A survey of the mycobiota of a natural Karoo pasture

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ABSTRACT

The survey of a natural Karoo pasture from 1978 to 1982 showed that a wealth and variety of fungi were present in the semi-arid environment. Hyphomycetes and Coelomycetes represented 45.8% and 34.6% respectively of the taxa identified. A total of 135 genera was identified of which Alternaria alternata, Cladosporium spp. and Fusarium spp. of the Hyphomycetes, Phoma spp., Ascochyta spp. and Camarosporium spp. of the Coelomycetes and Leptosphaerulina spp., of the Ascomycetes represented the most prevalent fungi in this order. This survey has shown conclusively that Pithomyces chartarum, which is associated with photosensitivity diseases of sheep, can always be recovered from the veld if the correct isolation techniques are employed. A number of new records for South Africa, as well as undescribed species, have been found, highlighting the necessity of correct methods and intensity of approach.

The original vegetation of the survey area, which according to Acocks (1979, 1988) was grassland, has largely been replaced by karroid veld, and there is general agreement that the process of deterioration is continuing with desertification advancing towards the northeast.

The flora of the region is rich in species (Acocks 1988) but the habitat is unpredictable with patches of temporary pioneer vegetation (Southwood 1977), comprising species such as Tribulus terrestris L., which become established when the first early summer rains fall.

‘Geeldikkop’, the hepatogenous photosensitivity disease of mainly sheep, was first described by Hutcheon (1886). Theiler (1918) showed that ingestion of T. terrestris, especially wilted material, was directly implicated in the aetiology of the disease. He reported the presence of a Colletotrichum sp. on such material and linked it to the disease as a possible cause. According to Watt & Breyer-Brandwijk (1962) the ingestion of T. terrestris causes a condition similar to ‘geeldikkop’ called ‘big head’ reported from Colorado and Texas. The plants are high in saponins and thus inherently toxic. In New Zealand (Thornton & Percival 1959; Thornton & Ross 1959) Pithomyces chartarum from ingested grasses proved to be responsible for the development of hepatogenous photosensitivity and facial eczema, which is very similar to ‘geeldikkop’. Very few researchers have been successful in reproducing ‘geeldikkop’ under field conditions (Van Tonder et al. 1972). Kellerman et al. (1980) were able to show that the combination of P. chartarum and T. terrestris gave histopathological lesions similar to those found during natural outbreaks of the disease.

Fungi from litter in the Karoo have received little attention. Doidge (1950) reported only a few fungi from the Karoo, mainly collected by MacOwan in the Eastern Cape. On Lycium spp., amongst others, Puccinia lycii Kalchbc. was recorded. No fungi were recorded on Tribulus terrestris L. Pithomyces karoo Marasas & Schumann (1972) was published after a study of litter from the Karoo.

Relatively few surveys of mycobiota have been published from South Africa. Eicker (1973) studied the mycobiota of a natural Karoo pasture...
cobiota of *Eucalyptus maculata* leaf litter. Papendorf & Jooste (1974) described five species of fungi from wheat field debris after isolation by the dilution plate method. Eicker (1976) studied the mycobiota of *Panicum coloratum* associated with an outbreak of photosensitivity of sheep for an 11 month period.

Bezuidenhout (1977) studied the hyphomycetes (mitotic fungi, Hawksworth et al. 1995) associated with *Cenchrus ciliaris* L., a fodder grass, over an 11 month period. Van der Merwe et al. (1979) studied the aerospora of an *Eragrostis curvula* (Schrad.) Nees pasture in South Africa.

An interim report based on the present survey of *Pithomyces chartarum* was published stating that a further 315 isolates were tested for sporidesmin production in culture of which most did not produce the toxin (Annual Report 1981).

The present survey was initiated to determine the incidence of *P. chartarum* in natural Karoo pasture at a time when 'geeldikkop' was likely to occur. The original scope of this study was increased considerably when it became apparent that much valuable information could be gained if a general survey of the mycobiota of the area was done.

### 2. MATERIALS AND METHODS

#### 2.1. Sampling, monitoring sites and dates

The survey was conducted at the Grootfontein Agricultural College Farm, Middelburg, Eastern Cape Province. Sampling and monitoring were done over a period of four seasons during which weekly or fortnightly samples were collected. The sampling procedure involved taking samples from up to seven different plants as well as litter, at three points (1978/79) and later in two camps of a hectare each from 1979 onwards (Table 1).

![FIGURE 1.—Weather data for the period 1980/82, Middelburg. Grass minimum temperature, rainfall and wind velocity per day.](image)

**1978/79 survey**

Three sampling points, A, B and C were chosen after completion of a botanical survey of an area where *Tribulus terrestris* occurred. The nature of the communities at the sampling points varied significantly regarding crown cover, basal cover and density.

Point A was situated in a community with a reasonably high density of perennial Karoo bushes. Therefore, the crown cover was such that wind movement between the individual bushes was possible. The basal cover of *T. terrestris* was fairly high but decreased with time.

Point B was situated in a very dense community of perennial Karoo bushes which allowed virtually no wind movement at soil level. Very few *T. terrestris* and other pioneer plants, such as *Galenia sarcophylla*, were present.

