FUNGAL SUCCESSION AND SUBSTRATE UTILIZATION ON THE LEAVES OF THREE SOUTH GEORGIA PHANEROGAMS

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ABSTRACT. Fungi were isolated from washed leaf discs from three sub-Antarctic phanerogams (two grasses and a dwarf shrub) during the summer season of 1979–80. The majority of isolations from both green leaves and litter were of sterile mycelia. A fungal succession was evident as leaves senesced and dried out. The composition of the airspora did not differ markedly from that of the phylloplane and litter. The airspora beneath a grass canopy was shown to contain more representatives of the litter fungi than that above the canopy. The five most commonly isolated fungi (excluding sterile mycelia) were shown to be cold-adapted mesophiles, capable of growth at 1°C on agar. All common phylloplane species except *Mucor hiemalis* produced extracellular cellulase and protease. *Mucor* produced pectinase and *Botrytis, Chaetophoma* and *Acremonium* showed possible lignolytic ability.

Fungi appear to be the major group of cellulolytic organisms in polar and subpolar environments (Dowding and Widden, 1974). The two comprehensive reviews on the external and internal colonization of leaves and litter (Preece and Dickinson, 1971; Dickinson and Preece, 1976) indicate clearly that very limited information is available on plants growing in the tundra regions. Most of the studies made on fungi from the colder regions of the world have concentrated on species from the Arctic and Alpine areas (Holding, 1981) and apart from contributions by Tubaki (1961), Corte and Daglio (1963 and 1964), Heal and others (1967), Dennis (1968), and Pegler and others (1980) relatively little is known about microfungi from the sub-Antarctic and Antarctic regions. Yet data on the species composition and activity of Antarctic fungal populations are important for an understanding of nutrient cycling in the tundra ecosystem.

As part of a continuing project by the British Antarctic Survey on nutrient cycling in tundra ecosystems, studies have been made of production and decomposition in communities of the more important plant species on the sub-Antarctic island of South Georgia (lat. 54–55°S, long. 36–38°W). A recent synthesis of available information (Smith and Walton, 1975) indicated that the lack of data on fungal composition hampered a proper understanding of nutrient fluxes. The present study provides basic information on the species composition of the phylloplane mycoflora for three ecologically important angiosperms together with data on some aspects of the growth and substrate utilization for several common fungal species. The term phylloplane is used in this paper to describe the ecological niche at the surface of any leaf either living or dead (Singleton and Sainsbury, 1978).

MATERIALS AND METHODS

Leaves were collected from pure stands of the host plants at three sampling sites. *Acaena magellanica* (Lam.) Vahl was collected from an almost pure stand at a site

5–10 m above sea level, on a slight north-east facing slope underlain by wet peat, in King Edward Cove. *Festuca contracta* T. Kirk was collected from open dry grassland near a fresh-water lake, 70 m above sea level on a relatively flat and exposed site underlain by a brown earth soil near Maiviken. *Poa flabellata* (Lam.) Hook. f. was collected from a pure stand in an area 10 m above sea level on the south-facing slope of scree and mineral soil at Hope Point, King Edward Cove.

Leaves were excised at their bases with a sterile knife and placed in sterile polythene bags for transport to the laboratory. There they were sorted into four age categories on a clean, alcohol-swabbed bench:

(1) Newly expanded leaves with no discoloration or visible growths of micro-organisms
(2) Mature green leaves showing no signs of senescence
(3) Senescent, standing, attached leaves
(4) Fallen litter.

Pieces were cut from the central portion of each leaf lamina using a sterilized 5-mm cork borer and 25 pieces of each species/age class were placed into Universal bottles and serially washed using autoclaved and cooled tapwater (Harley and Waid, 1955). Five washed leaf pieces per dish were plated on to Cellulose Agar (CA) (Eggins and Pugh, 1962), or Potato Dextrose Agar (PDA) in 9-cm Petri dishes, with five replicate dishes per leaf age class. Incubation was at 25 or 5°C with examinations at two and four weeks. Common phylloplane and litter species were sub-cultured on to Malt Extract Agar (MEA) and returned to England at 5°C. These cultures were used for growth rate and enzyme measurements.

