are likely to be retained in the still boundary layer of a relatively immobile leaf, although rain may remove spores by washing. Electrostatic charges on both leaves and spores, or the morphology of the lamina, may influence spore deposition (Gregory, 1971).

Conidia make a large contribution to the air spora, mostly originating from living and dead vegetation. The air spora comprises spores in the process of dispersal following release from mycelia, and prior to deposition on new substrates.

The initial phase in ecology is one of description (Griffin, 1972). Gochenaur and Wittingham (1967) showed that no fewer than 500 isolates per site were required to provide adequate sampling of the prevalent fungi capable of growing under the experimental conditions used. Direct observation plays an important part in sampling methods. Leaf washing can be used to identify "casual" leaf fungi, and as a preparatory treatment to remove non-living or surface matter. Surface sterilisation can be used to distinguish between surface and internal fungi. Damp chamber incubation has been used to encourage fungal sporulation for those species already growing on a leaf

surface (Lindsey, 1976). Frankland *et al.* (1981) showed that the fluorescent antibody technique can be used to identify positively a given microorganism in its chosen habitat.

It has long been known that fungal successions occur on plant substrates. Burges (1939) defined pioneer saprophytic colonisers as a group capable of utilising sugars. Garrett (1963) proposed a generalised pattern for successions on a leaf lying on the soil surface. Weak parasites were present on senescent tissue, followed by saprophytic sugar fungi, cellulose decomposers and finally, lignin decomposers. Webster (1956; 1957) described the colonisation of cocksfoot over a two year period following flowering. He concluded that Mucorales play little part in the above-ground phase of decomposition, an observation which agrees with the results gained in this study.

Hudson and Webster (1958) followed the succession on *Agropyron repens* and found a similar pattern to that of Webster. Their study suggested that physiological changes in the leaves, associated with the flowering process, do not solely determine the mycoflora distribution.

Hudson (1968) stated that only fungi which are capable of resisting the fluctuating water contents of senescent leaves and upper internodes (of monocotyledons) would be capable of growth on these substrates.

Eventually on the phylloplane, an overlapping of mycelia may restrict growth and encourage competition. Production of fungal metabolites and yet further leaf leachates, and the eventual damage of leaf cells by pathogens, brings about further possibilities for fungal colonisation by cellulose oxidising species.

The situation existing at any time on the leaf surface is a complicated one, and yet it is constantly changing throughout the life of the leaf, and even after its death as the leaf enters the litter layer. In the litter, cellulose oxidising fungi complete their utilisation of this substrate, and eventually ligninolytic species become the dominant mycoflora component. Lignin and humic acids are the final substrates in the majority of successions, mainly because of the stable chemical structure and resistance to microbiological attack (Sarkanen and Ludwig, 1971). It is still unclear at present to what extent fungi actively decompose the lignin materials in plant litter.

Relatively few studies have been made of microfungi in the sub-Antarctic regions (Tubaki, 1961; Corte and Daglio, 1963; 1964; Heal *et al.* 1967; Latter and Heal, 1971; Kerry, 1979; Wynn-Williams, In press).

Both the species composition and activity of the

mycoflora need to be investigated further, particularly during the harsh winter months and under snow cover. To this end, leaf samplings and washings were made over a five month period during the 1979 - 1980 Antarctic summer season, and airspora counts were made at intervals during the season. No large scale attempt was made to quantify the soil mycoflora, as this is presently being undertaken elsewhere, and was not the object of study.

Materials and Methods

1. Leaf sampling

At the beginning of the study it was decided that the following four categories of leaf age would be most applicable to the three plant species tested, providing an arbitrary but practical range whilst removing observer bias.

1) Newly expanded leaves with no discolouration or marking of surface by micro-organisms.
2) Standing mature leaves retaining green colouration.
3) Yellowed, or dried and decaying standing leaves.
4) Litter.

Leaves were collected from pure stands of the host plants at three separate sampling sites (Fig. I). *Acaena magellanica* was collected from a research site 5 - 10 metres above sea level, on a slight north-east facing slope (site 2). *Festuca contracta* was collected 4 kilometres from the research station, near a fresh-water lake 70 metres above sea level on a relatively flat and exposed moor (site 3). *Poa flabellata* was collected from an area 10 metres above sea level and 100 metres from the research station on a south-facing slope of shale and mineral soil (site 4).

Leaves were cut at their base with a previously sterilised knife and placed in sterile polythene bags for transport to the laboratory. The leaves

were then arranged on a cleaned, and alcohol-swabbed bench surface in a draught-free room and sorted into age categories. Discs were cut from the leaves using a sterilised 5 millimetre diameter cork borer and plastic cutting board, and 25 each were placed into Universal bottles. The pieces were then serially washed using autoclaved and cooled tapwater according to the technique of Harley and Waid (1955), with successive decantations and additions of fresh water. Following a number of pilot studies and plots of fungal colonies growing from the washes (Fig.20), it was decided to adopt six washings as an effective number. Further tests in midsummer and autumn gave no reason to change this number. Washed leaf pieces were plated onto Cellulose Agar (CA) (Eggins and Pugh, 1962), and Potato Dextrose Agar (PDA), five leaf pieces per petri dish, with three or five replicate dishes per leaf age class. Incubation was at 25°C and 5°C for two weeks followed by examination, re-incubation and a second examination for slowly-growing species after four weeks.

2. Airspora

a) This was sampled at roughly two week intervals at midday by placing five plates of PDA and five of CA on poles at a height of 1.5 metres above ground

Fig. 20 RESULTS OF THREE WASH SERIES - COLONIES REMOVED PER WASH

WASH NUMBER	SERIES				
	1	2	3	Mean	S.E.
1	29	7	0	12.00	2.00
2	10	7	1	6.00	1.414
3	0	6	0	2.00	0.82
4	0	3	0	1.00	0.58
5	1	4	0	1.67	0.75
6	0	0	0	0.00	0.00
7	1	2	1	1.33	0.67
8	0	1	0	0.33	0.33
9	0	2	0	0.66	0.47
10	0	3	0	1.00	0.58

for periods of ten minutes exposure (i.e. dish lids removed). Plates were incubated at 25°C for two weeks, examined and left for a further two weeks at 5°C to allow growth of more slowly-growing species.

b) At the same site as that used for part (a) above, (i.e. a stand of *Poa flabellata* tussocks), a measure of the airspora both inside and below the canopy was attempted. Five poles were placed at a height of 0.3 metres, inside the leaf canopy such that petri dishes could be placed amongst the leaves. A further five poles were embedded in the litter layer at the base of the tussocks, flush with the litter layer. At noon, petri dishes containing PDA and CA were placed on the poles, and these were exposed to the atmosphere by careful removal of the lids for five or ten minute periods. Incubation was as in (a) above.

c) Once in late spring and once in autumn, a diurnal airspora count was taken. This aimed to quantify changes, if any, which occurred over a 24 hour period. Once every hour a set of five, 8 cm. petri dishes of PDA was exposed to the air for five minutes. This was continued for twenty four hours whilst note was taken of windspeed and precipitation.

3. Moss core microfungi

In an attempt to estimate qualitatively the mycoflora, cores from a moss site (*Polytrichum alpestre*) on the island were taken at the end of the summer season. Using sterilised instruments in a draught-free room, the cores were cut open, and 10 g samples were then excised from the surface and from five depths through the core. A 10^{-3} dilution was made of each sample with thorough shaking. $0.1cm^3$ of diluted spore suspension was spread onto PDA plates containing aureomycin at $60mgl^{-1}$. Four replicate plates were made at each depth. Incubation was at 5^oC for two and four weeks.

RESULTS

1. Results of the isolation work using leaf discs are given in Appendix 3 . Because of the sampling and recording techniques used and the number of variables involved, these data are not amenable to statistical analysis of any depth. Rather, it is simply a check-list of those species isolated from leaf age classes, giving a generalised view of the more common species of fungi at any stage in the age of the leaves. By far the most frequently isolated fungi were sterile mycelia, comprising 41% of the total number of phylloplane fungi isolated. *Chrysosporium pannorum* was the most commonly isolated identifiable fungus, comprising 14% of the total number of isolations, and present as either spores or mycelia on all leaf age classes of each of the three host plant species except new leaves of *Festuca contracta*. The bulk of *Chrysosporium pannorum* isolations were from the two monocotyledon litters, suggesting the importance of this species in the decomposition process. Where relatively low numbers of isolations are recorded for a specific leaf age-class, it may occasionally be possible that these result from 'carry-over' of spores, although this is a factor which the serial washing technique is designed to prevent.

Mucor hiemalis proved to be a common isolate from the litter layers of all three species, and also from standing dead leaves of *Poa flabellata*.

An unidentified species of *Chaetophoma* was the third most common recognisable genus, rare on *Acaena magellanica (Acaena)* green leaves, but isolated in abundance from *Acaena* litter and standing dead, and from monocotyledon leaves of all age classes.

Botrytis cinerea was isolated mainly from senescent leaves and litter of *Acaena*, with lower numbers being isolated from *Festuca* leaves at the senescent stage and onwards.

Various species of *Penicillium* were isolated throughout the season, although this genus was relatively rare. Where *Penicillium* species were isolated, they often occurred on one sampling date only, suggesting that the isolation numbers were raised by the large numbers of spores resulting from a single colony growing within the phylloplane.

Cladosporium sphaerospermum was isolated from almost all leaf age classes, being more common on the earlier three age classes of the two monocotyledon hosts.

Other species isolated occurred at frequencies of less than 3% of the total number of isolates. *Mortierella* species was common on new *Acaena* leaves and in the litter of *Poa flabellata* and *Leptosphaeria* sp. (probably *Leptosphaeria silvatica*) was commonly isolated from *Poa* standing dead

HOST PLANT	*ACAENA MAGELLANICA*				*FESTUCA CONTRACTA*				*POA FLABELLATA*				*TOTAL*
SPECIES LEAF CLASS	1	2	3	4	1	2	3	4	1	2	3	4	
Acremonium terricola	-	-	-	-	-	-	-	-	-	1	3	9	12
Alternaria sp.	-	-	-	8	-	-	15	-	-	-	9	2	34
Aureobasidium pullulans	-	-	-	37	-	-	4	14	-	-	-	-	55
Ascomycete	-	-	-	1	-	9	-	-	-	-	-	26	36
Botrytis cinerea	7	-	25	68	-	10	9	11	-	1	2	-	133
Chaetophoma spp.	-	1	13	18	12	50	14	47	11	55	21	9	251
Chrysosporium pannorum	13	27	12	19	-	4	36	114	5	3	10	143	386
Cladosporium herbarum	-	-	-	-	-	14	-	-	-	-	1	-	15
Cladosporium sphaerospermum	5	3	-	5	17	18	5	1	17	12	20	1	104
Doratomyces nanus	-	-	-	-	-	-	-	-	-	-	-	7	7
Fusarium lateritium	1	-	-	2	-	5	-	-	-	3	-	-	11
Leptosphaeria spp.	-	-	-	-	-	-	-	-	-	8	55	-	63
Mortierella sp.	39	16	-	-	-	-	-	-	-	-	-	17	72
Mucor hiemalis	1	3	-	68	-	-	6	128	2	-	25	43	276
Penicillium spp.	4	-	24	-	5	5	4	-	2	3	-	71	118
Peyronellea sp.	-	-	1	-	-	-	-	-	-	-	12	-	13
Phialophora sp.	-	-	11	6	-	-	13	-	-	-	-	-	30
Sterile Mycelia	34	79	112	30	151	70	130	4	109	232	171	16	1138
TOTAL:	104	129	198	262	185	185	236	319	146	318	329	344	2755

Fig. 21 . Summed isolation results. Total number of isolations per leaf age class.

(-) denotes no isolation.

leaves. Fig.21 gives all isolations grouped together under the leaf age categories. 5°C and 25°C incubation results and monthly results have been summed for any given species/age class combination to give an overall view of fungal species frequency. From this, Fig. 22 (a - c) has been drawn, representing those species which comprised up to 70% of the total isolations from any age class. It must be remembered that these diagrams (a - c) assume no changes in fungal frequency are caused by the time of isolation through the season. The figures on the species lines represent the percentage of total isolations for that specific age class.

It is not easy to discern many obvious trends in the number of species isolations which may be caused by time in the season.

From personal observation it was noted that, early in the season, a large number of leaf pieces were quickly colonised by yeasts,(notably *Candida saké*) and psychrophilic bacteria. New leaves were particularly susceptible to this colonisation. It was particularly noticeable that leaf discs incubated in damp chambers, which showed a high rate of colonisation by yeasts and bacteria, did not yield many filamentous fungi.

Fig. 22 (a - c) Generalised Succession Patterns on Leaves.
(Species comprising 70% of isolates - %age in parentheses)

A. ACAENA

age class 1 2 3 4

(37.5) *Mortierella sp.*

(20.9) *Chrysosporium pannorum*

Botrytis cinerea (12.8) (25.9)

Aureobasidium sp. (14.1)

Mortierella sp. (25.9)

Sterile (32.7) (61.2) (56.6) (11.5)

B. FESTUCA

age class 1 2 3 4

Chaetophoma sp. (27.8)

Cladosporium sphaerospermum (4.7)

Chrysosporium pannorum (15.3) (35.7)

Chaetophoma sp. (14.7)

Mucor hiemalis (40.1)

Sterile (81.6) (37.8) (55.1)

Fig. 22 (cont'd.)

C. POA

age class	1	2	3	4
Leptosphaeria sp.			(16.7)	
Mucor hiemalis			(7.6)	(12.5)
Chrysosporium pannorum				(41.6)
Penicillium spp.				(21.6)
Sterile	(74.6)	(73.0)	(52.0)	

2. Airspora

a) Airspora spot tests

The occurrence of species in the airspora spot tests below and above the canopy is given in Fig.23 . There does appear to be a change in composition of the airspora as the summer progressed. *Aspergillus* species is a definite contaminant resulting from stores from a ship, as it was not isolated from any other natural materials on South Georgia at any time throughout the season, and the genus is not regarded by other workers as a common fungus in the sub-Antarctic (Bailey and Wynn-Williams, In press).

Botrytis cinerea was isolated only in early summer, above the *Poa* canopy. *Chrysosporium pannorum* spores were only found once in late summer above the leaf canopy, whilst below the canopy it was relatively abundant. The airspora beneath the leaf canopy reflects the mycoflora of the soil, litter and lower leaves. It has a similar species composition to the airspora above the canopy but contains genera such as *Doratomyces* and *Phoma* species, as well as an Ascomycete species. Possibly the spores of these fungi are not as mobile as those of other species, or are trapped in the boundary layer of slower air during wind. This species composition does not alter greatly during the summer when the environment below

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the canopy may be more constant. Again, sterile mycelia are relatively abundant in the airspora both above and below the leaf canopy. They may arise from hyphal fragments, or from spores which do not include a conidial stage in their life cycle.

b) Diurnal airspora tests

Results of these two tests, expressed as mean number of colonies per petri dish, are given in Fig. 24 (a - b). Significant peaks are present at sunrise and sunset as spores are released from colonies, presumably due to changes in humidity levels. The peaks are composed of several species,

During both of these tests, windspeed was zero to slight breeze, reducing any complications from this factor.

Fig. 23 Species found during airspora tests

a) Above canopy

Date	1979 DEC 5	1979 DEC 9	1979 DEC 28	1980 JAN 2	1980 JAN 11	1980 JAN 16	1980 FEB 13	1980 MAR 20	1980 APR. 11
Acremonium terricola								*	
Aspergillus sp.	**								
Botrytis cinerea	**	**	**	***	**				
Chaetophoma sp.						**			
Chrysosporium pannorum								*	
Cladosporium sphaerospermum				**		***	***		*
Mortierella sp.									**
Mucor hiemalis							*		
Penicillium sp.				**				***	***
Peyronellea sp.	***								
Trinacrium sp.									*
Sterile mycelia	**					***	**	***	***

* denotes presence; ** frequent; *** abundant

Fig. 23 continued

b) Below canopy

Date	1980 JAN 2	1980 JAN 11	1980 JAN 16	1980 FEB 13	1980 MAR 20
Acremonium terricola	*				
Ascomycete (unknown)				*	
Botrytis cinerea	***				***
Chaetophoma sp.	**	***	**	**	
Chrysosporium pannorum	***		*	***	***
Cladosporium sphaerospermum	***		*	***	***
Doratomyces nanus	*	*			
Mucor hiemalis	***			***	
Penicillium sp.	**	***	**	***	
Phoma sp.	*				
Sterile mycelia	***	**		***	***

* denotes presence; ** frequent; *** abundant

Fig. 24 Occurrence of Species in Diurnal Airspora Count - 16th January, 1980

(Occurrences on five plates)

	TIME (In hours)																						
	0000	0100	0200	0300	0400	0500	0600	0700	0800	0900	1000	1100	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300
PENICILLIUM spp.			1									4	5	7	2		16	12			7	45	23
BOTRYTIS CINEREA	3				16	6	2	2		4	3	1	1	3	1		5		1			1	
PIGMENTED STERILE MYCELIUM								1										3		4	1		
CLADOSPORIUM SPHAEROSPERMUM						21	2					1	1		1		2						
HYALINE STERILE MYCELIUM	61							2	2		3	3	4						1		1	1	
CHRYSOSPORIUM PANNORUM		2						1				3	13		1		5					1	
ACREMONIUM TERRICOLA		6							1				2				1			1	1		
MONOCILLIUM sp.										1													
MUCOR sp.													3										
UNIDENTIFIED															5		1						
BISPORA sp.		1													1								
ULOCLADIUM sp.																						2	

Fig.**24** Occurrence of Species in Diurnal Airspora Count - 11th April, 1980.

(Occurrences on five plates)

	TIME (In hours)																							
	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	0000	0100	0200	0300	0400	0500	0600	0700	0800	0900
CLADOSPORIUM SPHAEROSPERMUM	1	4		1	5		1						2			1	1			1				2
HYALINE STERILE MYCELIUM	1		7	7	3	12	3	2	2	2	4	1				2		3	1	2			8	2
PENICILLIUM spp.		2		3	2	1	2			2	3	3	10	2	2	1								
TRINACRIUM sp.			1		1													1						
BOTRYTIS CINEREA					1																			
ACREMONIUM TERRICOLA					1										1									
PEYRONELLEA sp.						1		1																
CHAETOPHOMA spp.															1									
PHIALOPHORA sp.															1									
CHRYSOSPORIUM PANNORUM	4		1		17								2	1	1				1	1				
MUCOR spp.	1			1	3			2																
AUREOBASIDIUM sp.			1	1								1												
PIGMENTED STERILE MYCELIUM					2	2							2		3									

3. Moss core microfungi

Only *Penicillium* species and *Mucor hiemalis* were isolated from the moss cores in appreciable numbers, although it is improbable that these two genera are the sole components of the moss mycoflora. Two colonies of *Chrysosporium pannorum* and four sterile mycelia were also found. The suggestion is that Basidiomycetes and bacteria are performing the majority of decomposition processes within the moss layer. Basidiomycetes were not isolated using the agars involved in this test, and a study should be made with the appropriate agars. *Penicillium* species were apparently more common in this surface layers of the moss core although this is not statistically significant, whilst *Mucor hiemalis* was common in the lower layers, (see **Fig.25**).

Fig.25 . Distribution of fungal colonies isolated from a moss core (10^{-3} dilution).

Depth (cm)	Mean colony number per plate ($\pm$ S.D.) @ 10^{-3}	
	Penicillium sp.	*Mucor hiemalis*
0	3.25 $\pm$ 0.96	1.00 $\pm$ 0.82
2	3.00 $\pm$ 2.58	1.50 $\pm$ 1.00
5	3.00 $\pm$ 3.83	3.00 $\pm$ 2.16
10	6.75 $\pm$ 6.70	3.25 $\pm$ 3.20
15	1.00 $\pm$ 1.15	4.25 $\pm$ 2.87
20	0.00	7.75 $\pm$ 2.87

DISCUSSION

Those fungi which are found growing on substrates of higher water content are probably restricted to that habitat by their high moisture requirements. When stems collapse or are collapsed prematurely, it has been shown that such fungi spread along the collapsed stem to areas which were previously relatively dry.

Webster and Dix (1960) showed that their "group 1" fungi (initial colonisers) mostly had a more rapid growth and germination and a better ability to grow at low relative humidity (RH) values, than did fungi from other groups. These abilities favour establishment and growth in the fluctuating environment of the upper internodes.

Pugh (1958), studying *Carex paniculata*, found Ascomycetes common on the dry outer leaves of the tussock. *Cladosporium herbarum* and *Cephalosporium acremonium (Acremonium strictum)* were also present on dry leaves, decreasing towards the wetter interior of the tussock. *Alternaria tenuis*, *Botrytis cinerea*, *Mortierella ramanniana* and *Penicillium* spp. were regarded as late colonisers, being common in the wet tussock.

The results of this study showed new leaves of *Poa flabellata* to be dominated by sterile mycelia with a small number of *Cladosporium sphaerospermum* and *Chaetophoma* sp. The situation is similar on standing infected leaves, but with an increased number of isolations. This reflects, in part, a decreasing incidence of yeasts and bacteria as the season progressed and leaves matured, and also the increasing time

available for leaf colonisation and spore germination. As leaves died and the water content decreased, the number of isolations of *Leptosphaeria spp.* increased. This is in agreement with the results of Pugh (1958) that *Leptosphaeria sp.* and *Metasphaeria cumana* (both Pyrenomycetes) were only found on the drier parts of leaves and dried dead leaves. The standing dead leaves of *Poa* had a relatively low water content (Chapter 3). It may be that the immersed pseudothecia confer a greater resistance to water stress due to their structure and to the protection afforded by the dead plant cells during drier periods. A small number of isolations of *Peyronellea sp.* a coelomycete, seem to support this hypothesis. Such fungi, growing on drier leaf parts, are escapers as defined by Pugh (1980), i.e. poor competitors under the conditions imposed by the green leaves and standing mature (class 2) leaves, where competition from more quickly growing species is high, but able to grow satisfactorily at lower RH values when competition is decreased.

Webster and Dix (1960) also studied the effect of RH upon germination of spores. Their secondary colonisers were more susceptible, whilst primary colonisers had lower cardinal RH vlaues for germination, these being genera such as *Alternaria, Cladosporium, Leptosphaeria* and *Pleospora.*

It appears that one of the more important factors determining species successions and the succession on *Poa*

is the Relative Humidity of the leaf boundary layer, i.e. the leaf water content, and the availability of the water to the fungal flora.

Many of the more important genera recorded by Pugh (1958) on *Carex* leaves have been isolated from *Poa flabellata* in this study, suggesting a degree of similarity in the substrates involved and the techniques used. The effect, if any, of climate is to alter the species involved on either host plant, whilst the genera remain almost identical.

Relatively few sterile mycelia were isolated from the litter layer of *Poa flabellata* , which was dominated by *Chrysosporium pannorum* and *Penicillium spp.*, in conjunction with *Mucor hiemalis*. Analysis of the litter for water- and ethanol-soluble carbohydrates (see Chapter 2) showed very low levels of sugars. *Mucor hiemalis* is incapable of cellulose oxidation and was present as a member of the 'secondary sugar fungi' group (Chapter 5), utilising the simple sugars resulting from the breakdown of cellulose by cellulolytic species such as *Chrysosporium pannorum* and the *Penicillium spp.* (Tribe, 1966; Hudson, 1968; Frankland, 1969).

The influence of moisture on the breakdown of litter has been demonstrated by a number of workers. In periods of drought, decomposition of mull and mor litter was retarded and the numbers of sarcophagous animals in the litter were reduced (van der Drift, 1963).

In Arctic conditions, moisture and temperature were

important factors influencing field rates of carbon dioxide evolution (Holding *et al*. 1974). Wynn-Williams (In press), studying maritime Antarctic peat *in vitro* found changes in oxygen uptake which correlated well with temperature and moisture variations similar to those monitored in the field. Respiration rate was used as a measure of aerobic decomposer activity. Supplemental sugars indicated the dependence of microbial respiration upon the availability of dissolved organic carbon.

Weight losses of tree litters were correlated with moisture content (Witkamp, 1963). Witkamp (1966) reported that microbial respiration in litter bags was influenced in decreasing order by temperature, bacterial density, moisture and age of litter. In a laboratory study, Floate (1970) found that moisture content variations had only minor effects on the mineralisation of grass litters.

The oxygen content of litter is a factor directly associated with moisture content. The most limiting effects on decomposition occur in waterlogged litter, where anaerobic conditions may occur (Williams and Gray, 1974). Anaerobic conditions maybe prevented to some extent in a waterlogged Antarctic environment because of low field respiration rates resulting from relatively low mean temperatures. In such a situation, the possibility exists that gaseous diffusion through waterlogged litter is sufficient to maintain aerobic conditions. Dennis (1968) noted the presence of many basidiomycete sporophores in

waterlogged ground near rivers on South Georgia.

The transition from standing dead leaves to fallen dead, i.e. litter layer, leaves is perhaps one of the more abrupt changes for phylloplane inhabitants. Newly fallen leaves are liable to colonisation by members of the soil mycoflora, and accumulate a higher water content due to their position (see Chapter 2).

Sjörs (1959) showed that during the first few weeks of decomposition in the litter layer most leaves became less acidic, almost certainly due to the leaching of organic acids. The water soluble organic matter content of litter provides a readily available energy source for decomposers, and therefore has an important influence on the early stages of litter colonisation. Usually there is also a rapid loss of soluble organic matter due to microbial utilization and leaching (Williams and Gray, 1974). At least in the early stages of litter decomposition, a high nitrogen content has been shown to promote the degradation process. Grass litters usually possess between 0.5 - 1.5% nitrogen (w/w) (Bartholemew, 1965).