Point C was situated in an area where only one *Lycium cinereum* bush of 1.5 m in height was present besides *T. terrestris*. Virtually no other vegetation was present at this point at the onset of the survey.
TABLE 1.—Sampling dates and numbers of sampling units collected

| Summer | Winter | Summer |
|--------|--------|--------|
| Jan.   | Feb.   | Mar.   | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. | No. of sampling units per year |
| 1978   | 3 points |        |      |     |      |      |      | *     |     |      |     | 15 700 | 78/79   |
| 1979   |         | *       | *    | *   |      |      |      |       |     |      |     | 4 050   | 79/80   |
| 1980   | 2 camps |        |      |     |      |      |      | *     |     |      |     | 28 600  | 80/81   |
| 1981   |         | *       |      |     |      |      |      |       |     |      |     |         | discontinued + |

+ The survey was discontinued because of the third successive year of drought and the resulting deterioration of the vegetation.

Weekly samples of litter were collected, including *T. terrestris* when present, after rains had fallen during December 1978. A total of 34 samples of litter and 20 of *T. terrestris* plants were studied during this period of seven months.

**1979/80 survey**

Shifting of plant communities at the points previously chosen necessitated another approach. It was decided to establish two camps (A and B) of one hectare each; the one (A) with a fair cover of *T. terrestris*, the other (B) without. Five sheep were put into Camp A and the following plants were sampled: the Karoo bushes *Galenia sarcophylla*, *G. procumbens*, *Felicia muricata* and *Lycium cinereum* and the grasses *Eragrostis lehmanniana* and *Cynodon incompletus*. Other plant materials sampled were unidentified litter and *T. terrestris*. Initially Camp B contained very little *T. terrestris* and, because of well established stands of perennials such as *Felicia muricata* and *Lycium cinereum*, was less susceptible to invasion by *T. terrestris* and other pioneers. The density of the communities in Camp B was much higher than in Camp A, and *T. terrestris* was only found in the corner of the camp adjacent to Camp A. Very few *Pentzia* spp. and other typical Karoo bushes grew in the two camps.

Sampling took place from December 1979 to the end of March 1980 on a fortnightly basis.

**1980/81 survey**

This survey started in September 1980 and was continued through 1981. A total of 52 weekly samples was collected and studied from each of the two camps. This time every plant species named in the 1979/80 survey was, however, sampled and studied individually. Thus four species of bushes, two species of grasses, litter and, when available, *T. terrestris* were sampled for a full calendar year.

**1981/82 survey**

This survey was a continuation of the 1980/81 survey, and continued to the end of March 1982.

2.1.1. Sampling methods employed

Samples were taken up to a height of 150 mm which corresponds to the vertical zone grazed by merino sheep. Care was taken to lift litter from the soil surface so as to pick up as few eelworms as possible. The camps were sampled at random to obtain representative samples. If wet, due to rain or dew, the samples were sun-dried before packing into paper bags, every sample from each plant species packed separately, and locality, date and species were noted. Samples were then posted to Pretoria which took approximately 10 days.

The sampling units used were individual leaves, leaflets and 10 mm lengths of stems and grass blades. The material was sorted and samples from as many different leaves and stems as was possible were taken. Fifty units from each of the samples were planted out directly on potato carrot agar (PCA) (Johnston & Booth 1983) to which 125 mg/l Albamycin T (Upjohn) had been added prior to autoclaving. Initially some samples from Camps A and B were first washed by shaking in tap water mixed with Teepol (Shell Chemicals) 1:100 in a wrist shaker for 10 minutes to dislodge superficial conidia. The washed material was planted out directly after this treatment. The first five samples collected during the 1980/81 survey were studied this way.

The plates were incubated for a period of seven days at 24°C with intermittent mixed near-UV and daylight fluorescent light from a height of 300 mm on a 12 h/d cycle. The presence of fungi on the material studied was noted and isolations made of *P. chartarum* and other noteworthy fungi. Chemical assays for sporidesmin, the toxin produced by *P. chartarum*, were done according to the method of Marasas et al. (1972) on a number of the isolates. Some of these cultures were also used to produce bulk cultures with which to dose sheep.

2.1.2. Sampling methods which proved inappropriate

2.1.2.1. Spore trapping

A Burkard volumetric spore trap was operated from 26-01-1976 to 26-02-1976 on a 24 hour basis in the toxic camp. Only one conidium of *P. chartarum* was collected (Roux 1977). It was later found that the spore trap had to operate too high above the ground to pick up the conidia released at a much lower level. No spore trap functioning on a suction principle can operate in a sandy environment at a low level. The use of a spore trapping device was therefore not employed further.

2.1.2.2. Exposure of Petri dishes

This technique had the dual advantage that it gave the best indication of how many airborne conidia there were, and isolates obtained in this manner were alive and could be used for sporidesmin assays almost right away. However, the distance between the sampling site at Grootfontein and Pretoria made this an impracticable method. It was noted that under windy conditions the Petri dishes
FIGURE 2.—Hyphomycetes from the fungal survey of the Karoo. A, Cladorhinum foecundissimum: distinct collarette on phialide and conidia in mucilaginous ball; B, Beauveria bassiana: conidiogenous cells with denticles bearing conidia; C, Cerebella andropogonis: conidia with distinct basal pedicels; D, Helicoon sessile: hyaline helicospore on slender conidiogenous cell; E, Pithomyces chartarum: conidia confined to ascostromata of Leptosphaerulina chartarum on blade of Cynodon incompletus; F, Helicomyces rosetum: hyaline helicospores on conidiophore; G, Volatina concentrica: coelomycete-like fungus with stipe, setae and conidia; H, Gyrothrix flagella: flagellum-like recurved setae in whorls; I, G. flagella: conidiogenous cells at bases of setae; J, Taeniolella sp.: characteristically curved conidia; K, Curvularia tuberculata: conidiogenous cell bearing conidium with tubercles. Scale bar: 50 μm.
could be opened for 10 minutes whereas 20 minutes in quiet conditions were needed to give the required results. A larger variety in fungal species was picked up in open patches than amongst dense undergrowth. On the lee side of bushes much fewer conidia could be collected. *P. chartarum* was collected in every Petri dish exposed.