The airspora was sampled at midday at about two-week intervals both above and below the *Poa flabellata* canopy by exposing horizontally five dishes each of CA and PDA for ten minutes. Plates were incubated at 25°C and examined after two and four weeks.

Effects of temperature on growth rate

Aliquots of 20 cm^3 of Basic Liquid Medium (BLM) were dispensed into 100-cm^3 Ehrlenmeyer flasks, autoclaved at 121°C for 15 minutes and inoculated with a disc cut from the margin of an actively growing colony of the test species. Flasks were incubated at a range of temperatures between 5 and 30°C in an orbital incubator. Mycelia were harvested at intervals by suction filtration on to preweighed, dried, Whatman No. 1 filter papers, which were then dried to constant weight. Dry matter production was then calculated for the log phase of growth, determined from previously constructed growth curves. Four replicate flasks were used for each weight measurement.

Effect of temperature on linear extension rate

Discs were cut from the margins of young, actively growing colonies of each test species and inoculated centrally on to MEA in 9-cm Petri dishes. Five replicate plates were used for each species/temperature. Dishes were incubated at a range of temperatures from 1 to 30°C. At intervals, two measurements were made of the colony diameter at right angles to each other.

Substrate utilization

A range of media was prepared with the following major carbohydrate sources: cellulose (Eggins and Pugh, 1962), starch, casein, pectin, indulin (a synthetic lignin),

chitin and tannic acid (for polyphenol oxidase). Preparation followed the techniques of Kjøller and Struwe (1980).

Four replicate plates of each agar medium were centrally inoculated with discs cut from the margins of actively growing colonies on Water Agar supplemented with sucrose at $2\,g\,l^{-1}$. Control plates of a basal medium lacking a specific carbohydrate source were also inoculated. Plates were incubated at 20°C for 30 days, and examined every five days for linear growth.

Extracellular enzymes

Drop tests were performed on species to test for extracellular cytochrome oxidase, and laccase (p-diphenol oxidases). Colonies were grown on MEA at 20°C for ten days before testing.

(a) Cytochrome oxidase: 20 mg of tetramethyl-p-phenylene diamine dihydrogen chloride was dissolved in $10\,cm^3$ of a solution of 15 parts per million ascorbic acid in water. A blue discoloration is given by reaction with cytochrome oxidase within 30 minutes.

(b) Laccase: 0.1M α-naphthol in 96% ethanol. Presence of laccase is indicated by a purplish discoloration of the mycelium.

Effect of temperature on clearing rate of starch agar

Starch agar (10 g starch, 1 g casein, 0.5 g proline, 0.5 g asparagine, $2\,g\,K_2HPO_4$, $0.5\,g\,MgSO_4$, $0.5\,g\,NaCl$, $10\,mg\,FeSO_4$ in 1 litre distilled water at pH 7) plates were centrally inoculated with discs cut from the margins of four- to seven-day-old, actively growing colonies of *Botrytis, Chaetophoma, Chrysosporium* and *Mucor*. Incubation was at six temperatures between 5 and 30°C for four to seven days. Plates were then flooded with iodine in potassium iodide solution, and the diameter of starch clearing was measured along two lines at right angles on the base of the colony. Three replicate plates were used for each species/temperature.

Results

All data on the number of isolations of individual fungi have been amalgamated in Table I to give an overall view of the distribution of species and their frequency of isolation. The fungi are listed in order of descending frequency of isolation. By far the most common fungi were the sterile mycelia, which accounted for 41% of the total number of fungi isolated. *Chrysosporium pannorum* was the most common identifiable species, providing 14% of the total number of isolations and present on all leaf age classes of each of the three host plant species except new leaves of *Festuca*. The bulk of the isolations of *Chrysosporium pannorum* were from the two grass litters, suggesting that this species is especially important in their decomposition.

Mucor hiemalis (10% of the total isolations) proved to be a common fungus on the litter of all three host plants and on standing dead leaves of *Poa flabellata*.

Unidentified species of *Chaetophoma* were quite common (9% of the total isolates). They were rare on *Acaena* green leaves but were frequently isolated from *Acaena* litter and standing dead leaves, and from all grass leaves.

Botrytis cinerea was isolated mainly from senescent leaves and litter of *Acaena*, with fewer isolates from *Festuca* leaves at the senescent stage and onwards. It contributed 4.8% of the total isolates.