The colonisation of *Festuca* leaves was very similar to that of *Poa*. New leaves were dominated by sterile mycelia, which remained until the litter stage. *Chaetophoma sp.* was abundant on standing mature (class 2) leaves, together with a range of other cellulolytic species. On class 3 leaves *Chrysosporium pannorum* and sterile mycelia were the major components, whilst litter again was colonised

by *Chrysosporium pannorum* and *Mucor hiemalis*, with very few sterile mycelia and no *Penicillium* species. The absence of *Penicillium spp.* is interesting, and may be related to the different growth form of *Festuca contracta*, in that the protection and possible temperature elevation afforded by the *Poa* tussocks is absent. Further studies are needed to explore this hypothesis.

Acaena magellanica leaves and litter were previously shown to contain relatively large amounts of sugars (Chapter 2), when compared with *Poa* leaves. The new leaves of *Acaena* supported a mycoflora composed of *Mortierella sp.* , sterile mycelia and *Chrysosporium pannorum*, with low numbers of isolations of *Botrytis cinerea* and *Cladosporium sphaerospermum*. The presence of *Mortierella sp.* on new leaves suggests a high level of freely available sugars on the phylloplane, as this species is incapable of cellulose oxidation. Mature leaves (class 2) were colonised by *Chrysosporium pannorum*, sterile mycelia, and a small number of *Mortierella sp. Botrytis cinerea* and *Penicillium spp.* were isolated from *Acaena* class 3 leaves, with sterile mycelia still dominating the mycoflora. *Acaena* litter had a combination of *Botrytis cinerea* and *Mucor hiemalis* as major components, together with sterile mycelia and *Aureobasidium pullulans* in smaller numbers. *Chrysosporium pannorum* and *Chaetophoma sp.* were also present, but isolated relatively infrequently.

Yadav (1966) studying hogweed (*Heracleum sphondylium)*, found a primary mycoflora which was similar to that of monocotyledonous substrates, but including *Botrytis cinerea,* a fungus known to cause soft rot of parenchymatous material. Although not necessarily an important primary coloniser, *Botrytis cinerea* was common on *Acaena* leaves at the senescent and dead stages. Again similar to the *Acaena* succession, Yadav often recorded the presence of Mucorales, notably *Mucor hiemalis,* on new leaf pieces.

Dickinson (1967) studied the colonisation of *Pisum sativum* and found that most spores on green leaves did not germinate. The majority of spores belonged to yeast-like fungi. McBride (1972) has demonstrated the possibility of cutin degradation by yeast cells (*Sporobolomyces roseus).* He concluded that not all fungi present upon leaf surfaces were phyllosphere saprophytes or fungi involved in leaf degradation, but that "casual inhabitants" were present due simply to deposition from the air spora.

Hill and Nelson (1976) have shown temperature adaptability in fungal isolates from cool environment and warm environment populations. Savile (1963) observed that a majority of fungal pathogens of Arctic plants have a persistent perennial mycelium which remains in the crown of the host making annual recolonisation by airborne inocula less critical. This is an important adaptive factor in view of the relatively short time available for

infection and growth.

There is evidence that sclerotium initiation in *Botrytis cinerea* has a lower temperature optimum than has growth (Harada *et al.* 1972). Sclerotium formation is promoted by high carbohydrate concentrations, but sclerotia do not mature until mycelial growth is checked by nutrient exhaustion or some metabolic change (Coley-Smith *et al.* 1980). The ability to form sclerotia is possibly an important factor in the survival of *Botrytis cinerea* in an Antarctic environment. *Aureobasidium* and *Cladosporium* species form microsclerotia which are able to withstand desiccation and probably other adverse environmental factors. The deeply-pigmented, thick-walled hyphae of *Cladosporium* species also help to ensure their survival (Dickinson, 1976).

It is highly probable, considering the geographical isolation of South Georgia, the prevailing wind direction, and the relatively low airspora, that all species growing on leaves and litter during summer are capable of overwintering successfully. It was noted that 5°C incubation promoted the growth of Dematiaceae, whilst 25°C allowed development of more hyaline species. There is now considerable evidence that fungal pigmentation prevents bacterial lysis of hyphal walls (Muirhead, 1981; Parbery, 1981). Overwintering hyphae, being exposed to bacterial and microfaunal degradation for long periods, would obviously gain an ecological and competitive advantage from melanisation.

With the melting of ice and snow cover new plant growth begins. The incidence of frosts can damage cells of newly exposed leaves and increase leaf leaching. The new leaves also contain high sugar concentrations. High leaf surface concentrations of carbohydrates supported large growths of mesophilic yeasts during the spring, followed by Mucorales as the season progressed. That the yeasts (mainly *Candida saké*) were mesophilic suggests that, even very early in the season, the leaf surface temperatures are relatively high compared to the ambient air temperature. The litter layer supported *Candida saké* and both psychrophilic and mesophilic bacteria at early stages in spring. *Candida saké* has been reported by other workers in Antarctic regions as a phylloplane yeast (Baxter and Illston, 1977), and is described as an ill-defined species with habitats ranging from soil and sea-water to breweries (Kirsop, pers. comm.).

During the spring isolations many leaf pieces produced no fungal growth at all, whilst others were covered by the yeasts and bacteria. This suggests that the air spora inoculum is important, especially on new leaves. It is probable that most microfungi are present in the litter layer as resting bodies or mycelia, and sporulate following the spring thaw.

The presence of large numbers of sterile mycelia tends to mask the underlying pattern of succession on age classes 1 - 3, but nevertheless, a pattern is apparent. The following chapters investigate some environmental

and competitive reasons for the successions of the more important phylloplane species.

CHAPTER 4

FACTORS AFFECTING SPORE GERMINATION

INTRODUCTION

Spore germination is a term used in mycology to describe a wide range of processes. Burnett (1976) describes germination as the essential process of the restoration of normal metabolic and physiological activity after a period during which these processes have been reduced, changed or have almost ceased. The consequence of germination in most fungi is the production of one or more germ tubes, which give rise to either a normal vegetative mycelium or a further sporangial phase as in the Mucoraceae.

In many spores the initial visible change is an increase in spore size. New wall material is produced, and net wall thickness often increases (Bartnicki-Garcia, 1969; Gull and Trinci, 1971). During or after swelling of the spore, the germ tube is produced, associated with localised accumulations of vesicles near the site of its emergence. A common feature is the production and elongation of mitochondria (Lowry and Sussman, 1968).

Sussman (1968) described the various mechanisms of dormancy, which essentially are grouped into exogenous and constitutive mechanisms. Exogenous dormancy is imposed by factors external to the spore, whilst constitutive dormancy is innate, resulting from metabolic blocks within the spore. The borderline between these groupings can be diffuse. Thick and impermeable spore walls are often linked with constitutive dormancy, specific nutrient deficiencies, or internal inhibitors may be depressing

metabolic activity until such time as they are removed or degraded.

Factors of obvious significance in the induction of exogenous dormancy include water activity, temperature, pH and inhibitors released from adjacent mycelia (Burnett, 1976). Most spores have a low water content, and hydration is usually an essential step for the onset of germination. Spores of Peronosporales are reported to germinate only in free water (Schnathorst, 1965), whilst free water is not always necessary for other species, these being capable of absorbing water vapour over a range of water activities.

Respiration and protein synthesis are implicated in germination. Ekundayo (1966) showed a glucose requirement for germination in members of the Mucorales, independent of the osmotic pressure of the glucose solution. Activation of spores of *Neurospora crassa* has been achieved using heat shocks, heterocyclic compounds and organic solvents, suggesting a blocking system linked with the spore membrane (Burnett, 1976). The check to germination of spores of *Aspergillus niger* seems to be due mainly to the degree of hydration of the spores. In this species a two-stage process of swelling occurs. Yanagita (1957) showed the first stage of swelling to be unaffected by temperature, being probably a simple physical process; the second stage is temperature sensitive and involves uptake of carbon dioxide. Presumably, the initial

hydration relieves a threshold limitation on the metabolic activities of the spores, allowing growth.

Dickinson and O'Donnell (1977) showed that water activities of 0.97 or greater were required to achieve maximum germination and germ tube growth with spores of *Alternaria alternata* and *Cladosporium cladosporioides*. The rate of respiration of recently-hydrated spores was shown by Mozumder and Caroselli (1966) to be dependent upon water activity. Decreasing the water activity around spores increases the latent period for germination, until at some level germination will not occur (Griffin, 1972).

Dickinson (1981), reviewing the biology of *Alternaria alternata* and *Cladosporium cladosporioides*, discusses the effects of water activity on germination. Dickinson and Bottomley (1980) have shown that the response of conidia of *Alternaria alternata* to water activity varies with temperature , low temperatures causing a reduction of germination at the lower A_W values tested. These workers also showed a wide range in the germination responses of spores to temperature, with optima ranging from 15° to 25^oC. Blakeman (1975) examined seven isolates of *Botrytis cinerea* and found one which was almost unable to germinate in water, whilst others exhibited germination of between 34% and 85%.

Water activity and availability of free water on plant surfaces are often thought of as the most important

single factors influencing infection. Snow (1949) demonstrated that conidia of *Botrytis cinerea* could only germinate at water activities between 1.00 and 0.93, and were thus considered to possess a high moisture requirement for germination in comparison with other fungi tested. This requirement results in part from the low water content of their spores, determined by Yarwood (1950) as 17% of the fresh weight. Spores of other air-disseminated species tested had water contents of 6 - 25%, whilst conidia of powdery mildews had a water content of 52 - 75%. Yarwood suggested that the high water content of these mildews explains why germination can occur at lower water activities.

The presence of water in a spore which is subjected to freezing could significantly reduce viability by the formation of ice crystals and by structural damage to enzymes. Vanev (1965) found that if temperatures fluctuated above and below zero, viability of *Botrytis cinerea* spores was markedly affected. He concluded that spores were unlikely to survive for long periods in the field under these conditions. Harrison (1979) found that mycelia of *Botrytis fabae* can overwinter in crop debris, although survival by this means is less important than in the form of sclerotia. In the dry state, spores are likely to remain viable for a longer time than if wetted and dried frequently. In the latter case a rapid decrease in viability may occur as a result of carbohydrate dep-

letion because of a combination of increased respiration and solute leakage (Brodie and Blakeman, 1975). Simon (1974) suggested that repeated rehydration and drying of spores could induce solute leakage through the cell membrane because of rapid reorganisation of the lipid layers.

Barnes (1930) exposed conidia of *Botrytis cinerea* to sublethal temperatures. Of 520 conidia, 424 died, 20 developed normal colonies, and 78 showed strong or slight morphological changes. All but four of these variants gradually reverted to a normal growth form.

Water activity and temperature appear to be the most important factors governing germination, but various other physical factors are probably of some importance in the field. Hennebert and Gilles (1958) showed that exposure to direct sunlight accelerated the decline of *Botrytis cinerea* spore viability. This effect was alleviated to some extent by the degree of hydration. Ultraviolet light promotes the formation of free radicals within the cell, these radicals then may react with metabolically important molecules. Water is thought to be the main source of radicals within cells (Bainbridge, 1966). There are also suggestions that ultraviolet light promotes sclerotium formation and inhibits conidiation in *Botrytis cinerea* (Coley-Smith *et al.* 1980). A combination of low temperatures and the inherent desiccation of a dormant

spore may help to combat damage induced by ultraviolet irradiation, and it has been shown that lowering the water content of spores mitigates the lethal effect of temperature and possibly light extremes (Sussman and Halvorsen, 1966; Muirhead, 1981). Melanised spores were more resistant than hyaline spores to ultraviolet light in laboratory studies (English and Gerhardt, 1946; Markert, 1953), and this has been used as an explanation for the melanisation of many common epiphytes (Parbery and Emmett, 1977).

Much work has been done on the responses of fungi to pH changes (reviewed by Sussman and Halvorsen, 1966). It is known that most fungi will tolerate a large range of pH in the growth medium, and inhibition of growth is usually sharply defined at the limits of this range. The optimum is often less clearly defined, but most fungi appear to grow best around pH 7(Hawker, 1950). As the field studies for this work covered three quite different host plant species, and as the pH values of the leaf litters were significantly different, it was appropriate to include a test to determine whether or not any differences in germination rates could be attributable solely to pH.

Antagonism from other members of the phylloplane microflora can have profound effects on germination. A species of *Bacillus* reduced germination, promoted appressorium formation and caused the lysis of all unmelanised

fungal tissues of *Colletotrichum gloeosporioides* and *Colletotrichum musae* (Muirhead, 1979).

Surfaces of plants are rich in epiphytic bacteria (McCracken and Swinburne, 1980), some of which produce enzymes which lyse hyphae (Lenné and Parbery, 1976). There is direct evidence that phenolic compounds in melanised hyphal walls prevent lysis by bacteria through an inhibition of enzyme action (Kuo and Alexander, 1967; Bull, 1970).

Two species of *Pseudomonas* were shown to reduce germination in *Colletotrichum acutatum* (Blakeman and Parbery, 1977). Blakeman and Parbery believed the effect to be caused by the induction of nutrient stress in the fungal spores, as they were able to produce a similar effect through leaching nutrients from germinating spores. Blakeman (1972) showed that leakage of nutrients, both from leaves and from the conidia of *Botrytis cinerea* affected numbers of bacteria, and in turn the amount of inhibition of spore germination. As the host plants aged, leakage of amino acids and carbohydrates increased, stimulating a *Pseudomonas* species and inhibiting still further the germination of *Botrytis* spores. Leaching of *Botrytis cinerea* spores with water to induce nutrient stress also inhibited germination, but the inhibition was overcome by addition of a nutrient solution. Further studies were carried out using ^{14}C labelled culture media (Brodie and Blakeman, 1975). Within the first few minutes of sus-

pension of spores in water there was a substantial leakage to the external solution ranging from 2.5 to 20% of the total ^{14}C originally present on the spores. Again, this is thought to be due to membrane reorganisation on hydration (Simon, 1974). Tukey (1971) demonstrated that leaching initially lowers the nutrient status of plant surfaces. The nutrient level rises when leaching ceases, and spores on such surfaces would be conditioned to germinate by wetting, also receiving a stimulus to germination by the rising level of nutrients (Parbery, 1981).

Barash *et al.* (1963) studied the effect of plant leachates on germination. Leachates from flowers had 2.5×10^{-2}M reducing sugars, whilst leaf leachates contained only 1.0×10^{-4}M. In flower leachates 95% of conidia of *Botrytis cinerea* germinated in only two hours, whereas in leaf leachates there was only 1% germination after sixteen hours. Flowers were readily infected by *Botrytis cinerea* , which used leachates as a substrate during the early stages of infection.

Recent work with chelating agents, notably dihydroxybenzoic acid, has shown these to be important factors during spore germination and growth. It appears that these agents lessen or remove any phytoalexin responses exhibited by a host plant in response to a spore (Swinburne, 1981). The mode of action of chelating agents in this case is, as yet, unclear.

Once germination has been initiated and a germ tube

has formed, the phylloplane mycelium either develops at the expense of endogenous reserves of lipids and carbohydrates, or absorbs nutrients from the leaf surface (Chapter 2). Nutrient levels on the phylloplane are relatively low, and there is considerable circumstantial evidence that microbial antagonism in the phyllosphere is based on nutrient competition (Fokkema, 1981).

It is possible that newly-developed mycelium attaches itself to the leaf. The tips of germ tubes of *Botrytis cinerea* have been reported to be securely fastened to the leaf by mucilage (McKeen, 1974), such that formation of a penetration peg can occur without formation of an appressorium.

Direct penetration of leaf surfaces by germ tubes has been observed in many host-parasite combinations (Coley-Smith *et al.* 1980). Ward (1888) studied the infection process of *Botrytis elliptica* on lilies, and concluded that germ tubes dissolved a pathway in the host tissue. Clark and Lorbeer (1976) observed germ tubes penetrating directly into intact, undamaged cells of onion leaves, usually *via* cell wall junctions. Kunoh (1981), reviewing infections of *Erisyphe*, stated that the tips of penetration pegs apparently lack cell walls, and the host cell wall is dissolved only in the region of the tip when viewed by electron microscopy. Rijkenberg *et al.* (1980) studied the infection process of *Botrytis cinerea* on tomato fruits, and showed areas of cuticle dissolution

in the region of the germ tube tip.

Hyphae need not necessarily penetrate the cuticle. Many species produce mycelia which gain entry to leaves *via* stomata (Millar, 1981), or through damaged areas of cuticle. This chapter investigates the effects on germination of various physical factors, and the processes of growth and penetration on the cuticle of the host plants.

MATERIALS AND METHODS

1) Effect of Water Activity on Spore Germination

4 - 5 day old cultures of test fungi were grown on Malt Extract Agar (MEA) at 20^oC. $10cm^3$ aliquots of sterile distilled water were pipetted onto each culture, which was then gently scraped using a sterile loop to dislodge spores. The spore suspension was transferred to sterile Universal bottles and shaken thoroughly. The spore density was determined using a haemacytometer, and then diluted to obtain a final spore concentration of $1 - 2 \times 10^5$ spores cm^{-3}. Using the solutions of Solomon (1951) (see Appendix 4), a range of water activity (A_w) values were established in glass petri dishes, the lids of which were made airtight with Petroleum jelly at their rims.

One drop of spore suspension (approximately 0.05 cm^3) was pipetted onto a flamed microscope slide, which was then placed on a Z-shaped glass rod lying in the appropriate A_w solution, such that the slide was above the level of the solution. Each of the five most commonly occurring phylloplane fungal species was tested at a range of five A_w values from A_w = 100 to A_w = 0.85. Following incubation

for 24 hours at 20°C, the slides were removed from the petri dishes and enumerated for germination by microscopic examination. Germination was taken to be the point when the germ tube length was equal to or greater than the length of the spore. A minimum of 200 spores were counted per slide, and three replicate petri dishes were made per A_w value. Large aggregations of spores were avoided during the counting procedure.

2. Effect of Temperature on Germination

Spore suspensions were prepared of the five major phylloplane species as outlined in Section 1. Drops of the suspension (approximately 0.05cm^3 volume) were pipetted onto petri dishes containing MEA. Five drops of suspension were placed on each dish, and dishes were incubated for 24 hours at a range of temperatures from 5 - 30°C. Three replicate plates were made for each temperature. Spores were examined by microscope for germination as described in Section 1.

3. Effect of pH on Germination

Petri dishes containing MEA buffered at pH 6.7 and pH 4.5 (using sodium citrate buffer) were inoc-

ulated with drops of spore suspensions and incubated for 24 hours at 20°C. Three replicate plates were made for each pH value. Five counts of at least 200 spores were made per petri dish.

4. Effect of Ultraviolet Light on Germination

Spore suspensions were prepared of each of the five major phylloplane fungi, as previously described. 0.05 cm^3 aliquots of the suspensions were pipetted onto tap water agar (TWA), five drops per plate. Plates were then exposed to a standard ultraviolet source* for a range of times from 0 - 60 minutes. Three replicate plates were made per treatment. Plates were then incubated for 24 hours at 20°C, followed by microscopic examination for germination as previously described.

* Gallenkamp LCF 870 bench lamp. 125W mercury discharge at 30 - 35 cm distance . (Irradiation peak at 365 nm).

5. Scanning Electron Micrographs of Germination

Using leaf material excised from active plants on South Georgia and returned to England in cold storage ($5^{o}C$) , 2 - 3 cm lengths of young leaves were cut and washed six times by the serial washing technique of Harley and Waid (1955) (previously described). Leaves were then briefly immersed in a 5% solution of mercuric nitrate and rinsed in four changes of sterile distilled water.

Leaf pieces were transferred to damp chambers in petri dishes, and inoculated with drops of spore suspensions from the five test species. Excess water was removed from the damp chambers and then leaf pieces were incubated for 4 days and 8 days at $20^{o}C$. Pieces were then removed from the damp chamber and immersed in 5% glutaraldehyde for one minute for fix hyphae and spores. The leaf pieces were placed in a desiccator for two days and then attached to specimen stubs using Electrodag 915 high conductivity paint. Specimens were then sputter coated with gold in a vacuum chamber and examined by a Cambridge Stereoscan S150 microscope.

6. Effect of Exogenous Glucose Supply upon Germination

Spore suspensions were prepared from young active colonies of each test fungus as previously described (Section 1). The suspensions were vacuum filtered through 0.22 µm cellulose acetate filters in order to separate spores and solution. Spores were rinsed with sterile distilled water, and the spores re-suspended in sterile water in Universal bottles. Glucose was added to one set of suspensions of each test species, with control bottles of sterile water without glucose. The final glucose concentration was 0.5M. Two replicated bottles of spore suspensions, from separate strains, were used per treatment. Drops of approximately 0.05cm^3 volume were pipetted from spore suspensions onto Tap Water Agar (TWA) in petri dishes. Incubation was for 5°, 15° and 25°C for 24 hours, following which spores were counted for germination using a microscope.

7. Effect upon germination of one freeze-thaw cycle

As a preliminary study of the effect of freeze-thaw conditions upon growth and germination, spores were subjected to one freeze-thaw cycle of $-10^{o}C$ to $+5^{o}C$ over a forty-eight hour period.

Spore suspensions were prepared of the five fungal test species, and suspensions were adjusted to approximately 50,000 spores cm^{3} in sterile distilled water.

$5cm^{3}$ aliquots of the suspensions were pipetted into sterile Universal bottles, and these were placed at $-10^{o}C$ for twenty-four hours. After this time, the bottles were removed to $+5^{o}C$ until thawed, and drops of suspensions were plated onto petri dishes of MEA. Dishes were incubated for twenty-four hours and forty-eight hours at $+5^{o}C$, and removed to $+15^{o}C$ for a further twenty-four hours. After each time period plates were scored for spore germination, using a microscope. Germination was defined as that stage when germ tube length exceeded spore length. Controls consisted of spores maintained at $+5^{o}C$. At least 200 spores were counted for each measurement, with five replications.

RESULTS

1. Effect of Water Activity on Germination

Results are given in Figs. 26 and 27 .

Botrytis cinerea showed no germination at $A_w = 1.00$ (i.e. in liquid water), and maximum germination at $A_w = 0.97$. Lower A_w values significantly reduced germination, and no germination was recorded at $A_w = 0.90$.

Chaetophoma sp. appeared to germinate only in liquid water and would not germinate at $A_w = 0.97$.

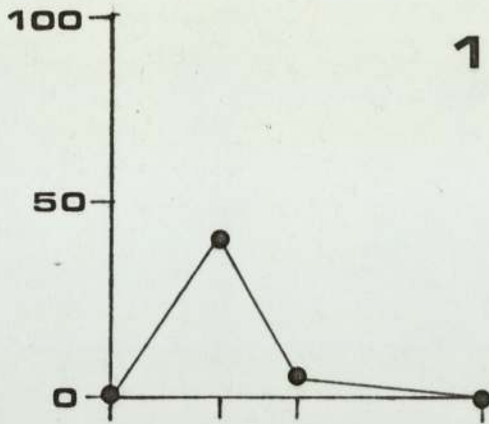
Chrysosporium pannorum showed a surprisingly high tolerance of lower A_w values, nearly 10% of the spores germinating at $A_w = 0.90$. Of the values tested, $A_w = 0.97$ caused maximum germination. This ability to germinate at lower A_w values is interesting in view of the relatively high incidence of *Chrysosporium pannorum* on almost all leaf classes.

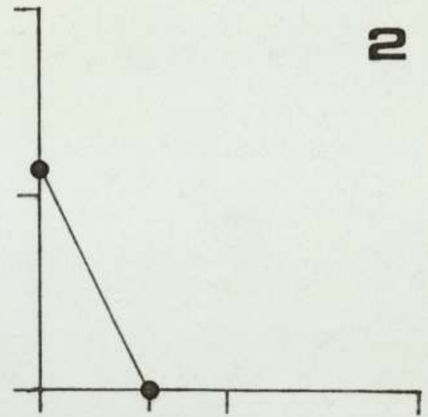
Mucor hiemalis germinated at a lower overall percentage than the other species tested, with maximum germination at $A_w = 0.97$. No germination was recorded at $A_w = 0.90$, and it seems likely that no germination occurs below $A_w = 0.94$.