2.2. Identification of fungi

The fungi were initially identified at magnifications of 25 x and 50 x using a Zeiss dissecting microscope. Verification of identifications was done with a similar make of research microscope. Material was mounted in lactophenol (Johnston & Booth 1983) but from 1980 the coelomycetes (mitotic fungi, Hawksworth et al. 1995) were mounted in ammonium hydroxide with 3.5% erythrosin (Sutton 1980) to facilitate identification based on conidiogenesis. Photomicrographs were obtained using an Olympus microscope camera and Ilford Pan F film.

The fungi were identified using standard monographs (Booth 1971; Ellis 1971, 1976; Subramanian 1971; Sutton 1980; Sivanisan 1987).

2.3. Meteorological data

Members of the Agricultural Meteorological Division of the Soils and Irrigation Research Institute stationed at Grootfontein recorded and monitored the weather from a casual station in the vicinity. This was equipped with a Stevenson Screen housing a thermohygrometer to record the daily minimum and maximum temperature, an anemometer, manual and automatic rainfall meters and a grass minimum thermometer.

2.4. Veterinary services

Veterinarians stationed at the Regional Diagnostic Laboratory of the Division of Veterinary Services inspected the sheep from time to time for clinical signs of 'geeldikkop'.

2.5. Flowering plants sampled

*Tribulus terrestris* L. (Zygophyllaceae): known as caltrop in the USA, three-cornered jack in Australia and also as Mexican sand-burr (Watt & Breyer-Brandwijk 1962); is notorious for causing disease in sheep and goats; is a thorny nature; when grazed heavily this species is similar to the smaller Karoo bushes.

*Cynodon incompletus* Nees (Poaceae): a stoloniferous perennial with a sprawling habit similar to *T. terrestris* and *G. sarcophylla*; under adverse conditions the plant is an annual.

*Eragrostis lehmanniana* Nees var. *lehmanniana* (Poaceae): an erect tussock grass which is intensively grazed; usually perennial but it may be annual under adverse conditions.

2.6. Sporidesmin assays

A total of 1005 isolates of *P. chartarum* were made for toxin production testing. Of these, 437 isolates were selected and grown on semisynthetic broth (Di Menna et al. 1970) for three weeks under near-UV and daylight fluorescent tubes on a 12 h/d cycle from a height of 300 mm at 20°C. The extraction procedure described by Masras et al. (1972) was used.

3. RESULTS

3.1. Fungi recorded

3.1.1. From material directly planted out

All mycobiota identified during this survey are listed in the Appendix. Records of genera and species that were new for South Africa are marked.

The main groups and their incidence in relation to the seasons during the 1980/81 survey are given in Table 2. The total number of genera identified and the percentage representation of classes is given in Table 3. Tables 5, 6 and 7 give complete information regarding the percentage occurrence of the majority of identified fungi on particular substrates for the surveys from 1978 to 1981.

Some of the more unusual fungi identified have been illustrated in Figure 2 (Hyphomycetes) and Figure 3 (Coelomycetes). Conidia of *P. chartarum* localized on the ascostromata of *Leptosphaerulina chartarum* are especially noteworthy (Figure 2E).

Weather data recorded from October 1980 to April 1982 are shown on Figure 1. Seasonal fluctuations characterized most of the more prevalent fungi recorded. The seasonal incidences have been summarized in Table 2 where fungi which occurred continuously can be identified as having a peak in a particular season, e.g. summer or winter, as well as on what substrate they occurred. *P. chartarum* occurred frequently during the first years of...
FIGURE 3.—Coelomycetes from the fungal survey of the Karoo. A, Chaetospermum chaetosporum: conidia with hilum and appendages on apical and basal ends of conidium; B, Melanophoma sp.: conidia with distinct epispor; C, Dinemasporium sp.: conidigenous apparatus with collarette on phialide; base of conidium protruding; D, Dinemasporium striogseud: conidia showing apical and basal appendages; E, Septotella junct: conidium with apical mucilaginous appendages and septa clearly visible; F, Pyrenochaeta sp.: longitudinal section through pycnidium showing setae surrounding ostiole; G, Pseudoseptoria sp.: falcate conidia; H, Pseudoseptoria sp.: conidiogenous cell showing developing conidia and (inset) characteristically long neck with multiple annellations; I, cf. Tetranacrium sp.: conidium with more than usual number of divergent arms; J, Sarcinulella sp.: pycnidium with characteristic tendril of conidia enveloped in a mucilaginous tube; K, Sarcinulella sp.: detail of conidial tendril with constriction caused by individual sac (arrowed); L, Pestalotiopsis sp.: conidium with apical three-armed appendage and single basal appendage. Scale bars: A, F, G, J, 50 μm; B–E, H, I, K, L, 10 μm.
the survey, reaching numbers of more than 80% but de-
clined steadily as the drought continued. It could still,
however, be isolated from material in each camp. Ga-
enia procumbens, Felicia muricata and Cynodon in-
completus were the hosts with the highest numbers of Leptosphaerulina sp. recorded throughout the 1980-1981 sea-
son, reaching peaks during the winter months. The
weather kept to the same pattern over the entire survey
and is shown in the record for the period October 1980
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and is shown in the record for the period October 1980
and is shown in the record for the period October 1980
to April 1982 in Figure 1.