Cladosporium sphaerospermum was found on almost all leaf age classes, being

Table I. Number of isolations of fungi from the leaves and litter of three plant species.

	Leaf age classes												
	Acaena magellanica				*Festuca contracta*				*Poa flabellata*				
Fungal species	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*Total*
Sterile mycelia	34	79	112	30	151	70	130	4	109	232	171	16	1138
Chrysosporium pannorum	13	27	12	19	–	4	36	114	5	3	10	143	386
Mucor hiemalis	1	3	–	68	–	–	6	128	2	–	25	43	276
Chaetophoma spp.	–	1	13	18	12	50	14	47	11	55	21	9	251
Botrytis cinerea	7	–	25	68	–	10	9	11	–	1	2	–	133
Cladosporium spp.	5	3	–	5	17	32	5	1	17	12	21	1	119
Penicillium spp.	4	–	24	–	5	5	4	–	2	3	–	71	118
Mortierella spp.	39	16	–	–	–	–	–	–	–	–	–	17	72
Leptosphaeria spp.	–	–	–	–	–	–	–	–	–	8	55	–	63
Aureobasidium pullulans	–	–	–	37	–	–	4	14	–	–	–	–	55
Ascomycete	–	–	–	1	–	9	–	–	–	–	–	26	36
Alternaria sp.	–	–	–	8	–	–	15	–	–	–	9	2	34
Phialophora sp.	–	–	11	6	–	–	13	–	–	–	–	–	30
Peyronellea sp.	–	–	1	–	–	–	–	–	–	–	12	–	13
Acremonium terricola	–	–	–	–	–	–	–	–	–	1	3	9	13
Fusarium lateritium	1	–	–	2	–	5	–	–	–	3	–	–	11
Doratomyces nanus	–	–	–	–	–	–	–	–	–	–	–	7	7
Total	104	129	198	262	185	185	236	319	146	318	329	344	2755

more common on the younger age classes of the two grasses. It was far more abundant than *C. herbarum*, which was isolated mainly from *Festuca* mature leaves (only 14 isolations).

Various species of *Penicillium* were recorded throughout the season, but overall, this genus was relatively rarely encountered. Where *Penicillium* species were isolated, they often occurred on one sampling date only, suggesting that the isolations were increased by abundant sporulation of a single colony.

Other species occurred at frequencies of less than 3% of the total number of isolates. *Mortierella* spp. were common on new *Acaena* leaves and in the litter of *Poa flabellata*. *Leptosphaeria* spp. (probably including *L. sylvatica*) were commonly recorded from *Poa* standing dead leaves.

In order to explore the possibility that succession occurred on the three higher plants, those fungi which constituted up to 70% of the total isolations from any age class were used to produce Fig. 1. These diagrams assume that no changes in fungal frequency are caused by time of isolation through the season.

Early in the season, a large number of the incubated leaf pieces (especially those from new leaves) yielded yeasts (notably *Candida saké*) and bacteria which grew at 5°C. It was particularly noticeable that any leaf pieces incubated in damp chambers which showed a high frequency of yeasts and bacteria, did not yield many filamentous fungi.

Compared with those of more temperate climates, the South Georgian airspora was found to be sparse. Spot tests of airspora are not significant in quantitative terms, but they do provide data on the presence or absence of spores. The most commonly isolated leaf fungi were all represented in the airspora. A comparison of the airspora above and below a canopy of *Poa* indicated that the litter species were better represented in the below-canopy airspora, as would be expected. The below-canopy