Cladosporium sphaerospermum showed a very high tolerance to A_w over the complete range of values tested. This

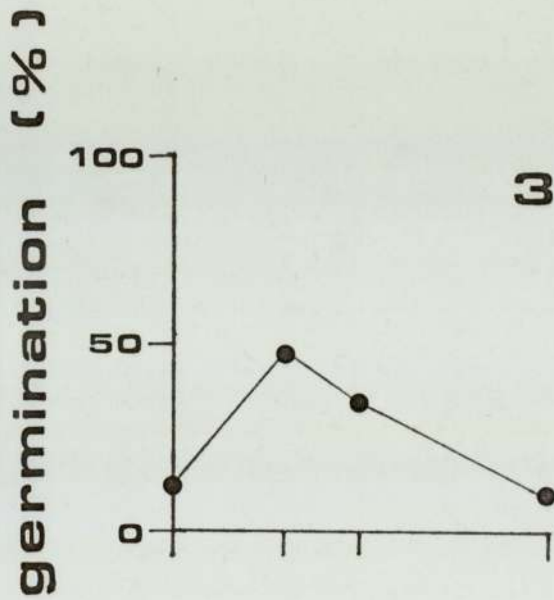
Fig 26 Effect of water activity upon germination

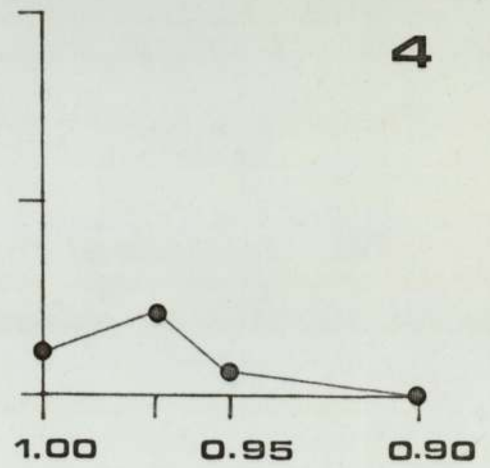
1. *Botrytis cinerea*
2. *Chaetophoma sp.*
3. *Chrysosporium pannorum*
4. *Mucor hiemalis*
5. *Cladosporium sphaerospermum*

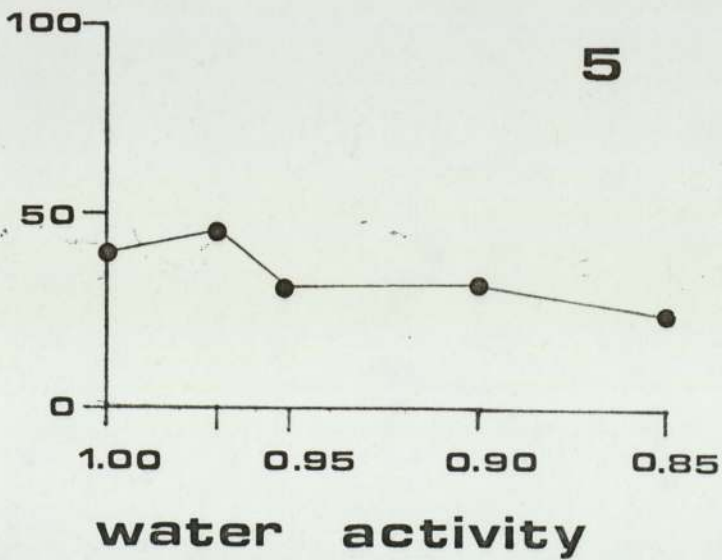




germination (%)







water activity

Fig. 27 Effect of Water Activity on Germination (% $\pm$ S.D.)

SPECIES	A_w				
	1.00	0.97	0.95	0.90	0.85
Botrytis cinerea	0.0	42.6 $\pm$ 13.8	4.7 $\pm$ 2.6	0.0	0.0
Chaetophoma sp.	58.0 $\pm$ 24.8	0.0	0.0	0.0	0.0
Chrysosporium pannorum	11.7 $\pm$ 5.1	47.9 $\pm$ 3.9	34.7 $\pm$ 17.4	9.6 $\pm$ 6.1	0.0
Cladosporium sphaerospermum	40.6 $\pm$ 7.2	46.2 $\pm$ 22.1	10.4 $\pm$ 9.7	33.5 $\pm$ 23.3	37.3 $\pm$ 18.4
Mucor hiemalis	11.1 $\pm$ 2.4	22.4 $\pm$ 5.1	5.6 $\pm$ 3.2	0.0	0.0

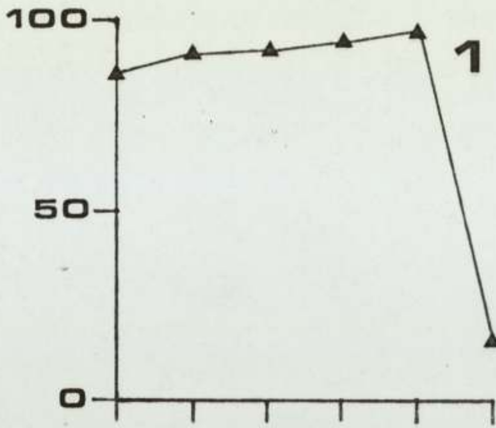
is in accordance with similar studies on *Cladosporium herbarum*, a closely related species which is tolerant to values as low as $A_W = 0.76$ (Hawker, 1950; Burnett, 1976). This ability to germinate in a relatively dry environment may be a reason for the predominance of *Cladosporium sphaerospermum* on aerial plant parts in this study.

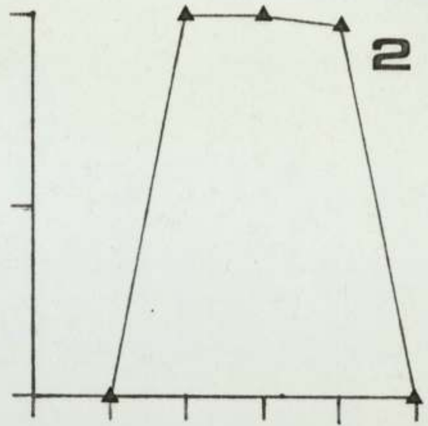
2. Effect of Temperature on Germination

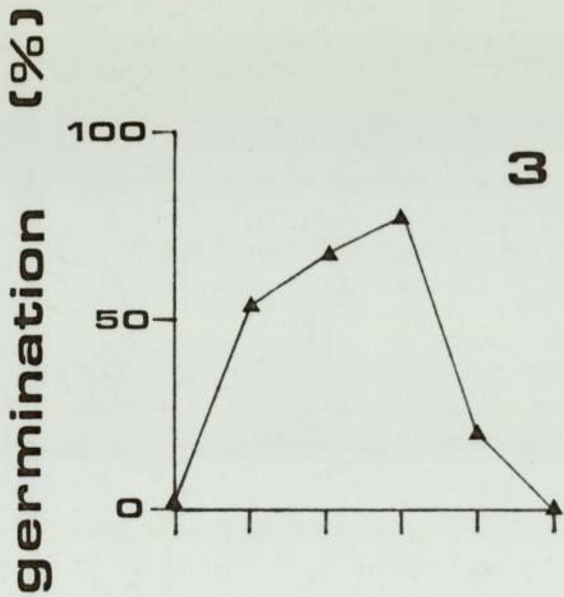
Results are given in Figures 28 and 29. *Botrytis cinerea* germinated at around 90% after 24 h incubation over the range 5 - 25°C, but showed a significant decrease ($p > 0.05$) at 30°C, where germination was 15.5% ± a standard deviation of 7.4% *Chaetophoma sp.* only germinated between 15°C and 25°C, with figures of almost 100% germination at these temperatures. The graph shows an apparent threshold effect of temperature for the germination process, and the temperature range for germination is the smallest of all of the five fungal species tested. *Chrysosporium pannorum* showed almost no germination at 5°C (approximately 1%), but no germination at 30°C. The optimum temperature was around 20°C, agreeing with the results of Kuthubutheen (1977) using an English isolate of *Chrysosporium pannorum*. *Cladosporium sphaerospermum* again exhibited an apparent

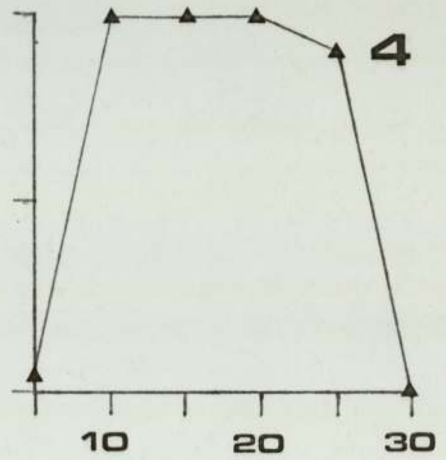
Fig. 28 <u>Effect of temperature upon germination</u>

1. *Botrytis cinerea*
2. *Chaetophoma sp.*
3. *Chrysosporium pannorum*
4. *Cladosporium sphaerospermum*
5. *Mucor hiemalis*


100
50
0
1


2


germination (%)
100
50
0
3


4
10
20
30

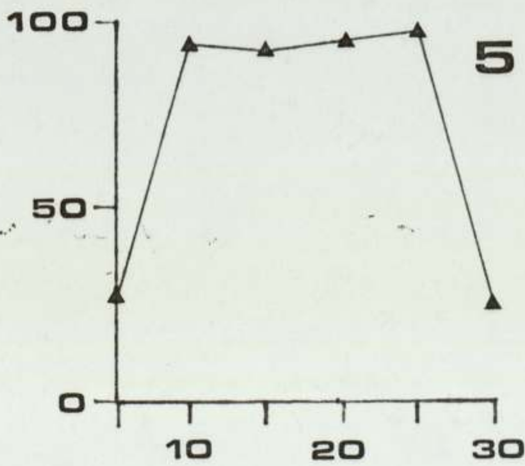

100
50
0
5
10
20
30
temperature (°C)

Fig. 29 Effect of Temperature on Germination (% ± S.D.)

SPECIES	TEMPERATURE (°C)					
	5	10	15	20	25	30
Botrytis cinerea	86.0 ± 23.0	91.5 ± 42.0	93.0 ± 38.4	94.5 ± 34.7	98.0 ± 48.7	15.5 ± 7.4
Chaetophoma sp.	0.0	0.0	98.0 ± 27.1	98.0 ± 61.6	98.0 ± 79.3	0.0
Chrysosporium pannorum	1.0 ± 0.2	54.5 ± 22.0	68.0 ± 24.6	77.0 ± 15.1	20.0 ± 12.2	0.0
Cladosporium sphaerospermum	3.5 ± 0.7	98.0 ± 21.6	98.0 ± 24.5	99.0 ± 46.0	90.0 ± 24.8	0.0
Mucor hiemalis	25.0 ± 9.7	94.2 ± 59.8	92.6 ± 24.8	95.0 ± 37.1	97.1 ± 16.9	25.1 ± 4.7

threshold effect, with either very few spores or all viable spores germinating, and did not germinate at 30°C.

Mucor hiemalis had a temperature range of 10 - 25°C for optimum germination, in which the majority of spores germinated. This species was more tolerant of 30°C than was any of the others, and showed a relatively high percentage germination at 5°C.

3. Effect of pH on Germination

No significant changes were detected between the percentage germination of spores at the two pH values tested. At each pH germination percentage was not significantly different from that in the previous experiment at 20°C incubation. In addition to the more common phylloplane species, Antarctic isolates of *Acremonium terricola*, *Doratomyces nanus*, *Fusarium lateritium* and a second species of *Chaetophoma* were tested for the effect of pH on germination, with no significant differences in each case. These results suggest that germination is a process not affected by hydrogen ion concentration within the range tested, i.e. in the pH range found within tundra litter layers and on leaves in South Georgia.

4. Effect of Ultraviolet Light on Germination

No significant change in percentage germination resulted from irradiation for 60 minutes in each species tested.

5. Microscopic Examination

Botrytis cinerea showed rapid and extensive growth on washed leaf surfaces, but there was no evidence of cuticle penetration by hyphae or germ tubes. It has been demonstrated, however, that temperate isolates of this species are capable of direct entry through the cuticle by enzymatic action. The fact that this isolate of *Botrytis cinerea* displayed a relatively strong cellulase activity suggests that penetration of the epidermis can occur.

Chaetophoma species developed thick appressorium-like structures suggesting cuticle penetration. Hyphae tended to grow parallel to the ridging in leaf venation, and entry of germ tubes through stomata was visible.

Chrysosporium pannorum growth was very restricted on washed new leaf surfaces, but strong on class 3 leaves, where superficial degradation of the epidermal layer was apparent and germination was heavy after incubation for seven days.

Cladosporium sphaerospermum grew quickly on washed new leaves and was observed growing out of leaf pieces from within the leaf after one week. Sporulation was heavy after ten days. The mode of entry is unclear.

Mucor hiemalis was incapable of growth on washed new leaves and spore germination was relatively low.

6. Effect of Exogenous Glucose Supply on Germination

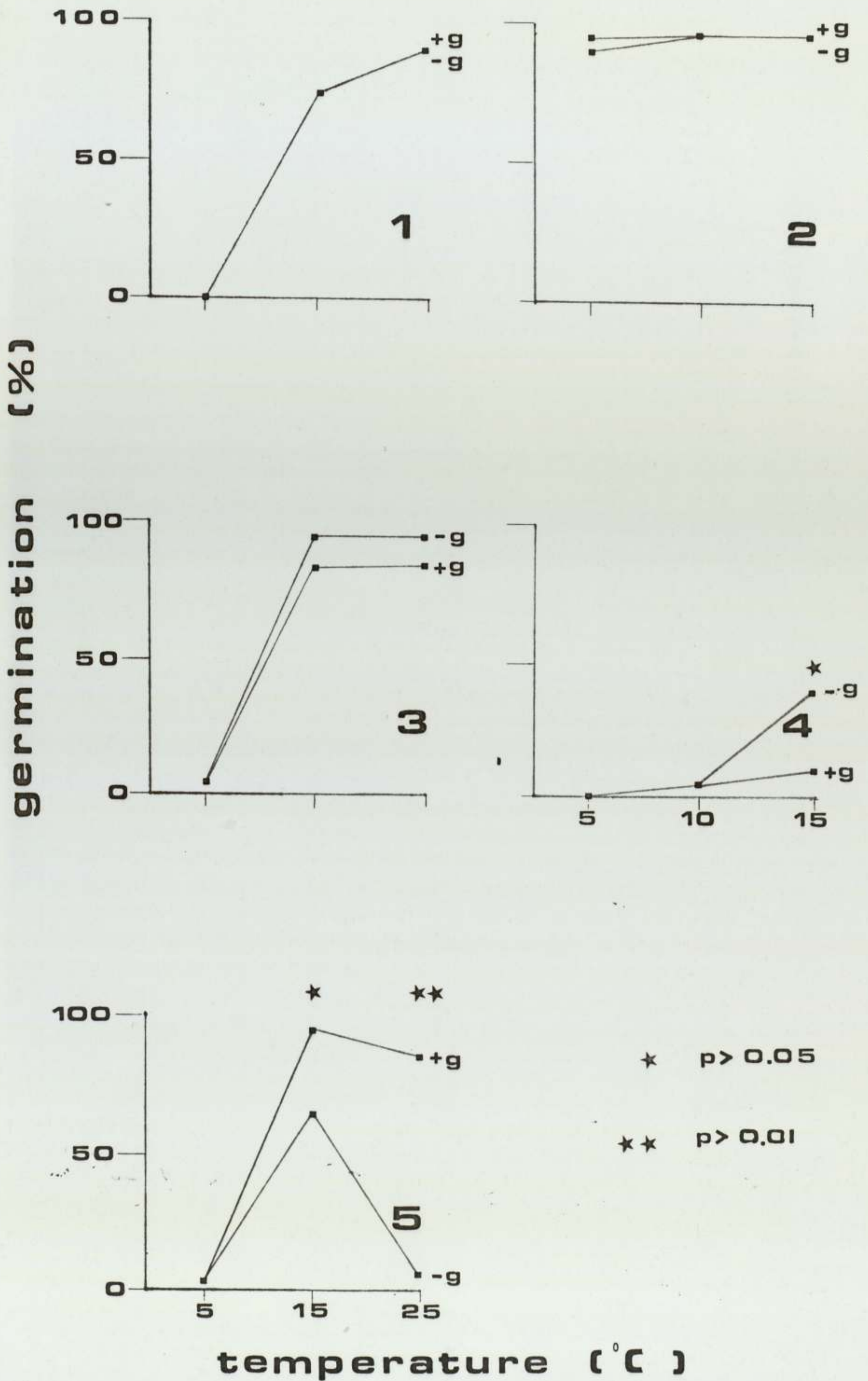
Results are shown in Fig. 30 and Appendix 5 . No significant difference in germination of *Botrytis cinerea*, *Chrysosporium pannorum* or *Cladosporium sphaerospermum* resulted from addition of glucose at any incubation temperature. Germination of *Chaetophoma* species was significantly reduced ($p > 0.05$) by addition of glucose, probably as a result of osmotic effects. *Mucor hiemalis* displayed increased germination at both $15^{o}C$ ($p > 0.05$) and $25^{o}C$ ($p > 0.01$) upon addition of glucose.

7. Effect of One Freeze-Thaw Cycle on Germination

The effects of this treatment show, in many cases, a significant slowing of the germination process. The germination rate of *Botrytis cinerea* was apparently unaffected by passage through the cycle. It is probable that the lower temperatures slowed germination by retarding action on the metabolism of the spores. An incubation temperature of $5^{o}C$ was used in the regime because this enabled the time course to be studied more effectively. All species except *Chaetophoma* had been shown to be capable of germination at this temperature. *Chaetophoma* species does not germinate below $15^{o}C$, which was one reason for the inclusion of this incubation temperature. Results are given in Fig. 31.

Fig. 30 Effect of exogenous glucose source upon germination after 24 hours

1. *Chrysosporium pannorum*
2. *Botrytis cinerea*
3. *Cladosphorium sphaerospermum*
4. *Chaetophoma sp.*
5. *Mucor hiemalis*



germination (%)
100
50
0
+g
-g
1
2
-g
+g
3
4
5
10
15
*
**
p> 0.05
p> 0.01
25
temperature (°C)

FIG. 31 EFFECT OF FREEZE-THAW CYCLE UPON GERMINATION (% $\pm$ S.D.)

* Significantly different from control ($p > 0.05$)

** Significantly different from control ($p > 0.001$)

SPECIES	TREATMENT	24hrs @ 5°C	48hrs @ 5°C	72hrs (to 15°C)
BOTRYTIS CINEREA	F	44.1 ± 5.8	79.4 ± 5.1	81.7 ± 13.3
	C	38.8 ± 9.3	85.7 ± 10.9	92.1 ± 1.1
CHRYSOSPORIUM PANNORUM	F	0.00 **	0.00 **	77.8 ± 9.2
	C	10.0 ± 1.7	10.0 ± 2.1	81.8 ± 9.5
CLADOSPORIUM SPHAEROSPERMUM	F	0.00 **	0.00 **	69.4 ± 10.2 *
	C	47.8 ± 10.5	78.0 ± 5.5	89.0 ± 4.1
MUCOR HIEMALIS	F	0.00 **	10.0 ± 1.1 **	98.0 ± 1.0
	C	37.2 ± 3.9	51.0 ± 6.7	98.0 ± 1.0
CHAETOPHOMA sp.	F	0.00	0.00	0.00 **
	C	0.00	0.00	93.0 ± 5.0

F = ONE FREEZE-THAW CYCLE   C = CONSTANT +5°C (CONTROL)

DISCUSSION

Of the various factors studied, water activity and temperature had the most significant effect on spore germination. The mechanisms for these responses are presumably metabolic processes within the germinating spore which are related to gene expression and the mobilisation of the lipid nutrient reserves.

There is no reason to suspect that low temperatures will directly affect spore viability; rather the rate of metabolism and mobilisation within the spore will be reduced such that germination takes place over a longer time period.

Rapid wetting and drying have been suggested to cause nutrient depletion in spores due to leakage, and it is now accepted that increased leaching from spores due to freeze-thaw cycles can reduce viability or increase the lag phase of germination as increasingly more complex carbohydrate reserves need to be mobilised (Vanev, 1965; Cheng *et al.* 1971; Brodie and Blakeman, 1975; Blakeman and Parbery, 1977).

Low water activities may stop germination by removal of essential water molecules from within the cell. If attraction to solute molecules in the media is greater than that of hydrated molecules in the spore, it is probable that dehydration will occur. In the case of those molecules whose structure is determined by the

inclusion of water, dehydration may be irreversibly damaging to the structure and functioning of the molecules.

The induction of sporulation by near ultraviolet light is a common phenomenon exhibited by many Ascomycetes and Deuteromycetes (Leach, 1971) but little is known at present of the quantitative metabolic changes occurring subsequent to irradiation. There seems no doubt that such light can alter fungal metabolism to at least a small extent. There are, however, very few reports on the effect of naturally encountered levels of ultraviolet irradiation on growth or linear extension rate. It would be expected that strong irradiation would be required to cause damage to cytoplasmic components, protected as they are by the hyphal wall and possibly trichomes and shading in the field situation.

A buffer effect to pH changes has been noted in leachates from *Acaena* and *Poa* leaves, tending to maintain pH at the original value even following the growth of inoculated fungi in such media. It is possible that such an effect is also present on the leaf surface, preventing large scale fluctuations in pH. Spores seem insensitive to pH changes over the values tested. Discussing the active transport of K^+ across the cell membrane, Burnett (1976) stated that this process is highly sensitive to pH, being greatly reduced below about pH 5.0. This process, in common with many other transport mechanisms, is

dependent upon exchange of hydrogen ions from the cell, and is therefore altered by the ambient hydrogen ion concentration. Active transport may not be an important factor during the early stages of germination, but for whatever reason, pH seems relatively unimportant at this stage of the life cycle.

Ekundayo and Carlile (1964) and Ekundayo (1966) showed that glucose is often required for swelling to occur during the first stages of germination in the Mucoraceae. This process was shown to be independent of the molarity of the glucose solution and suggests that a non-osmotic, energy requiring process may be involved. A similar glucose requirement in *Fusarium* supports this view (Marchant and White, 1966). The results of the application of glucose to spore suspensions have shown a significant promotion of germination in *Mucor hiemalis* ($p > 0.01$) which was more pronounced at $25^{o}C$ ($p > 0.001$) than at $15^{o}C$.

No other test species showed a significant promotion of germination, but germination of *Chaetophoma* species was significantly reduced ($p > 0.01$) by the glucose solution. This is probably an osmotic effect, enhanced by the relatively small size of the pycnidiospores (1 - 3μm x 2μm) and hence their low water content. This is in agreement with the results of the effect of water activity on germination, which showed that germination of *Chaetophoma* sp. occurred only at 1.00 water activity.

This glucose requirement may have important implications for the distribution of *Mucor hiemalis* in the ecosystem. This species was found mainly in litter in conjunction with cellulolytic species, and on new leaves with relatively high carbohydrate concentrations. Being a member of the Mucoraceae, *Mucor hiemalis* is able to utilise high sugar concentrations very rapidly, exhibiting a relatively high linear extension rate.

An important aspect of the glucose requirement is the fact that it acts as an inhibitory system until the habitat is amenable to successful germination and growth. The suggestion here is that in litter, *Mucor hiemalis* is inhabiting a microhabitat with relatively high carbohydrate levels. *Chrysosporium pannorum* probably provides a high glucose concentration in the proximity of its hyphae because of the efficiency of its C_x cellulase system, and carbohydrates resulting from leakage, guttation and freeze-thaw action on all cell types will contribute to *Mucor* growth in the litter.

Water activity is apparently a factor of major significance in determining germination of spores on leaf surfaces. Further studies are needed concerning the microenvironmental conditions on the phylloplane with respect to water activity and the effect that leaf age has upon this factor.

CHAPTER 5

FACTORS AFFECTING COLONY GROWTH

Introduction - Growth of Colonies; Factors Affecting Growth

The aim of this chapter is to outline the responses of the five most commonly isolated phylloplane fungi to various environmental factors which would be expected to exert an effect under field conditions.

The first parameter studied was water activity (A_w), and its effect on colony extension rate. A full explanation of water activity is given by Scott (1956). Water activity is the water vapour pressure of a solution expressed as a fraction of the vapour pressure of pure water at the same temperature, and is numerically equal to relative humidity expressed as a fraction.

The model system used in these experiments is fungus growing on agars of defined water activity. In this system it is assumed that, at a given water activity, the amount of energy required by the fungus to remove water molecules which are bound to solute molecules is similar to the amount of energy required by the fungus to remove water molecules from soil at an equivalent relative humidity or matric water potential (Dubé *et al.* 1971).

The use of solutes to produce a medium with defined water activity was suggested by Scott (1953) and depends upon hydration of solute ions or molecules. The force with which water molecules are bound to a solute molecule decreases with each successive hydration shell, and more concentrated solutions have less space for hydration shells,

with correspondingly higher A_w values. Fungi growing in such solutions compete directly with solute molecules or ions for the available water. In a closed container the water activity of the atmosphere is equal to that of the medium.

The water activity or water potential of a system can have marked effects on fungal activity. Rosswall (1974) showed that, in an Arctic tundra, moisture contents of less than 50% of fresh weight caused drastic reductions in rates of decomposition of litter.

The degree of hydration of any given enzyme also depends upon its ability to compete with the solute for water molecules. Skujins and McLaren (1967) have clearly shown that the change in rate of a reaction of urease with water potential follows closely the water sorption isotherm of the enzyme. Activity seems to depend on the degree of hydration of the enzyme.

The nutrients necessary for fungal growth can reduce water activity by osmotic means, and organisms are likely to have evolved so that their most rapid growth occurs at water activities commonly found in their substrates. Some other reasons put forward for the growth-limiting effect of low water activities are; the possibility of slowed formation of high-energy phosphate centres (ATP); and the fact that at low water activities the DNA base pairs are dehydrated and reversible helix distortion is caused (Griffin, 1972).

There is a direct relationship between the osmotic potential of xerophiles and their ability to grow at low water potentials due to the relative ease of transfer of water into hyphae which are not at an osmotic potential difference with a medium (Pugh, 1974).

Trinci (1969) studied the relationship between the radial growth rates of colonies on agar and in liquid culture. He concluded that linear extension rate is a reliable parameter to determine the optimum temperature for growth of a fungus unless the morphology is altered.