Average occurrences of the dominant fungi at the vari-
ous sampling points and areas are presented for the Hy-
phomycetes (Figure 4), for the Coelomycetes and the
genus Leptosphaerulina (Figure 5), the only ascomycete
which occurred continuously for the periods 1978/79, 1979/80
and 80/81.

Sudden fluctuations can be attributed to personal sam-
ping error when someone other than the regular sampler
had collected the samples.

3.1.2. From material planted out after washing (Table 4)

P. chartarum does not, under normal circumstances,
colonize living leaves in the Karoo and usually occurs as

superficial conidia on exposed plant surfaces. Surface ster-
ilization is therefore not an appropriate technique when
looking for this organism. However, the fact that it can
occur as an endophyte would add another dimension to
its versatility as it is already known as a pathogen of rice
(Sutton & Gibson 1977) and a saprophyte.

3.2. Sporidesmin assays

A total of 36 isolates or 7.5% of the 1 005 isolates of
P. chartarum was positive, and the highest yield was 40
mg/l sporidesmin. Most isolates, however, gave 10 mg/l
or less sporidesmin under these conditions. The tele-
morph Leptosphaerulina chartarum also produced 10
mg/l sporidesmin under the standard conditions.

3.3. Photosensitization

Although Merino sheep were kept in at least one sam-
ping area at a time, no photosensitization on a clinical
level was reported. This is supported by the weather data
obtained, which confirmed that no 'danger period' for the
outbreak of photosensitization had occurred according to
the conditions given by Crawley & Woolford (1965).

The Basidiomycetes were not included in the calculation because identification to genus level was not possible.

### TABLE 2.—Main groups of fungi recorded in the 1980/81 survey

| Winter fungi      | On Lycium in Camp A |
|-------------------|---------------------|
| Alternaria spp.   | High on all substrates except Tribulus and litter |
| Aureobasidium spp.| High on Galenia procumbens in Camp A |
| Camarosporium spp.| High on all substrates in Camp B |
| Cladosporium spp. | On Cynodon in Camps A & B |
| Epicoccum purpurascens | High on G. procumbens |
| Leptosphaerulina spp. | High on litter from April onwards in Camp B |
| Rhizoctonia spp.  |                     |

| Autumn fungi      | High on F. muricata and lower plants (Fig. 1) in Camp B |
|-------------------|------------------------------------------------------|
| Fusarium spp.     | On all except Tribulus, Cynodon and Felicia in Camp A; disappeared after autumn in Camp B |
| Metarhizium anisopliae | Peak in late summer in Camps A & B; low on litter, peak on Tribulus in late summer in Camp A |
| Myrothecium spp.  | In Camp A low close to the soil on Galenia sarcophylla and litter; in Camp B high on G. procumbens; lowest on prostrate plants, viz. G. sarcophylla and litter |
| Leptosphaerulina spp. |                     |

| Summer fungi      | Highest on Galenia procumbens, peak in mid-summer, consistent on litter in Camp B; different patterns on the different substrates; in Camp A, the lowest on litter all year round |
|-------------------|------------------------------------------------------|
| Canarosporium spp.| On all substrates except Lycium cinereum in Camp B; slightly higher in winter in Camp A |
| Mycosphaerella spp.| Inconsistent on most substrates, high on Lycium in Camp B |
| Fungi always present |                                             |
| Drechslera spp.   | High on Cynodon, low but present on other substrates in Camps A & B |
| Pithomyces chartarum | Higher in Camp A; always present on all substrates but at very low levels |
| Phoma spp.        | Consistent in Camp B; lowest on G. procumbens in Camp A |
| Stauronema spp.   | Consistent on litter, peaks on Felicia, Eragrostis, Cynodon in Camp B; inconsistent in Camp A |

| TABLE 3.—No. of genera identified and percentage representation of classes during entire survey |
|---------------------------------------------|
| Taxa* | No. of genera | % of total |
|-------|---------------|------------|
| Myxomycetes | 4 | 3.25 |
| Zygomycetes | 5 | 4.07 |
| Ascomycetes | 11 | 8.94 |
| Hyphomycetes | 55 | 44.72 |
| Coelomycetes | 45 | 36.59 |
| Mycelia Sterilia | 3 | 2.44 |
| Total | 123 | |

* The Basidiomycetes were not included in the calculation because identification to genus level was not possible.
4. DISCUSSION

4.1. Fungi recorded

A significant finding of this survey was that the Coelomycetes were abundant and diverse and that the number of genera found was nearly equal to that of the Hyphomycetes (Table 5). The 46 genera of identified Coelomycetes (Appendix) and 63 genera of the Hyphomycetes included 24 genera of the Coelomycetes and four genera and 14 species of Hyphomycetes newly recorded for South Africa (see Appendix). Two new records of Ascomycetes were noted, including one new species, *Leptosphaerulina chartarum* Cec.Roux, which is the teleomorph of *Pithomyces chartarum* (Roux 1985a).