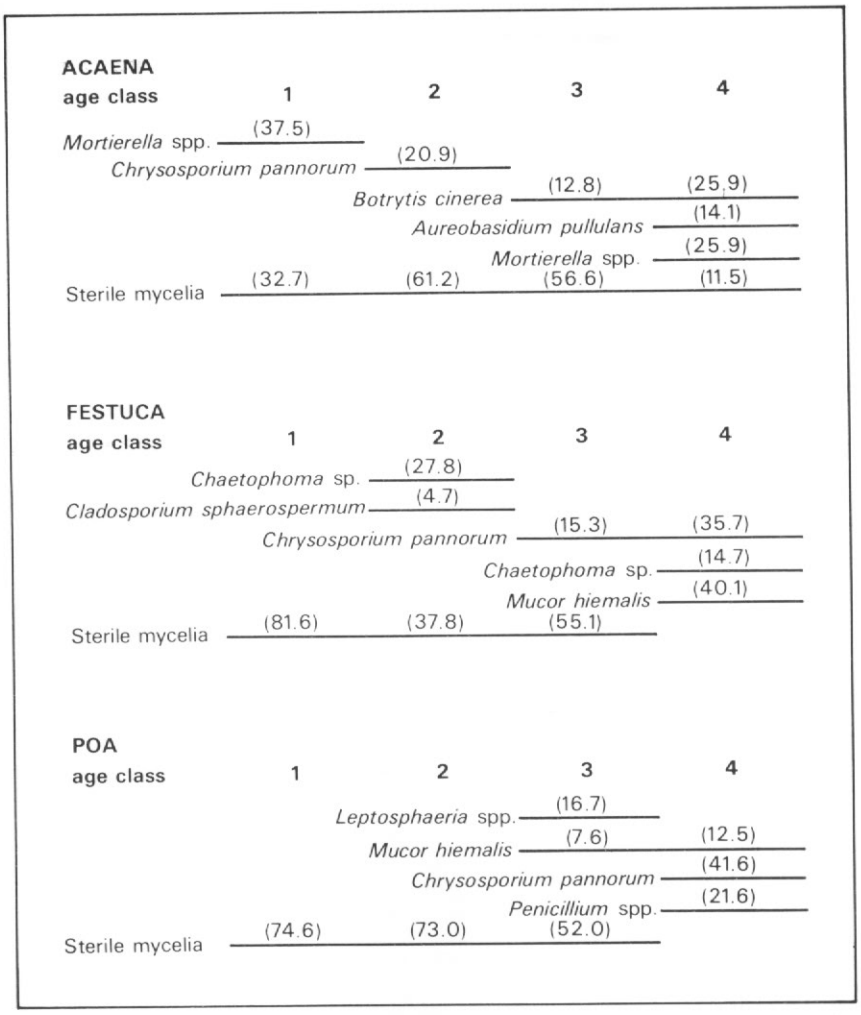

Fig. 1. Generalized succession patterns on leaves. Frequency of isolation of species in parentheses.

airspora generally contained many more species than the above-canopy airspora, presumably due to a high localized concentration of fungal colonies.

Effects of temperature on growth

The five identifiable fungi isolated most commonly all showed growth optima between 15 and 25°C, and only *Mucor hiemalis* grew at 30°C in liquid culture (Fig. 2a). Fig. 2b shows the linear extension rates of the five fungi on MEA, and indicates that growth continues at 1°C. Although *Botrytis cinerea* shows high dry matter production at its optimum temperatures, its lower extension rate suggests that it is likely to utilize its substrate in a way quite different from *Mucor hiemalis*.

Substrate utilization and extracellular enzyme production

The results of growth on specific substrates and the drop tests are tabulated in Table II. All of the most commonly isolated species, except *Mucor hiemalis*,