It could be expected that in a field situation such as South Georgia, with relatively low mean field temperatures, a psychrophilic mycoflora would develop. It was therefore necessary to determine the effect of temperature on the most commonly isolated species. By measurement of both the dry weight growth rate and the linear extension rate, it was hoped that a clearer picture would be gained of the fungal responses to temperature. For example, it was noted early in the study that whilst *Mucor hiemalis* was quite capable of growth on agar, its colonies were so sparse that dry weight measurements would indicate almost no growth.

The responses of fungi to pH changes and ultraviolet irradiation were discussed in the previous chapter. Moses (1955) showed that at pH values of around 7.0, *Zygorhynchus moelleri* was unable to assimilate and oxidise

various intermediates of the citric acid cycle, whilst pH 3.4 allowed all test substrates to be oxidised. Further discussion of the effect of pH will be made in the final section of this chapter.

Baxter and Illston (1977; 1980) have noted the incidence of the yeast *Candida saké* in soils from low-temperature habitats and on frozen foodstuffs. They found the yeast to have a temperature optimum of 15°C, and therefore classified it as a psychrophile. In view of the abundance of this yeast on new leaves early in the South Georgian spring, it seemed appropriate to measure the effect of temperature on growth of this species. Appendix 6 includes data concerning the assimilative capacity of the yeast, as measured by the National Collection of Yeast Cultures in Norwich.

Flanagan and Scarborough (1974) studied the enzyme complements of various fungi isolated from Arctic tundra sites and Britain. To determine fungal potential in decomposition it was assumed that if a fungus could oxidise a substrate *in vitro* it may be able to perform this function in the field. It was also assumed that the *in vitro* response would emulate the response in the field.

As plant senescence progresses, phylloplane fungi possessing cutinase, pectinase and cellulase can penetrate the cuticle and begin cell wall degradation. Although many saprophytes may be classed as cellulolytic, only a

few can degrade native cellulose. Reese *et al.* (1950) and Reese and Mandels (1967) proposed that cellulolytic activity involves three groups of enzymes:-

C_1, an exoenzyme converting cellulose to shorter chains of β-glucose units.

C_x, a second exoenzyme which hydrolyses these chains to cellobiose, -triose or -tetraose.

These glucosides are then further hydrolysed by β-glucosidase and utilised in metabolism.

Fungi are undoubtedly the most important decomposers of plant material (Anderson and Domsch, 1975). No single fungal species can use all plant components and it is now established that a succession of different groups of fungi will appear on different substrates (Kjøller and Struwe, 1980). Ideally, a determination of succession patterns should be accompanied by a study of fungal enzymes.

Koenigs (1972) reported the production of extracellular hydrogen peroxide by wood-rotting fungi. Reese (1975), reviewing the cellulase system, stated that H_2O_2 could complement the action of the enzyme by a preliminary catabolic action on cellulose fibrils. It therefore seemed appropriate to study the possibility that South Georgia fungal isolates could produce H_2O_2 to aid the decomposition process and hence increase nutrient cycling in the short summer season available for growth.

Of fourteen species isolated from Devon Island in

northern Canada, all could utilise humic acid as a substrate (Rosswall and Heal, 1975). Although at present no single enzyme has been found or even proposed for the decomposition of lignin, it is currently assumed that fungi capable of utilising gallic acid, tannic acid or humic acid and cellulose, are probably capable of some lignin degradation (Hurst, Burges and Latter, 1962). The Bavendamm reaction to test for polyphenol oxidase can help to clarify any lignin degradative capacity possessed by a fungus.

Microarthropods have been shown to have potentially important effects on organic matter decomposition and nutrient cycling. It is now generally accepted that the decomposition of litter involves both leaching and biotic processes (Parkinson, 1981). Although microarthropods can act as primary decomposers of litter *via* cellulolytic and ligninolytic enzymes in the gut passage (Neuhauser and Hartenstein, 1978), the major roles of these members of the soil fauna are presumed to be physical and chemical, e.g. fragmenting organic matter and altering the chemical nature of litter as it passes through the gut.

As *Chrysosporium pannorum* was a commonly isolated litter fungus, and has been isolated in abundance from Arctic and Antarctic litters alike, (Ivarson, 1973; 1974; Holding *et al.* 1974) a study was included of the effect of this fungus on litter decomposition rates.

It is hoped that the studies described in this chapter can lead to a better understanding of the decomposition process.

MATERIALS AND METHODS

1. Effect of Water Activity on Linear Extension Rate

Cultures of the five test fungi used for inoculum were grown at 20°C on one fifth strength MEA with supplemental agar at 5g $^{-1}$. Plugs of agar cultures were cut from margins of young, active colonies (4 - 7 days old), and these discs were then transferred to agars of defined water activity and subsequent growth measured from the base of each petri dish. Two measurements of colony diameter were made at right angles for each dish. Care was taken to prevent water loss by covering solutions while autoclaving, pouring plates only with cooled agar, and sealing plates with tape immediately after pouring.

All media used for assessing water activity effects had a nutrient base of KH_2PO_4 0.68g, K_2HPO_4 1.4g, $MgSO_4.7H_2O$ 0.12g, sucrose 4.00 g, $NaNO_3$ 0.68g, added to one litre of distilled water with 1% agar w/w.

Scott (1953) demonstrated that this concentration of agar had negligible effect on reducing water activity.

Water activities were adjusted using the solutions of Solomon (1951) and glycerol at varying concentrations, (See Appendix 4).

2. Effect of Temperature on Linear Extension Rate

Discs were cut from the margins of young active colonies of each test species and inoculated centrally

onto plates of Malt Extract Agar (MEA). Plates were incubated at a range of temperatures from 1°C to 30°C, and measured at intervals of one or two days until the colony grew to the edge of the petri dish. Two measurements were made of the colony diameter at right angles to each other on the base of the dish.

3. Effect of pH on Linear Extension Rate

Petri dishes were prepared containing Malt Extract Agar (MEA), buffered with sodium citrate and citric acid at pH 6.7 and 4.5 after autoclaving. Plates were centrally inoculated with discs cut from the margins of 4 - 7 day old, active colonies growing on MEA. Linear extension (colony diameter) was measured as described in the previous section at intervals until the side of the petri dish was touched. Incubation was at 20°C, three replicate plates were made per species.

4. Effect of Ultraviolet Light on Linear Extension Rate

Petri dishes containing MEA were centrally inoculated with discs cut from the margins of young, active colonies of the test species and incubated for 2 - 4 days at 20°C. Plates were then exposed to ultraviolet light* for a range of times from 0 - 60 minutes. Three replicate plates were made per treatment. Following irradiation, plates were returned to incubation at 20°C, and colony

*(See section 4 in chapter 4)

diameter was measured at intervals as previously described.

5. <u>Effect of Temperature on Growth Rate</u>

100 cm^3 Ehrlenmeyer flasks containing 20 cm^3 of Basic Liquid Medium (BLM) (See Appendix 7) were autoclaved at 121°C for 15 minutes and allowed to cool. Each flask was inoculated with a disc cut from the margin of a young, active colony of the test species, and flasks were incubated at a range of temperatures between 5° and 30°C on an orbital incubator and also in still culture. For dry weight measurements, mycelia were harvested by suction filtration onto preweighed, dried Whatman No. 1 filter papers. Papers and mycelia were then dried to constant weight, and the weight of mycelium calculated by subtraction. Growth rates were calculated during the log phase of growth, determined from previously constructed growth curves. At least three replicates were included for each weight measurement.

6. <u>Effect of Temperature on Growth Rate of Phylloplane Yeast</u>

The predominant yeast present on the phylloplane during the South Georgia spring was *Candida saké*. The growth rate (cell numbers) of this yeast was determined at a range of temperatures in order to estimate its optimum temperature.

100 cm^3 Ehrlenmeyer flasks containing 20 cm^3 aliquots of Yeast Extract Peptone Dextrose (YEPD) medium[1], were each inoculated with 0.1 cm^3 of a suspension of 1.1×10^7 cells cm^{-3}. Incubation was for a range of times from 3 - 50 hours at temperatures from 5° - 30^oC. Three replicate flasks were made per treatment. Counts of total cells were made using a haemacytometer. Viable cell numbers were estimated via a dilution plate count at 10^{-4} and 10^{-6} dilution factors on MEA at 20^oC incubation.

[1] Appendix 8

7. Substrate Utilisation and Extracellular Enzymes

A range of agars was prepared with the following single carbohydrate sources (see Appendix 8); cellulose (Eggins and Pugh, 1962) starch, casein, pectin, indolin (a synthetic lignin), chitin and tannic acid agar.

Four replicate plates of each agar were centrally inoculated with discs cut from the margins of actively growing colonies on Tap Water Agar supplemented with sucrose at $2gl^{-1}$. Control plates of basal agars without the sole carbohydrate source were also inoculated. Plates were incubated at $20^{o}C$ for thirty days,and examined for linear growth. The following sub-Antarctic isolates were tested; *Acremonium terricola, Botrytis cinerea, Chaetophoma spp.* (two species), *Chrysosporium pannorum, Cladosporium sphaerospermum, Fusarium lateritium* and *Mucor hiemalis.* Two isolates of *Chrysosporium pannorum* from Swedish soil were also included in the tests.

Drop tests were performed on the test species to test for extracellular cytochrome oxidase, peroxidase and laccase (p-diphenol oxidases). Colonies were grown on MEA at $20^{o}C$ for ten days before testing. Several different tests could be performed on one plate, as most of the reagents do not react.

a) Cytochrome oxidase, 20 mg of tetramethyl-p-phenylene diamine dihydrogen chloride was dissolved in $10cm^3$ of 15 parts per million ascorbic acid in water. Upon addition

to the colony margin, a blue discolouration is given by reaction with cytochrome oxidase. The test must be read before thirty minutes have elapsed, as atmospheric oxidation then rapidly takes place. Cytochrome oxidase and cytochrome reductase are present in varying amounts in every species. No colour reaction within two hours is regarded as a positive result for cytochrome reductase.

b) Peroxidase; Equal parts of 0.4% H_2O_2 and 1% pyrogallol in water, freshly prepared. A yellow-brown colouration after thirty minutes is a positive reaction.

c) Laccase; 0.1M α-naphthol in 96% ethanol. Presence of laccase is indicated by a purplish discolouration of the mycelium.

8. Effect of Temperature on Clearing Rate of Starch Agar

Starch agar (Appendix 8) plates were centrally inoculated with discs cut from the margins of 4 - 7 day old, active colonies. Incubation was at a range of temperatures for 4 - 7 days. Plates were then flooded with iodine in potassium iodide solution, and the area of starch clearing measured at two radii at right angles on the base of the colony. Three replicate plates were made for each treatment level.

9. Effect of Temperature on C_x Cellulase Activity

Aliquots of $30cm^3$ of cellulase production medium (see Appendix 7) in $100cm^3$ Ehrlenmeyer flasks were autoclaved and cooled. Flasks were inoculated with discs

cut from the margins of actively growing, young colonies on MEA, and incubated for nine days at 15°C on a rotary shaker at 80 - 100 r.p.m. Four replicate flasks were made per test fungus, using different colonies for each replicate. After incubation, colonies were removed from the medium by vacuum filtration, the medium was then centrifuged and filtered through cellulose acetate filters with 0.47 μm pore size. The method used by Gascoigne and Gascoigne (1960) was used to assay cellulase activity of filtrates.

$1cm^3$ aliquots of filtered medium were incubated with $9cm^3$ of carboxymethylcellulose (CMC) substrate (1% CMC in 0.055M sodium-citrate buffer at pH 5.4), in test tubes at a range of temperatures from 1° - 30°C. A control set of boiled medium, and one of medium to which $0.1cm^3$ of 0.5M Trichloroacetic acid had been added, were also incubated. Following incubation for twenty-four hours, the tubes were shaken thoroughly. $1cm^3$ of the enzyme-substrate mixture was removed from each tube and tested for reducing sugars using the dinitrosalicylic acid (DNSA) assay (Appendix 2). $3cm^3$ of DNSA reagent were added to the $1cm^3$ of enzyme-substrate mixture and boiled in a waterbath for fifteen minutes. The resulting colour was tested for absorbance at a wavelength of 540nm using a Beckman DB spectrophotometer, and the concentration of reducing sugar calculated from a previously constructed calibration curve of D-glucose in aqueous solutions.

The original, filtered media were also tested for reducing sugar concentrations. All solutions were read against a reagent blank.

10. Test for Extracellular Hydrogen Peroxide Production

Following the procedure of Koenigs (1972), extracellular production of H_2O_2 was detected by the growing of the test species on a heated blood medium similar in its blood component to a chocolate agar used to detect H_2O_2 with anaerobic bacteria; peroxide production was inferred when a green discolouration developed beyond or beneath colonies growing on the blood medium. The basal medium consisted of 2% malt extract, 2% glucose, 0.1% peptone and 2% agar, in 950 cm^3 distilled water. The medium was autoclaved for fifteen minutes and cooled to 80°C, and 50cm^3 of fresh, defibrinated blood was added, held at 80°C for ten minutes, and poured with constant stirring into petri dishes. (Medium pH was adjusted to 5.4 with concentrated HCl before autoclaving). Plates were centrally inoculated with discs cut from margins of actively growing colonies of the test fungi, and incubated at 20°C in the dark to prevent peroxide formation in the medium by light. Plates were examined after ten, twenty, forty and sixty days.

11. Effect of Temperature on Lag Phase of Colonies on Malt Extract Agar

Following the procedure outlined in part 2 of this

section, petri dishes of MEA were inoculated with discs of mycelium and incubated at 1°C. Measurements of colony diameter were made at intervals for a period of forty-eight days. Linear extension rate was calculated and plotted for each fungus over the growth period. Four replicate petri dishes were made per species, using different isolates for each replicate.

12. Effect of Litter Fungi upon Litter Decomposition Rate

Following the procedure of Ivarson (1974), wet litter collected from beneath *Poa* tussocks was placed into pre-weighed 100cm^3 conical flasks, roughly 15 - 20g per flask. The litter contained an active mycoflora and microfauna, as previously determined by microscopic examination and isolations on agar. Flasks were loosely sealed with aluminium foil and autoclaved at 121°C for fifteen minutes. A set of flasks was left unsterilised in order to assess the decomposition rate of active litter. Pilot tests had previously been made to determine the amounts of free carbohydrate present in the litter before and after autoclaving, and showed no significant difference to be caused.

Sets of flasks containing sterilised litter were inoculated with spores of:-

1) *Chrysósporium pannorum;* 2) *Chrysosporium pannorum* and *Mucor hiemalis*. A further set of flasks was incubated in a sterile condition. 5cm^3 aliquots of sterile distilled

water were added to each flask, and flasks were surrounded with plastic film to minimise water losses. Incubation was for eight months at 5°C, 20°C and in a 24hr freeze-thaw cycle of +5° to -5°C. Eight replicate flasks were made per treatment. Following incubation, flask contents were removed and examined, and then dried to constant weight at 60°C.

RESULTS

1. Effect of Water Activity on Linear Extension Rate

Results are shown in Fig. 32 and Appendix 9 . In general, all species displayed a higher linear extension rate on glycerol media than on the salts media. This indicates the influence of a factor or factors other than water activity having a effect on growth. Nevertheless, the results are comparable for both media types, in that the curves have similar forms, and the lower A_w limits are within 0.02 A_w of each other in each case except *Cladosporium* , which continued growth beyond the lower A_w limit of the experiment (i.e. 0.90). With the exception of *Cladosporium sphaerospermum*, all fungi showed a lower A_w limit for L.E.R. of between 0.90 and 0.92. *Mucor hiemalis* appears to be the least tolerant of low A_w values, showing no growth at A_w 0.94 on the salts media and none at 0.92 on glycerol.

Tests on *Cladosporium* have shown this fungus to be capable of growth at A_w 0.85 on glycerol media, but not on salts media. Studies have also shown that spore germination occurs at A_w 0.85 (Chapter 4).

2. Effect of Temperature on Linear Extension Rate

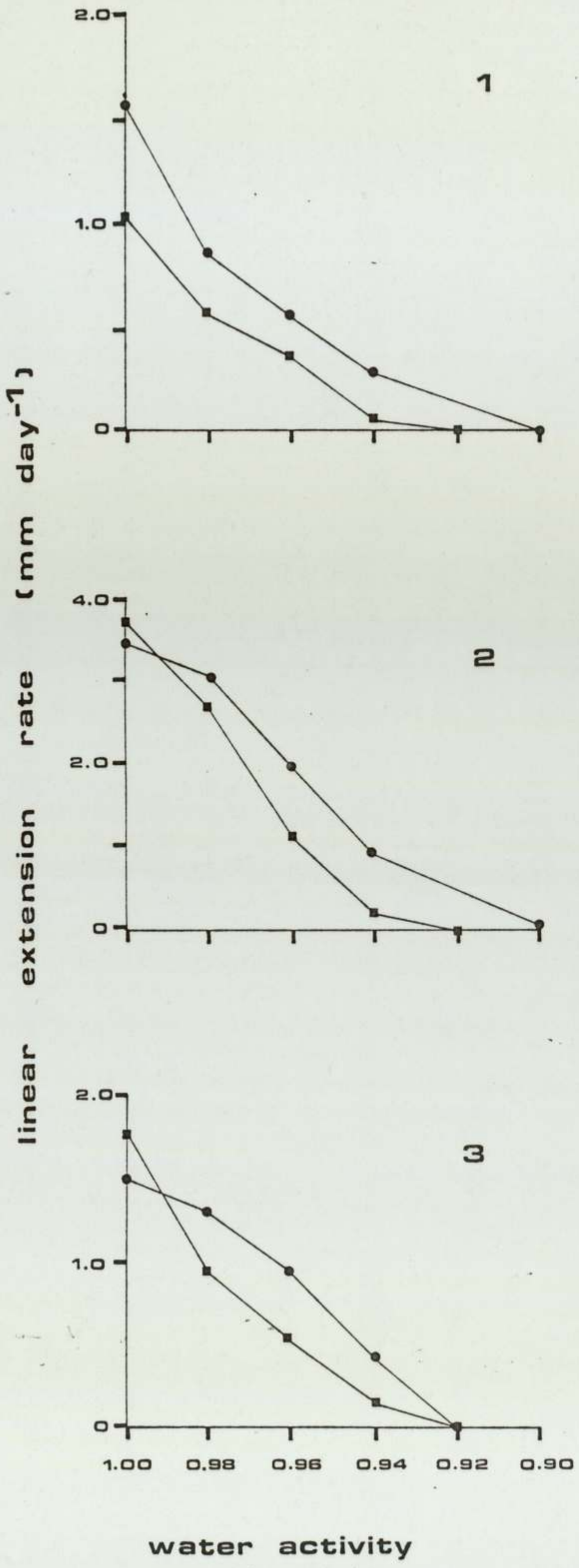
Results are shown in Fig. 33 and Appendix 10. Relatively low linear extension rates were recorded for *Chaetophoma* species, *Chrysosporium pannorum* and *Cladosporium sphaerospermum* over the temperature range tested, and rates

Fig. 32 Effect of Water Activity on Linear Extension Rate

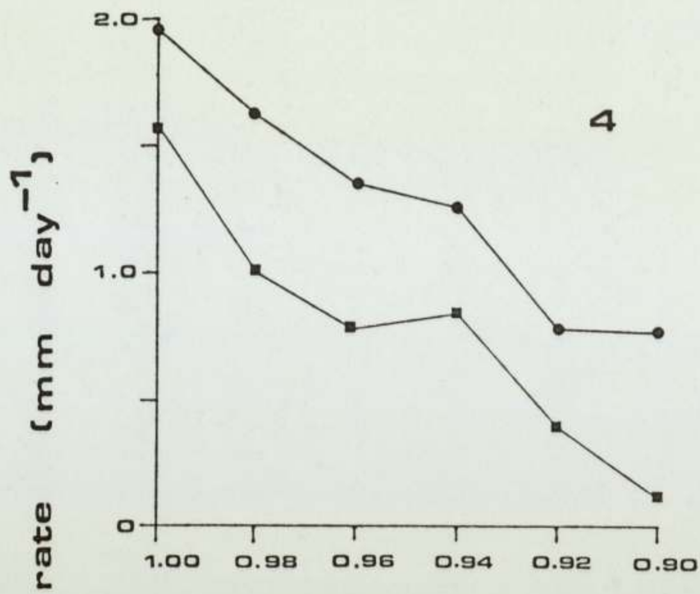
■ Salts media

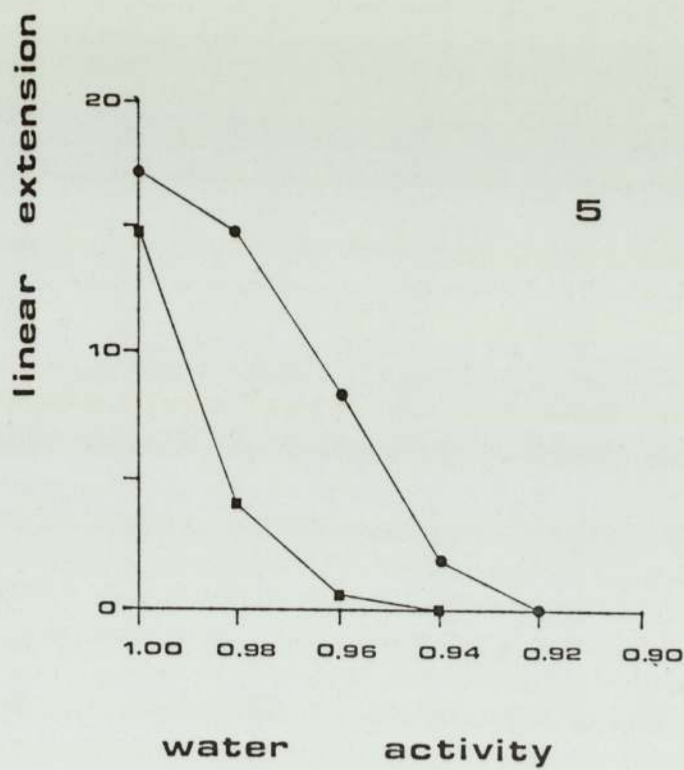
● Glycerol media

1. *Botrytis cinerea*
2. *Chaetophoma sp.*
3. *Chrysosporium pannorum*
4. *Cladosporium sphaerospermum*
5. *Mucor hiemalis*



2.0
1
1.0
0
4.0
2
2.0
0
2.0
3
1.0
0
1.00
0.98
0.96
0.94
0.92
0.90
linear extension rate (mm day^{-1})
water activity


2.0
1.0
0
1.00
0.98
0.96
0.94
0.92
0.90
4
linear extension rate (mm day^{-1})


20
10
0
1.00
0.98
0.96
0.94
0.92
0.90
5
water activity

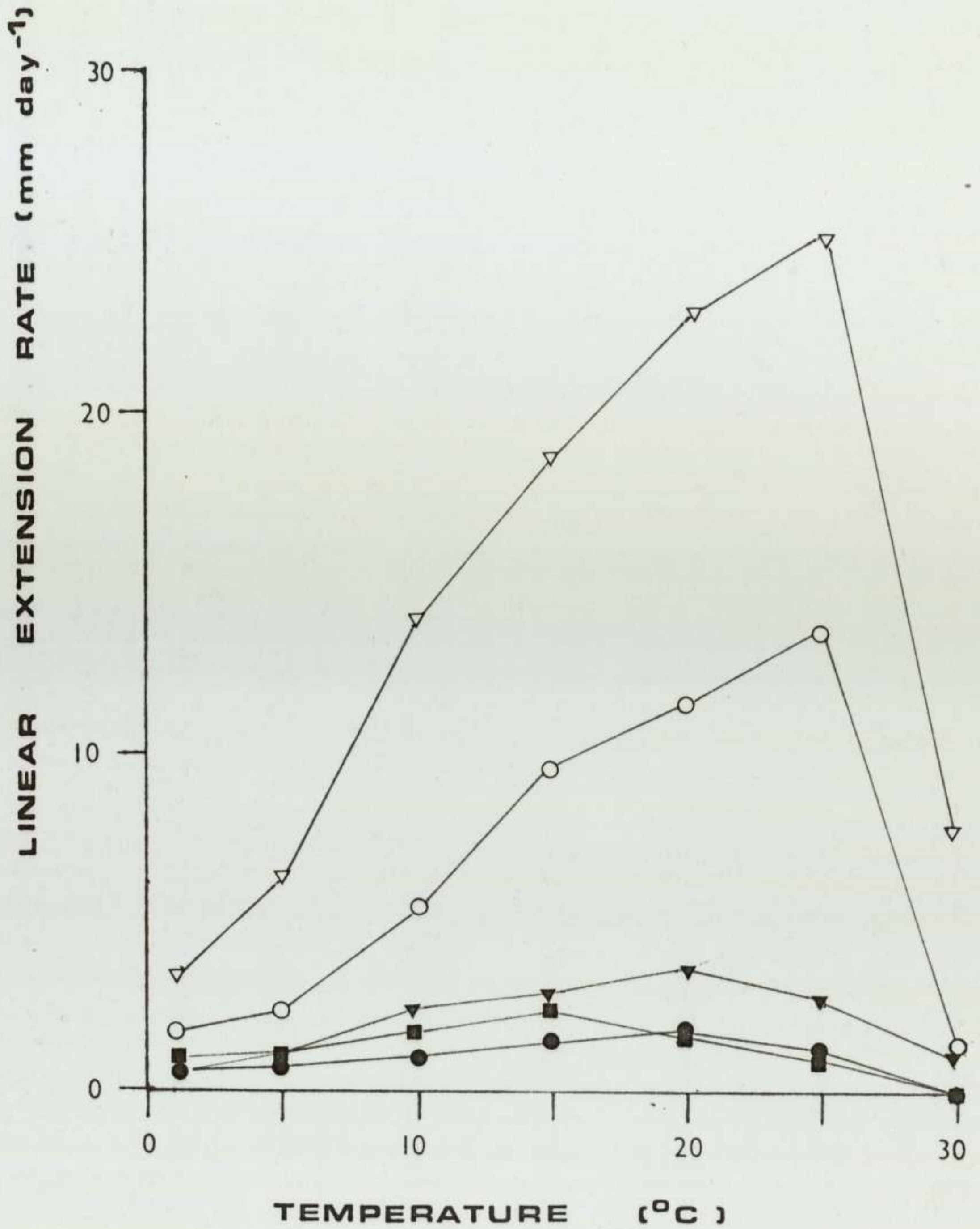


Fig.33 Effect of temperature on linear extension rate

○ *Botrytis cinerea*

▼ *Chaetophoma sp.*

● *Chrysosporium pannorum*

■ *Cladosporium sphaerospermum*

▽ *Mucor hiemalis*

were similar for each of these species. These three species, with relatively low extension rates, each exhibit a flattened growth curve with optimum growth temperatures not clearly defined. *Botrytis cinerea* and *Mucor hiemalis* have relatively high linear extension rates with sharper peaks near to their optimum temperatures. Both of the latter species have optima for linear extension at 25°C. Neither *Chrysosporium* nor *Cladosporium* were capable of linear extension at 30°C, whilst the remaining three species exhibited significant growth at this temperature. All species were capable of growth at 1°C, although lag phases were pronounced at this temperature (see section **11** , this chapter). Kuthubutheen (1977) found English isolates of *Chrysosporium pannorum* to have a temperature optimum for linear extension of 18°C, corresponding with these results.