The total of 63 known genera of Hyphomycetes found in this survey is not as low as it would appear when compared with other surveys, for example that of Bezuidenhout (1977), which were done on either irrigated lands or under temperate conditions. The fungi in this survey were collected under conditions not usually considered conducive to the maintenance of an extensive fungal population.

The fungi with consistently high counts were *Phoma* spp., *Alternaria alternata* and *Cladosporium* spp. (Figures 4 & 5). Pugh & Mulder (1971) also encountered *Alternaria tenuis*, *Aureobasidium pullulans*, *Cladosporium herbarum*, *Epicoccum nigrum* and *Phoma typharum* as initial colonizers of *Typha latifolia* L. Populations of *Phoma* spp. increased over the years which could be due to their being better adapted to the increasingly dry conditions. *Ascochyta* spp. and *Camarosporium* spp. increased with time and then levelled off. The incidence of *Bipolaris* spp. (including related genera such as *Drechslera* and *Exserohilum*), *Epicoccum nigrum* and *Pithomyces chartarum* declined over the study years, although these organisms still occurred consistently. The only Ascomycete which occurred consistently was the genus *Leptosphaerulina* which also declined eventually (Table 7). It is possible that *P. chartarum*, which was also present throughout the survey, could have been produced by *L. chartarum*, which was then counted as *P. chartarum* rather than as *L. char-
Asperchyta, Camarosporium, Diplodia, Phoma.

1978/79

Asperchyta
Camarosporium
Diplodia
Phoma

1979/80

Asperchyta
Camarosporium
Diplodia
Phoma

1980/81

Asperchyta
Camarosporium
Diplodia
Phoma

Leptosphaerulina

1978/79

Point A

Point B

1979/80

Point C

Camp A

Camp B

1980/81

LEGEND

0 10 20 30 40 50 60 70

FIGURE 5.—Most prevalent Coelomycetes recorded from 1978–1981 including Leptosphaerulina spp., the only consistent Ascomycete.

tarum, when considering the nature of the sporulation straight from the ascospores. The correlation between the incidences of these two fungi, the ana- and teleomorph (mitotic and meiotic, Hawksworth et al. 1995) states, was therefore most significant.

The plant communities studied contain a wealth of fungi, many previously unrecorded. Noteworthy was the occurrence of albino strains of the common species Alternaria alternata, Cladosporium cladosporioides and Sta-chybotrys chartarum.

The Hyphomycetes (Table 5) occurred widely and were not as restricted regarding substrate as the other groups encountered. Unusually low incidences were, however, noted for species of Aspergillus, Penicillium and Trichoderma.

Coelomycetes (Table 6) recorded on a wide range of substrates were the following: Ascochyta spp., Camarosporium spp., Diplodia spp. and Phoma spp.

The highest incidence of the most prominent genera was noted during autumn and winter (Table 2). This could be explained by the fact that free water in the form of dew and rain was available for longer periods, thus enhancing the growth of fungi. Grass minimum temperatures recorded were substantially lower in winter than in summer. Highest rainfall occurred during late summer and autumn, seasons in which the wind tended to subside (Figure 1), thus reducing evaporation.

Nematophagous fungi, such as Dactylella and Candelabrella spp., were found. Large numbers of eelworms were inadvertently picked up with some of the samples and interfered with the counting of the fungi present on the substrate studied.

The entomophagous fungi Beauveria bassiana and Metarhizium anisopliae were frequently found but only in small numbers. B. bassiana is an important component of a complex of natural enemies of the Karoo caterpillar Loxostege frutalis Zeller (Möh 1982). During the survey the Middelburg District experienced drought for three successive years. Consequently the ground cover decreased drastically and the unstable sandy soil was disturbed by wind and hoof action. The conidea of B. bassiana, associated with the early subterranean pupal stage of the karoo caterpillar, were therefore set free into the atmosphere in increasing numbers.

The increase in the number of species of Hyphomycetes from 1980 onwards can also be attributed to the worsening drought conditions which resulted in greater amounts of litter being deposited. The litter became very rich in fungi which would otherwise probably not have been isolated, as the litter fraction represented all the plant material available at the various sampling points and thus included all plant species not sampled separately. It is, therefore, understandable that mycobiota of litter should be much more varied than those of single plant species.

Aspergillus flavus deserves special mention. This toxicogenic fungus was very common in animal feeds from all over South Africa examined for mycotoxicological fungi during the entire survey period (Roux 1985b), but it was not recorded in the present survey during the normal rainy season of 1978/79.

In the initial trial run during which material was planted out after washing, P. chartarum was found to be an endophyte. This is even more significant in the light of the subsequent discovery of the teleomorph. Thus P. chartarum, or L. chartarum as it should now be known, can survive unsuitable conditions protected by the leaves of live plants and possibly sporulate when they die. The fact that the conidial stage of L. chartarum was found in tissues from all live plants studied is most significant.

Due to the large number of samples and the primary emphasis on Pithomyces chartarum, species of common genera such as Fusarium, Bipolaris and Leptosphaerulina were not recorded separately. The most common species of Fusarium was F. moniliforme followed by F. subglutinans. In the Bipolaris group the following species were identified: B. cynodontis, B. halodes, B. hawaiensis, B. papendorfii, B. zeicola, Drechslera phlei and Exserohilum rostrata. B. halodes was the most prevalent.