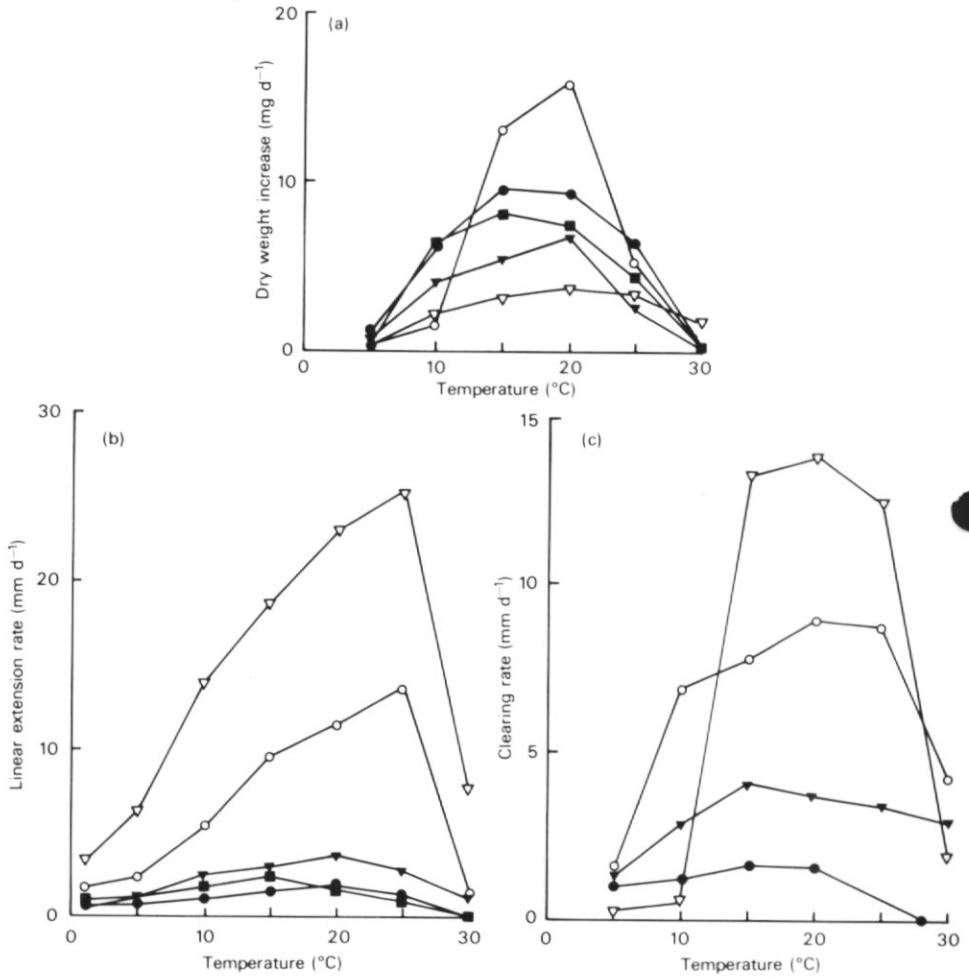

Fig. 2. a, Effect of temperature on dry matter production of fungi in shake cultures. b, Effect of temperature on linear extension rate of fungal colonies. c, Effect of temperature on the clearing rate of starch agar by fungal activity. ○ *Botrytis cinerea*, ▼ *Chaetophoma* sp., • *Chrysosporium pannorum*, ■ *Cladosporium sphaerospermum*, ▽ *Mucor hiemalis*.

produced 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. *Cladosporium* showed only a very weak growth on starch agar, similar to that on the control medium, and was presumed not to possess α-amylase. Amylase activity in *Mucor hiemalis* was very strong but very weak in *Chrysosporium pannorum*. All species possessed protease, indicating an ability to utilize proteins within plant cells if these are made accessible through the action of other enzymes.

Mucor hiemalis showed strong pectinase activity indicating a capacity to degrade 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 position in the general succession, after the early

Table II. Substrate utilization and production of extracellular enzymes.

Species	*Cellulase*	*Amylase*	*Protease*	*Pectinase*	*Polyphenol oxidase*	*Indulin growth*	*Laccase*	*Chitinase*	*Cytochrome oxidase*
Acremonium terricola	+	+	+	+	+	N.T.	+	N.T.	−
Botrytis cinerea	+	+	+	(+)	+	+	−	+	+
Chaetophoma sp. 1	+	+	+	+	+	+	−	+	+
Chaetophoma sp. 2	+	+	+	+	N.T.	N.T.	N.T.	+	−
Chrysosporium pannorum	+	+	+	(+)	+	−	−	−	+
Cladosporium sphaerospermum	+	−	+	N.T.	+	−	−	−	N.T.
Fusarium lateritium	+	+	+	+	+	N.T.	−	N.T.	N.T.
Mucor hiemalis	−	+	+	+	−	−	−	(+)	−

+ Activity or growth; (+) weak activity or growth; − no activity or growth; N.T. denotes not tested.

spring dominance of yeasts, as leaf surface carbohydrates become more scarce. Pectinase activity was very poor for these isolates of *Botrytis cinerea* and *Chrysosporium pannorum*.