3. <u>Effect of pH on Linear Extension Rate</u>

Results are presented in Appendix **11** . There was no significant difference between linear extension rates at each pH, rates being similar to those at 20°C in the previous section (agar at pH 4.5).

4. <u>Effect of Ultraviolet Light on Linear Extension Rate</u>

Results of linear extension rates of colonies following sixty minutes exposure to the ultraviolet source are shown in Appendix **12** . No significant differences resulted

from this exposure, which was equivalent to sixty minutes of exposure to strong sunlight, i.e. around 150 mw of ultraviolet light per square centimetre.

5. Effect of Temperature on Growth Rate

Results of growth rates in still flasks are given in Fig. 34 , whilst Fig.35 shows growth rates in agitated cultures. Tabulated data for both graphs is given in Appendix 13 . Aeration of cultures by agitation appears to affect growth markedly, reducing growth rates of some species and increasing those of others.

Botrytis cinerea appears to have an optimum temperature for dry weight growth of between 15° and 20°C. This is lower than its optimum for L.E.R., which was near 25°C. *Chaetophoma* species has a dry weight growth optimum of 20°C in both cases, but exhibits a significantly higher growth rate in still culture ($p > 0.01$). These optima correspond with the linear extension optimum of 20°C.

Chrysosporium pannorum showed maximum growth at 20°C in still culture, and between 15° and 20°C in shake culture, agreeing with the optimum temperature for linear extension rate.

Cladosporium sphaerospermum grew at a rate similar to *Chrysosporium* in both types of culture, and showed an optimum of 20°C in both cases.

Mucor hiemalis had a relatively low dry weight growth rate in agitated culture with an optimum at 20°C, whilst

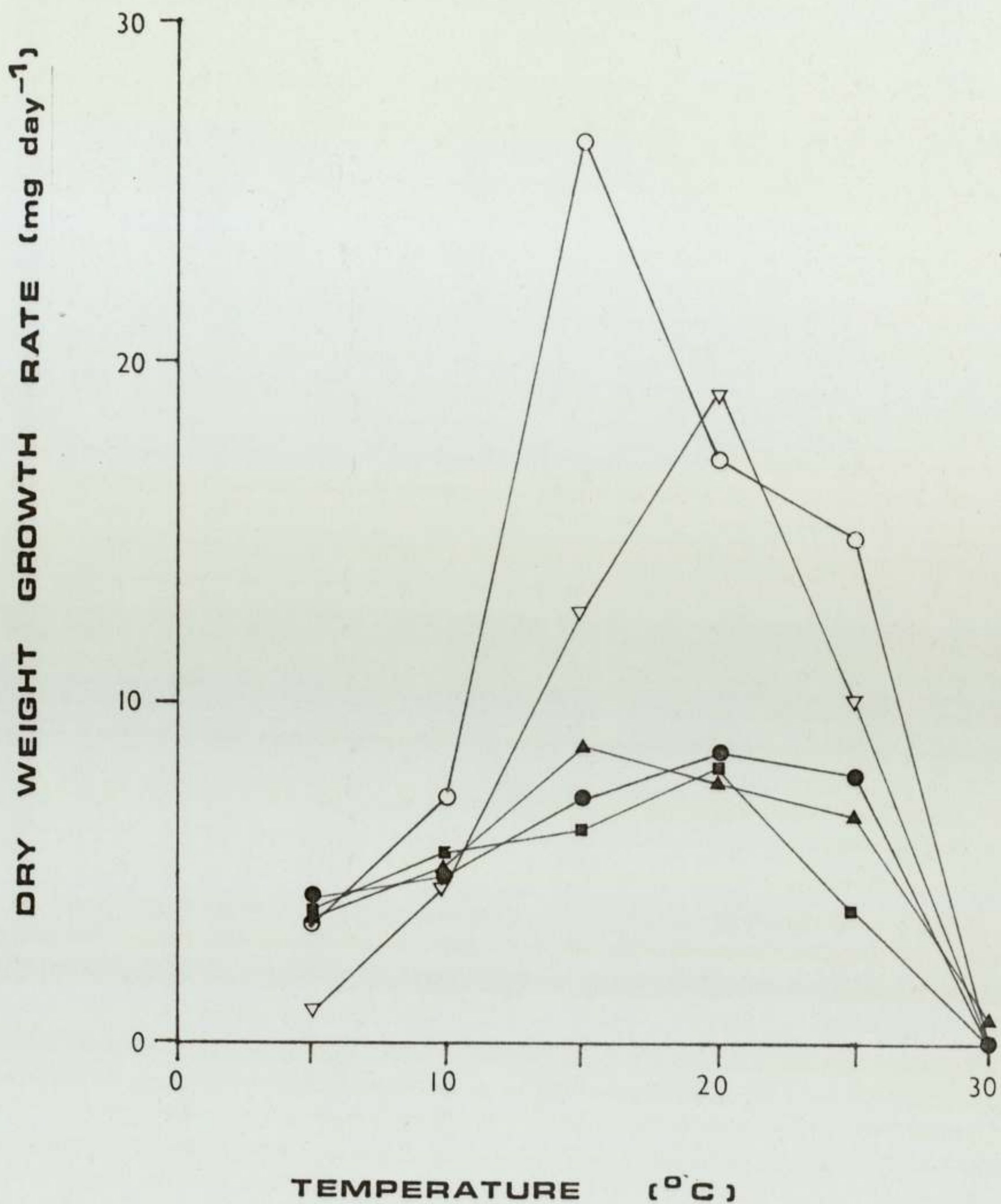


Fig. 34 Effect of temperature on growth rate - still culture

○ *Botrytis cinerea*

▲ *Chaetophoma sp.*

● *Chrysosporium pannorum*

■ *Cladosporium sphaerospermum*

▽ *Mucor hiemalis*

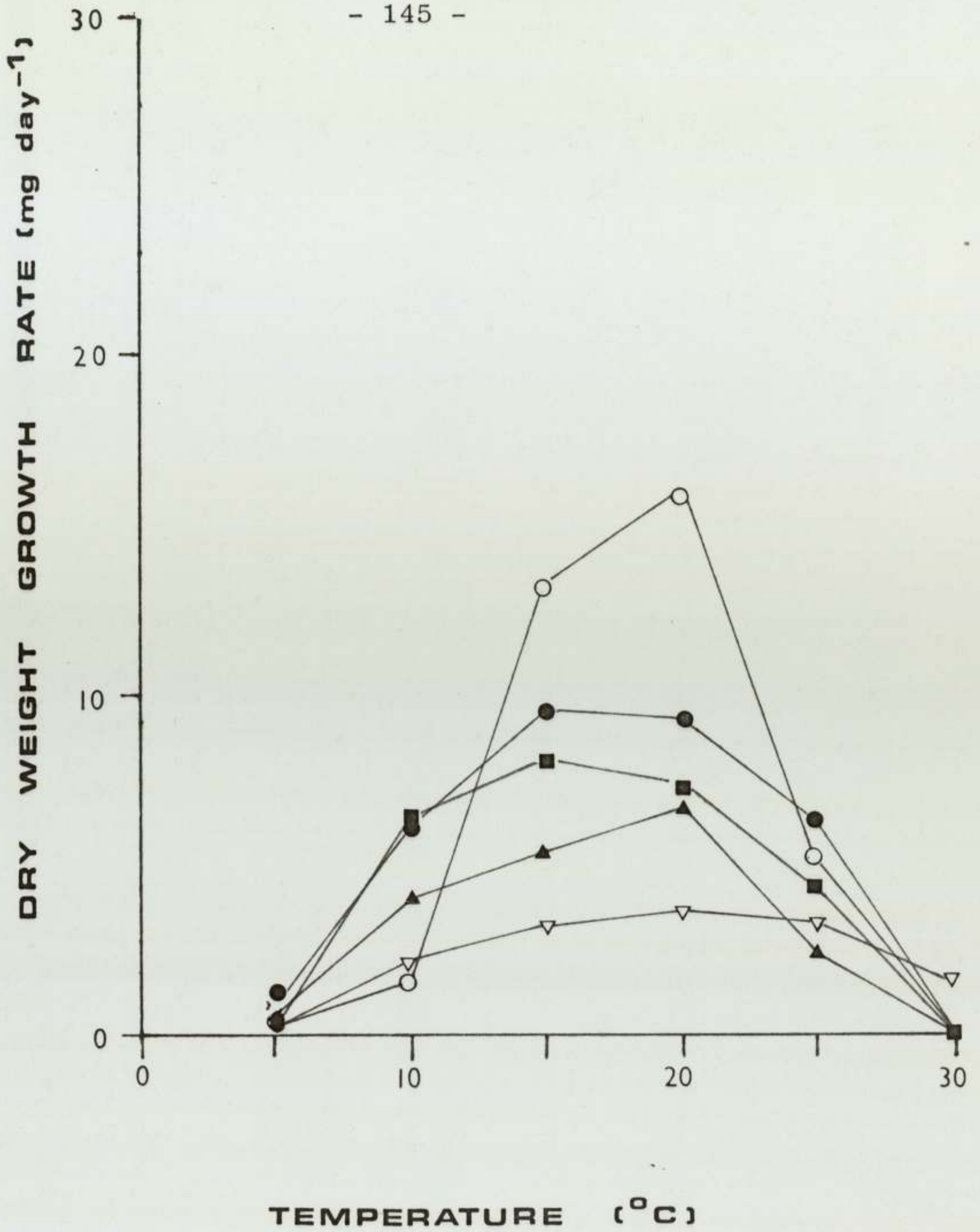


Fig. 35 Effect of temperature on growth rates in shake culture

○ *Botrytis cinerea*

▲ *Chaetophoma sp.*

● *Chrysosporium pannorum*

▽ *Mucor hiemalis*

■ *Cladosporium sphaerospermum*

in still culture the optimum was apparently near 15°C, with a growth rate comparable to that of *Chysosporium* and *Cladosporium*.

All fungi except *Mucor hiemalis* were incapable of growth at 30°C.

6. Effect of Temperature on Growth Rate of *Candida saké*

Total cell number and viable cell number after fifteen hours are plotted in Fig. 36 , and tabulated in Appendix 14.

Optimum growth by both measurements was between 20° and 25°C, although significant growth was apparent at 5°C. These results indicate that the yeast is mesophilic and is probably adapted to growth at relatively low temperatures.

7/8 Substrate Utilisation and Extracellular Enzymes

The results of growth on agars and the drop tests are tabulated in Fig. 37 , and Fig. 38 shows the clearing rates on starch agar of the four species tested. *Cladosporium* showed only a very weak growth on starch agar, similar to that on the control medium, and was presumed not to possess alpha amylase. Amylase activity in *Chrysosporium pannorum* was present but very weak.

All of the most commonly isolated species except *Mucor hiemalis* possessed both C_1 and C_x cellulase, and the fact that the majority of sterile mycelia isolated were capable of clearing cellulose agar suggests that these too possess the cellulase system. All species

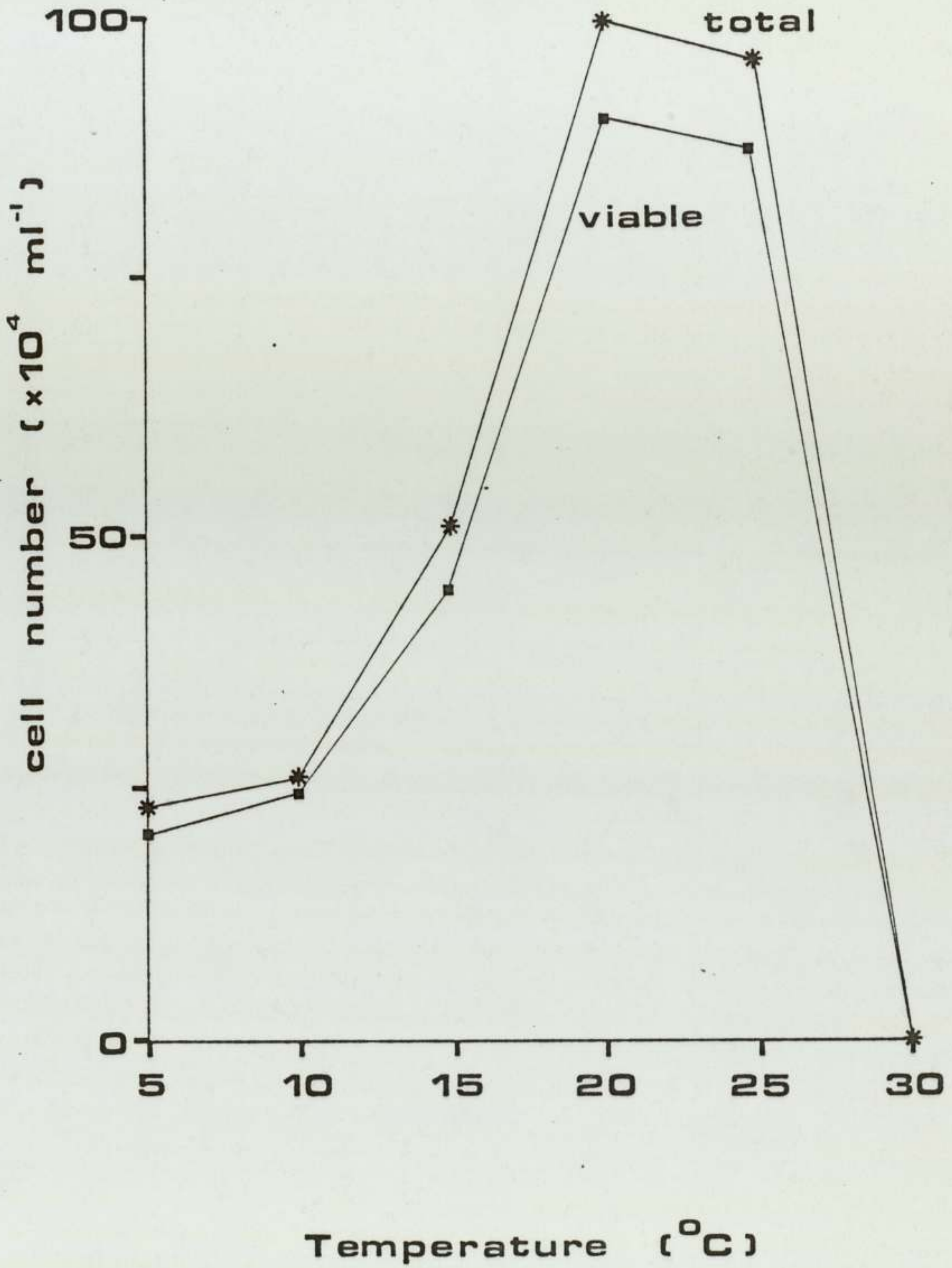


Fig.36,Effect of temperature on growth of *Candida saké*.

FIG.37 SUBSTRATE UTILISATION AND EXTRACELLULAR ENZYMES

SPECIES	CELLULASE	ALPHA AMYLASE	PROTEASE	PECTINASE	TANNIC ACID GROWTH	CYTOCHROME OXIDASE	LACCASE	PEROXIDASE	CHITINASE	INDOLIN GROWTH
ACREMONIUM TERRICOLA	+	+	+	+	+	-	+	-	N.T	N.T.
BOTRYTIS CINEREA	+	+	+	(+)	+	+	-	-	+	+
CHAETOPHOMA SP.1	+	+	+	+	+	+	-	+	+	-
CHAETOPHOMA SP.2	+	+	+	+	N.T.	-	N.T.	+	+	N.T.
CHRYSOSPORIUM PANNORUM (SWEDISH)	+	(+)	+	(+)	+	N.T	N.T	N.T	N.T	N.T.
CHRYSOSPORIUM PANNORUM (ANTARCTIC)	+	+	+	(+)	+	+	-	N.T.	-	-
CLADOSPORIUM SPHAEROSPERMUM	+	-	+	N.T.	+	N.T.	-	N.T.	-	-
FUSARIUM LATERITIUM	+	+	+	+	+	N.T	-	-	N.T	N.T
MUCOR HIEMALIS	-	(+)	+	+	-	-	-	-	(+)	-

+, ACTIVITY OR GROWTH; -. NO ACTIVITY OR GROWTH; (+), WEAK ACTIVITY OR GROWTH;

N.T. DENOTES NOT TESTED

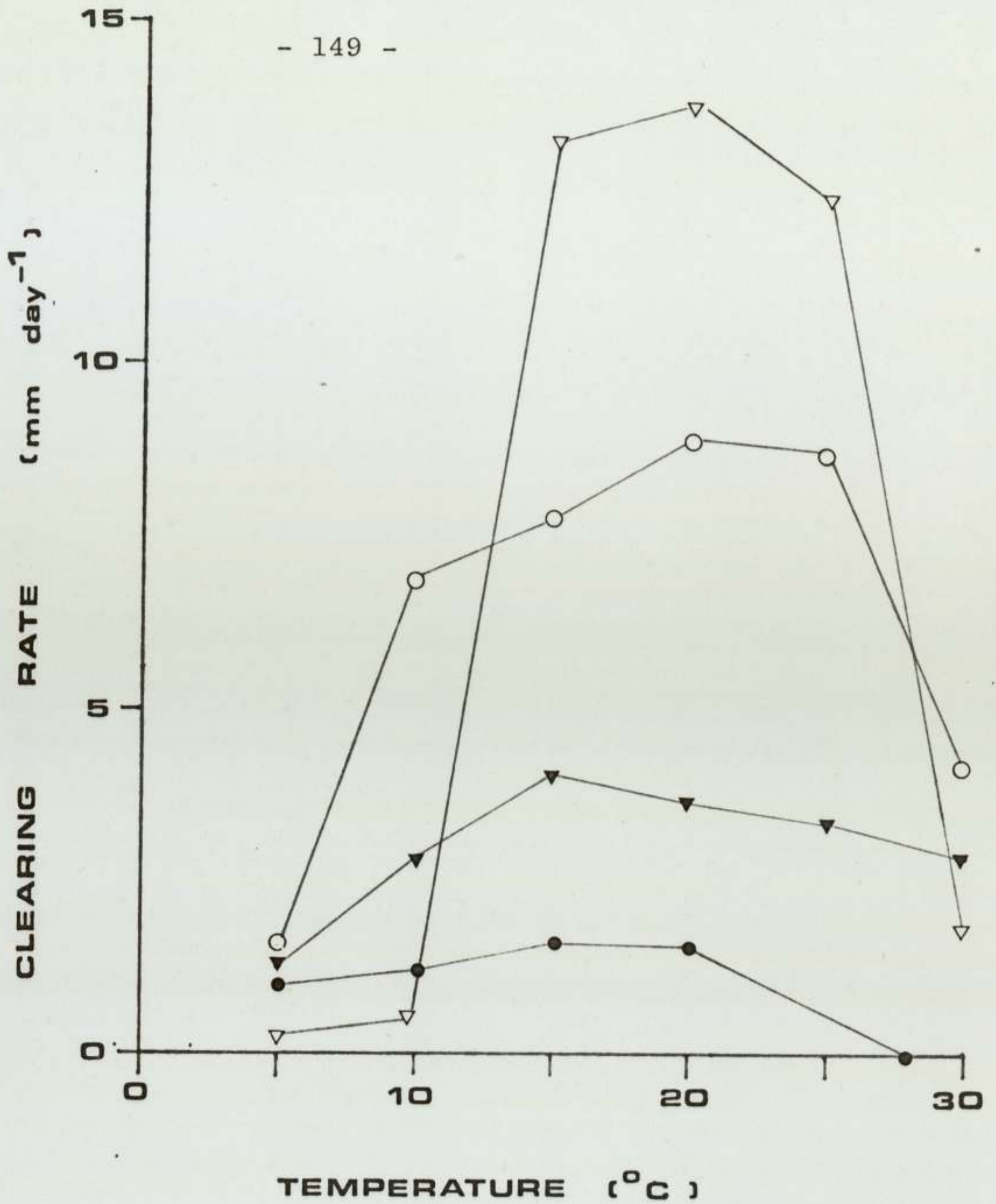


Fig. 38 Effect of temperature on clearing rate of starch agar

○ *Botrytis cinerea*

▼ *Chaetophoma sp.*

● *Chrysosporium pannorum*

▽ *Mucor hiemalis*

Fig. 39. Clearing Zones on Starch Agar ($mm.day^{-1} \pm S.D.$)

SPECIES	TEMPERATURE °C					
	5	10	15	20	25	30
Botrytis cinerea	1.53 ± 0.10	6.81 ± 0.32	7.74 ± 1.97	8.93 ± 1.42	8.71 ± 1.01	4.14 ± 1.17
Chaetophoma sp.	1.33 ± 0.46	2.83 ± 0.68	4.00 ± 0.28	3.67 ± 0.15	3.33 ± 0.47	2.83 ± 0.82
Mucor hiemalis	0.25 ± 0.01	0.50 ± 0.68	13.18 ± 3.85	13.75 ± 2.78	12.38 ± 4.83	1.75 ± 0.26
	TEMPERATURE °C					
	5	10	18	25	28	
Chrysosporium pannorum	0.96 ± 0.03	1.16 ± 0.36	1.55 ± 0.61	1.47 ± 0.89	0.00	

Cladosporium showed very weak amylase activity and growth

possessed protease, indicating an ability to utilise proteins within plant cells if these are made accessible through the action of other enzymes or plant damage.

Mucor hiemalis showed strong pectinase activity, a surprising result for this species but indicating the capacity to degrade the middle lamellae. This combination of pectinase and protease activity in *Mucor* suggests that the fungus can exhibit growth within the lamina, although the mechanism of penetration of the cuticle remains unclear. The enzyme complement of this species may account for its succession after the early spring dominance of yeasts as leaf surface carbohydrates become more scarce.

Botrytis cinerea, Cladosporium sphaerospermum and *Acremonium terricola* gave a positive reaction to the Bavendamm test, although only *Acremonium terricola* gave a positive result for the laccase drop test, and only *Botrytis cinerea* was capable of clearing indolin agar. No species was capable of growth on tapwater agar and supplemental indolin. These results suggest that *Botrytis cinerea* can degrade lignin, and there is a strong probability that *Acremonium terricola* also has this capacity, although further tests are required. *Botrytis cinerea* was commonly isolated from litter of *Acaena magellanica*, and *Acremonium terricola* was present in the grass litters. Both litters contain significant amounts of lignin, woody stems of *Acaena* having relatively high lignin contents.

Botrytis cinerea and *Chaetophoma* species had strong chitinase activity, clearing liquid culture media completely of chitin, and *Mucor hiemalis* showed a weak but definite chitinase activity.

9. Effect of Temperature on C_x Cellulase Activity

Figs. 40 and 41 show the responses of the C_x enzyme of each species to temperature, expressed as reducing sugar concentration and as concentration per unit dry weight respectively. *Botrytis cinerea* and *Chrysosporium pannorum* show high cellulase activity relative to other species tested, and all species exhibit a significant activity at $1^{o}C$. The temperature optima for the cellulases of *Botrytis cinerea* and *Chrysosporium pannorum* are reasonably well defined, and correspond well with the respective optimum temperatures for growth. Results are tabulated in Fig. 42.

10. Extracellular Hydrogen Peroxide Production

None of the species tested gave a positive result for the production of peroxide. *Chaetophoma* species, *Chrysosporium pannorum* and *Cladosporium sphaerospermum* cleared the agar after five days, indicating some protease activity and the ability to degrade cytoplasmic components.