Hering (1965) stated that though he had isolated a number of Ascomycetes and Coelomycetes, they failed to grow on the isolation medium. Experience obtained during this study showed that any bacteriostatic agent other than a few drops of lactic acid per Petri dish could completely inhibit the growth of some Coelomycetes. This could explain why the numbers of the Coelomycetes re-
## TABLE 5.—Percentage of the total number of samples taken for the Hyphomycetes (1978–1981)

| Year    | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C | Point A | Point B | Point C |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1978/79 | 15 700  |         |         | 4 050   |         |         |         | 28 600  |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
### TABLE 5: Percentage of the total number of samples taken for the Hyphomycetes (1978–1981) (continued)

| Year | Point A | Point B | Point C | Camp A | Camp B | Camp A | Camp B |
|------|---------|---------|---------|--------|--------|--------|--------|
| 1978/79 | 15,700 | - | - | - | - | - | - |
| 1979/80 | 4,050 | 1,5 | 1,0 | - | - | - | - |
| 1980/81 | 28,600 | - | - | - | - | - | - |

| Hyphomycete | 1978/79 | 1979/80 | 1980/81 |
|-------------|---------|---------|---------|
| Monacrosporium sp. | 0.4 | 0.1 | 0.7 |
| Monilia stiophila | - | - | - |
| Myrothecium spp. | 23.9 | 17.8 | 10.7 |
| Nigrospora oryzae | 0.3 | 0.1 | 0.1 |
| Oedoeclaphalum sp. | 1.2 | 1.5 | 0.4 |
| Paecilomyces sp. | 0.2 | 0.1 | 0.1 |
| Parapericonia augusti | - | - | - |
| Penicillium spp. | 0.7 | 0.8 | 1.4 |
| Periconia spp. | 4.0 | 0.2 | 0.9 |
| Pithomyces atro-olivaceus | - | - | - |
| charrarum | 2.1 | 25.1 | 4.1 |
| cynodontis | - | - | - |
| graminicola | - | - | - |
| karoo | 0.5 | 0.1 | 2.1 |
| maydicus | - | - | - |
| Rhinocladiella spp. | 0.1 | 0.4 | 0.1 |
| Scopulariopsis brevicaulis | - | - | - |
| Spegazzinia parkeri | - | - | - |
| tesratha | 0.4 | 0.1 | 0.5 |
| Stachybotrys chartarum | - | - | - |
| Stemphylium botryosum | - | - | - |
| borysium | - | - | - |
| vesicularium | 0.2 | 0.6 | 0.1 |
| Taeiocladiella spp. | 3.0 | 1.1 | 0.8 |
| Tetráploa elissi | - | - | - |
| Torula herbarum | 0.3 | 0.3 | 0.6 |
| Trichoderma sp. | 0.1 | 0.4 | 0.4 |
| Trichothecium roseum | - | - | - |
| Ulocladium atrum | - | - | - |
| chartarum | 0.1 | 0.1 | 0.7 |
| Volutella colletotrichoides | 1.5 | 0.7 | 0.6 |
| Volutina sp. | 0.3 | 0.1 | 0.5 |
| Unknown | - | - | - |
| CR 20 | 0.1 | - | - |
| Hyphomycete no. 1 | - | - | - |

CR20: Percentage of the total number of samples taken for the Hyphomycetes (1978–1981) (continued)
| Species                          | 1978/79 | 1979/80 | 1980/81 |
|---------------------------------|---------|---------|---------|
| **Amerosporium sp.**            | 0.3 2.9 | 0.7 0.5 | 0.2 0.4 |
| **Ascochyta sp.**               | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Bartalinia sp.**              | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Camposporium sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Chaetodiplodia sp.**          | 2.1 2.2 | 1.6 1.1 | 1.5 1.1 |
| **Chaetospermum sp.**           | 1.9 1.4 | 1.6 1.1 | 1.5 1.1 |
| **Diplodia sp.**                | 1.9 1.4 | 1.6 1.1 | 1.5 1.1 |
| **Eriopsis sp.**                | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Eriopsis sp.**                | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Gelatinosporella sp.**        | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Hendersonia sp.**             | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Hendersonia sp.**             | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Idioceras sp.**               | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Macrophomina sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Mellinonema sp.**             | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Neotitiospora sp.**           | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Pestalotiopsis sp.**          | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Phoma sp.**                   | 34.9 90.5 | 34.9 90.5 | 34.9 90.5 |
| **Phomopsis sp.**               | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Pleurothrysm sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Polyspora sp.**               | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Pyrenochaeta sp.**            | 1.9 1.4 | 1.6 1.1 | 1.5 1.1 |
| **Septoria sp.**                | 1.9 1.4 | 1.6 1.1 | 1.5 1.1 |
| **Septoria sp.**                | 1.9 1.4 | 1.6 1.1 | 1.5 1.1 |
| **Sphaerotheca sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Stagonospora sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Sturnospora sp.**             | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Tetranychium sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
| **Triatrichium sp.**            | 0.3 2.9 | 0.4 0.7 | 0.2 0.4 |
TABLE 7.—Percentage of the total number of samples taken for the Ascomycetes, Zygomycetes, Mycelia Sterilia, Myxomycetes and unknown fungi (1978–1981)

| Year    | Samples taken | Camp A      | Camp B      | Camp A      | Camp B      | Camp A      | Camp B      | Camp A      | Camp B      |
|---------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 1978/79 | 15,700        | 0.7-0.1     | 0.5-0.7     | 0.4-0.4     | 0.3-0.2     | 0.7-0.7     | 0.1-0.3     | 0.4-0.4     | 0.4-0.4     |
| 1979/80 | 4,050         | 2.2-1.9     | 3.0-1.9     | 0.7-1.9     | 0.1-0.3     | 2.2-10.0    | 0.4-0.4     | 1.2-0.2     | 0.7-0.7     |
| 1980/81 | 28,600        | 1.1-0.4     | 2.1-0.7     | 1.9-0.7     | 0.1-0.3     | 3.0-1.5     | 1.4-0.7     | 1.2-0.2     | 0.1-0.3     |