All species, except *Mucor hiemalis*, gave a positive reaction to the tannic acid test (polyphenol oxidase), although only *Acremonium terricola* gave a positive result for the laccase (p-diphenol oxidase) drop test, and only *Botrytis cinerea* and *Chaetophoma* were capable of clearing indulin agar. No species was capable of growth on water agar and supplemental indulin. These results suggest that *Botrytis cinerea* and *Chaetophoma* could degrade lignin, and there is a strong probability that *Acremonium terricola* may also have this capacity. *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 and the woody stems of *Acaena* have relatively high lignin contents. *Botrytis cinerea* and *Chaetophoma* species showed strong chitinase activity, destroying all the chitin in a liquid culture medium. *Mucor hiemalis* also showed a weak but definite chitinase activity. Cytochrome oxidase results were unusual with no activity being detected for *Mucor*, *Chrysosporium* or *Acremonium*.

The ability to utilize starch varied considerably between the species tested (Fig. 2c). The optimum temperature range for amylase activity was 15–20°C with relatively little activity being shown at 5°C. *Mucor* and *Botrytis* both appeared to produce much larger quantities of extracellular amylase than *Chaetophoma* and *Chrysosporium*. Of the three host species only *Acaena* stores large quantities of starch, the two grasses favouring fructosans for carbohydrate storage.

Discussion

A comparison between the species recorded in this study and those listed in the most recent checklist for South Georgia shows a number of interesting points. Pegler and others (1980) record three species of *Leptosphaeria*, including *L. sylvatica*, none of them on *Poa flabellata* but one of them on *Festuca contracta*. *Pleospora heleocharidis* Karst., *Wentiomyces inconspicuous* Spooner, *Phyllachora graminis* (Pers. ex Fr.) Fckl., and *Hymenoscyphus chloophilus* Spooner are all reported from *Poa flabellata* leaves or litter. *Hysteropezizella festucae* Dennis and *Didymella antarctica* Spooner are reported from *Festuca contracta* material. The importance of these species in the course of fungal succession on host plant material is not known.

The results of this study showed new leaves of *Poa flabellata* to be colonized by sterile mycelia together with a limited population of *Cladosporium* and *Chaetophoma*. A similar situation dominated matured leaves but with an increased number of isolations. This reflects, in part, a decreasing incidence of yeasts (Hurst and others, in press *b*) and bacteria as the season progressed and leaves matured, and also the increasing time available for leaf colonization and spore germination. As leaves died and the water content decreased, the number of records of *Leptosphaeria* spp. increased. This is in agreement with the results of Webster (1957), who found *Leptosphaeria* on upper leaves and inflorescences of moribund *Dactylis glomerata*, and of Pugh (1958), who found that *Leptosphaeria* sp. and *Metasphaeria cumana* were only recorded on the drier parts of living leaves and dried dead leaves of *Carex paniculata*. The standing dead leaves of *Poa* had a relatively low water content (Hurst and others, in press *a*) and it may be that an immersed pseudothecial structure is advantageous under conditions of water stress. 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 on the green leaves where competition from more quickly growing species is severe, but able to grow satisfactorily at lower water potentials where competition is decreased.

Relatively few sterile mycelia were isolated from the litter of *Poa flabellata*, which was dominated by *Chrysosporium pannorum* and *Penicillium* spp. in conjunction with *Mucor hiemalis*. This is similar to the findings of Ivarson (1975) in the Canadian Arctic. Analysis of *Poa* litter for water- and ethanol-soluble carbohydrates showed very low levels of sugars, *c.* 2 $\mu g\,mg^{-1}$ when compared with *c.* 15 $\mu g\,mg^{-1}$ in *Acaena* litter (Hurst and others, in press *a*). *Chrysosporium* and *Penicillium* attack cellulose but *Mucor hiemalis* is incapable of using cellulose and is a 'secondary sugar fungus' (Frankland, 1974) utilizing the sugars which result from the breakdown of cellulose by cellulolytic species (Tribe, 1966; Frankland, 1969; Hurst and Pugh, in press).