11. Colony Growth at $1^{o}C$

Results are shown in Figs. 43 and 44, the latter comparing Swedish and Antarctic isolates of *Chrysosporium*

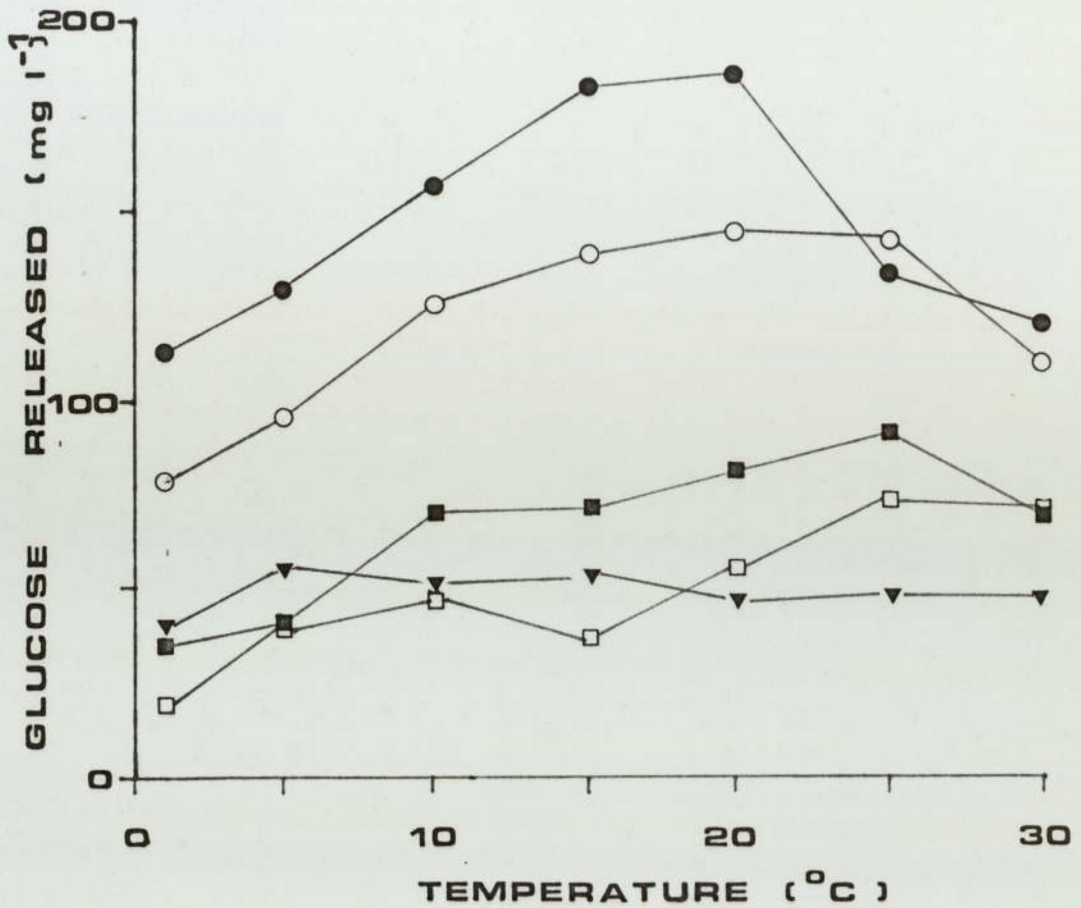


Fig.40 Effect of incubation temperature on C_x cellulase activity.

○ - *Botrytis cinerea*

■ - *Chaetophoma sp.1*

▼ - *Chaetophoma sp.2*

● - *Chrysosporium pannorum*

□ - *Cladosporium sphaerospermum*

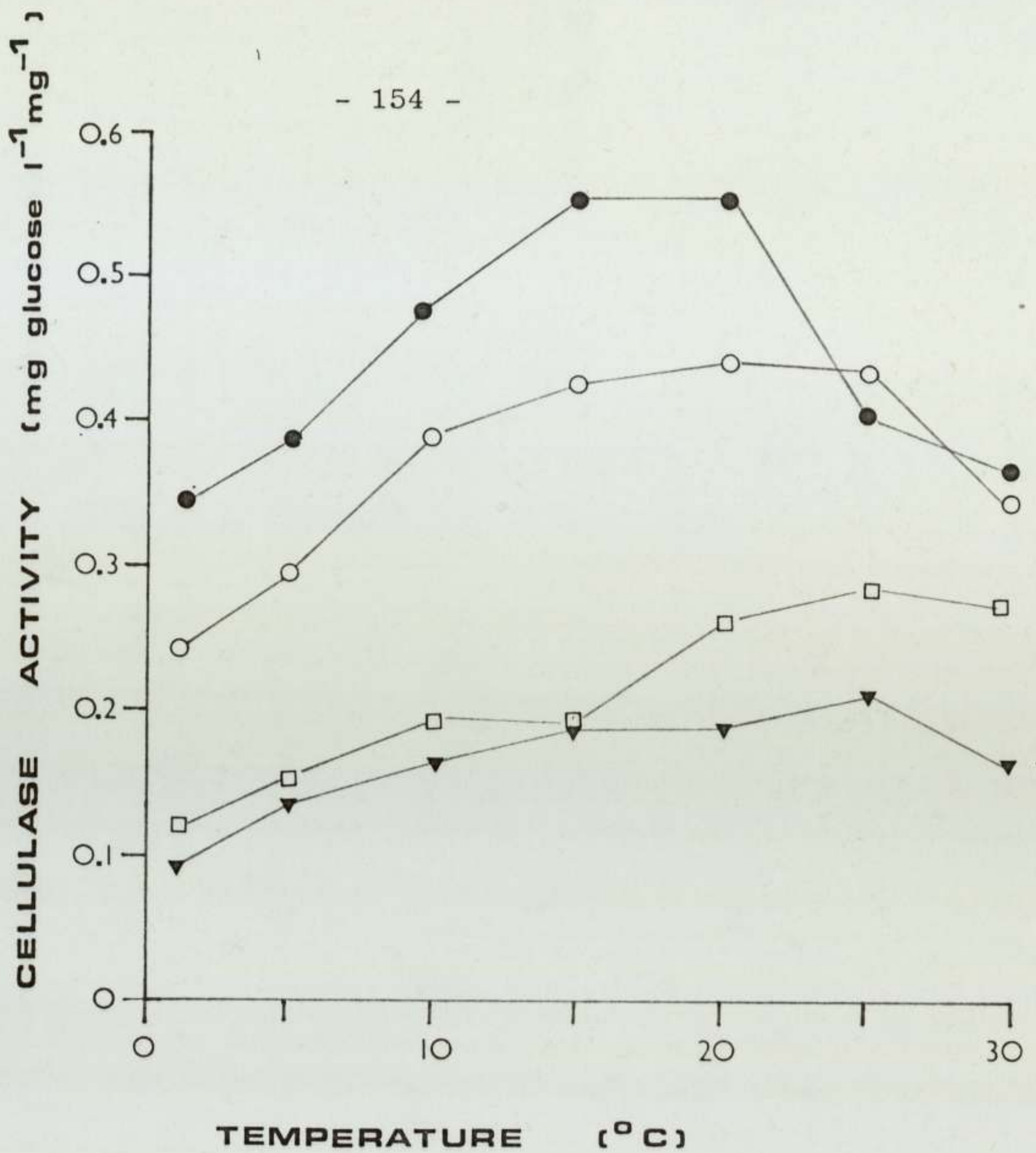


Fig. 41 Effect of temperature on C_x cellulase activity

○ *Botrytis cinerea*

▼ *Chaetophoma sp.*

● *Chrysosporium pannorum*

□ *Cladosporium sphaerospermum*

Fig.42 , Effect of temperature upon cellulase activity (mg glucose released ml^{-1} medium)($\pm$ S.D.)

SPECIES		INCUBATION TEMPERATURE (^{o}C)						
		1	5	10	15	20	25	30
Botrytis cinerea	mean	79.0	95.8	126.6	137.6	144.0	142.6	108.4
	S.D.	22.89	34.11	42.11	51.75	51.78	62.38	20.79
Chrysosporium pannorum	mean	112.4	128.4	156.8	181.0	185.4	133.0	119.2
	S.D.	7.23	24.64	9.55	8.31	8.47	9.11	8.81
Cladosporium sphaerospermum	mean	20.0	40.33	47.33	37.0	55.33	72.67	70.00
	S.D.	34.64	35.22	41.19	82.05	49.65	22.03	14.53
Chaetophoma sp.	mean	34.60	40.80	69.20	71.60	80.60	90.60	69.60
	S.D.	31.68	38.22	13.52	44.52	22.48	28.11	8.96
Chaetophoma sp. (S140)	mean	40.0	56.4	49.60	53.60	46.00	48.00	46.8
	S.D.	0	1.82	8.79	7.67	8.22	11.51	9.34

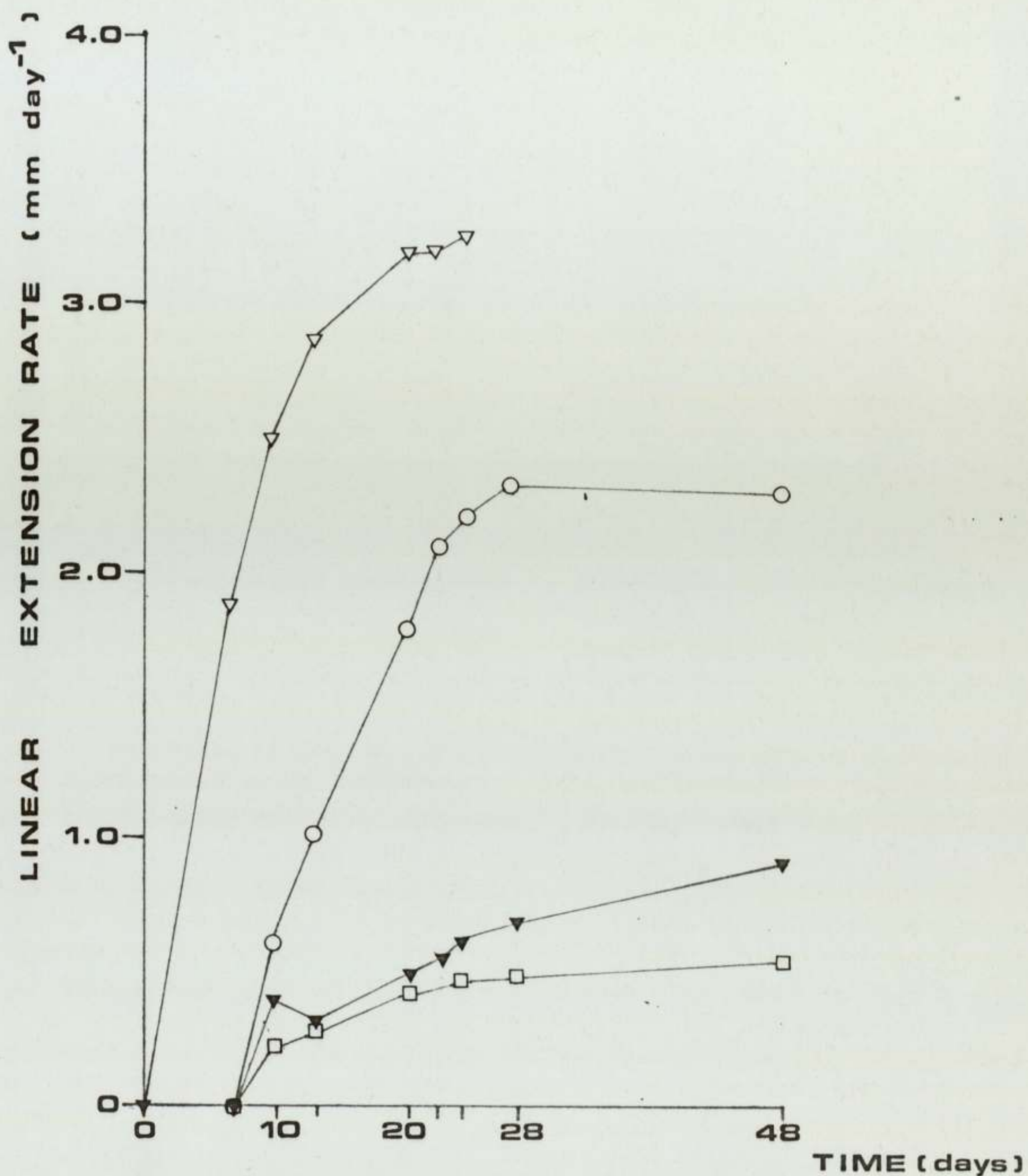


Fig. 43 LINEAR EXTENSION RATES AT 1°C

○ *Botrytis cinerea*

▼ *Chaetophoma sp.*

□ *Cladosporium sphaerospermum*

▽ *Mucor hiemalis*

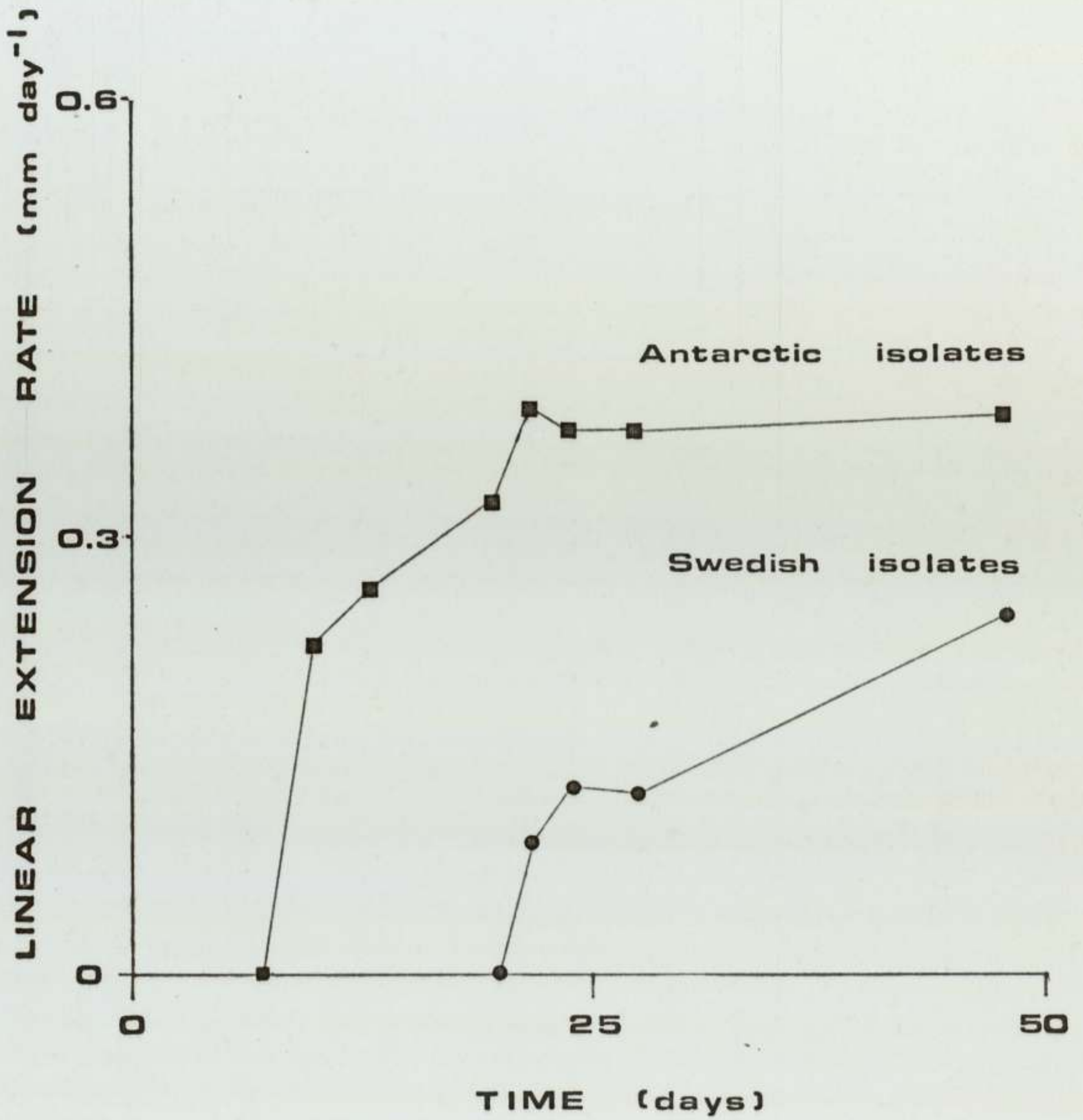


Fig. 44 <u>Comparison of Temperate and Antarctic strains of *Chrysosporium pannorum*.</u>
Growth at 1°C.

pannorum. Results are tabulated in Appendix 14. After ten days all Antarctic species were growing. *Botrytis cinerea*, *Cladosporium sphaerospermum* and *Mucor hiemalis* showed maximum growth after approximately twenty-eight days, whilst *Chaetophoma* had not reached maximum extension rate after forty-eight days. The Antarctic strains of *Chrysosporium pannorum* reached maximum extension rate at twenty-five days, whilst the Swedish strains had lag phases of at least twenty days, and were not showing maximum extension rates after forty-eight days at 1°C. These species all had lag phases of less than two days at 20°C, and all attained maximum extension rate after three days.

12. Effect of Litter Fungi upon Decomposition Rate

The results are tabulated in Fig. 45, and show a decrease in litter dry weight for cultures inoculated with *Chrysosporium pannorum* which is not significantly different from that of non-sterile litter at 20°C. Addition of *Mucor hiemalis* to cultures of *Chrysosporium pannorum* on sterile litter caused no significant increase in decomposition rate above that of non-sterile litter. Due to experimental difficulties the results of the study of freeze-thaw action cannot be quantitatively expressed. It was noted, however, that those flasks containing *Chrysosporium pannorum* and flasks of non-sterile litter were apparently in a further state of decomposition

FIG.45 EFFECT OF *CHRYSOSPORIUM PANNORUM* AND *MUCOR HIEMALIS* ON DECOMPOSITION OF *POA* LITTER

TREATMENT	TEMP. (^{o}C)	PERCENTAGE LOSS OF DRY WEIGHT ($\pm$ S.D.)
1. *CHRYSOSPORIUM PANNORUM*	20	67.88 $\pm$ 5.30
	5	71.95 $\pm$ 1.85
2. *C. PANNORUM/MUCOR HIEMALIS*	20	70.60 $\pm$ 2.23
	5	67.06 $\pm$ 3.66
3. STERILE LITTER	20	6.86 $\pm$ 13.32
	5	9.71 $\pm$ 27.50
4. NON-STERILE LITTER	20	68.04 $\pm$ 2.64
	5	64.89 $\pm$ 2.77

relative to other flasks. Litter was matted, amorphous and wetter than control (non frozen) litter. Extensive further study of freeze-thaw action is required.

Chrysosporium pannorum on sterilised litter showed a significantly higher decomposition rate than non-sterile litter at 5°C incubation ($p > 0.05$). In each of the treatments, incubation temperature produced no significant effect on decomposition.

All of the non-sterile and inoculated treatments were significantly different from controls ($p > 0.001$).

DISCUSSION

Of the species tested, only *Cladosporium sphaerospermum* can be classed as moderately tolerant of low water activity, in agreement with results of other workers, and similar to the response of the closely related species *Cladosporium cladosporioides* and *Cladosporium herbarum* (Hawker, 1950; Dickinson, 1981). Possibly, this ability to survive in relatively dry, hostile habitats could be a major factor in the survival of *Cladosporium sphaerospermum* in the phylloplane as the water content of dying leaves decreases. *Chrysosporium pannorum* has a tolerance of lower A_W values than the remaining species, and this could be of importance in the drier litter fractions in tussocks and on standing dead leaves.

Only fungi which are capable of resisting the fluctuating water contents of dead leaves will be able to grow there. Fungi growing on material with high water contents are probably restricted there by their high water requirement (Hudson and Webster, 1958; Hudson, 1968).

Webster and Dix (1960) showed that their group of initial colonisers usually had a more rapid germination and growth and a better ability to grow at lower relative humidities than did fungi from other groups. These abilities favour establishment and growth on substrates with fluctuating water contents. The salt concentrations used in these media to adjust A_W appeared to have a det-

rimental effect on growth, probably by ionic interactions across the cell membrane and a disruption of active transport processes. Nevertheless, the responses of the test species were comparable on both agar types, and the results seem justified.

The responses of the test species to incubation temperature indicate that all are mesophilic but psychrotolerant. All could grow at $1^{o}C$, and all could survive freezing to $-5^{o}C$. As the mean tundra field temperature during the summer months is approximately $5^{o}C$ on South Georgia, it was originally thought that psychrophiles would be predominant. Other Arctic and Antarctic studies have provided results similar to those found in this report, i.e. maximum growth in tundra fungi is generally at $20^{o}C$, suggesting cold tolerance rather than cold adaptation (Latter and Heal, 1971; Rosswall and Heal, 1975). It appears that the phylloplane species are capable of growth at low temperatures, but can take advantage of periods of relatively high temperature when they occur, to produce maximum growth. It is possible that psychrophilic fungi, with their inherently slower overall growth rates, are unable to compete with cold tolerant mesophiles, and are, therefore, ousted, especially during the summer months (Ingraham and Stokes, 1959). Beneath snow cover and ice during the winter months, there is a possibility that any psychrophiles which are present can dominate

the mycoflora until spring, but as yet no studies have been made on this topic. Latter and Heal (1971) studied growth rates of fungi from temperate, Arctic and Antarctic sites and concluded that most cold tolerant fungi were capable of adaptation such that their growth rate at $1^{o}C$ was at least 10% of that at the optimum temperature, in accordance with the results obtained in this study.

Fungi seem relatively tolerant of pH changes, and usually show sharp decreases in growth at their pH limits (Hawker, 1950). When pH does effect fungal growth, it is apparently due to an alteration of active transport processes over the cell membrane (Burnett, 1976). This study has shown no effect of either pH or ultraviolet irradiation upon germination and growth within the range of values found for these parameters in the field, and it is suggested that they are not of significance in determining species composition on the phylloplane.

The dominant phylloplane yeast, *Candida sakē*, was shown to have optimum growth at 20° - $25^{o}C$ and is best classed as a mesophile by this result. This yeast has previously been reported from a high altitude environment in New Zealand, with a temperature optimum of 15° - $20^{o}C$ (Baxter and Illston, 1977; 1980).

The most commonly isolated phylloplane species exhibit a range of enzyme complements sufficient to degrade almost all components of the substrate upon which they were growing. Cellulase was possessed by almost all

species, including the ubiquitous sterile mycelia, and appears to be an important factor in determining those species which can compete on the phylloplane. Fungi are the most important decomposers of plant material. No single species is able to utilise all components, and it is now well established that a succession of fungal groups will appear on leaf substrates with ageing (Anderson and Domsch, 1975; Kjøller and Struwe, 1980).

Any attempted determination of succession should be accompanied by a study of the enzymes active in the decomposition of the substrate. Garrett (1963) uses the exploitation of carbon compounds as the basic explanation for his scheme of fungal succession, and this trend shows in most later reports on succession (Gray and Williams, 1971; Widden and Parkinson, 1973; Flanagan and Scarborough, 1974; Visser and Parkinson, 1975).

Numerous studies have been made of nutrient loss from Arctic tundra litter (Dowding, 1974; Rosswall, 1974; Heal and French, 1974), but little work has been reported on a typical Antarctic tundra (Allen *et al.* 1967; Northover and Allen, 1967).

Walton and Smith (1979) made a study of chemical composition of new leaves and standing dead leaves of various higher plants from South Georgia, from which protein contents could be calculated. They showed that the protein levels in standing dead leaves of *Acaena* and *Festuca* remained constant throughout the summer. *Acaena*

new leaf protein content declined steadily as summer progressed. In all cases the protein content of new leaves was higher than that of dead leaves, and *Acaena* leaves had a higher protein content than that of either monocotyledon host species.

Kjøller and Struwe (1980) found that cellulose decomposing ability was always associated with the ability to degrade either pectin or starch. They found pectin utilisation to be the most common feature of their phylloplane species.

Although the role of fungi in decomposing fungal mycelium is uncertain (Domsch, 1960) some *Mortierella* species were found by Kjøller and Struwe to be capable of chitin utilisation. Four of the species tested in this study exhibited extracellular chitinase. This could be an important factor in antagonism and in chitin degradation in the litter layer.

Cellulase activity at relatively low temperatures was significantly high, indicating an ability to utilise this resource even under a cold temperature regime. This is an important factor, coupled with the capacity for growth at low temperature, which probably enables the mycoflora to continue litter degradation during winter months.

Chrysosporium pannorum exhibited a capacity for litter degradation which equalled that in non-sterile litter

at 5°C, and there is no reason to suppose that degradation by this psychrotolerant species should not continue throughout the year, thereby providing an efficient system for the prevention of peat accumulation. Peat formation in such an ecosystem may have a profound effect on nutrient availability as nutrient turnover is decreased. It appears that the ecosystem has developed to produce the maximum rate of nutrient cycling in order to conserve resources.

The South Georgia microfungal community is adapted to achieve maximum degradation under the relatively cold conditions of the tundra, but can take advantage of high temperatures in order to increase decomposition in summer.

CHAPTER 6

COLONY INTERACTIONS

INTRODUCTION

It has long been known that many fungal species possess the ability to inhibit the growth of other colonies (Brian, 1960). More recently, studies have been made on the nature of the inhibitors themselves, and their modes of action (Fries, 1973; Harman *et al.* 1980). Such studies require a far greater understanding of membrane and cell-wall synthesis in the fungal hypha before a complete picture may be drawn.

In the context of competition and growth on the phylloplane, fungal inhibition could be an important factor determining succession or the maintenance of dominance of a particular species after its optimal growth conditions have ceased.