Ascomycetes
- Ceratocystis sp.
- Chaetomium spp.
- Leptosphaeria spp.
- Leptosphaerulina spp.
- Microsporaella spp.
- Ophiobolus circis
- Platyphora pirumia
- Pleospora herbarum
- Sarcobolus sp.
- Sordaria fimicola

Zygomycetes
- Cunninghamella sp.
- Mortierella spp.
- Mucor spp.
- Rhizopus stolonifer
- Rhizomucor sp.

Mycelia Sterilia
- Papulospora sp.
- Puccinia graminis
- Ustilaginales

Unknown
- Ascomycetes
- Discomycetes
- Coelomycetes sp. 1
- Coelomycetes sp. 2
- Coelomycetes sp. 3
- Coelomycetes sp. 4
- Coelomycetes sp. 5
- Coelomycetes sp. 6
- Mycelia Sterilia

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ported in other surveys of fungal populations are negligible.

Dickinson (1967) could correlate an increase in frequency of Stemphylium botryosum with records of its perfect state, Pleospora herbarum, on Pisum leaves. In the present survey the relation between Leptosphaerulina chartarum and its anamorph only became clear after conclusion of the sampling programme. All specimens of Leptosphaerulina were not identified to species level. It can, however, be assumed that L. chartarum was more prevalent at times when incidences of Pithomyces chartarum reached peaks, e.g. late summer and early winter (February to May), seeing that P. chartarum and Leptosphaerulina spp. were more prevalent then.

4.2. Photosensitization

Crawley & Woolford (1965) stipulated a minimum temperature of 12.2°C or more on three consecutive days together with 3.76 mm of rain as a danger period for the development of the facial eczema in sheep. The same conditions were assumed to be necessary for the development of 'geeldikkop' in local sheep. No such conditions were recorded and no cases of photosensitization on the sampled pastures were reported.

Another factor which could play a role was the presence of saponins in the T. terrestris plants (Watt & Breyer-Brandwijk 1962). Aas & Ulvund (1989) speculated that P. chartarum, especially the sporidesmin present on bog asphodel and saponins, may be involved in the aetiology of alveld (a hepatogenous photosensitivity) in Norway. Since then, Kellerman et al. (1991) have shown that saponins on their own are able to induce hepatogenous photosensitivity in some sheep. The importance of sporidesmins has, however, not diminished as all sheep in that trial did not react positively. Kellerman et al. (1991) found that fresh T. terrestris, both on its own and with sporidesmin, caused 'geeldikkop' in sheep.

5. CONCLUSIONS

The survey highlights the wealth and variety of fungi found in this inhospitable environment. The large numbers of genera found is due to the wide range of materials sampled. A peculiarity was that virtually the same number of genera of Coelomycetes and Hypothromycetes was found. Nag Raj (1981) noted that Coelomycetes were more prevalent in dry climates, a fact which has been confirmed here. This phenomenon can be attributed to the adaptation of the fungus in shielding its conidiogenous cells and hyaline conidia from the high UV-radiation in the predominantly cloudless Karoo region by developing a conidioma. Very few synnematous genera of the Hypothromycetes were recorded. An analogue in the Hyphomycetes is the protective mechanism of melanin, because a great proportion of the species present have melanized conidia.

This is the first survey in southern Africa in which such a high proportion of fungi identified belonged to the Coelomycetes. The invidual genera could be determined to a great extent using Sutton's keys (1980). Numerous new records for South Africa were registered.

The suitability of litter as a substrate for fungal growth, even under these harsh climatic conditions, was an indication of the role fungi play as agents in the breakdown of organic matter. The wide spectrum of fungal genera noted on the litter gave an indication of what was present on substrates not sampled separately.

This survey demonstrated the persistent presence of Pithomyces chartarum on various substrates in the Karoo. This is a very important finding in view of its toxicity. The teleomorph of this fungus, Leptosphaerulina chartarum, was found during this study (Roux 1985a). P. chartarum was recovered from T. terrestris leaves without lesions. This possible endophytic symbiosis of certain strains may indicate its mycotoxicological, opposed to pathogenic (Haware & Sharma 1973) nature and also of the existence of purely saprophytic strains. This survey illustrates the importance of intensive studies of fungal populations.

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The actual fungus which was recorded is cited, not the sexual phase (teleomorph) if it was not found, as is recommended in the Rules of Nomenclature. *New genera for South Africa. **New species for South Africa

**HYPHOMYCETES**

Acremoniella atra (Corda) Sacc. vernucosa Tognini

Alternaria alternata (Fr) Keissl. zinniae M B Ellis

Arthrobotrys superba Corda

Arthrinium saccharicolana (Speg.) M B Ellis

Aspergillus candidus Link

flavus Link

nidulans Eidam

niger Tiegh.

tereus Thom. spp.

Aureobasidium pullulans (de Bary) Arnaud spp.