The pattern of colonization of *Festuca* leaves was very similar to that of *Poa*. New leaves were colonized by sterile mycelia, which remained until the litter stage. *Chaetophoma* spp. were abundant on mature leaves, together with a range of other cellulolytic species. On standing dead leaves *Chrysosporium pannorum* and sterile mycelia were the major components, whilst litter was again colonized by *C. pannorum* and *Mucor hiemalis*, with very few sterile mycelia and no *Penicillium* species. The absence of the latter genus is interesting, and may be related to the different growth form of *Festuca contracta* which does not give the protection and possible temperature elevation afforded by the *Poa* tussocks (Walton and Hurst, in press).

Living and dead leaves of *Acaena magellanica* have been shown to contain relatively large amounts of sugars compared with *Poa* leaves (Hurst and others, in press *a*), thus the presence of the saccharophilic *Mortierella* sp. on new leaves is not unexpected. Nitrogen levels are also much higher in living leaves of *A. magellanica* than in other South Georgia species (Walton and Smith, 1979) and this may be important in the rapid rate of decomposition.

Differences between the leaves of the three species in susceptibility to decay may be related to anatomy, water-holding capacity, nitrogen levels, carbohydrate content or fibre content. Standing dead leaves of the two grasses normally remain erect and in a relatively dry state, with occasional wetting, over long periods of time (Walton and Hurst, in press), and their water contents are normally sufficiently low to restrict fungal growth significantly. Only when such leaves reach the litter layer do they remain moist for long periods and even here water availability may frequently be a limiting factor for litter at the top of the layer.

In contrast to this situation, *Acaena* leaves are held for relatively short periods on stems (usually less than twelve months) before entering the litter layer where decomposition is rapid, over 90% of lamina material disappearing in under two months in the summer (Walton, 1977).

The most commonly isolated sporing fungal species were mesophilic, but were capable of growth at 1°C on MEA. This is in accordance with the results of similar studies in the Arctic and sub-Arctic which have indicated the predominance of mesophilic filamentous fungi in tundra ecosystems (Holding and others, 1974). Some such filamentous fungi have low temperature optima for cellulase activity and tundra populations of obligatory psychrophilic yeasts have been found (Holding, 1981). In this context it is interesting to note that the survey by Rosswall and Clarholm (1974) showed no significant differences in physiology between tundra and non-tundra bacteria. It is possible that the tundra mycoflora has adapted to growth at lower temperatures whilst maintaining a relatively high temperature optimum for growth. Such an adaptation would allow growth during the winter, but would confer upon the fungi the ability to exploit warmer periods as and when they occur. In this respect,

cold-adapted mesophiles may out-compete any psychrophiles, at least during the summer months, due to the inherently slower growth rate of the latter group. Comparison of Figs. 2a and 2b shows that *Botrytis cinerea* is able to grow quickly both in terms of colony diameter and in dry weight production. *Mucor hiemalis* colonies showed greater rates of radial growth, but a much smaller increase in dry weight. It was isolated approximately twice as frequently as *Botrytis*, and this may indicate that it is a better competitive saprophyte in the initial stages of colonization of a substrate. Wastie (1961) showed that there was a good correlation between saprophytic colonization and relative growth rates of competitors on agar plates.

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 produced 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, especially of dead leaves. Kjøller and Struwe (1980) found that cellulose decomposing ability was always associated with the ability to degrade either pectin or starch. They found pectin utilization 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 (1980) to be capable of chitin utilization. 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 low temperatures was significantly high. Cellulase production by *Botrytis* at 1°C was 70% of its maximum; comparable values for other species were for *Chrysosporium pannorum* 60%, for *Chaetophoma* 50% and for *Cladosporium sphaerospermum* 40%, all at 1°C (Hurst, 1982). This indicates a marked ability to utilize cellulose even under a cold temperature regime. This factor, coupled with the capacity for growth at low temperature, probably enables the mycoflora to continue litter degradation during winter months. Evidence for significant enzyme production by colonizing fungal species on young leaves would be especially interesting.

Recent attempts at synthesis of tundra data on decomposition (Holding, 1981; Heal and others, 1981; Bunnell and others, 1980) have demonstrated that the role of extracellular enzymes, the ecology of undersnow decomposition and habitat specificity of decomposers are all subjects yet to be properly investigated. Although there is a general understanding of nutrient cycling in tundra ecosystems more detailed information on decomposition and decomposer ecology is needed to explain process limitations.

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