Harman *et al.* (1980) found that as little as 200 parts per billion of 2, 4 - hexadienal in the atmosphere stimulated germination of *Alternaria alternata* spores in a soil-imposed stasis. Compounds similar to those stimulating germination also inhibited sporulation of thalli at higher concentrations. Clearly, the presence of inhibitors in a field ecosystem, albeit as yet undemonstrated, could have profound effects upon fungal growth or species composition.

In this part of the study, efforts have been made to determine the presence or absence of both volatile

and non-volatile inhibitors produced by the five main species, and their effects upon the growth and germination of other colonies. The methods used are similar to those used by previous investigators in this field (e.g. Dennis and Webster, 1971a).

MATERIALS AND METHODS

1. Effect of fungal filtrates on spore germination

Spore suspensions were prepared as described previously, from young, active colonies of each fungus, and adjusted to approximately 50,000 spores cm^{-3} by dilution. Colonies of each species were grown on Basic Liquid Medium for seven days at $15^{o}C$ in shake culture. Colonies were then harvested onto Whatman number 1 filter papers by vacuum filtration and then discarded. The filtrates from four culture flasks were pooled, and filtrates were ultra-cleaned using 0.22 μm cellulose acetate filters under vacuum filtration in aseptic conditions. 0.1 cm^3 of cleaned filtrate and 0.1 cm^3 spore suspension were mixed on a glass slide in a damp chamber (petri dish and filter paper) and incubated for 24 hours at $15^{o}C$. Following incubation a coverslip was placed over the suspension and the slide was removed for microscopic examination. Five replicates were made per test interaction.

2. Production of non-volatile antibiotics - dry weight increase

Filtrates of the five main species were prepared as described in the previous section. 5 cm^3 aliquots of either autoclaved or unautoclaved filtrate were added to 100 cm^3 conical flasks containing 20 cm^3 of fresh Basic Liquid Medium (BLM). Flasks were inoculated with discs cut from the margins of young, active colonies growing

on MEA, and incubated for six days at 15°C. Colonies were harvested onto Whatman No.1 filter papers, rinsed with distilled water, and the dry weight determined. Five replicates were made per treatment.

3. Production of volatile antibiotics - linear extension

Following the procedure of Dennis and Webster (1971c), cultures were grown on MEA in petri dishes for two to six days. Plates were inoculated centrally with agar discs cut from the margins of young, active colonies. After the initial incubation period, the lid of each dish was replaced by the base of a second dish of MEA inoculated with a test fungus. The two dishes were taped together with adhesive tape. Lids of control plates were treated in the same way, but replaced with uninoculated bases. Test plates and controls were set up in triplicate. After a further two, and five days of incubation at 20°C, the colony diameters of the test fungi were measured, and compared with controls.

4. Colony Interaction on MEA

Malt Extract Agar plates were inoculated with two discs cut from margins of young, active colonies. The discs were placed 3 cm apart along a line drawn through the diameter of each plate. Incubation was at 15°C until the colonies met on the plate diameter. Colonies were examined by microscope, and any apparent interaction was described.

RESULTS

1. Effect of Filtrates on Spore Germination

Results are shown in Fig.46 , and in many cases indicate a significant effect of the filtrates on germination. When the experiment was repeated using buffered medium at pH 4.6, no significant differences in germination resulted from addition of the buffered filtrates. Neither was any significant change recorded when host plant exudates were utilised as media for production of filtrates (host plant exudates were found to buffer pH changes when used as media). The pH of unbuffered Basic Liquid Medium following colony growth for seven days was found to be as low as pH 2.8 in many cases, whilst plant exudates maintained a pH of 5.5 - 6.5 with comparable fungal activity.

2. Production of Non-Volatile Antibiotics

Neither autoclaved nor unautoclaved filtrate caused any significant change in dry weight increase over control flasks at the concentrations used.

3. Production of Volatile Antibiotics

Results are shown in Fig.47 after five days of growth. No significant differences in linear extension rate resulted with *Chrysosporium pannorum*, *Cladosporium sphaerospermum* or *Mucor hiemalis* in conjunction with other colonies. *Chaetophoma* species appears to be autoinhibitory, and *Botrytis cinerea*

Fig.46 EFFECT OF UNBUFFERED FUNGAL FILTRATES ON SPORE GERMINATION (% GERMINATION ± S.D.)

FILTRATE	SPORES				
	CHRYSOSPORIUM PANNORUM	*BOTRYTIS CINEREA*	*CLADOSPORIUM SPHAEROSPERMUM*	*CHAETOPHOMA* Sp.	*MUCOR HIEMALIS*
CHRYSOSPORIUM PANNORUM	0.00	85.0 ± 7.54	99.0 ± 0.00	2.75 ± 0.20	17.2 ± 1.81
BOTRYTIS CINEREA	0.00	13.15 ± 1.77	81.5 ± 0.71	1.00 ± 0.20	0.00
CLADOSPORIUM SPHAEROSPERMUM	0.00	61.5 ± 3.54	0.00	0.00	0.00
CHAETOPHOMA SPECIES	0.00	43.5 ± 13.44	66.5 ± 17.68	1.00 ± 0.51	0.00
MUCOR HIEMALIS	0.00	97.0 ± 1.40	91.0 ± 1.41	1.00	1.00
BASIC LIQUID MEDIUM	51.0 ± 1.40	98.0	98.0	25.00 ± 0.70	98.5 ± 0.71

FIG. 47 EFFECTS OF VOLATILE INHIBITORS ON LINEAR EXTENSION

TEST SPECIES	ANTAGONIST	(mm) COLONY DIA. ± S.D.	% REDUCTION	SIG.
1. *CHRYSOSPORIUM PANNORUM*	*BOTRYTIS*	12.67 ± 6.00	9.5	all n.s.
	CHRYSOSPORIUM	13.67 ± 1.53	2.3	
	CLADOSPORIUM	13.67 ± 1.53	2.3	
	MUCOR	12.00 ± 0.00	14.0	
	CHAETOPHOMA	13.50 ± 0.57	3.6	
	CONTROL	14.00 ± 1.73	0.0	
2. *BOTRYTIS CINEREA*	*BOTRYTIS*	53.00 ± 1.00	32.1	*
	CHRYSOSPORIUM	61.00 ± 6.00	21.8	*
	CLADOSPORIUM	53.00 ± 2.00	32.1	*
	MUCOR	48.67 ± 3.21	37.6	**
	CHAETOPHOMA	60.67 ± 1.00	22.2	*
	CONTROL	78.00 ± 2.00	0.0	
3. *CLADOSPORIUM SPHAEROSPERMUM*	*BOTRYTIS*	9.00 ± 2.65	0.0	all n.s.
	CHRYSOSPORIUM	10.33 ± 1.53	14.8 inc.	
	CLADOSPORIUM	9.00 ± 1.15	0.0	
	MUCOR	7.33 ± 1.53	18.6	
	CHAETOPHOMA	9.33 ± 0.58	3.7 inc.	
	CONTROL	9.00 ± 1.12	0.0	

* p is greater than 0.05

** p is greater than 0.01

FIG.**47** Continued

TEST SPECIES	ANTAGONIST	(mm) COLONY DIA. ± S.D.	% REDUCTION	SIG.
4. *MUCOR HIEMALIS*	*BOTRYTIS*	85 +	0.0	all n.s.
	CHRYSOSPORIUM	85 +	0.0	
	CLADOSPORIUM	85 +	0.0	
	MUCOR	83.70 ± 3.21	1.5	
	CHAETOPHOMA	85 +	0.0	
	CONTROL	85 +	0.0	
5. *CHAETOPHOMA* SPECIES	*BOTRYTIS*	20.00 ± 1.00	13.0	
	CHRYSOSPORIUM	20.33 ± 0.57	11.6	
	CLADOSPORIUM	20.33 ± 0.58	11.6	
	MUCOR	17.00 ± 3.61	26.1	*
	CHAETOPHOMA	19.33 ± 0.58	15.9	*
	CONTROL	23.00 ± 1.00	0.0	

appears to be significantly susceptible to inhibition by all of the other test species.

4. Colony Interaction on MEA.

Results are shown in Fig. 48, using a scale of 1 - 4 for the interactions viewed:-

1. Denotes mycostasis of the colony to which the number refers.
2. Denotes significant hyphal lysis ($p > 0.05$).
3. Denotes altered colony morphology.
4. Denotes overgrowth of the other colony.

All interactions were scored relative to controls, except number 4. In the majority of interactions mutual mycostasis or mycostasis of one colony resulted. Hyphae of *Botrytis cinerea* were often observed to coil around those of *Chaetophoma* species and *Cladosporium sphaerospermum,* and some hyphal lysis of the latter two species often resulted.

Mucor hiemalis, in the presence of *Chaetophoma* sp., frequently exhibited a growth form which resembled the budding of yeast cells, in that constricted junctions formed between hyphal units. Occasionally, these junctions were sufficiently weak to cause separation of hyphal apices from the parent mycelium, forming yeast-like bodies.

In the vicinity of *M. hiemalis* , the morphology of colonies of *Chrysosporium pannorum* was apparently altered, such that the normally dense, low growth habit was replaced by floccose aerial hyphae. Subsequently, overgrowth of the *Chrysosporium* colony by the *Mucor* colony was observed.

FIG.48 OUTLINE RESULTS OF COLONY INTERACTION AT 20°C

COLUMN / ROW	*BOTRYTIS*	*CHAETOPHOMA*	*CHRYSOSPORIUM*	*CLADOSPORIUM*	*MUCOR*
BOTRYTIS	1 / 1	3 / 1	1 / 2	3 / 1	1 / 2
CHAETOPHOMA		4 / 4	1 / 1	1 / 1	1 / 3
CHRYSOSPORIUM			1 / 1	1 / 1	3 / 4
CLADOSPORIUM				1 / 1	1 / 4
MUCOR					4 / 4

1. MYCOSTASIS
2. HYPHAL LYSIS RESULTED
3. MORPHOLOGY ALTERED
4. OVERGROWTH OF OTHER COLONY

(1 - 3 are relative to controls)

DISCUSSION

The factors controlling growth and differentiation in fungi are not well known. Endogenous, hormonelike substances are known to regulate the development of some fungi (Macko *et al.* 1978). A complex lipoidal fraction from pea seeds inhibited sporulation of many Ascomycetes and Deuteromycetes, (Pfleger and Harman, 1975), and fatty acids and their volatile peroxidation products have been shown to inhibit sporulation and promote germination, (Harman *et al.* 1980).

Fries (1973) reviewed the current information regarding growth regulators in fungi, and gave some examples of possible modes of action of such organic compounds. As yet, however, very little is known about the action of inhibitors or promotors of growth and sporulation. The study of germination factors present in fungal filtrates showed some apparently strong and highly significant results, but it is highly probable that this was solely an effect of low pH values resulting in the media. At present it is thought unlikely that filtrates have a significant effect on the spores of species used in this study.

The fact that spores were inhibited by low pH values may be of some importance if the situation arose in the phyllosphere or litter layer where low localised pH values were caused by adjacent mycelia, and further

studies of the effect of colonies on the pH of leaf material may be beneficial.

No discernible effect could be established of filtrates upon the growth of other fungi. This result is interesting in that, in the light of evidence from other results in this study, an effect would be expected. Fries (1973) reported that *Coniophora cerebella* was inhibited by volatile factors when measured in terms of linear extension on agar, and yet was stimulated when measured as dry weight.

Fries has quoted many examples of the effects of volatile inhibitory compounds on linear extension rates of colonies. Dennis and Webster (1971 b) tentatively identified acetaldehyde as an inhibitor resulting from colonies of *Trichoderma viride* , and discussed the possible nature of other inhibitors. The results obtained from this study indicate that some strong inhibitors are produced by certain of the test fungi.

Botrytis cinerea is apparently very susceptible to volatile growth inhibitors, and *Chaetophoma* species is apparently auto-inhibitory.

Volatile growth inhibitors are perhaps of more importance to fungi than are non-volatile inhibitors, because of the longer distance over which they may be active and the ease of their diffusion and perhaps dissolution in water around the substrate. Non-volatile

inhibitors, unless capable of appreciable diffusion through plant tissue, would presumably tend to remain in areas in which the fungus had already grown, utilised the available resources, and left staling products. Volatile antibiotics prevent linear growth of hyphae into areas of fresh nutrients, which areas are obviously of greater ecological importance to survival of the colonies. This may explain their apparently widespread distribution.

Dennis and Webster (1971 c) described the coiling of hyphae of *Trichoderma viride* around hyphae of various test fungi, and the occasional rupturing of the 'host'. It is feasible that nutrients may either be absorbed via the host exoenzymes acting upon the substrate, or by destruction of the host hypha. It seems quite probable that such activity would provide a competitive advantage to the parasitic hyphae.

The observation of hyphal interaction on agar plates showed the usual result of confrontation between colonies of different species to be a mutual mycostasis. Only *Botrytis cinerea* was capable of lysis of other hyphae, and those hyphae susceptible to lysis were the unpigmented species, *Chrysosporium pannorum* and *Mucor hiemalis*. Lysis possibly results from chitinase production or a poisoning of the host metabolism, and hence alteration of the critical balance involved in cell wall production. *Botrytis*

hyphae were observed to curl around hyphae of *Cladosporium* and *Chaetophoma* species, as described by Dennis and Webster (1971 c) with *Trichoderma viride.*

Chrysosporium pannorum grown in the presence of *Mucor hiemalis* apparently changed its morphology via a volatile factor which stimulated elongation of aerial hyphae. (This phenomenon had previously been observed upon plates during isolation work.) The normal growth of *Chrysosporium pannorum* on MEA or other such rich media is a compact, dense, low colony with no visible aerial hyphal strands. This result is interesting in view of the fact that these species coexist in the litter layer of the monocotyledons on South Georgia. Although no growth stimulation occurs, the production of a more diffuse colony by *Chrysosporium pannorum* enables the *Mucor* species to gain access to larger surface areas of the 'host' hyphae in order to absorb the products of cellulose degradation. This may be another example of the complex systems which these fungi have evolved in order to exist in such an ecosystem.

In the presence of *Chaetophoma* species, some *Mucor hiemalis* hyphae apparently lose the normal configuration and grow into a form similar to a protoplast structure or large yeast cells. Whether or not this is a yeast form of *Mucor* , or simply the result of hyphal damage via chitinase or a hormonal factor, remains to be established.

Apart from these interactions, the only notable

result of the growth tests on agar was the overgrowth of all other colonies (except *Botrytis cinerea*) by *Mucor hiemalis.* This species seems largely immune to antibiotic effects, and may use its high growth rate to obtain nutrients from other colonies by overgrowth of their hyphae.

Colony interactions seem to be important in an *in vivo* situation. Even the phenomenon of mycostasis can be expected to have profound effects upon species growing on the phylloplane. Parasitic or antagonistic effects can presumably increase the competitive advantage of a species to a large extent, and many more studies are required on this subject.

GENERAL DISCUSSION

Phylloplane microfungi play an important role in the initial stages of leaf decomposition. Any assessment of nutrient turnover in an ecosystem must take account of the decomposition processes and the effect of climatic parameters upon rates of organic matter degradation.

The relative humidity of air surrounding plant leaves in the South Georgia ecosystem often falls to levels below those which will support growth of the most commonly isolated species tested in this study (Walton, pers.comm.). Whether the effect of leaf boundary layers significantly influences phylloplane fungal growth remains to be established, but the existence of a humidity gradient from stomata to atmosphere is now accepted, and the presence of trichomes on the monocotyledon leaves studied here will maintain and extend this layer of moist air in the microenvironment of the cuticle (Burrage, 1976).

Pre-initiation of inflorescences of *Poa flabellata* begins in May and continues throughout winter for flowering in January (Walton, pers.comm.). Present evidence suggests that young plant material will be more susceptible to damage from winter and spring frosts, especially following early-season melting of the snow cover. The tussock growth habit exhibited by the monocotyledonous host plants studied may aid survival by the protection from frosts which is afforded by the dead outer leaves.

There is also a possibility of temperature elevation within the tussocks as a result of microbial activity. Such warming could be an important factor in increasing localised rates of decomposition in the ecosystem.

Gunn (1976) found relatively high levels of carbohydrates in both recently-initiated and young leaves of *Poa flabellata*. The present investigation has indicated that active leaves and litter of *Acaena magellanica* also contain high carbohydrate levels, which are readily available to the mycoflora through leakage. As leaves senesce, their carbohydrate levels decline to such an extent that the litter of *Poa flabellata* cannot support growth of *Mucor hiemalis* in pure culture.

Leakage rates from moribund leaves and dry standing dead leaves placed in water have been shown to be sufficiently great to allow a loss of 90% of total available reducing sugars after only 6 - 8 hours. This corresponds to the findings of other researchers who have indicated that even active green leaves of some plant species are prone to high leakage rates (Tukey, 1971). Such leakage rates may provide relatively high levels of soluble carbohydrates on leaf surfaces as substrates for fungal growth.

Freeze - thaw action upon cellular structures is currently thought to play an important role in plant nutrient release from leaves, and freeze - thaw action on litter may be a vital factor in the degradation process

in such ecosystems by physical disruption of substrates, thereby increasing the surface area available for colonisation (Cheng *et al.* 1971; Holding *et al.* 1974). Studies of freeze-thaw action over long periods and the effects of such action on *in vitro* decomposition rates may prove rewarding. The action of freeze-thaw cycles on young leaves, particularly during the Antarctic spring when cellular carbohydrate levels are known to be relatively high, could be a factor of major significance to the activity of the mycoflora. Stimulation of litter respiration rates by addition of exogenous soluble carbohydrate sources has already been demonstrated (Wynn-Williams, In press), The deposition of such carbohydrates leached from leaves into the litter layer may promote microbial activity early in the summer season with accelerating effects on the rate of decomposition.

The availability of nitrogen to a phylloplane mycoflora is not thought to be a major limiting factor at present. Leachates are known to contain amino acids in proportions which approximate to their relative concentrations within the leaf (Tukey, 1971), and aerial deposition of nitrates from ocean spray, guano and dust particles is currently thought to be a major source of nitrogen to sub-Antarctic island ecosystems (Lindeboom, 1979). The carbon to nitrogen ratio of an age sequence of *Poa flabellata* leaves has been studied and shows young

leaves to have ratios of around 20:1, increasing to 30 or 40:1 in mature leaves, 40 - 50:1 in senescing leaves, and rising to 90:1 in dead leaves. As leaves become incorporated into the litter the ratio falls and then remains relatively constant at between 40:1 and 60:1 (Walton, pers.comm.).

The species composition of the tundra phylloplane mycoflora is in some ways similar to that of temperate habitats. The absence of *Trichoderma* and *Aspergillus* species in the litter has been noted by other researchers in the Antarctic and is currently assumed to be due mainly to the prevailing cold temperatures (Bailey and Wynn-Williams, In press).

Latter and Heal (1971) demonstrated cold adaptation in many species isolated from Signy island, a maritime sub-Antarctic ecosystem. The mycoflora had been selected by, or had become adapted to, the climate.

Large numbers of sterile mycelia were isolated from leaves and litter in South Georgia. In this respect, the findings of similar studies of the mycoflora in sub-polar regions correspond to those of this project (Ivarson and Bullen, 1971; Latter and Heal, 1971; Hayes and Rheinberg, 1974). The high incidence of such mycelia in interpreted as the result of a high stress environment (Pugh, 1980). Spores are known to contain high levels of nutrient reserves, usually in the form of lipids, which are mobil-

ised during germination (Weete, 1980). The accumulation of such nutrient reserves at the expense of growth can be considered detrimental to survival of the colony. This strategy has been discussed with respect to Arctic plant communities by Savile (1963).

The high lipid contents and, in many cases, the low water contents of spores will confer a resistance to damage resulting from freezing, as the possibility of the formation of ice crystals is reduced. It has been established that metabolite leakage from spores often results from repeated drying and rehydration, and from alternate freezing and thawing owing to temporary disruption of the cell membrane (Simon, 1974).

Further research is required on the responses of spores to freeze-thaw cycles with respect to leaching and viability. It is possible that the existence of microhabitats which are protected from climatic extremes can provide refuge for overwintering spores, and the protection afforded by snow cover is significant (Walton, pers. comm.).

All of the tested species isolated from leaf discs were capable of growth at $1^{o}C$, indicating that mycoflora activity may continue beneath snow cover during winter. Cellulase activity also continues at such low temperatures, suggesting the continuation of litter degradation, although a thorough survey of those species which are active in

the winter months is required for a reliable estimate of winter decomposition rates. Those species most commonly isolated during the summer collectively possessed enzyme complements sufficient to degrade the main organic litter components.

Of the fungi studied during this project, only one was susceptible to volatile antibiotics produced by other species. Gibbs (1967) demonstrated that an ability to produce or tolerate antibiotics could determine the pattern of colonisation and succession on pine logs. Such antibiotic effects could play a role in the determination of patterns of succession on leaves. The curling of hyphae around the hyphae of other species was observed, as was lysis, possibly as a result of chitinase activity or an effect on cell wall synthesis.

The relationship between *Mucor hiemalis* and *Chrysosporium pannorum* in the litter layer is an interesting case for further study in terms of decomposition rate and a possible reduction of cellulase repression. There is a suggestion that the morphology of *Chrysosporium pannorum* is altered by a volatile factor released by *Mucor hiemalis*, increasing the surface area of the former colony.

The purpose of this investigation was to provide information concerning the species composition of the phylloplane mycoflora on the dominant South Georgian plants, and the activity of these fungi under the condi-

tions prevailing in the tundra. The mycoflora has been found to consist of large numbers of cellulolytic sterile mycelia and a number of cold-adapted hyphomycetes, in common with the results of Arctic studies. A succession of both sterile and conidial fungi occurs as the leaves age, probably as a result of changes in water and carbohydrate contents of leaves (Chapter 2 and Chapter 3).

The mean summer field temperature is around 5°C, and yet those species studied had temperature optima for growth which were between 15°C and 25°C. The fact that the mycoflora does not have a lower general optimum for growth suggests that this system has developed in order to remain active at mean field temperatures and be capable of rapid growth during the transient warm periods of summer. This strategy thereby ensures efficient nutrient cycling within the ecosystem.

FURTHER RESEARCH

1. Localised warming by microbial activity may play an important role in increasing localised decomposition rates in tussocks and litter.
2. Interactions between colonies and the role of antibiotics in fungal succession need to be investigated in more detail.
3. The effect of the mycoflora upon leaf senescence, and the possibility of the production of antimicrobial substances by plant cells in response to infection need to be investigated.

4. More information in required concerning the process of penetration of the cuticle.
5. It is probable that decomposition can occur at low temperatures, but further studies are necessary concerning the mycoflora composition during the winter months.
6. Studies of the action of freeze-thaw cycles on new leaves and on the decomposition of litter are vitally important for a better understanding of the decomposition process.

APPENDICES

APPENDIX 1. Abridged Climatic Data - Summer, 1979 - 80

	NOV	DEC	JAN	FEB	MAR
Maximum daily insolation (hours)	14.5	14.0	14.4	12.3	9.4
Total insolation (hours)	157.2	163.0	151.3	146.6	122.3
Mean insolation per day (hours)	5.24	5.25	4.88	5.06	3.95
Mean maximum daily temp. (^{o}C)	4.80	6.30	8.19	9.45	7.03
Mean minimum daily temp. (^{o}C)	-1.0	0.8	2.5	2.89	0.84
No. of days with subzero temperatures	23	13	1	2	8
Maximum monthly temperature (^{o}C)	9.8	18.3	16.0	17.0	12.0
Minimum monthly temperature (^{o}C)	-4.4	-3.9	-1.0	-2.0	-2.0

APPENDIX 2

Colour Reagent for Phosphate Assay

16% Ammonium molybdate in 5M H_2SO_4	4 cm^3
Ferrous sulphate	2 g
Distilled Water	35 cm^3

Colour Reagent for Reducing Sugar Assay

3.5 dinitrosalicylic acid	1 g
Phenol	0.2 g
Na_2SO_4	0.05 g
NaOH	1 g
Sodium potassium tartrate	20 g
Distilled Water	100 cm^3

APPENDIX 3

ISOLATION RESULTS FOR DECEMBER, 1979 TO APRIL, 1980

(Numbers indicate number of leaf pieces on or from which
a given species was seen to be growing.
Maximum 50 pieces.)

- denotes no isolations.

NT denotes not tested.