Beauveria bassiana (Bals.-Criv.) Vuill.

Bipolaris cynodontis (Marjorani) Shoemaker

hawaiensis (M B Ellis) Uchida & Aragaki

papendorfii (Alcorn) Alcorn

reicola (Stout) Shoemaker

Botrytis state of Sclerotinia fuckeliana (de Bary) Fuckel sp.

Candelabrella sp.

Cephalosporium sp.

Cercospora sp.

Cerebella andropogonis Ces.

Chrysosporium sitophila (Mont.) Arc (= Monilia sitophila Mont.)

Chlororhocha andropogonis Ces

Cladorrhinum foecundissimum

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Cladosporium
cladosporioides (Fresen.) G.A.de Vries
herbarum (Pers.) Link
variable (Cook) G.A.de Vries

Sporidesmium
Spegazzinia

cf. Septofusidium elegantulum
Scopulariopsis brevicaulis

Oedocephalum glomerulosum

Dactylella sp.
Dichotomophysora portulacae Mehrl. & Fitzp. ex M.B.Ellis*

Doratomyces
stemonites (Pers. ex Fe) F.J. Morton & G. Sm.
phlei (Graham) Shoemaker

Epicrocum nigrum Link
Exserohilium rostratum Leonard & Suggs
Fusariella cf. obsipha (Pollack) S.Hughes

Fusarium
acuminatum Ellis & Everh. sensu Gordon
equesti (Corda) Sauz. sensu Gordon
moniliforme E.Shelden
semitectum Berk. & Ruvanel
stoveri Booth*

subglutinans (Wollenw. & Reinking) P.E.Nelson, Tousson & Marasas

Gliocladium
penicillioides Corda
roseum Bainier

Gonatobotrys simplex

Hyalodendron lignicola

Helicomyces roseus

Hendersonia sp.

Idiocercus macarangae

Hendersonia sp.

Trichoderma

Hyphomycetes

carmicheli Grev.
cinctum (Corda) Sauz.**

Hydendron lignicolae (Diddens) de Hoog
cf. Lacellina macrospora (Berk. & Broome) Petch**

Mesorhizium sindicoi (Metchn.) Simón

Monacrosporium sp.

Monasaniella sp., conidial state

Monilia sp., conidial state

Myrothecium

Penicillium

Parapericonia angustii M.B.Ellis*
Penicillium

Periconia

Phaeosphaeria
byssoides Pers. ex Mérat
cookei E.W.Mason & M.B.Ellis
cf. madreyana Subram.

Phomopsis

Pleurothecium

Pleurothecium
tuberculatum (Preuss) E.G.Simmons

Pleurotus
cinctum (Pers. & Fr.) Grove

gloeosporioides (Pers.) Sauz.

Polystigmina rubrum

Polystigmina rubrum

Pseudomonas

Pseudomonas

Pseudomonas

Pseudomonas

Pseudomonas

Phaeosphaeria

Phaeosphaeria

Phaeosphaeria

Polystigmina rubrum

Polystigmina rubrum

Phoma

Pleurothecium
tuberculatum (Preuss) E.G.Simmons

Pleurotus
cinctum (Pers. & Fr.) Grove

gloeosporioides (Pers.) Sauz.

Polystigmina rubrum

Polystigmina rubrum

Phoma

Pleurothecium
tuberculatum (Preuss) E.G.Simmons

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Pleurotus
cinctum (Pers. & Fr.) Grove

gloeosporioides (Pers.) Sauz.
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Sarcinulella cf. banksiae B.Sutton & Alcorn*
Seimatosporium sp.*
Septoria sp.
Septoriaella*
jugi (Desm.) B.Sutton**
sp.**
Sphaeropsis sp.
Stagonospora sp.
Stauronema spp.*
cf. Tetraneurium gramineum H.J.Huds. & B.Sutton*
Tiarosporella graminis (Piroz. & Shoemaker) Nag Raj var. karoo B.Sutton & Marasas
Tryblidophyhcis sp.*
Urohendersonia platensis Spec.*

MYCELIA STERILIA (Agonomycetes)
Papulospora sp.
Rhizoctonia sp.
Sclerotium rolfsii Sacc.

ZYGOMYCETES—Mucorales

Actinomucor elegans Shostakowitz
Cunninghamella echinulata (Thwct.) Thaxt.
Mortierella sp.
Mucor sp.
Rhizopus stolonifer (Ehrend.:Fr.) Vaill. var. stolonifer
Rhizomucor spp.

ASCOMYCETES

Ascotricha sp.
Ceratocystis sp.

Chaetomium
globosum Kanze
sp.
Leptosphaeria spp.
Leptosphaerulina
briosiana (Poll.) J.H.Graham & Luttrell
chartarum Cec.Roux**

Mycosphaerella
tassiana (Desm.) Johnson
sp.
Ophiobolus sp.

Pezizales (unidentified)
Platyospora plendula (Cooke) Wehm. = Comoclathris Clem.**
Pleospora
herbarum (Pers. ex Fr) Rabenh.
sp.
Saccobolus minimus Vel.
Sordaria fimicola (Roberge) Ces. & De Not.

BASIDIOMYCETES

Agaricoles
Aphyllophorales
Coprinus spp.
Puccinia graminis Pers.
Ustilaginales

MYXOMYCETES

Didymium sp.
Physarum cinereum (Batch.) Pers.
cf. Reticularia sp.
Stemonitis cf. smithii T. Macbr.