1. *ACAENA NEW LEAVES*

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) 25°C Incubation					
Botrytis cinerea	-	-	-	4	NT
Chrysosporium pannorum	-	-	7	2	NT
Mucor hiemalis	-	-	-	1	NT
Penicillium sp.	2	-	-	-	NT
Sterile Mycelia	2	1	7	7	NT
Sterile discs	31	35	27	19	NT
b) 5°C Incubation					
Botrytis cinerea	NT	-	3	-	NT
Chrysosporium pannorum	NT	-	4	-	NT
Cladosporium sphaerospermum	NT	3	-	2	NT
Fusarium lateritium	NT	1	-	-	NT
Mortierella sp.	NT	16	13	10	NT
Penicillium sp.	NT	-	2	-	NT
Sterile Mycelia	NT	6	3	8	NT
Sterile discs	NT	5	8	4	NT

2. *ACAENA MATURE LEAVES*

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) 25^oC Incubation					
Chaetophoma spp.	-	-	1	-	NT
Chrysosporium pannorum	-	-	17	6	NT
Cladosporium sphaerospermum	-	-	-	2	NT
Mucor hiemalis	-	-	-	3	NT
Sterile Mycelia	-	6	-	4	NT
Sterile discs	50	19	8	12	NT
b) 5^oC Incubation					
Chrysosporium pannorum	NT	-	1	2	1
Cladosporium sphaerospermum	NT	1	-	-	-
Mortierella sp.	NT	-	-	-	16
Sterile Mycelia	NT	16	12	24	17
Sterile discs	NT	18	17	15	7

3. *ACAENA STANDING DEAD*

SPECIES	*DEC.*	*JAN.*	*FEB.*	*MARCH*	*APRIL*
a) 25°C Incubation					
Botrytis cinerea	NT	4	8	-	NT
Chaetophoma spp.	NT	-	9	-	NT
Chrysosporium pannorum	NT	-	2	3	NT
Penicillium spp.	NT	19	2	1	NT
Phialophora sp.	NT	-	6	-	NT
Sterile Mycelia	NT	-	12	16	NT
Sterile discs	NT	23	3	28	NT
b) 5°C Incubation					
Botrytis cinerea	NT	-	13	-	-
Chaetophoma spp.	NT	-	4	-	-
Chrysosporium pannorum	NT	5	2	-	-
Penicillium spp.	NT	-	2	-	-
Peyronellea sp.	NT	-	1	-	-
Phialophora sp.	NT	-	5	-	-
Sterile Mycelia	NT	15	18	17	34
Sterile discs	NT	26	13	6	1

4. ACAENA LITTER

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) <u>25°C Incubation</u>					
Alternaria sp.	4	-	4	-	NT
Ascomycete (unknown)	1	-	-	-	NT
Botrytis cinerea	16	31	14	7	NT
Chaetophoma sp.	-	-	7	11	NT
Chrysosporium pannorum	-	-	4	-	NT
Fusarium lateritium	2	-	-	-	NT
Mucor hiemalis	11	6	2	4	NT
Phialophora sp.	-	-	-	6	NT
Sterile Mycelia	-	-	2	4	NT
Sterile discs	1	5	1	5	NT
b) <u>5°C Incubation</u>					
Aureobasidium sp.	NT	12	3	11	11
Chrysosporium pannorum	NT	2	1	-	12
Cladosporium sphaerospermum	NT	1	4	-	-
Mucor hiemalis	NT	5	15	-	25
Sterile Mycelia	NT	-	6	13	5
Sterile discs	NT	2	5	4	2

5. *FESTUCA NEW LEAVES*

SPECIES	*DEC.*	*JAN.*	*FEB.*	*MARCH*	*APRIL*
a) 25°C Incubation					
Chaetophoma sp.	NT	NT	-	10	NT
Sterile Mycelia	NT	NT	50	35	NT
Sterile discs	NT	NT	-	7	NT
b) 5°C Incubation					
Chaetophoma sp.	NT	1	1	-	NT
Cladosporium sphaerospermum	NT	6	1	10	NT
Penicillium sp.	NT	1	1	3	NT
Sterile Mycelia	NT	8	16	42	NT
Sterile discs	NT	7	7	-	NT

6. *FESTUCA MATURE LEAVES*

SPECIES	*DEC.*	*JAN.*	*FEB.*	*MARCH*	*APRIL*
a) 25°C Incubation					
Ascomycete	NT	5	-	-	NT
Botrytis cinerea	NT	-	3	-	NT
Chaetophoma sp.	NT	26	15	-	NT
Penicillium sp.	NT	-	5	-	NT
Sterile Mycelia	NT	8	27	35	NT
Sterile discs	NT	11	6	-	NT
b) 5°C Incubation					
Ascomycete	NT	-	-	4	11
Botrytis cinerea	NT	-	-	7	-
Chaetophoma sp.	NT	9	-	-	-
Chrysosporium pannorum	NT	-	-	2	2
Cladosporium herbarum	NT	-	-	3	11
Cladosporium sphaerospermum	NT	5	13	-	-
Fusarium lateritium	NT	5	-	-	-
Other	NT	-	-	5	-
Sterile discs	NT	1	4	6	8

7. *FESTUCA STANDING DEAD LEAVES*

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) <u>25^oC Incubation</u>					
Alternaria sp.	NT	5	10	-	NT
Chaetophoma sp.	NT	10	-	4	NT
Chrysosporium pannorum	NT	-	-	8	NT
Cladosporium sphaerospermum	NT	1	-	-	NT
Mucor hiemalis	NT	-	2	-	NT
Penicillium sp.	NT	4	-	-	NT
Sterile Mycelia	NT	10	24	44	NT
Other	NT	3	-	-	NT
Sterile discs	NT	4	5	-	NT
b) <u>5^oC Incubation</u>					
Aureobasidium sp.	NT	3	-	1	-
Botrytis cinerea	NT	-	-	9	-
Chrysosporium pannorum	NT	-	-	19	9
Cladosporium sphaerospermum	NT	3	-	1	-
Mucor hiemalis	NT	-	-	4	-
Phialophora sp.	NT	-	-	-	13
Sterile Mycelia	NT	15	10	20	7
Sterile discs	NT	5	10	6	1

8. FESTUCA LITTER

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) 25°C Incubation					
Aureobasidium sp.	NT	3	-	11	NT
Botrytis cinerea	NT	1	-	7	NT
Chrysosporium pannorum	NT	6	39	19	NT
Cladosporium sphaerospermum	NT	1	-	-	NT
Mucor hiemalis	NT	16	30	16	NT
Sterile Mycelia	NT	4	-	-	NT
Sterile discs	NT	4	-	7	NT
b) 5°C Incubation					
Botrytis cinerea	NT	-	-	3	-
Chaetophoma sp.	NT	10	2	18	17
Chrysosporium pannorum	NT	-	-	27	23
Mucor hiemalis	NT	17	11	24	14
Sterile discs	NT	11	7	-	-

9. POA NEW LEAVES

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) $25^{o}C$ Incubation					
Chaetophoma sp.	-	-	6	-	NT
Chrysosporium pannorum	-	-	2	2	NT
Cladosporium sphaerospermum	-	-	-	3	NT
Sterile Mycelia	9	-	36	26	NT
Sterile discs	36	29	6	14	NT
b) $5^{o}C$ Incubation					
Chaetophoma sp.	NT	-	-	5	NT
Chrysosporium pannorum	NT	-	-	1	NT
Cladosporium sphaerospermum	NT	-	7	7	NT
Mucor hiemalis	NT	-	-	2	NT
Penicillium sp.	NT	-	-	2	NT
Sterile Mycelia	NT	-	29	9	NT
Sterile discs	NT	5	15	10	NT

10. POA MATURE LEAVES

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) 25°C Incubation					
Chaetophoma sp.	NT	20	4	5	NT
Chrysosporium pannorum	NT	-	1	1	NT
Cladosporium sphaerospermum	NT	-	1	1	NT
Penicillium sp.	NT	1	1	-	NT
Sterile Mycelia	NT	21	38	42	NT
Sterile discs	NT	4	6	5	NT
b) 5°C Incubation					
Acremonium terricola	NT	1	-	-	-
Botrytis cinerea	NT	-	1	-	-
Chaetophoma sp.	NT	5	8	11	2
Chrysosporium pannorum	NT	-	-	-	1
Cladosporium sphaerospermum	NT	3	2	3	2
Fusarium lateritium	NT	2	1	-	-
Leptosphaeria spp.	NT	4	4	-	-
Penicillium	NT	-	1	-	-
Sterile Mycelia	NT	28	16	37	50
Sterile discs	NT	-	-	-	-

11. POA STANDING DEAD LEAVES

SPECIES	DEC.	JAN.	FEB.	MARCH	APRIL
a) 25°C Incubation					
Acremonium terricola	NT	3	-	-	NT
Alternaria sp.	NT	4	3	1	NT
Chaetophoma sp.	NT	7	2	-	NT
Chrysosporium pannorum	NT	2	-	-	NT
Leptosphaeria sp.	NT	1	-	-	NT
Mucor hiemalis	NT	4	-	-	NT
Peyronellea sp.	NT	2	-	-	NT
Sterile Mycelia	NT	12	15	48	NT
Other	NT	4	-	-	NT
Sterile discs	NT	5	5	-	NT
b) 5°C Incubation					
Alternaria sp.	NT	1	-	-	-
Botrytis cinerea	NT	-	-	-	2
Chaetophoma sp.	NT	-	-	12	-
Chrysosporium pannorum	NT	-	-	-	8
Cladosporium herbarum	NT	1	-	-	-
Cladosporium sphaerospermum	NT	16	-	-	4
Leptosphaeria spp.	NT	17	7	14	16
Mucor hiemalis	NT	4	2	5	10
Peyronellea sp.	NT	10	-	-	-
Sterile Mycelia	NT	11	16	34	35
Other	NT	11	-	4	-
Sterile discs	NT	2	1	-	-

12. POA LITTER

SPECIES	*DEC.*	*JAN.*	*FEB.*	*MARCH*	*APRIL*
a) 25°C Incubation					
Alternaria sp.	-	2	-	-	NT
Ascomycete	21	5	-	-	NT
Chaetophoma sp.	-	6	-	-	NT
Chrysosporium pannorum	-	-	7	34	NT
Mortierella sp.	2	-	15	-	NT
Mucor hiemalis	-	1	-	3	NT
Penicillium spp.	-	19	10	26	NT
Sterile Mycelia	5	7	-	-	NT
Other	-	2	-	-	NT
Sterile discs	4	1	3	-	NT
b) 5°C Incubation					
Acremonium terricola	NT	9	-	-	-
Chaetophoma sp.	NT	3	-	-	-
Chrysosporium pannorum	NT	9	16	33	44
Cladosporium sphaerospermum	NT	1	-	-	-
Doratomyces sp.	NT	-	-	-	7
Mucor hiemalis	NT	9	6	7	17
Penicillium sp.	NT	-	-	15	1
Sterile Mycelia	NT	3	-	1	-
Other	NT	-	-	-	1
Sterile discs	NT	2	5	6	-

APPENDIX 4. Molal concentrations of salts required for adjusting medium of A_w 0.999 to various values of A_w at 25°C with a 5:3:2 mixture of NaCl, KCl and Na_2SO_4 (Modified from Scott, 1953)

FINAL A_w in MEDIUM	NaCl (Molal concentration)	NaCl (gms/200 ml of medium)	KCl (molal concentration)	KCl (gms/200 ml of medium)	Na_2SO_4 (molal concentration)	Na_2SO_4 (gms/200 ml of medium)
0.990	0.1293	1.5115	0.0776	1.1571	0.0517	1.4687
0.980	0.2789	3.2603	0.1673	2.4947	0.1116	3.1705
0.960	0.5805	6.7860	0.3483	5.1938	0.2322	6.5968
0.940	0.869	10.1586	0.521	7.7691	0.348	9.8866
0.920	1.149	13.4318	0.690	10.2892	0.460	13.0686
0.900	1.418	16.5764	0.851	12.6901	0.567	16.1084
0.880	1.663	19.4404	0.998	14.8821	0.665	18.8916
0.860	1.921	22.4564	1.153	17.1935	0.768	21.8188

Appendix 5. Effect of Exogenous Glucose on Germination

a) Percentage Germination after 24 hours with Additional 0.5M Glucose ($\pm$ S.D.)

SPECIES	TEMPERATURE (^{o}C)		
	5	15	25
Botrytis cinerea	90.50 $\pm$ 5.9	98.40 $\pm$ 1.10	98.00 $\pm$ 2.00
Chaetophoma sp.	0.00	1.50 $\pm$ 0.10	9.30 $\pm$ 2.00
Chrysosporium pannorum	0.00	75.60 $\pm$ 12.30	89.20 $\pm$ 4.40
Cladosporium sphaerospermum	0.00	83.80 $\pm$ 8.90	87.40 $\pm$ 2.40
Mucor hiemalis	0.26 $\pm$ 0.46	91.00 $\pm$ 5.70	83.40 $\pm$ 14.40

Appendix 5 . Effect of Exogenous Glucose on Germination

b) Percentage Germination after 24 hours without Additional Glucose (± S.D.)

SPECIES	TEMPERATURE (^{o}C)		
	5	15	25
Botrytis cinerea	93.96 ± 5.30	98.00 ± 1.10	98.00 ± 2.00
Chaetophoma sp.	0.00	0.00	38.30 ± 11.20
Chrysosporium pannorum	0.00	76.50 ± 6.60	88.20 ± 9.80
Cladosporium sphaerospermum	1.90 ± 1.93	91.20 ± 4.90	91.30 ± 3.90
Mucor hiemalis	1.35 ± 1.60	63.40 ± 7.80	4.70 ± 2.80

Appendix 6 Fermentation and Assimilation of Carbon Compounds by *Candida saké*

a) Fermentation

Dextrose	+
Maltose	+
Fructose	+
Sucrose	+
Trehalose	-
Cellobiose	-
Soluble starch	-

b) Assimilation of Carbon Compounds

Glucose	+
Sucrose	+
Maltose	+
Trehalose	+
Cellobiose	-
Starch	-
Mannitol	+
Sorbitol	+

Appendix 7. MEDIA

1. Basic Liquid Medium

D - glucose	10g
Malt Extract	20g
NH_4Cl	0.5g
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.5g
Microelement solution*	2 cm^3
$FeCl_3$ 1% solution	0.5 cm^3
Distilled water	1000 cm^3
pH	4.6 before autoclaving

2. Cellulase Production Medium (Mandels and Reese, 1957)

KH_2PO_4	2.0g
$(NH_4)_2SO_4$	1.4g
Urea	0.3g
$MgSO_4.7H_2O$	0.3g
$CaCl_2$	0.3g
$FeSO_4.7H_2O$	5.0mg
$MnSO_4.H_2O$	1.6mg
$ZnCl_2$	1.7mg
$CoCl_2$	2.0mg
Peptone	1.0g
Whatman CF11 cellulose powder	10.0g
Distilled water	1000 cm^3
pH	5.3 before autoclaving

* Microelements

$Fe(NO_3)_3.9H_2O$	723.5mg
$ZnSO_4.4H_2O$	439.8mg
$MnSO_4.4H_2O$	203.0mg
Distilled water	1000 cm^3

Appendix 8. MEDIA

1. STARCH AGAR

Starch	10g
Casein	1g
L - Proline	0.5g
L - Asparagine	0.5g
$MgSO_4.7H_2O$	0.5g
NaCl	0.5g
$FeSO_4.7H_2O$	0.01g
K_2HPO_4	2.0g
Agar	15g
Distilled Water	1000 cm^3
pH	7.0

2. CASEIN AGAR

Casein	10g
Yeast Extract	5g
Bacto Agar	20g
Tapwater	1000cm^3

3. PECTIN AGAR

Yeast Extract	1g
Agar	15g
Pectin	5g
Microelement sol'n.	500cm^3
Distilled water	500cm^3

pH 7 for pectate lyase.

4. TANNIC ACID AGAR

Glucose	10g
NH_4Cl	0.5g
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.5g
Malt Extract	2.5g
Agar	15g
Distilled water	1000cm^3
Tannic acid	500 mg, added after autoclaving (filter sterilised)

Positive result is brown colouration around colonies.

Appendix 8

5. CHITIN AGAR

Acid hydrolysed chitin	10g
Na NO_3	2g
K Cl	0.5g
KH_2PO_4	1.0g
Mg SO_4	0.5g
Fe SO_4	0.01g
Agar	15g
Distilled Water	1000 cm^3
pH	5.4

6. INDOLIN AGAR

Sucrose	30g	Solution A
Na NO_3	2g	1% Fe Cl_3
K Cl	0.5 g	1% K_3 $Fe(CN)_6$
KH_2 PO_4	1.0g	(mix immediately prior to use)
Mg SO_4	0.5g	
Fe SO_4	0.01g	
Yeast Extract	0.5g	
Agar	12g	
Distilled Water	1000 cm^3	
pH	6.5	

Plates flooded with solution A for ten minutes, avoiding strong light. Agar coloured green where lignin is utilised.

Appendix 8 (continued) MEDIA

YEAST EXTRACT PEPTONE DEXTROSE

Yeast Extract	10g
Peptone	20g
Dextrose	20g
Distilled water	1000cm^3

MALT EXTRACT AGAR

Malt Extract	20g
Peptone	15g
Agar	15g
Distilled water	1000cm^3

APPENDIX 9 <u>Effect of Water Activity on Linear Extension Rate</u> (mm $day^{-1} \pm$ S.D.)

a) <u>Glycerol media</u>

SPECIES	A_W					
	1.00	0.98	0.96	0.94	0.92	0.90
Botrytis cinerea	15.80 ± 0.48	8.60 ± 0.30	5.50 ± 0.13	2.80 ± 0.32	NT	0.00
Chaetophoma sp.	3.50 ± 0.32	3.11 ± 0.12	1.89 ± 0.12	0.83 ± 0.40	NT	0.08 ± 0.01
Chrysosporium pannorum	1.50 ± 0.11	1.33 ± 0.20	0.94 ± 0.07	0.42 ± 0.01	NT	0.00
Cladosporium sphaerospermum	1.96 ± 0.80	1.62 ± 0.24	1.35 ± 0.30	1.26 ± 0.20	0.80 ± 0.80	0.77 ± 0.12
Mucor hiemalis	17.4 ± 0.70	14.77 ± 0.60	8.43 ± 0.24	1.90 ± 0.30	NT	0.00

NT denotes not tested

APPENDIX 9. Effect of Water Activity on Linear Extension Rate (mm day^{-1} $\pm$ S.D.)

b) Salts media

SPECIES	A_W					
	1.00	0.98	0.96	0.94	0.92	0.90
Botrytis cinerea	10.24 $\pm$ 0.40	5.75 $\pm$ 0.40	3.61 $\pm$ 1.10	0.53 $\pm$ 0.30	0.00	0.00
Chaetophoma sp.	3.77 $\pm$ 0.24	2.65 $\pm$ 0.20	1.14 $\pm$ 0.24	0.20 $\pm$ 0.20	0.00	0.00
Chrysosporium pannorum	1.77 $\pm$ 0.40	0.93 $\pm$ 0.90	0.53 $\pm$ 0.28	0.15 $\pm$ 0.12	0.00	0.00
Cladosporium sphaerospermum	1.57 $\pm$ 0.28	1.00 $\pm$ 0.07	0.78 $\pm$ 0.14	0.83 $\pm$ 0.34	0.39 $\pm$ 0.12	0.11 $\pm$ 0.02
Mucor hiemalis	14.83 $\pm$ 0.30	4.08 $\pm$ 0.40	0.25 $\pm$ 0.01	0.00	0.00	0.00

APPENDIX **10** <u>Effect of Temperature on Linear Extension Rate</u> (mm $day^{-1} \pm$ S.D.)

SPECIES	TEMPERATURE (°C)					
	5	10	15	20	25	30
Botrytis cinerea	2.40 ± 0.23	5.34 ± 0.42	9.60 ± 0.97	11.46 ± 0.57	13.70 ± 1.04	1.60 ± 0.91
Chaetophoma sp.	1.17 ± 0.21	2.45 ± 0.95	2.95 ± 0.51	3.67 ± 0.42	2.78 ± 0.61	1.00 ± 0.26
Chrysosporium pannorum	0.66 ± 0.50	1.06 ± 0.48	1.45 ± 0.27	1.82 ± 0.92	1.36 ± 0.47	0.00
Cladosporium sphaerospermum	1.16 ± 0.03	1.71 ± 0.53	2.41 ± 0.79	1.78 ± 0.61	1.03 ± 0.42	0.00
Mucor hiemalis	6.33 ± 0.83	14.00 ± 2.63	18.67 ± 5.26	22.90 ± 5.31	25.33 ± 4.22	7.71 ± 2.40

APPENDIX 11 Effect of pH on Linear Extension Rate (mm $day^{-1} \pm$ S.E.)

SPECIES	pH	
	4.5	6.7
Botrytis cinerea	11.51 ± 2.55	11.78 ± 4.92
Chaetophoma sp.	3.82 ± 1.71	4.32 ± 1.61
Chrysosporium pannorum	2.30 ± 0.59	3.12 ± 0.55
Cladosporium sphaerospermum	1.70 ± 0.98	2.00 ± 0.42
Mucor hiemalis	21.87 ± 7.34	21.62 ± 5.57

Appendix 12. Effect of Ultraviolet Light on Linear Extension (mm day^{-1} $\pm$ S.D.)

SPECIES	CONTROL	IRRADIATED *
Botrytis cinerea	12.46 ± 1.03	13.05 ± 2.25
Chaetophoma sp.	3.78 ± 0.30	2.98 ± 0.84
Chrysosporium pannorum	1.82 ± 0.97	1.71 ± 1.03
Cladosporium sphaerospermum	1.78 ± 0.77	1.48 ± 0.71
Mucor hiemalis	22.90 ± 2.43	19.73 ± 3.86

* 150 mW cm^{-2} for 120 minutes

Appendix 13. Dry weight growth increase (mg dry wt. day^{-1}).(± S.D.)

a) Still Culture

Temp. °C	*Botrytis*	*Chaetophoma*	*Chrysosporium*	*Cladosporium*	*Mucor*
5	3.41 ± 1.16	0.98 ± 0.17	4.29 ± 1.66	3.88 ± 2.07	3.58 ± 1.76
10	7.27 ± 1.99	4.67 ± 2.23	4.84 ± 1.27	5.64 ± 0.66	5.18 ± 2.00
15	28.46 ± 2.31	12.78 ± 8.30	7.09 ± 2.44	6.30 ± 0.00	8.76 ± 0.47
20	17.14 ± 1.94	19.23 ± 2.50	8.52 ± 1.20	8.25 ± 0.00	7.66 ± 1.29
25	14.85 ± 0.16	10.02 ± 0.90	7.86 ± 1.36	3.875± 0.00	6.76 ± 0.33
30	0.00	0.00	0.00	0.00	0.71 ± 0.10

b) Shaken Culture

Temp. °C	*Botrytis*	*Chaetophoma*	*Chrysosporium*	*Cladosporium*	*Mucor*
5	0.26 ± 0.10	0.53 ± 0.32	0.67 ± 0.28	2.59 ± 1.20	0.29 ± 0.04
10	1.60 ± 0.71	4.09 ± 0.88	6.19 ± 2.01	6.01 ± 0.66	2.20 ± 0.50
15	13.19 ± 5.69	5.43 ± 1.96	9.62 ± 2.00	8.09 ± 2.38	3.08 ± 0.94
20	16.16 ± 8.57	6.71 ± 3.71	9.39 ± 2.22	9.34 ± 1.16	3.64 ± 1.84
25	5.34 ± 0.87	1.47 ± 1.22	6.36 ± 2.36	3.62 ± 1.93	3.36 ± 2.30
30	0.00	0.00	0.00	0.00	1.41 ± 0.66

APPENDIX 14

EFFECT OF TEMPERATURE ON GROWTH OF *CANDIDA SAKÉ*

TEMPERATURE (^{o}C)	TOTAL CELL NUMBER $(\times 10^4)ml^{-1} \pm$ S.D.	VIABLE CELL NUMBER $(\times 10^4)ml^{-1} \pm$ S.D.
5	23.0 ± 2.16	20.93 ± 3.21
10	25.75 ± 6.13	24.70 ± 8.64
15	50.10 ± 5.88	44.45 ± 7.79
20	101.75 ± 37.53	90.56 ± 30.29
25	95.75 ± 23.64	87.25 ± 17.68
30	0.00	0.00

SPECIES	INCUBATION TIME (Days)			
	7	10	13	20
BOTRYTIS CINEREA	0.000	0.600 ± 0.141	1.038 ± 0.077	1.775 ± 0.05
CHAETOPHOMA SP.	0.000	0.400 ± 0.010	0.308 ± 0.010	0.488 ± 0.048
CHRYSOSPORIUM PANNORUM (Antarctic Strain)	0.000	0.225 ± 0.05	0.269 ± 0.44	0.325 ± 0.065
CHRYSOSPORIUM PANNORUM (Swedish Strain)	0.000	0.000	0.000	0.000
CLADOSPORIUM SPHAEROSPERMUM	0.000	0.225 ± 0.050	0.269 ± 0.099	0.400 ± 0.092
MUCOR HIEMALIS	1.857 ± 0.117	2.50 ± 0.013	2.865 ± 0.038	3.20 ± 0.01

Appendix **15** LINEAR EXTENSION RATES AT 1°C (mm day ± S.D.)

SPECIES	INCUBATION TIME (Days			
	22	24	28	48
BOTRYTIS CINEREA	2.076 ± 0.070	2.195 ± 0.064	2.32 ± 0.129	2.30 ± 0.010
CHAETOPHOMA SP.	0.568 ± 0.025	0.625 ± 0.034	0.688 ± 0.018	0.911 ± 0.026
CHRYSOSPORIUM PANNORUM (Antarctic Strain)	0.386 ± 0.026	0.375 ± 0.048	0.375 ± 0.046	0.380 ± 0.031
CHRYSOSPORIUM PANNORUM (Swedish Strain)	0.091 ± 0.010	0.125 ± 0.012	0.119 ± 0.020	0.243 ± 0.012
CLADOSPORIUM SPHAEROSPERMUM	0.455 ± 0.074	0.448 ± 0.063	0.482 ± 0.046	0.531 ± 0.015
MUCOR HIEMALIS	3.216 ± 0.108	3.250 ± 0.083	0.000	0.000

Appendix 15 continued. LINEAR EXTENSION RATES AT 1°C
(mm day ± S.D.)

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